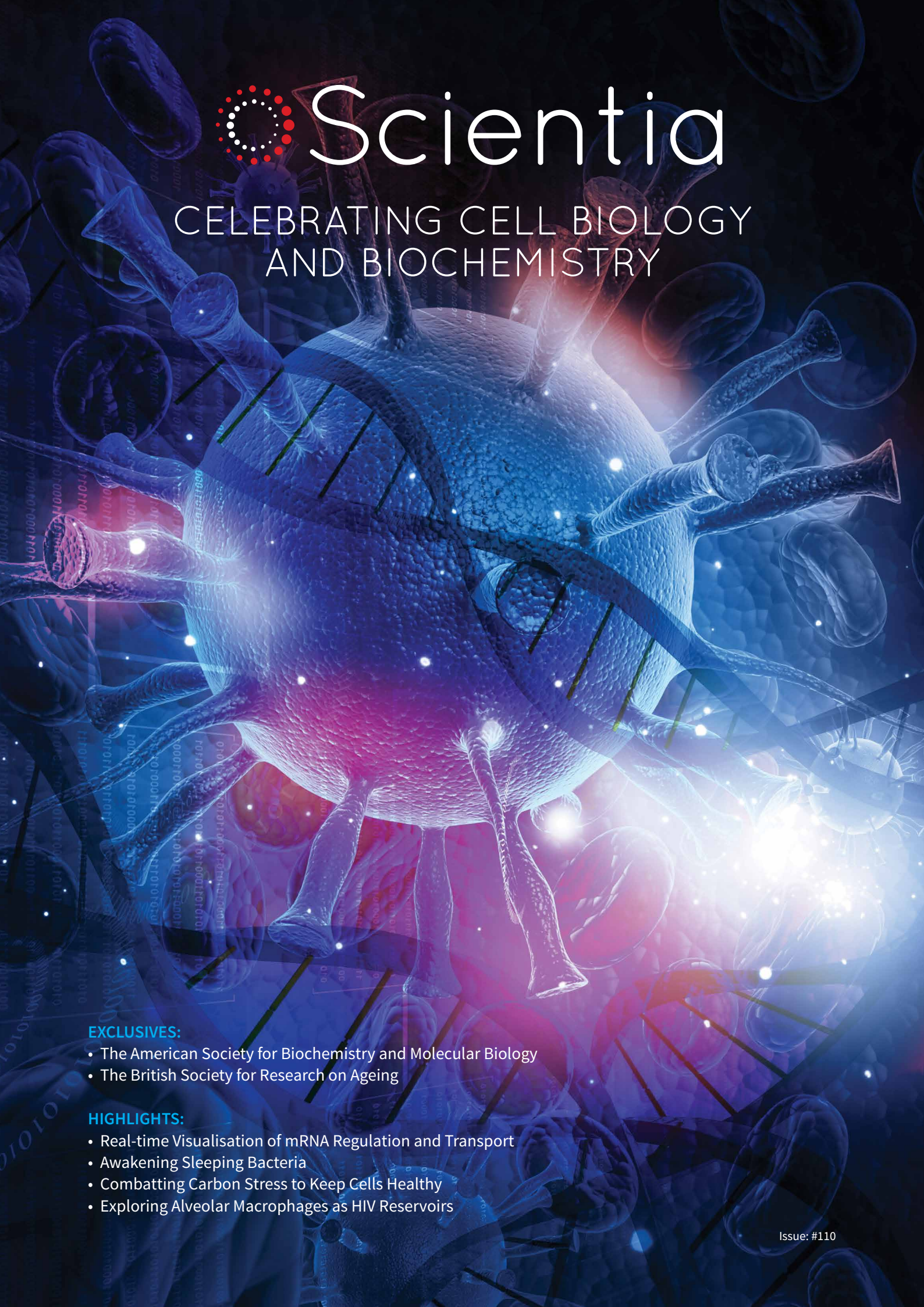


# Scientia

## CELEBRATING CELL BIOLOGY AND BIOCHEMISTRY

**EXCLUSIVES:**

- The American Society for Biochemistry and Molecular Biology
- The British Society for Research on Ageing

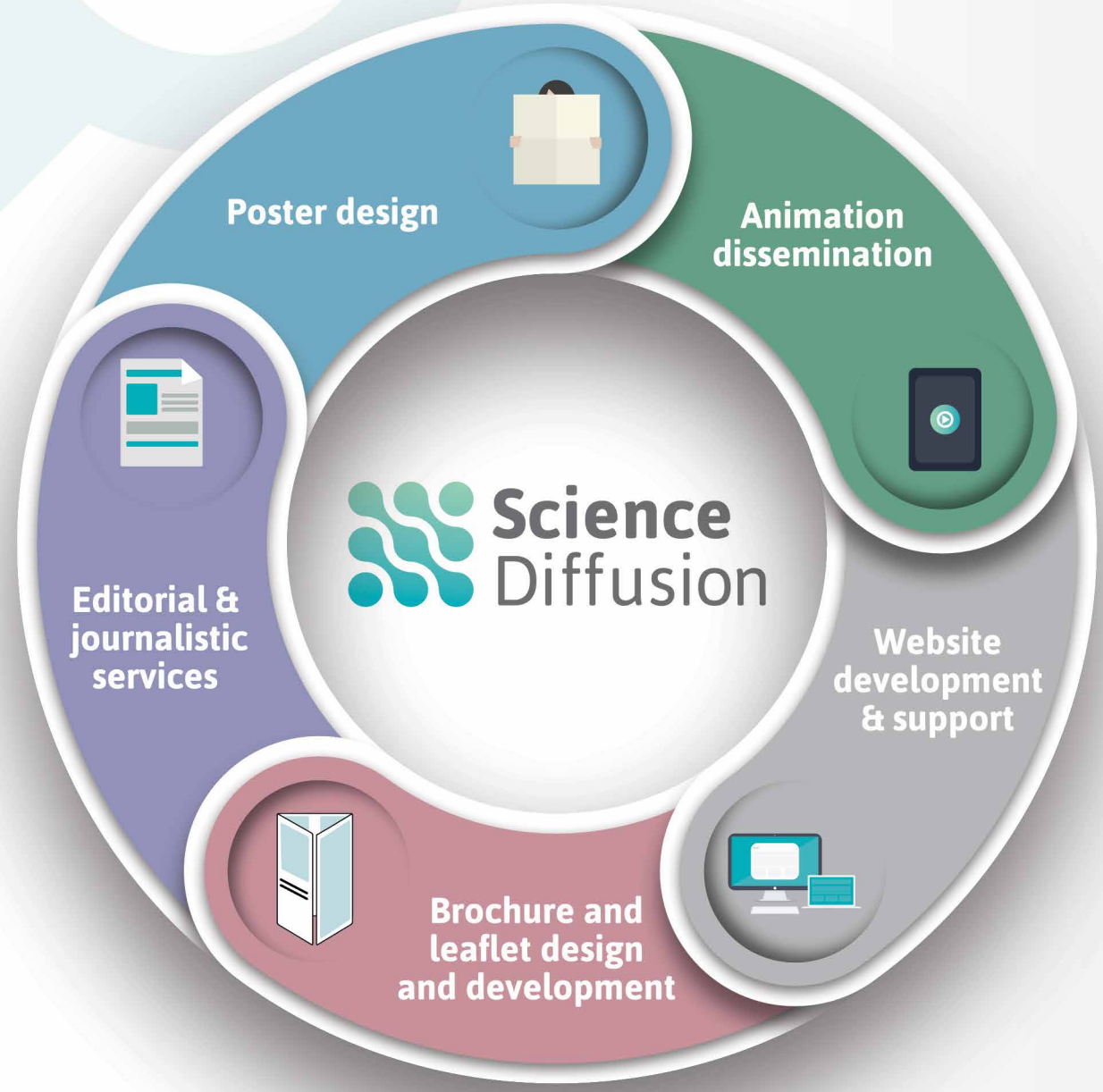
**HIGHLIGHTS:**

- Real-time Visualisation of mRNA Regulation and Transport
- Awakening Sleeping Bacteria
- Combatting Carbon Stress to Keep Cells Healthy
- Exploring Alveolar Macrophages as HIV Reservoirs

Issue: #110

# Do you want to increase the **visibility** and **accessibility** of your **research?**

**Contact us today** to discuss your dissemination needs and find out how we can help.




**W:** www.sciencediffusion.com **E:** info@sciencediffusion.com

# WELCOME...

Cell biologists, biochemists and molecular biologists alike have illuminated many of the microscopic mechanisms essential to life on Earth. Because of their tireless efforts, we now understand the most fundamental processes that characterise living cells, such as the replication of DNA and its translation into proteins with specific amino acid sequences. This insight has given us a molecular basis for how the first unicellular life forms evolved into the vast multitude of different species we observe today. Furthermore, by elucidating the human genome, scientists have greatly enhanced our ability to pinpoint molecular aberrations responsible for even the most complex of genetic diseases.

Beyond the genetic code, a deep understanding of the structure and function of proteins has also enabled us to discover how many diseases can arise, such as sickle cell anaemia. Similarly, knowing the biochemistry of lipids has enlightened our understanding of atherosclerosis, while a molecular understanding of carbohydrate metabolism has further elucidated diseases like diabetes. Insight into these complex processes has made the rational design of new drugs possible, such as molecules that inhibit the enzymes required for viruses to replicate. Furthermore, the ability to detect certain biomolecules associated with disease has greatly enhanced our medical diagnostics.

In this fascinating edition of Scientia, we celebrate the ways in which these disciplines have illuminated our understanding of life on Earth in addition to advancing our healthcare. To open the issue, we have had the pleasure of speaking with Dr Erica Siebrasse of the American Society for Biochemistry and Molecular Biology (ASBMB), who discusses the organisation's dedication to accelerating research in these fields. Next, we feature an article about the Dresden International Graduate School for Biomedicine and Bioengineering (DIGS-BB) – a PhD training program based at TU Dresden that combines cutting edge research projects with innovative mentoring strategies.

Following an interview with our charity partner, Trees for Cities, we then move on to highlight the latest in genetics research. Here we feature eight fascinating projects, ranging from investigating the genes responsible for parenting behaviour, to developing new DNA fingerprinting techniques for ensuring quality in medicinal cannabis production. Next, we showcase the work of three researchers, each dedicated to improving our health into old-age, by investigating the biochemistry behind the ageing process. Our final two sections in the magazine highlight ground-breaking discoveries in the fields of both breast cancer and HIV research. By investigating the nuts and bolts of these diseases at the cellular and molecular levels, the scientists behind these projects are opening the door to targeted therapies.

Science Diffusion

## CONTACT

**Published in the UK, by**
**Science Diffusion ltd**

ISSN 2059-8971 (print)
ISSN 2059-898X (online)

**E:** info@sciencediffusion.com
**W:** www.sciencediffusion.com
**W:** www.scientia.global

@scientia_social
www.facebook.com/sciencediffusion/

Barrier Free

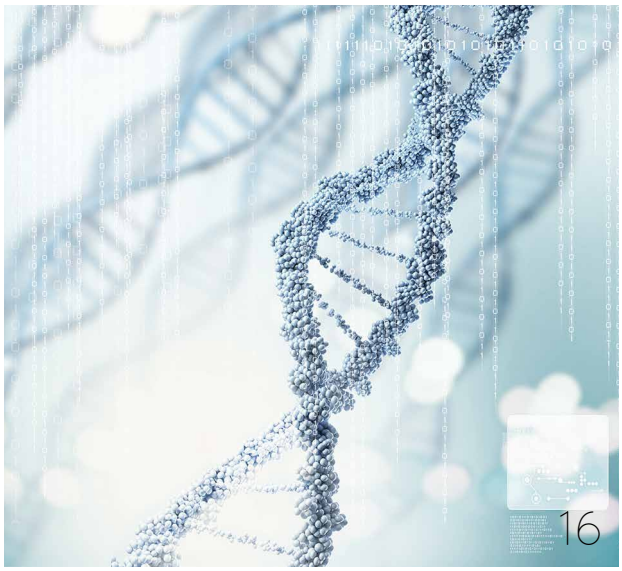


# Meet The Team...

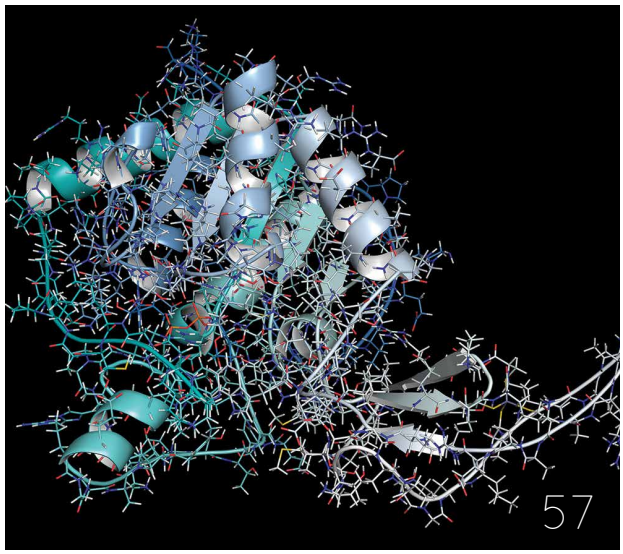
# CONTENTS

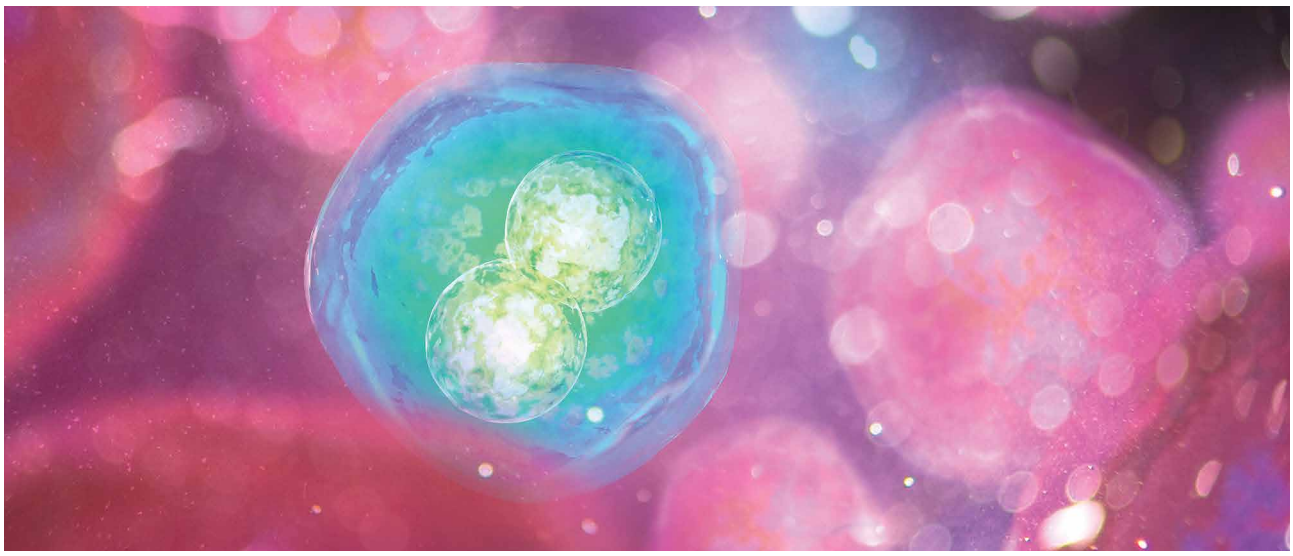
ISSUE : #110











# THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

Representing over 12,000 members worldwide, the American Society for Biochemistry and Molecular Biology (ASBMB) has been advancing the fields of biochemistry and molecular biology for the last 100 years. Over the next few pages we have had the pleasure of speaking with **Dr Erica Siebrasse,** Education & Professional Development Manager at the ASBMB, who tells us all about the organisation. From disseminating the latest scientific research to increasing science literacy and promoting cultural diversity in these disciplines, here we gain great insight into the organisation's activities.

**To start, please tell us what ASBMB is all about. How was the society founded and what is its mission?**

The ASBMB is a non-profit scientific and educational organisation with over 12,000 members. It was founded in 1906 as the American Society of Biological Chemists by a small group of biochemists, many of whom had participated in the launch of the Journal of Biological Chemistry in 1905. The society advances the science of biochemistry and molecular biology by publishing scientific journals, organising scientific meetings, advocating on Capitol Hill for basic research, supporting science education, and promoting the diversity of individuals in the discipline.

**What are the benefits of being an ASBMB member? Are there any grants and scholarships available that our readers might be interested in applying for?**

The ASBMB has supported the careers and research of biochemists and molecular biologists for more than 100 years. All members receive access to top research through subscriptions to the society's three journals and the member magazine, and regular members receive substantial publication discounts. All members also receive registration discounts to society meetings, including the annual meeting, specialised small meetings and career workshops. The ASBMB offers substantial and numerous travel awards to the annual meeting and connects thousands of undergraduate students and faculty through its Student Chapters program. Student Chapters provides grants and scholarships to support education, research and outreach. Finally, the society offers members excellent online and in-person career resources.

**Tell us about ASBMB's goal to increase cultural diversity within the fields of Biochemistry and Molecular Biology, and give examples of how the society is actively achieving this.**

The Minority Affairs Committee (MAC) leads the ASBMB's efforts to promote diversity in biochemistry and molecular biology. The MAC sponsors a National Science Foundation-funded annual grant-writing workshop for early-career scientists preparing for independent positions, a transition shown to be a critical step in long-term success. The committee supports underrepresented students through travel awards to the annual meeting and undergraduate scholarships for students who enhance the diversity of science. The MAC also highlights scientists from underrepresented backgrounds in the 'Research Spotlight' interview series and connects those interested in promoting diversity within the community through the 'Partnership for Diversity'. Finally, the committee recognises scientists committed to diversity and effective mentorship of underrepresented students with the annual Ruth Kirschstein Diversity in Science award.

**Tell us a bit about your journals, and the types of research you publish in each one.**

The ASBMB publishes three journals: the Journal of Biological Chemistry, Molecular & Cellular Proteomics and the Journal of Lipid Research. The JBC is one of the top-cited journals in the world and is the most significant contributor to the field of biochemistry and molecular biology. It also boasts a rapid review time. MCP fosters the development and applications of proteomics in both basic and translation research. It showcases cutting-edge advances in proteomics, metabolomics and bioinformatics. Finally, the JLR focuses on the science of lipids in health and disease. It publishes new insights into mechanisms of lipid function and metabolism and/or genes regulating lipid metabolism. All three journals have no submission fees, and authors receive fair, thorough and constructive peer review from practicing scientists.

**What is the Public Affairs Advisory Committee (PAAC) of the ASBMB, and what exactly do they do?**

The PAAC advocates on behalf of ASBMB members to lawmakers and federal science agencies and advises society leadership on public affairs issues. The committee supports and trains ASBMB members to engage with their congressional representatives by sponsoring an annual Capitol Hill day and the 'August is for Advocacy' event, among other events. It also supports science-policy training through its fellowship program, which brings PhD-level scientists to work with the public affairs team at our headquarters. The society maintains a policy blog and contributes regular articles in the member magazine to keep members informed of policy issues affecting them. Finally, the PAAC supports the Howard K. Schachman Public Service Award, which recognises an individual who best demonstrates dedication to public service in support of biomedical science.

**Describe some of the many other ways that ASBMB supports Biochemistry and Molecular Biology research and education in the US and further afield.**

Beyond the many member benefits, publications, meetings, advocacy and diversity efforts already mentioned, the ASBMB is a leader in biochemistry and molecular biology education, professional development and community outreach.

The ASBMB Education and Professional Development Committee leads several initiatives that demonstrate the society's commitment to education, including the accreditation and concept-driven teaching programs. The ASBMB accredits departments and programs that offer bachelor's degrees in biochemistry, molecular biology or related subjects. ASBMB accreditation recognises programs whose features and infrastructure fulfil the basic expectations of the society. The society recently ended an NSF-funded concept-driven teaching project that brought together experienced and early-career educators to learn about and develop resources for course materials, assessment tools and teaching the foundational concepts of biochemistry and molecular biology. The ASBMB Student Chapters program, led by a steering committee of the same name, provides networking and career-development opportunities, access to research and science outreach, as well as grants and awards to facilitate these aims to more than 2,000 undergraduate student and faculty members.

## 'The ASBMB is a leader in biochemistry and molecular biology education, professional development and community outreach'

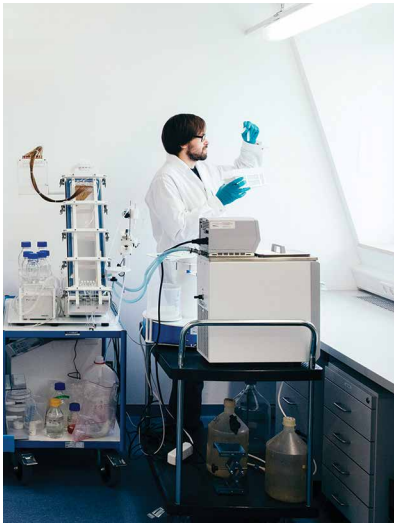
Over the past year, the EPD has created a new set of excellent career resources to better serve members' professional-development needs. The society maintains a job board and a careers blog that rounds up job opportunities in specific subfields. The EPD also offers career-development training through webinars, video tutorials, online courses, articles and in-person workshops, all of which are either complimentary or low cost. Those interested in careers in biochemistry and molecular biology can learn about jobs through a comprehensive set of online resources. Finally, the ASBMB maintains a national online database of summer research opportunities for undergraduates.

The ASBMB has shown its commitment to science literacy through the work of its Public Outreach Committee, which provides training, resources and opportunities for ASBMB members to get involved with informal education and science outreach in their local communities. In addition to providing funds to support outreach efforts, the committee maintains a database of outreach opportunities, hosts outreach and training events and offers communication training and online outreach resources.

**Finally, what do you see as the biggest challenges to Biochemistry and Molecular Biology research worldwide within the next 10 years?**

Consistent funding and support for basic biomedical research is necessary to maintain and build upon the excellent biochemistry and molecular biology research of the past. Economic issues have challenged many countries to adequately support basic science and its workforce. The need to continually write grants and publish papers constrains the amount of time investigators have to train the future generation of scientists, who are faced with a challenging job market. While unemployment rates for scientists remain low, the vast majority of PhD-level scientists will not land tenure-track positions, although most are still trained (sometimes exclusively) for this path. Efforts to broaden training experiences are increasing, especially in the US. Despite these challenges, the biochemistry and molecular biology community continues to produce ground-breaking discoveries.

# DIGS-BB: SETTING THE STANDARD FOR MODERN PHD TRAINING



Over the past decade, the **Dresden International Graduate School for Biomedicine and Bioengineering (DIGS-BB)** has risen to the forefront of PhD student training. The program combines cutting edge research projects with innovative mentoring strategies to train well-rounded, interdisciplinary scientists, poised to be leaders in the global scientific community.

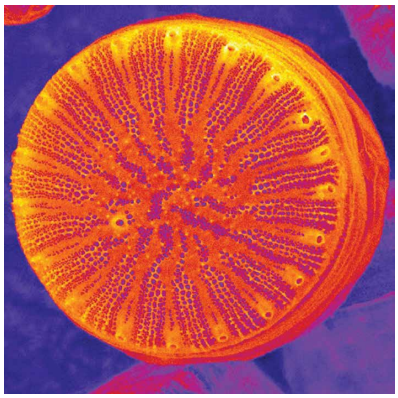
**The Formation of DIGS-BB at TU Dresden**

In 1828, the TU Dresden first opened its doors as the Saxon Technical School, a college devoted to the education of mechanics and engineers. Over the next 100 years, the college grew and diversified in subjects, officially becoming the Technische Universität Dresden (TU Dresden) in 1961. In 2006, the TU Dresden successfully applied to the German Excellence Initiative, a federal program aimed at promoting cutting-edge research and exemplary scholarly training for young scientists. The TU Dresden is now one of only 11 excellent ranked universities in Germany. One of the achievements to become an excellent university was the establishment of the Dresden International Graduate School for Biomedicine and Bioengineering (DIGS-BB). Since 2006, students of the DIGS-BB have generated over 792 publications with an average impact factor of 8.4. Of these publications, 462 had students as first authors and 263 were in collaboration with other research groups from the DIGS-BB or local partner PhD programs.

The DIGS-BB has quickly become a world leader in PhD training for biomedicine and bioengineering, coupling innovative research with unsurpassed mentoring and training. The program admits 15–25 new PhD students twice a year, and the current student body represents 27 nationalities covering a wide range of educational backgrounds. Students enrolled in the program benefit from extensive collaborative relationships with mentors and scientists in labs within the Dresden life science campus.

**Leading the Way in Biomedical and Bioengineering Research**

Research at the DIGS-BB focuses on six interdisciplinary fields: Developmental Cell Biology, Biomedicine, Regenerative Biology, Biophysics, Bioengineering & Biomaterials, and Computational Biology. These disciplines are tied together by the desire to understand how organisms form, function, and self-repair on different molecular scales, from individual cells to whole organisms, with the ultimate goal of translating findings into medical practice. This goal is accomplished by combining depth of expertise across multiple cutting-edge fields to accelerate interdisciplinary discovery and the development of new technologies. DIGS-BB researchers recognise that innovative scientific discovery is not an individual pursuit, but only possible



through the collaboration of many minds and perspectives. This belief in the power of collaboration instils an incredible sense of community among DIGS-BB students and faculty, that spills over into interactions with laboratories beyond TU Dresden walls.

The DIGS-BB offers graduate students state-of-the-art dedicated facilities and world class faculty mentors. The multidisciplinary research projects at the DIGS-BB often pull expertise from laboratories and experts in neighbouring fields, providing PhD students with a diverse offering of techniques and mentoring when approaching some of biology's most intricate problems. All students have access to cutting-edge research equipment and technologies through the Joint Technology Platforms. The program offers an open-door policy, whereby students can easily learn to utilise a multitude of techniques and technology from experts across the campus. Current student Ramya Ravindranathan describes her take on the program: 'What I like the most about DIGS-BB PhD program is how it has an international and dynamic work environment, providing students with access to state-of-the-art research facilities and exceptional resources. Every single student's intellectual potential can be fully explored owing to the rigorous training and constant mentorship one gets from program.'

**What is Unique About DIGS-BB's PhD Training Program?**

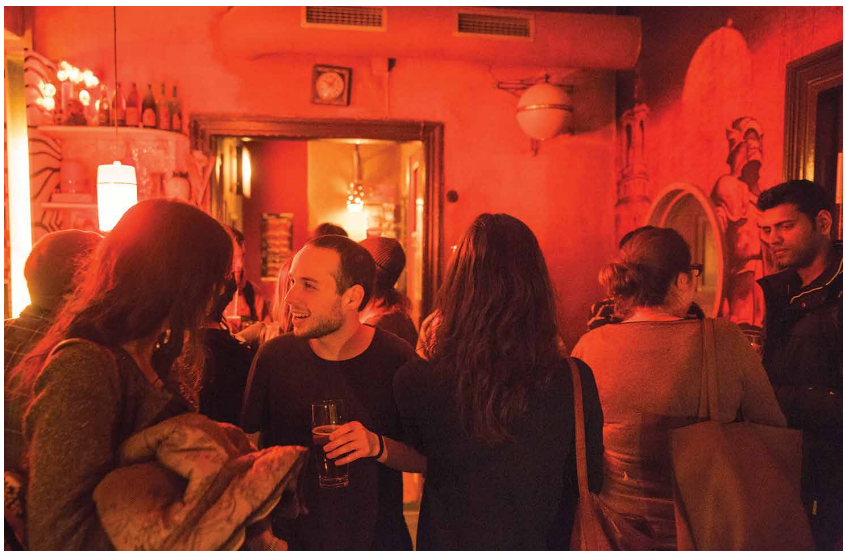
The DIGS-BB program combines novel research projects with advanced training courses to produce PhD students who are confident and capable of engaging in multidisciplinary research on the forefront of biomedical and bioengineering research. First year PhD students complete two obligatory courses, and students have the option to take additional elective courses during their PhDs to continue developing strengths that will contribute to the success of their research careers.

The mandatory first year curriculum is designed to bridge disciplines to promote multidisciplinary thought, introduce students to a range of techniques, faculty, and the scientific community at large, and build essential skills tailored to the unique demands of research careers. The *Introductory Predoc Course* exposes students to the wide range of laboratories and techniques available to them through the DIGS-BB's Dresden International PhD Program (DIPP) affiliation, which includes research groups from DIGS-BB and its neighbouring partner the Max Planck Research School for Cell, Developmental and Systems Biology (IMPRS-CellDevoSys). During the course, students complete four week-long practical rotations, choosing at least one practical in their field and at least one practical in another field. During each practical, students learn about a current topic within that field and practice working with relevant techniques. Past topics have ranged from stem cell techniques and cellular biophysics, to machine learning.

Scientific writing is the backbone of modern research and academic careers, so first year DIGS-BB students also complete a *Foundation Level Scientific Writing Course* aimed at developing the essential writing skills that will enable them to communicate their ground-breaking research discoveries. This course is the first component of the excellence in communication program, a thoughtfully designed program with the aim of helping students master scientific writing and communication, to the profit of their PhD thesis and research career as a whole. Through these courses, DIGS-BB prepares PhD students to be confident, well-rounded scientists in today's highly competitive job market.

For the remainder of the four-year program, students have the freedom to enrol in additional elective courses as needed while they work on their individual research projects. Students also have the option to participate in many programs designed to facilitate career development and position students for successful job placement upon completion of their PhD. The Research Exchange Program places students in prestigious laboratories worldwide to participate in collaborative research or to learn novel methods not available on the Dresden campus. Competitive awards are available to fund student conference travel and participation in specialised training workshops, and to recognise outstanding work. Students at the end of their PhD have the option to participate in Springboard-to-Postdoc and Wrap-Up programs, which provide additional support for the transition from PhD student to postdoctoral positions.

Mentoring at DIGS-BB begins with acceptance of a position in a DIGS-BB research group, attaching students to a core faculty mentor who will provide individualised one-on-one guidance and support for the student's independent research on their assigned PhD thesis project.



During the foundational first year, students also form a Thesis Advisory Committee (TAC) composed of three experienced faculty members. The TAC provides multidisciplinary mentorship and guidance to the PhD student throughout their thesis work. Students typically meet with and update their TAC formally once a year, but the TAC members are available to work with the student individually at any time during their PhD project when guidance is needed. Outside a student's TAC, every two years an ombudsperson is elected to act as a mentor for personal situations outside of the PhD project or delicate situations such as conflicts of interest with a supervisor. Quality mentorship is a critical component of a successful PhD project, and the DIGS-BB faculty members are dedicated to not only providing scientific direction, but also guidance to help students' personal development.

**It's Not Just About the Project**

When asked to imagine a PhD student, the common image that comes to mind is a downtrodden academic locked away in a lab. The DIGS-BB works to break this stereotype by encouraging students to step away from their thesis work and engage in social and community activities outside the laboratory. The campus is a supportive and friendly community for PhD students, providing social support for the rigors of graduate school while encouraging students to forge connections to build a strong scientific network that will profit them for the span of their careers. DIGS-BB student representatives are elected twice yearly to represent the interests of the PhD student community at program board meetings.

As student representative Jelena Popovic describes, 'I became a student representative as I am very interested in knowing all the small details of how our great program came to be. I wanted to help improve it even further, build a stronger bond with the PhD office and all predocs, including the very young new arrivals. And of course, being a part of the amazing group of people, which student representatives are!'

Student-driven activities, such as 'Science Goes to School', 'Ask the Expert', and 'Career Day Symposium', are at the heart of the DIGS-BB PhD student experience. Once a month, the 'Science Goes to School' program places a group of PhD students in local grade school classrooms to engage schoolchildren with science. This program ignites curiosity about science and science careers in children, while giving PhD students the opportunity to develop their skills in communicating science to a broad audience. Twice a year, PhD students have an opportunity to interact with academic, management and industry leaders at the 'Ask the Expert event'. The informal setting in which this event takes place allows students to learn about and discuss topics such as current conditions for scientists in academia or balancing a family with a science career. The annual 'Career Day Symposium' serves to further expose students to the wide range of career options available to them following graduation. This highly praised event provides students with networking opportunities across multiple scientific fields and industries.

For modern scientists, laboratory skills and academic knowledge are not enough to secure a successful career. To be competitive on the global scientific stage, scientists must also be compelling communicators and avid networkers. They must be able to work collaboratively with researchers across a wide range of cultures, while maintaining the ability to work independently, think critically, and approach challenges with an open mind and enthusiasm. Student-driven activities, coupled with innovative mentoring strategies, help ensure that PhD students in the DIGS-BB program grow both personally and professionally during their time in Dresden. The DIGS-BB's multifaceted approach to student development turns out well-rounded scientists that are both intellectually and emotionally prepared to become leaders in the scientific community at large.

**What Makes a DIGS-BB PhD Student?**

Staying at the cutting edge of innovative science and discovery requires an academic environment that values diversity in people and ideas. This is one of the reasons the DIGS-BB prides itself on having a culturally diverse student community, hosting doctoral students from many different countries, each bringing unique educational backgrounds and viewpoints. Over 75% of current PhD students hail from outside of Germany, and of the current 68 research groups, a third are led by international primary investigators. Interactions between students and research groups both within and outside of TU Dresden are encouraged as students engage in the DIGS-BB's dynamic interdisciplinary training.

The DIGS-BB wants to attract the brightest minds from all corners of the globe, and thus offers excellent support for students, both international and domestic. PhD students at the TU Dresden are offered competitive awards and do not pay any tuition or fees. Additionally, international students are offered extensive support with obtaining visas, finding housing, and all of the other potential difficulties that come with moving to a new country. While curriculum and research at the DIGS-BB is performed in English, first year international students have the option to complete an intensive German language course to help them adjust to life outside the lab in Dresden. DIGS-BB mentors foster a learning environment that champions both personal and professional development, ensuring that students graduate with the skills necessary to be both renowned scientists and leaders within the scientific community.

# Research Areas at DIGS-BB

## Developmental Cell Biology

Within the program on Developmental Cell Biology, we want to understand the nuts and bolts of how cells work and get together to generate complex tissues. The Dresden campus has a strong core of basic scientists working on a number of different model organisms including fly, fish, newt and mouse. Here, molecular and cellular approaches are integrated with evolutionary and developmental cell biology to understand how the differentiation of stem cells and the behaviour of their progeny function to build a complete body. The insights obtained within this research area feed extensively into other programs in Dresden to facilitate applied and translational science.

## Biomedicine

The goal of Biomedicine is to use a variety of life science research approaches to benefit human health. Basic research feeds into translational research, which extends into clinical trials and medical applications. Prominent biomedical research areas in Dresden include immunology, metabolism, tumour biology, neuroscience, bone research, and germ-cell biology, and there is a special emphasis on stem- and progenitor-cell research. To address the great complexity of basic and disease-related mechanisms and factors, we use a wide range of tools, including high-end microscopy and other imaging and cell-sorting methods, sophisticated mouse manipulation including humanised animal models, iPS and other stem cell technologies, molecular approaches, and innovative screening technologies.

## Regenerative Biology

Regeneration is the renewal of degenerated or lost cells, tissues or organs within an organism. Such regenerative processes are mainly based on the action of specific stem or progenitor cells that possess the capacity to proliferate and differentiate into the required cell-types. In Dresden, the mechanisms of regeneration and stem cell function are studied to understand general principles of cellular and tissue repair with the aim to use this knowledge to develop novel therapies. Diverse strategies for in depth genetic, molecular and cellular analysis are used to dissect fundamental pathways of tissue regeneration. Animals with high regenerative capacities are utilised to understand intrinsic mechanisms of regeneration as models to induce tissue repair also in mammals. Additionally, the controlled expansion and directed differentiation of stem cells towards specific target cell-types is assessed to develop cell replacement strategies for currently incurable diseases.

## Biophysics

Biophysics is an increasingly popular discipline that applies the approaches and methods of physics to unravel the underlying organisational principles of biological systems. Research in Dresden has a strong emphasis on investigating phenomena across all relevant scales – from individual molecules, to sub-cellular organisation, to cellular properties, and on to tissues, organs, and organisms. Our strength lies in the close collaboration between theoretical physicists, providing the conceptual and modelling framework, experimental groups, developing and applying state-of-the-art techniques to obtain quantitative data, and cell and developmental biologists, who contribute the necessary biological expertise.

## Bioengineering & Biomaterials

Bioengineering is the application of life science, physical science, mathematics and engineering principles to define and solve problems in biology, medicine, the environment, materials and other fields. In Dresden, we focus on the application of nanotechnological tools to broaden our understanding of biology and medicine, as well as using the wide variety of molecular functions provided by nature's 'nanomachines' as a basis for an innovative nanobiotechnology. The tools we use to characterise and engineer molecular-scale systems include single-molecule imaging and manipulation tools, cutting-edge technologies for the micro/nano-structuring of organic/inorganic materials, and a wide range of biomolecular synthesis techniques.

## Computational Biology

Computational Biology addresses problems in biology, biomedicine and ecology through image analysis, theory, computer simulations and data visualisation. In Dresden, we focus on dynamic processes in cells and embryos but also on biomedical questions like tissue regeneration. An overarching question is how complex system behaviour at a large scale can emerge from simpler physical and chemical interactions at smaller scales. In close collaboration with experimentalists, our research groups develop and apply computational tools including image analysis and image quantification algorithms, model-based image segmentation and cell tracking algorithms, adaptive particle methods for spatiotemporal simulations, parallel high-performance computing, multi-scale mechanistic model simulations and deep learning.

## Website:

**W:** http://www.digs-bb.de/

DIGS-BB

# TREES FOR CITIES

As the only charity working on an international scale to create greener cities, Trees for Cities has engaged over 70,000 people to plant over 650,000 trees in parks, streets, schools and housing estates across the UK and internationally. Trees for Cities helps to strengthen communities through volunteering opportunities and inspires people to connect with nature. Here, we have had the pleasure of speaking with David Elliott, Chief Executive of Trees for Cities, who tells us all about the charity's work in improving cities and communities in the UK and beyond.

**To start, please tell us how Trees for Cities was first established. What was the motivation behind setting up such a charity?**

Trees for Cities was founded in 1993 by a small group of 'guerrilla tree planters'. The founders were saddened by the lack of green and prevalence of grey on some of our capital's streets, and were determined to do something about it. They threw parties to raise money from their friends and contacts and set about planting trees where they felt there was a particular need. It was a visionary movement as the role and value of trees and high quality green spaces in our urban environments have only really started to move to the forefront of thinking and planning in recent years.

**Your mission is to plant trees and green cities worldwide – please tell us what cities you have been focusing on thus far, how many trees you have planted to date and what your goals for the future are.**

Trees for Cities was originally Trees for London and so in the early years our work was solely London-focused. As the appreciation of the role of urban trees and green spaces grew, our projects became more in demand and we expanded into cities across the country and internationally. In 2003, we became Trees for Cities to reflect this broadening of our work.

Since then we have worked in almost all major cities across the country. Each year the cities we work in vary depending on funding and project demand. Internationally, our

Trees for Cities
Breathing life into your neighbourhood

**www.treesforcities.org/**

focus tends to be East Africa – cities such as Nairobi, Dar es Salaam and Addis Ababa – but we have also worked in Peru, Nepal and other countries across the globe.

To date we have planted around 700,000 urban trees in the UK and internationally. Whilst we aim to reach a million trees by 2020, the numbers only tell part of the story as what is most important is ensuring that projects have the greatest impact on the communities and environments in the most deprived and grey parts of our cities.

**Describe some of the many benefits of having more trees in urban areas.**

Trees in our cities provide us with an amazing range of ecosystem services and benefits: they filter pollutants such as nitrous oxide gases and particulate matter from the air; they mask noise; they intercept rain and absorb water to reduce flooding; and they act as a natural carbon sink.

Of course, trees also provide a vital habitat for biodiversity – many birds, bats and beetles could not survive without them.

Trees for Cities delivers all of its projects through people: local communities help plan for, plant and maintain the trees. This brings people into the outdoors and fosters a connection with nature. Creating outside places and spaces that people spend more time in has enormous physical and mental health benefits.

Arguably above all, trees provide and create beauty and a sense of wellbeing that no human-made structure can come close to replicating.

**Trees for Cities has also created 50 'edible playgrounds' in several cities across the UK, please describe what these playgrounds involve.**

An Edible Playground is a high quality, custom-designed raised bed system that is created to make optimum use of underutilised areas of school grounds. Edible Playgrounds transform these areas into vibrant outdoor spaces that excite and teach children about growing and eating healthy food.

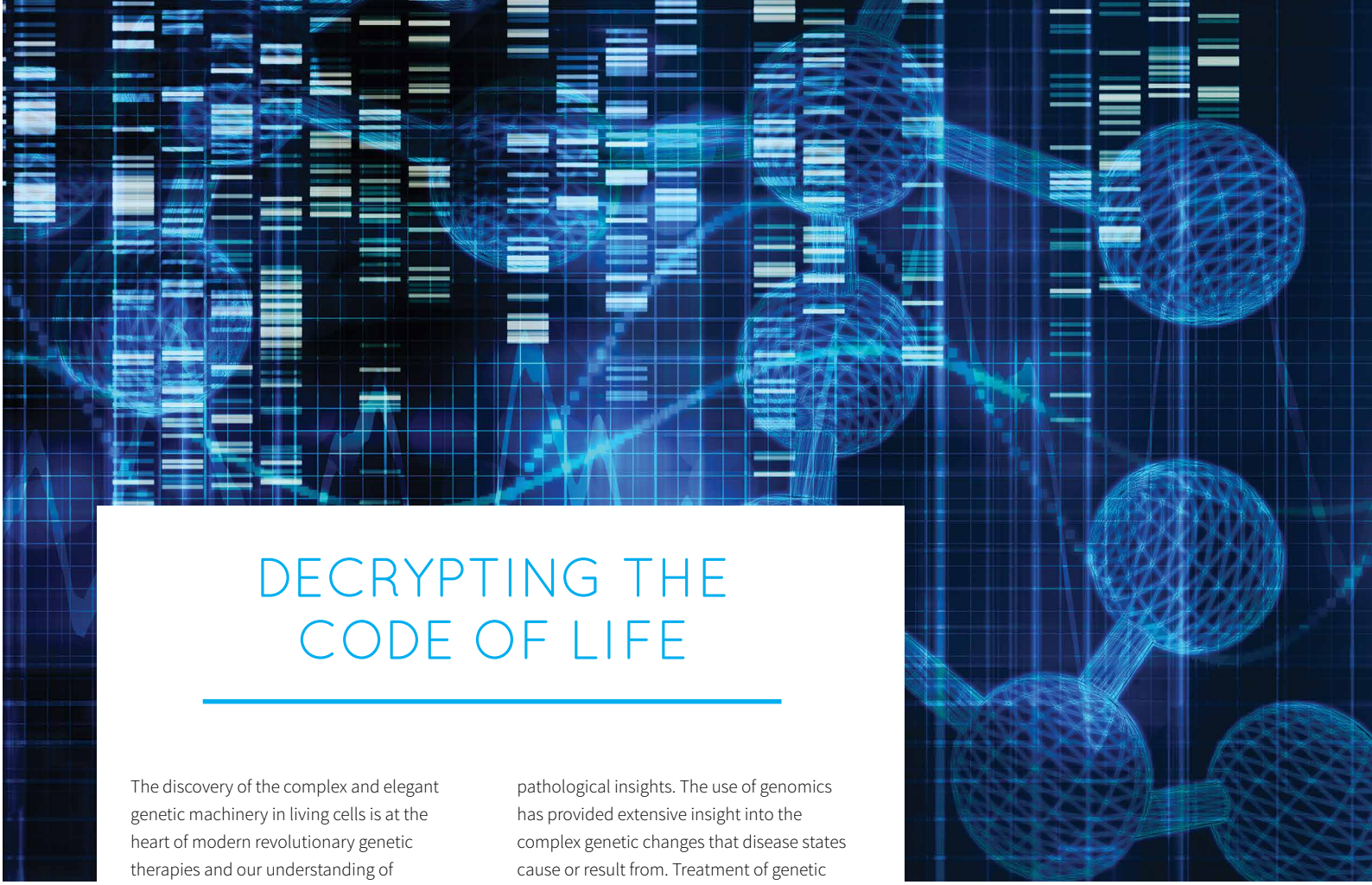
By instilling healthy eating habits at an early age, Edible Playgrounds can help to tackle obesity, food poverty and lack of access to nature head on, and provide a platform for fun and engaging lessons that support the school curriculum.

**Please share one or two achievements made by Trees for Cities that you are personally most proud of.**

Over recent years, Trees for Cities has been a key delivery partner for two major planting programmes. The first of these was the 'Big Tree Plant', a national campaign funded by the Forestry Commission to encourage people and communities to increase the number of trees planted in towns, cities and neighbourhoods throughout England. This programme successfully planted over one million new trees in urban areas. The second of these was the former Mayor of London's street tree programme, which put 20,000 new trees on the capital's streets over the Mayor's two terms in office.

**Finally, how can people donate to the cause, or get involved in the work you do?**

Despite the numerous benefits that trees provide to people and nature, our urban trees face huge threats and challenges. Many trees are being lost to developers and insurance claims, and entire key species are at risk from pests and diseases. Climate change threatens to change the face of our streets and parks. In light of budget cuts to local councils, we are faced with the huge challenge and responsibility to help plant, protect and promote trees in our cities. The support of individual donors and volunteers is crucial. You can make a massive difference by helping us in this work. Through our website at **http://www.treesforcities.org/** donate you can join us in our mission to plant trees and green cities worldwide.



# DECRYPTING THE CODE OF LIFE

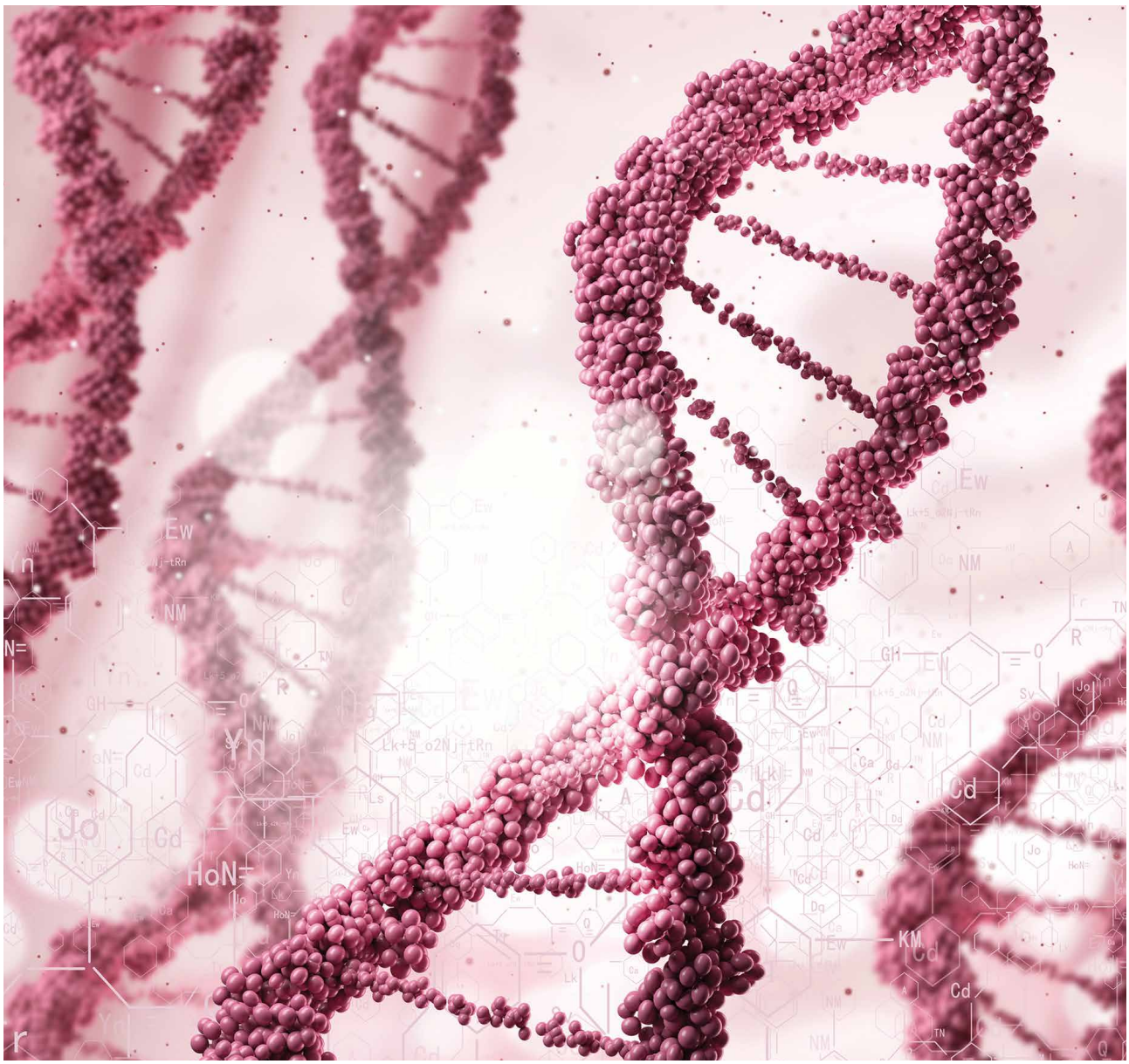
The discovery of the complex and elegant genetic machinery in living cells is at the heart of modern revolutionary genetic therapies and our understanding of evolutionary science. The base pairs in complementary strands of DNA encode our genome and are responsible for so much about us. Only four nucleotide bases make up this code – adenine, thymine, guanine and cytosine, and yet they code for the hugely diverse array of species on Earth.

So how do these nucleotide bases in our cells affect who, or indeed what, we are? Our genes, which are discrete sections of our genome, encode for specific proteins, which are generated through the process of protein synthesis. This happens when complex cellular machinery causes the DNA double helix in the nucleus of a cell to unwind, and the gene is transcribed onto a single complementary RNA strand. The RNA strand then travels outside of the nucleus where numerous enzymes and cellular machinery participate in translating the code into an equivalent strand of amino acids, otherwise known as a protein. Proteins go on to affect cellular signalling and function, and in this way, our genes influence who we are at the most basic level.

Since the discovery of DNA, we have begun to realise its potential in therapeutic interventions. Learning more about our genes, their protein end-products and the genes that are active or silent during specific diseases has provided significant pathological insights. The use of genomics has provided extensive insight into the complex genetic changes that disease states cause or result from. Treatment of genetic diseases is becoming a possibility, and given the important role of genetics in nearly every process in our cells, genetic therapies could provide fundamental and potent treatments for a whole host of diseases.

Our first article in this section of the magazine gives an overview of the OMIM knowledgebase, and introduces OMIM's scientific director, Professor Ada Hamosh of Johns Hopkins University. This easy-to-use database, which is used by researchers and clinicians worldwide, provides a map to the complex world of human genetics, linking genes with their associated traits. Also working in the complex field of genetics research is Professor Allen J. Moore of the University of Georgia, who has dedicated his career to uncovering the genes that underlie parental behaviours. Here we introduce his studies involving the burying beetle – a model parent used by Professor Moore to understand the genes behind parenting, in the hope of illuminating parenting's mysterious evolutionary origins.

From the burying beetle, we move on to the field of plant genetics, where we showcase the work of Professor Zamir Punja and his team at Simon Fraser University. The Punja laboratory applies DNA fingerprinting techniques to numerous strains of medicinal cannabis. Their work will make it much

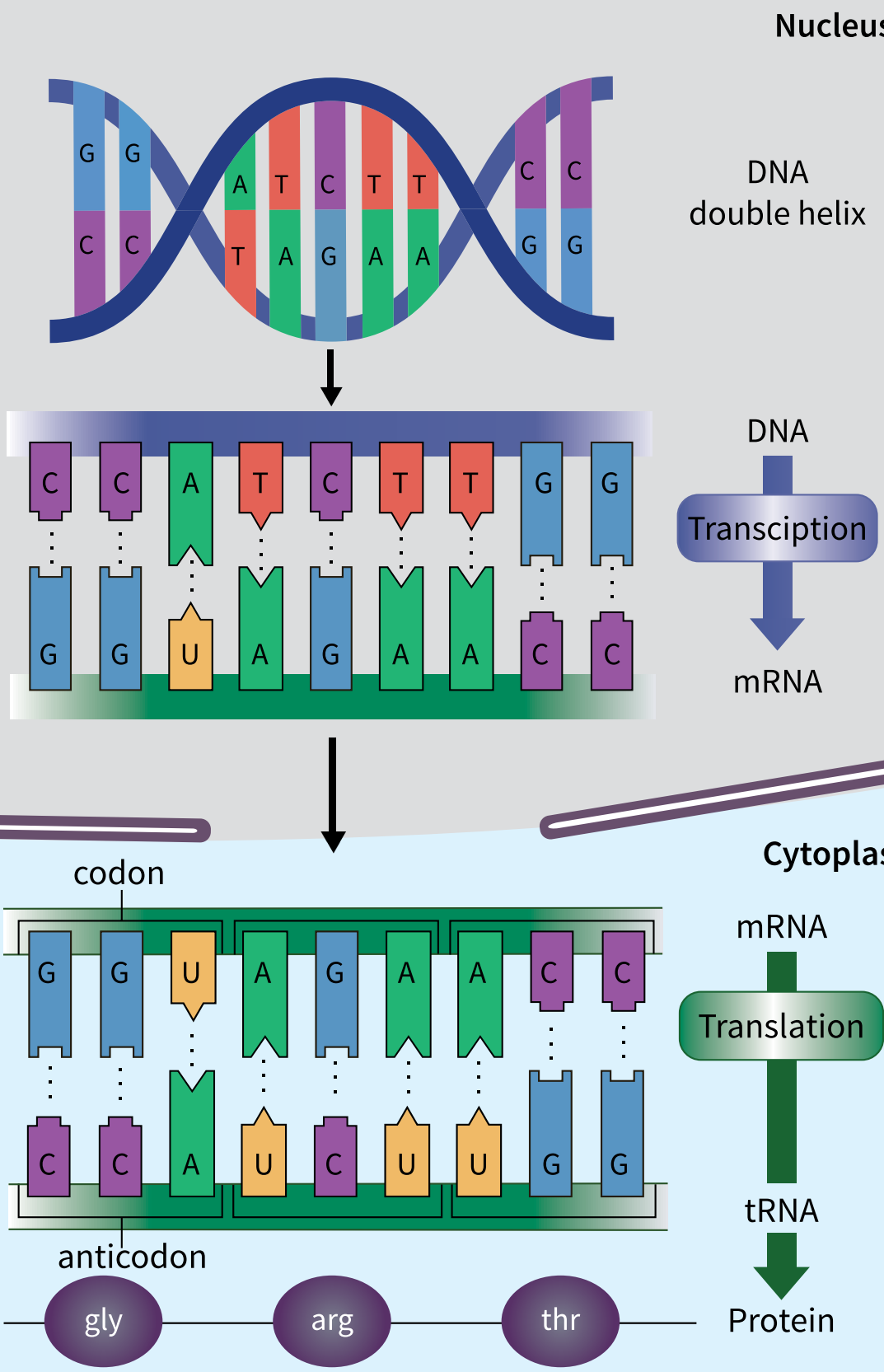


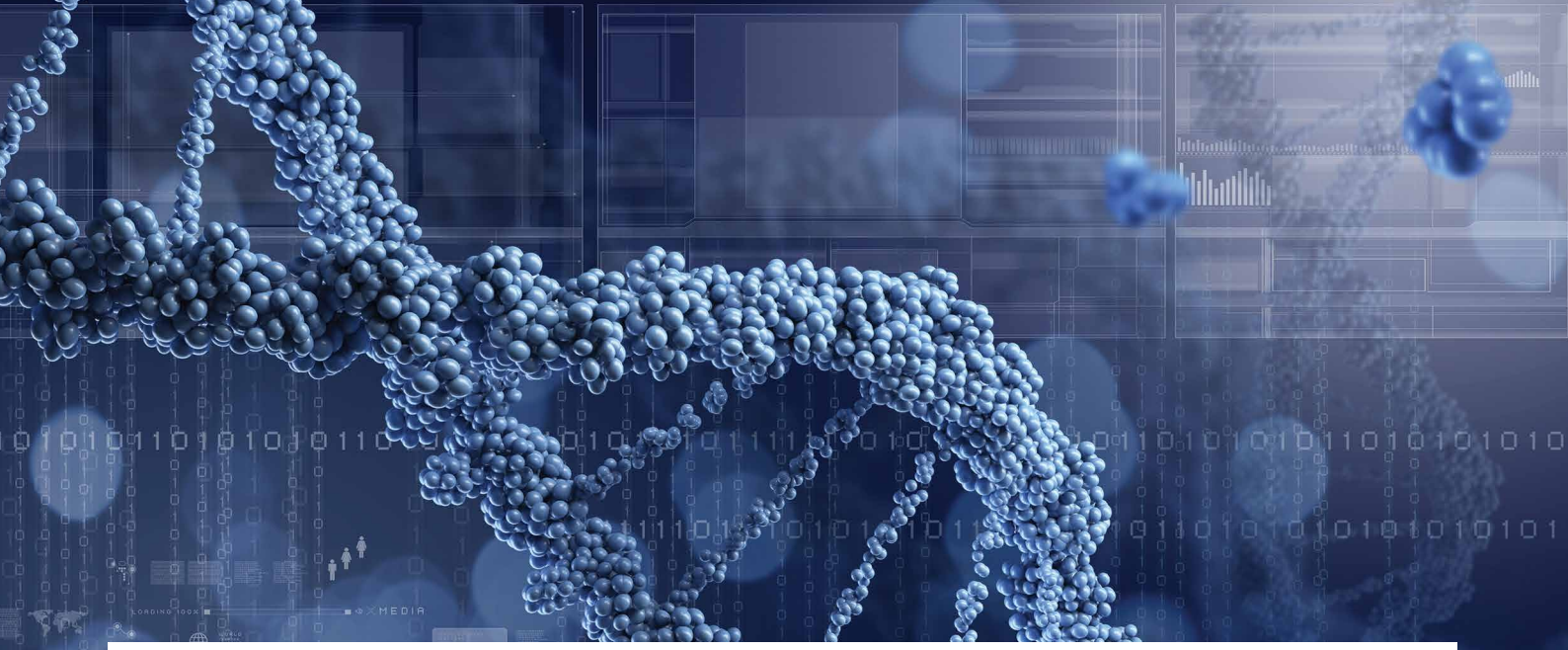
easier to identify different genetic strains of cannabis, thus helping to ensure the quality of plants intended for medicinal use.

Next, we feature the work of three scientists, each focusing on the activity of messenger RNA (mRNA) – the molecule which carries genetic information from DNA to the ribosome, where it can be translated into an amino acid sequence in a fabricated protein. In the first of these two articles, we introduce Dr Diana Bratu and her team of biologists at CUNY. Dr Diana Bratu's laboratory employs biophotonics to image the movement of mRNA and the proteins it interacts with during the development of the fruit fly egg. This fascinating work will inform research ranging from the fundamentals of mRNA transport to the development of targeted therapeutics. Also investigating the behaviour of mRNA is Professor William Marzluff and his colleagues at the University of North Carolina, who study the regulation of gene activity in animal cells. Specifically, his team are interested in the regulation of histone mRNA, both during the mammalian cell cycle and during early development in fruit flies (drosophila), frogs and sea urchins. Next, we explore mRNA in bacterial cells, and introduce the work Dr Harald Putzer at Université Paris Diderot, who studies RNases (enzymes that help to degrade RNA molecules). Quite amazingly, the Putzer lab has shown that RNases from two completely different types of bacteria have independently evolved similar structural properties and mechanistic features.

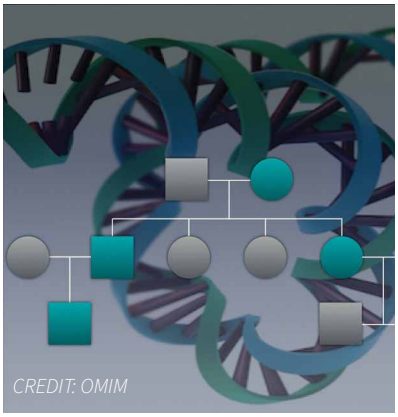
In keeping with the theme of bacterial genetics, our next article in this section describes the work of Professor Marc Bramkamp and his team at the Ludwig-Maximilians University in Munich. Professor Bramkamp is at the forefront of research into the growth, division and chromosome organisation of bacterial microorganisms. Last but not least, we showcase the research of Professor Karl Forchhammer and his colleagues at the University of Tübingen. His team explore how cyanobacteria can survive and recover from long periods of starvation. In their experiments, the team deprives bacterial cells of nitrogen, sending them into a dormant state. Upon re-addition of a nitrogen source, a genetically determined program is initiated that brings the cells back to life. During these experiments, the researchers have gained fascinating new insight into gene expression and DNA content in cyanobacterial cells during dormancy and resuscitation.

# TRANSCRIPTION AND TRANSLATION: FROM DNA TO PROTEIN





# OMIM® – THE ONLINE MENDELIAN INHERITANCE IN MAN KNOWLEDGEBASE: A WARDROBE FULL OF GENES


CREDIT: OMIM

The OMIM knowledgebase provides a map to the complex world of human genetics, linking genes and their associated traits in an easy-to-understand system that is an essential resource used by researchers and clinicians worldwide.

A commercial and technological revolution, the internet is often thought of as a source for fast communication, easy online shopping, and cute videos of baby elephants. However, the ability to access large amounts of information in public databases has led to vast changes in the manner in which science is performed. These changes are most noticeable in the field of life science, particularly genetics, where high-throughput biology yielded data that, when organised into databases, accelerated the understanding of the highly complex systems underlying biology and medicine.

Researchers and clinicians thus need a road-map to the complex pathways involved, such as a central database of genes and diseases and all of the pathways leading from one to the other. This is where the Online Mendelian Inheritance in Man (OMIM) knowledgebase comes into play, a continuously updated catalogue of human genes and their associated traits and disorders. Setting out to clarify the relationship between genes and phenotypes, OMIM is a vital resource for everyone involved in the steadily-growing field of medical genetics.

**Retro Denim**

OMIM is an extension of Mendelian Inheritance in Man (MIM), a catalogue of genetic disorders and genes which was published as a rather hefty book in 12 editions between 1966 and 1998. The original author and eventual chief editor of the book was Dr Victor McKusick, one of the pioneers of the medical genetics field. A man with an impressive degree of talent, he was the founder of the well-respected journal *Genomics,* recipient of the Lasker Award, and at one point held no less than four professorships simultaneously. Mendelian Inheritance in Man was one of his side projects, beginning as a catalogue of genetic traits and research thereof in the early 1960s but rapidly growing into a weighty tome which no self-respecting geneticist would be seen without.

The advent of the internet meant that information could be shared far more easily than before, and the scientific world jumped at the chance. Because MIM had been stored in electronic form since 1964, it was an ideal resource to be used in pioneering efforts by the National Library of Medicine to create a full-text search engine. The result was OMIM, which became open to internet visitors around the world in 1987, 10 years before the founding of Google. In 1995, OMIM was moved to the World Wide Web by NCBI, the National Center for Biotechnology Information, where it was brought alongside their many different medical and biological databases – the most well-known of these is the literature database PubMed, used by many researchers who need to keep up with publications in their field.

OMIM established their own website (the appropriately named OMIM.org) in 2011 to provide a greater level of control over the presentation of information. Since going online in 1987, OMIM has been updated daily

**With well-referenced entries for over 15500 genes and every known Mendelian disease, OMIM has become a cornerstone knowledgebase for the medical genetics field and increasingly all of medicine.**

by a small group of expert science writers and curators now under the guidance of Professor Ada Hamosh, of the Johns Hopkins University. Currently, OMIM has over 23,900 well-referenced entries describing over 15500 genes and every known Mendelian disease. Each entry includes copious targeted links to other online databases, and OMIM.org has become a cornerstone resource for almost everyone in the fields of medicine and genomics.

**Blue, Grey, Skinny, Flared?**

OMIM contains information on both genes and phenotypes, two related but nonetheless differing subjects. A phenotype is a constellation of observable clinical features describing an individual or a disorder – think of the blue eyes and blonde hair of your partner, the black hair of your teacher, your highly specific blood group, or your family member's early-onset Alzheimer disease. All of these observable attributes are related to genetic factors – variations or other alterations in specific genes that are inherited from your parents, i.e. your genotype. The genotype thus leads to the phenotype, although this process is exceptionally complex and spread across many different interacting factors. A Mendelian trait, the focus of OMIM, is one in which the phenotype is controlled by a single gene – mutations in one gene will lead to an altered state such as a disease (cystic fibrosis is a classic example here – mutations within the CFTR gene inherited from both parents leads directly to the disease).

The evolving knowledge of genes and phenotypes is evident in the content in the MIM books. The first edition of *Mendelian Inheritance in Man*, printed in 1966, had almost 1500 entries. The science of genetics was still in its infancy at that stage; the structure of DNA had been found a mere 13 years prior, while the first genetic sequence was still 6 years in the future. As such, almost every entry referred to a phenotype. By contrast the current database at OMIM has around 7,500 phenotypes alongside 15,500 genes – a clear indication of the rapid growth of genetic sequencing and associated information.

**Sorting the Collection**

Every entry within OMIM follows a standard format that enables researchers to easily find the required information. Particular attention is given to the relationship between genes and phenotypes. If mutations in a gene are known to be associated with a disease or trait, this is displayed at the top of the entry. Sometimes different mutations in a gene underlie different phenotypes. These allelic phenotypes are listed together in gene-phenotype tables. In other instances, mutations in different genes can lead to phenotypes with the same or highly overlapping clinical features. These phenotypes are brought together in phenotypic series. OMIM entries describing phenotypes are accompanied by a clinical synopsis – an anatomical overview of the clinical features associated with the phenotype. Cataloguing clinical features through both discursive text and synopses provides clinicians with a unique and powerful way to arrive at a diagnosis.

OMIM also has a tabular listing of genes and phenotypes. This genemap provides a way

to view all Mendelian phenotypes within a specified genomic region. Locations of genes and phenotypes are given in cytogenetic or genomic coordinates. The cytogenetic terminology refers to regions observed when viewing a chromosome under a microscope, and the genomic coordinates refer to the number of bases counting along the length of a chromosome. This is comparable to a street mailing address and the corresponding GPS coordinates.

Determining which gene causes a particular disease can be a difficult and time-consuming job. Years ago, geneticists would spend long hours taking samples from extended families with particular diseases and then trying to determine how those samples related to each other, over time building up a map of related data points that could be used to pick out where on the genome the disease-causing mutation must lie. The advent of full-genome sequencing changed this immensely – geneticists now sequence DNA from individuals with a disorder and compare it to online databases such as OMIM to identify genes with a high probability of causing the phenotype. Additional research is performed to validate the relationship between the gene and disease. As in many fields, the existence of a shared knowledgebase helps scientists to find results far faster than otherwise possible.

OMIM also plays a central role in providing a unified system of classifying Mendelian diseases and genes – essentially providing a unifying structure with stable number identifiers which allows researchers to unambiguously refer to each gene/locus/marker or phenotype. The system is fairly simple: each entry is given a unique six-digit number as an identifier: sex-chromosome-linked entries begin with 3 (X-linked) or 4 (Y-linked), and mitochondrial entries begin with 5. Autosomal entries created before 1995 begin with 1 or 2, those made afterwards begin with 6. Each entry number is preceded by a symbol (*, #, %) to indicate whether the entry is a gene or a phenotype and whether the phenotype has a known molecular basis or has not yet been associated with mutations in a specific gene. These numbers are regularly used in scientific literature, even in journals that do not directly relate to genetics, simply to ensure that everyone understands exactly what is being discussed.

**A Pair for Everyone**

OMIM is intended to be useful to a wide range of professionals, from researchers to clinicians, and students of many disciplines. From a clinical point of view, OMIM comes into its own as a reference for diagnoses – doctors can look at summaries of research on diseases associated with particular genes and then use that to decide how their own patients should be evaluated. OMIM also links to many clinical resources, including databases of laboratories across the world that provide genetic testing services, as well as databases for clinical management and clinical trials. For researchers, OMIM provides a comprehensive resource that can be surveyed for potential scientific correlation – either computationally via bioinformatics programs or by an individual leisurely browsing the entries and maps at OMIM.org. To facilitate research connections and stay current on a topic, OMIM users can tag entries and receive a notification whenever an update occurs. This service, MIMmatch, also connects researchers with similar interests together, as a sort of genetics-focused social media network.

The information in OMIM is sourced from the peer-reviewed biomedical literature accessed through journals and publication databases such as PubMed. Curators also keep a close eye on news feeds and life-science articles. This information is then organised and incorporated into OMIM by a team of science writers and curators headed by Professor Ada Hamosh. Only selected articles are included in OMIM. Additional articles on a topic are readily available through the ‘reference plus’ links at the end of each paragraph in an OMIM entry. Funding for the project comes from grants, in particular the National Human Genome Research Institute, from licenses to industry, and from public donations.

**A Weave for the Future**

Over the course of 50 years, OMIM has grown from a single printed book into a vast knowledgebase with thousands of entries describing genes and phenotypes. As research pushes the boundaries of genetic knowledge ever further, OMIM will grow alongside it, providing a map for scientists and medical professionals around the world. How will it look after the next 50 years? We’ll just have to wait to find out.

# Meet the researcher

**Ada Hamosh, MD, MPH**
Dr Frank V. Sutland Professor of Pediatric Genetics
McKusick-Nathans Institute of Genetic Medicine
Johns Hopkins University
Baltimore, USA

Professor Ada Hamosh began her medical genetics career with a series of publications covering cystic fibrosis, a prime example of a Mendelian disease. Her interests then broadened to cover the general field of Mendelian diseases, from which she progressed to the role of scientific director of one of the leading genetic knowledgebases, OMIM. Currently located at the Johns Hopkins University, where she is simultaneously a Professor of Paediatrics and Genetic Medicine, she mixes her work at OMIM with further research on discovery of novel Mendelian disease genes and the integration of genetics into general clinical medicine by building tools to facilitate the process.

## CONTACT:

**T:** (+1) 410 614 3313
**E:** ahamosh@jhmi.edu
**W:** www.hopkinsmedicine.org/profiles/results/directory/profile/0002818/ada-hamosh

## KEY COLLABORATORS

Joanna Amberger, Johns Hopkins University
Carol Bocchini, Johns Hopkins University
Alan F Scott, Johns Hopkins University
Francois Schiettecatte, Johns Hopkins University
David Valle, Johns Hopkins University
Nara Sobreira, Johns Hopkins University

## FUNDING

National Human Genome Research Institute

## REFERENCES

JS Amberger, CA Bocchini, F Schiettecatte, AF Scott and A Hamosh, OMIM.org: Online Mendelian Inheritance in Man (OMIM), an online catalog of human genes and genetic disorders, Nucleic Acids Research, 2015, 43, Database issue D789–D798.

JS Amberger, CA Bocchini and A Hamosh, A New Face and New Challenges for Online Mendelian Inheritance in Man (OMIM®) Human Mutation, 2011, 32, 564–7.

JOHNS HOPKINS MEDICINE

# UNCOVERING THE EVOLUTIONARY ORIGINS OF PARENTAL CARE

Parental care is relatively uncommon in the animal kingdom, and most young are left to fend for themselves at birth. However, parenting behaviours have evolved multiple times in the history of life and are seen in diverse groups of animals. In recent years, biologists have begun to understand the genetic underpinnings of parental behaviour in the hope of illuminating parenting's mysterious evolutionary origins.

**It's Expensive Being a Parent**

Human babies are decidedly the neediest young in the animal kingdom. They require intensive parental care for years, as they cannot walk, feed themselves, or protect themselves until much later in life. Often people find it surprising that this is seldom the case in other animals. In fact, parenting is a relatively rare phenomenon across the animal kingdom. The offspring of most species get no more than the genetic contributions of their mothers and fathers, before emerging into the world as independent beings required to feed and protect themselves to survive.

Why is parenting so rare? Because it's expensive! Any human parent knows that having a baby is no cheap endeavour, but in evolutionary terms, parenting is a costly behaviour across the animal kingdom. The currency of evolution is fitness – your ability to spread your genes. The more grandkids and great-grandkids you have, the more evolutionarily fit you are so taking care of your babies would seem to make sense. However, offspring that require care can cost parents food and energy resources, make them more vulnerable to predators, and limit their ability to reproduce again quickly. Taking care of your babies limits how many you can have.

While it may be rare, examples of parental care are scattered across almost every branch of the taxonomic tree. Once presumed to be unique to large brained mammals and birds, researchers have discovered forms of parental behaviour in animals as simple as insects. This indicates that parenting has evolved multiple times in the history of animal life on earth. Thus, while parenting may be an evolutionarily costly behaviour, for some species the benefits of parental behaviour outweigh the costs.

While biologists have a good grasp on 'why' parenting is expected to evolve in certain conditions, less is known about the 'how'. Knowing what has to change through evolution should also inform us on why it changes. Ultimately, what changes is genes as evolution is defined by the changes in genetic makeup of populations. But is it new genes that have to evolve, or does evolution change the existing genes? It has only been within the past 15 years that technological advances have begun to allow researchers to unravel the genetics underlying parental behaviour. Professor Allen J. Moore is one researcher who has dedicated his career to uncovering the genes that underlie parental behaviours and their evolution.

**A New Model Parent**

Historically, biologists have focused on mammals and birds when studying parental care, particularly in the limited research focused on the genetics of parenting behaviour. Mammals may seem like a logical choice since parenting is more widespread amongst this group. However, beyond laboratory rodents there are few mammalian species whose genetics we understand well enough to enable us to pin down the complex genetic contributions

to parenting behaviours. Mammalian studies using rodents tend to focus only on maternal behaviours, since females tend to do the heavy lifting in parenting as they provide the food. Many species of bird engage in balanced mother/father parenting workloads, because both are potentially equally capable of care, but their genetics are less clearly mapped out. Birds can also be more difficult to study in the laboratory, as many behaviours observed in the wild are altered in captivity. In addition to the potential for behavioural changes, both mammals and birds require large amounts of space, specialised care, and a great deal of time to observe multiple generations in the laboratory.

The ideal organism to study parenting behaviour evolution would need to meet several criteria. First, the animal needs to be a good parent, demonstrating a range of easily observable parenting behaviours. To understand sex differences, both males and females should have the potential to contribute to offspring care. In depth genetic information must be available for the chosen animal, so that unique genetic pathways can be analysed. Finally, it needs to reproduce quickly and reliably, with minimal behavioural effects of captivity. In a perfect world, this animal would also be easy and inexpensive to care for in a small space.

These criteria may sound impossible to meet, but Professor Moore found the ideal model organism for studying the evolution and genetics of parental behaviour: *Nicrophorus vespilloides*, the burying beetle. Burying beetles are excellent parents – before mating they find an ideal animal carcass, lay eggs nearby, then carefully go about preparing the carcass for their developing eggs by burying it underground and stripping it of its fur or feathers. Once larvae hatch, parents feed the begging babies pre-digested meat until they can feed themselves, while continuing to clean the carcass of mould and defend it from other insects. Burying beetle babies eat fresh – not rotting – meat, so the parents are essential. Moreover, the parents partially digest the carcass and regurgitate food to their begging young. Both males and females engage in parental care, sometimes as single mothers or fathers, and sometimes as a pair. Professor Moore's team has sequenced the complete genome of the burying beetle and has a full repertoire of genetic tools at their disposal. The beetle's entire life cycle from newly hatched egg to sexually mature adult is extremely fast, taking only about a month, and there is no observable difference in behaviour between beetles in the wild and beetles in the lab. To top it all off, burying beetles are simple to care for in even the most basic of facilities. Professor Moore and his team have been working to build this fascinating insect into a recognised 'model parent' in biology for nearly 20 years, and in doing so, have shed light on many novel facets of parenting genetics and evolution.

**Mother *vs.* Father**

One unique trait that burying beetles share with humans is flexibility in family structure. In most animals that show parenting behaviours, the roles of the mother and father are relatively set in stone, and alterations in the family structure cause the young to suffer. For many mammals, leaving the baby alone with the father can be a death sentence, as mammal fathers are often unable and/or unwilling to care directly for their young. Not so with burying beetles; it is common for a mating pair to work together to co-parent their larvae, but both single mothers and single fathers will dutifully care for a brood on their own if the other parent departs for any reason. In their research, Professor Moore and colleagues demonstrated that offspring raised by one parent are just as successful as offspring raised by both parents, and larvae raised by a single burying beetle dad do just as well as larvae raised by a single mother or both parents.

Instances of truly equal maternal and paternal contributions to offspring rearing are even rarer in the animal kingdom than parenting itself. While in some fish and bird species fathers take the primary care role, most often it is the mother that makes the biggest contribution to caring for young. In most mammalian species, offspring do equally well when cared for by the mother alone as when cared for by both parents. Because of this, it has been hypothesised that dual care evolved more as a way for males to protect and secure valuable females, than for the benefit of the offspring. While burying beetles appeared to be equal contributors at the surface, Professor Moore intuitively knew that family dynamics are often more complex, and sought to dig deeper into the family structure of burying beetles. He started by performing a large-scale behavioural and genetic study of over 250 beetle family units that would allow him to fully tease apart the roles of the mother and father in burying beetle parenting.

First, Professor Moore allowed beetles to freely pair and form family units, carefully observing behaviour and time spent parenting. Interesting behavioural patterns emerged: the level of attention that both paired mothers and single mothers gave their offspring was nearly indistinguishable, and single fathers were just as attentive as mothers. However, paired fathers spent much less time parenting than single dads, allowing the mother to do most of the work when she was available. While it was true that offspring of single males did just as well as those in other family situations, single father families were much less common that single mother families, because males were ten times more likely to abandon their families than females.

To understand what was going on beneath the surface, Professor Moore and his team looked at the gene expression

patterns of male and female burying beetles before, during, and after parenting, and compared this to the gene expression profiles of unmated beetles of the same age. They found that the beetles expressed genes differently while caring for offspring, but had similar expression patterns to non-parents before and after rearing young. This suggests that different genes are turned on and off when parenting. Unsurprisingly, there was little difference between the gene expression profiles of paired and single mothers. However, single and paired males were drastically different. Single father gene expression mirrored the profiles of mothers, while paired father profiles were more similar to unmated beetles. This means that single dads not only behave more like mothers on the outside – their gene expression shifts to support caring behaviour on the inside. Regardless of family structure, once their larvae mature, both sexes go back to pre-mating gene expression patterns. This finding highlights the flexibility of burying beetle family structure – males are biologically capable of rising to the occasion when necessary to ensure offspring survival.

**Becoming a Parent**

The discovery that the gene expression profiles of burying beetles is flexible with regard to parenting could shine light on how parenting evolved in burying beetles in the first place. Expression profiles of parents only differ while they are actively parenting, but before mating and after their larvae mature, their gene expression patterns are the same as non-parents. Since parenting itself encompasses a complex suite of behaviours, Professor Moore decided to focus on genes that are keystones in gene networks.

A key component of burying beetle parenting behaviour is regurgitating partially digested food for newly hatched larvae. Professor Moore hypothesised that to perform this behaviour, beetle parents would need to suppress their own urges to eat. To examine genetic pathways associated with this behaviour, Professor Moore opted to target neuropeptide F (NPF), a gene involved in regulating hunger with the capability to influence many other gene expression networks associated with both feeding and social behaviour. This gene is found in all organisms, including humans (where it is called neuropeptide Y), and plays a central role in the motivation to feed. As he predicted, they found that NPF expression was reduced during parenting stages, but returned to normal levels once offspring matured. This provides insight into how a highly complex behaviour may evolve simply by changing the expression of a single gene.

Through further analysis of the brain chemistry of burying beetles at different stages of parenting, Professor Moore and his team have identified additional candidate neuropeptides that have not previously been associated with parenting. Like NPF, these neuropeptides are predicted to play a role in pathways associated with complex behavioural changes. These findings demonstrate that it is possible to predict and identify likely targets of evolutionary selection, and reinforce the concept that changes in the expression of one gene are often sufficient to induce complex, novel behavioural states. These networks of genetic changes lend flexibility to behavioural systems and provide a basis for the dynamic range of behaviours an individual is capable of under different conditions.

Parenting is certainly a complex set of behaviours, but Professor Moore's work with the burying beetle has begun to reveal how such a multifaceted process may evolve through even minor changes in key parts of a gene network.

# Meet the researcher

**Professor Allen J. Moore**
Department of Genetics
University of Georgia
Athens
USA

Professor Allen Moore began his education at Arizona State University, graduating with a BSc in Zoology in 1982. He went on to complete a PhD in Environmental, Population, and Organismal Biology at the University of Colorado in 1988, during which he was awarded a Behavioural Genetics Trainee fellowship by the National Institute of Mental Health. He went on to complete postdoctoral research in neurobiology at both Northwestern University Medical School, Chicago, and Washington University School of Medicine, St. Louis. Professor Moore has served as a Professor at many prestigious universities, including the University of Kentucky, Lexington, the University of Manchester, UK, and the University of Exeter, UK, and worked as a Program Director in Population Biology for the National Science Foundation. He founded his laboratory at the University of Georgia in 2011, where he currently serves as a Distinguished Research Professor in the Department of Genetics.

## CONTACT

**E:** ajmoore@uga.edu
**T:** (+1) 706 542-4423
**W:** http://www.genetics.uga.edu/directory/allen-j-moore

## KEY COLLABORATORS

Dr Chris Cunningham, University of Swansea, UK
Professor Mike Ritchie, University of St. Andrews, UK

## FUNDING

NERC, UK
NSF, USA

## REFERENCES

CB Cunningham, M Badgett, RB Meagher, R Orlando and AJ Moore, Ethological principles predict the neuropeptides co-opted to influence parenting, *Nature Communications,* 2017, 8, 14225. DOI: 10.1038/ncomms14225

CB Cunningham, K VanDenHeuvel, DB Khana, EC McKinney and AJ Moore, The role of neuropeptide F in a transition to parental care, *Biology Letters,* 2016, 12, 20160158.

NJ Royle, SH Alonzo and AJ Moore, Co-evolution, conflict and complexity: what have we learned about the evolution of parental care behaviours?, *Current Opinion in Behavioral Sciences,* 2016, 12, 30–36.

DJ Parker, CB Cunningham, CA Walling, CE Stamper, ML Head, EM Roy-Zokan, EC McKinney, MG Ritchie, AJ Moore, Transcriptomes of parents identify parenting strategies and sexual conflict in a subsocial beetle, *Nature Communications,* 2015, 6, 8449.

UNIVERSITY OF GEORGIA

# ENSURING QUALITY ASPECTS OF MEDICINAL MARIHUANA

**Professor Zamir Punja,** an expert in plant pathogens, tissue culture and molecular biology at Simon Fraser University, turns his attention toward the under-studied, but medically important field of cannabis research.

Humans have consumed and cultivated cannabis (or marihuana) since the beginning of recorded history, with evidence for this dating back to as far as 7000 BC. In most countries, the consumption of marihuana has been prohibited for around 100 years, but relatively recently there has been renewed scientific interest in its medicinal properties for the treatment of various health conditions. One of the scientists at the forefront of this research is Dr Zamir Punja, a professor of plant biotechnology at Simon Fraser University in British Columbia, Canada. Over the course of his scientific career, Professor Punja has worked on various aspects of ensuring high-quality production of agricultural plants, such as blueberry, cucumber, tomato and ginseng, and studying the diseases which affect them. He has published over 250 papers and book chapters on this topic. His focus is now squarely on cannabis, as he believes that 'ensuring consistency and high quality of product will be essential to ensure the utility of cannabis for medical purposes.'

**The Medicinal Properties of Cannabis**

In some ways, marihuana is more complex than most drugs, because its effects do not result from any single compound produced by the plant. Over 100 cannabinoids – compounds which interact with a specific set of endocannabinoid receptors within our bodies – have been identified so far. These compounds can have distinct pharmacological properties. THC (delta-9 tetrahydrocannabinol) is the often the most prevalent of these molecules and is the principal cause of the plant's psychoactive effects, by interacting with receptors found in, among other places, the nervous system and brain. In contrast, the second most prevalent cannabinoid, CDB (cannabidiol) possesses a range of pharmacological activity, but is not psychoactive, in that it does not affect the brain directly. In addition, cannabis plants produce a complex group of compounds called terpenoids, which impart volatile scents and flavours similar to those found in a range of other plants including pine trees, lemon fruit and basil.

The quantity of these phytochemicals and their ratio relative to each other will induce different effects when the plant is consumed. Cannabinoid receptors, such as CB1 and CB2, have evolved to interact with endocannabinoids – cannabinoids produced by the body itself. The interactions between cannabinoids and these receptors are responsible for the pain-relieving, anti-inflammatory, anti-spasmodic, anti-emetic and psychoactive effects of cannabis. These properties make cannabinoids a promising and thus-far under-studied avenue for the treatment of diseases such as auto-immune disorders, multiple sclerosis, inflammatory bowel disease and post-traumatic stress disorder, to name a few. The quantity of these chemicals and their relative proportions are influenced by various factors, such as the plant's growth conditions, harvest practices, post-harvest handling techniques and storage conditions. One of the most influential variables though, is the genetic background of the plant.

**The Genetics of Cannabis**

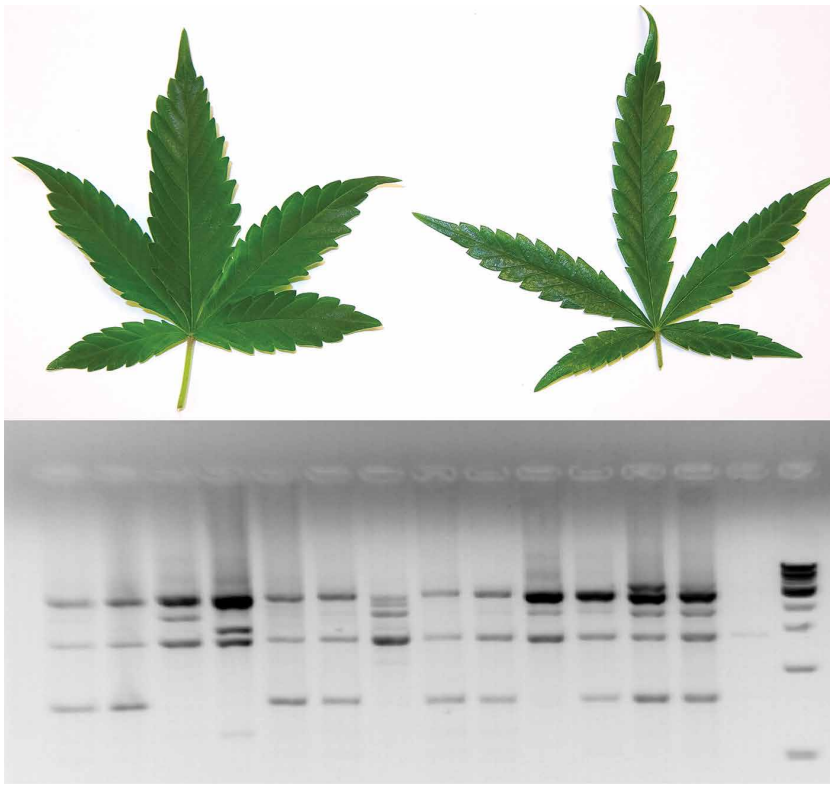
Like humans, cannabis exists either as male or female individuals, determined by the presence of either a pair of X chromosomes (female) or one X and one Y chromosome (male). Interestingly, under stressful conditions, plants can become hermaphroditic, meaning an individual plant which is genetically female (XX) for example, could still produce both male and female flowers. Exactly how the plant does this is not fully understood. Cannabinoids are produced within glandular trichomes which are most abundant in unfertilised, female flower buds (inflorescences). Therefore, male plants are undesirable for harvesting as marihuana, as they contain negligible levels of THC and CBD, and their presence is highly detrimental to development of female flowers, which will produce only small quantities of these chemicals once pollinated. Further research into this area will hopefully improve our knowledge of what causes genetically female plants to produce male flowers, and therefore facilitate more efficient marihuana production.

A huge number of cannabis strains exist, resulting from genetic crosses originally made between indica and sativa varieties, and subsequent crosses made between hybrids. These contain varying proportions of sativa and indica genes, and possess a range of pharmacological properties (as cannabis) or industrial usefulness (as hemp fibre). The names of these strains can offer clues to either geographical origin or source genetics. 'Kush' strains, for example, have ancestry from varieties collected from the Afghanistan/Pakistan region. Strains are often named after the parental strains used to produce them, so crossing 'Blueberry' with 'White Widow' for example, has produced a strain named 'Berry White'.

Many of these strains have been produced either by seed companies or by 'covert breeders', without much communication between them, oversight or agreed-upon system of nomenclature, leading to a great deal of uncertainty regarding the identity of particular strains. The Punja laboratory therefore is applying DNA fingerprinting techniques to a large number of strains, to help to clear up some of the uncertainty regarding genetic backgrounds. 'We want to apply methods in DNA fingerprinting to characterise those genetic strains that are widely used commercially to develop a

**‘The ultimate goal is to ensure high-quality and consistent production of marihuana for medical purposes using modern research methods’**

*Ensuring quality of medicinal marijuana requires management of various diseases. Upper left: seemingly healthy flower buds to be harvested and dried can contain high levels of Penicillium species (petri dish on the right), which can contribute to a high mould count and impart odours to the product. Lower left: a common disease affecting marijuana world-wide, namely powdery mildew. Lower right: viral infection can distort the growth of plants*



*Variation in morphological features is very common in marihuana strains, as shown in the two different leaf types (top photo) of ‘sativa’ type (right) and an ‘indica’ type (left). Bottom photo shows differences in DNA banding patterns between a number of different strains of marihuana.*

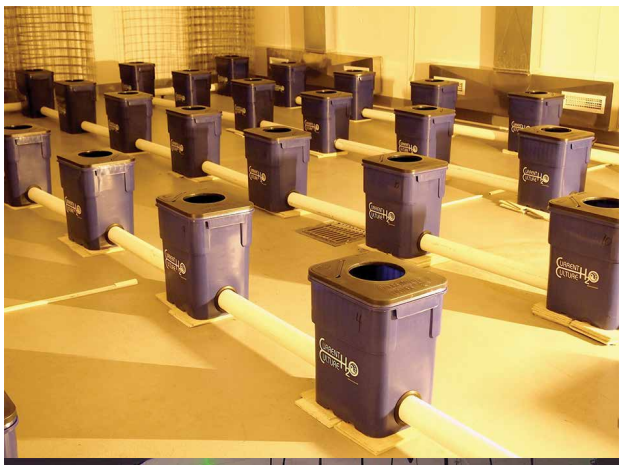
unique way to identify them,’ says Professor Punja. These techniques are very much like those used to forensically identify a suspect in a criminal case, by detecting differences in the DNA sequence at a large number of locations. When analysed together, these differences provide characteristic ‘fingerprints’ for individual strains.
Some of the strains the team has tested are genetically identical, despite have different names, while others have displayed genetic distinctions between plants of the same strain. As breeders work towards producing better cannabis strains for medicinal purposes, hemp, or recreational use, having a reliable starting point of knowing genetically which strain is being utilised, is essential.

**Growth Conditions and Flowering**

Control over the timing of flowering is crucial for cannabis growers, as flower buds are the major site of cannabinoid production. While it is well understood that photoperiod (the proportion of the day spent in light and darkness) greatly influence the timing of flowering, what is not well understood is the mechanism behind ‘automatic’ varieties – plants that flower after a certain period of time regardless of light cycle conditions. Studies are underway to investigate how these plants achieve this, in addition to research into the use of lighting in general for cannabis growth, and effects of soil composition, atmospheric carbon dioxide and a multitude of other variables. One of the approaches to enhance the growth and quality of medicinal marihuana plants is growing them in hydroponic systems – where plants grow in a nutrient rich solution, instead of soil. Hydroponic systems not only produce plants with enhanced growth and yield, but potentially can also give more consistent and repeatable results compared to other production environments.

**Distinguishing Male and Female Cannabis Plants**

When starting marihuana plants from seeds that are produced following fertilisation of female flowers with pollen, approximately half of them will be female with the remaining being male. Distinguishing between the sexes of these plants is currently only possible at the stage of flower formation, approximately 6–8 weeks following seed germination. This presents a problem with identifying male plants early enough to remove them from a production facility before pollen production ensues.



Working jointly with Agrima Botanicals, Professor Punja's lab developed procedures for a test kit that can be used to identify the gender of individual plants, allowing producers to rogue out or destroy male plants early in growth. This kit, called the GreenScreen Plant Sex ID Kit, is based on extracting DNA from seedling leaf tissue that is then used to specifically identify regions of the chromosomes unique to male and female plants. This allows rapid (24 hr) sexing of plants.

**Tissue Culture of Cannabis**

One interesting property of plants is that all of their cells have the potential to become stem cells, and give rise to all the different tissues of a plant. Large numbers of tissue samples can be taken from an individual plant and grown on a culture medium. In theory, a complete plant could be obtained from a living cell taken from anywhere on an existing plant – something which is not possible for animals. In practice, this procedure is not simple, and varies in difficulty from species to species. Initially, cell growth occurs as disorganised masses of cells called calluses, but under precisely controlled conditions, supplemented with specific nutrients and growth hormones, they can develop into roots and shoots. This process, known as organogenesis, allows the production of a large number of plants, which will be genetic clones, from a single individual without having to produce seeds. Propagation of marihuana plants can also be carried out using vegetative cuttings and root-inducing hormones, and this is the preferred method for large-scale commercial production. Not all strains respond in the same manner, however. An alternative process is micropropagation using tissue culture which has not been entirely perfected yet, and remains an active area of research. Professor Punja feels that since large-scale vegetative cuttings can be a potential route for the spread of pathogens, tissue culture and micropropagation may be alternatively used to reduce the introduction of diseases in marihuana growing facilities. Tissue culture micropropagation is routinely used in the horticulture industry for orchids, strawberry, blueberry, and many other crops.

**Creating GM Cannabis**

One useful technique in the study of plant genetic modification involves a pathogenic bacterium called *Agrobacterium*, which is responsible for crown gall disease in plants. *Agrobacterium* has the interesting capability to insert part of its own DNA into the genome of the plant, inducing tumour-like growths, or galls, on the plant tissue. Scientists realised soon after the discovery of this mechanism that the genes responsible for tumour formation could be disarmed and replaced with DNA from a different organism, providing an extremely useful system for genetic studies and engineering of plants.

In cannabis, genetic engineering is still in it's infancy. Research into this area has been hampered by legal restrictions on handling of plant material. Professor Punja's lab previously demonstrated that in hemp, a combination of the techniques of plant tissue culture and *Agrobacterium*-mediated genetic transformation can be used to produce genetically-transformed calluses, the first step in producing fully transformed plants of this species. Professor Punja's work has also focussed on applying transgenic techniques (introducing genes from other organisms) to carrot and ginseng, to enhance their resistance to disease. One such experiment involved inserting a gene from petunia into carrot, which produced an enzyme called chitinase. This enzyme breaks down a component of the fungal cell wall, chitin, and its presence in carrot was found to increase its resistance to fungal pathogens. The creation of GM cannabis could lead to improvement in the genetic traits but regulatory approval is likely to be difficult.

**Diseases of Cannabis**

Despite a large volume of scientific literature on pathogens affecting hemp, information on diseases that afflict marihuana specifically is much rarer. As the industry expands and marihuana is cultivated in larger areas, the consequences of this lack of knowledge will become increasingly severe. Previously unreported diseases are now appearing, caused by bacteria, fungi and viruses.

Certain fungal pathogens are known to cause root rot or bud rot, and are known to spread rapidly if introduced into an environmentally controlled indoor production facility. Research will need to be conducted into how these pathogens get inside cannabis growth facilities and how best to manage them when they do. Having worked on the subject of plant diseases in other species, such as wasabi, cucumber and ginseng, Professor Punja has demonstrated how environmental conditions can be controlled to influence the way pathogens interact with host plants. He has also studied the use of mycoviruses (viruses which infect fungi) as a control against fungal pathogens, in addition to using bacterial and fungal species as biocontrol agents. 'Our research will target some of the major fungal diseases affecting plant production and develop rapid screening methods for the pathogens and develop disease management tools,' he tells us.

The lab's continued work on cannabis pathogens, as well as research on transformation, tissue culture and optimisation of growth conditions, will no doubt be invaluable to producers as they find their way in this rapidly expanding industry.

# Meet the researcher

**Professor Zamir Punja**
Department of Biological Sciences
Simon Fraser University
Burnaby
Canada

Professor Zamir Punja developed his interest in plants at a young age, studying plant biology for his BSc at the University of British Columbia and later focussing on plant pathology for his MSc and PhD at the University of California Davis. During his studies, he gravitated towards studying fungal pathogens that cause diseases in food crops, plant tissue culture and plant molecular biology. After four years of private sector research and development in plant biotechnology at the Campbell Soup Company, he returned to academia at Simon Fraser University, where he now holds a position as Professor of Plant Pathology and Biotechnology.

## CONTACT

**E:** punja@sfu.ca
**T:** (+1) 778 782 4471
**W:** http://www.sfu.ca/biology/faculty/punja/

## KEY COLLABORATORS

Agrima Botanicals, Maple Ridge, BC

## FUNDING

Natural Sciences and Engineering Research Council of Canada, Engage and CRD grants
Agrima Botanicals

## REFERENCES

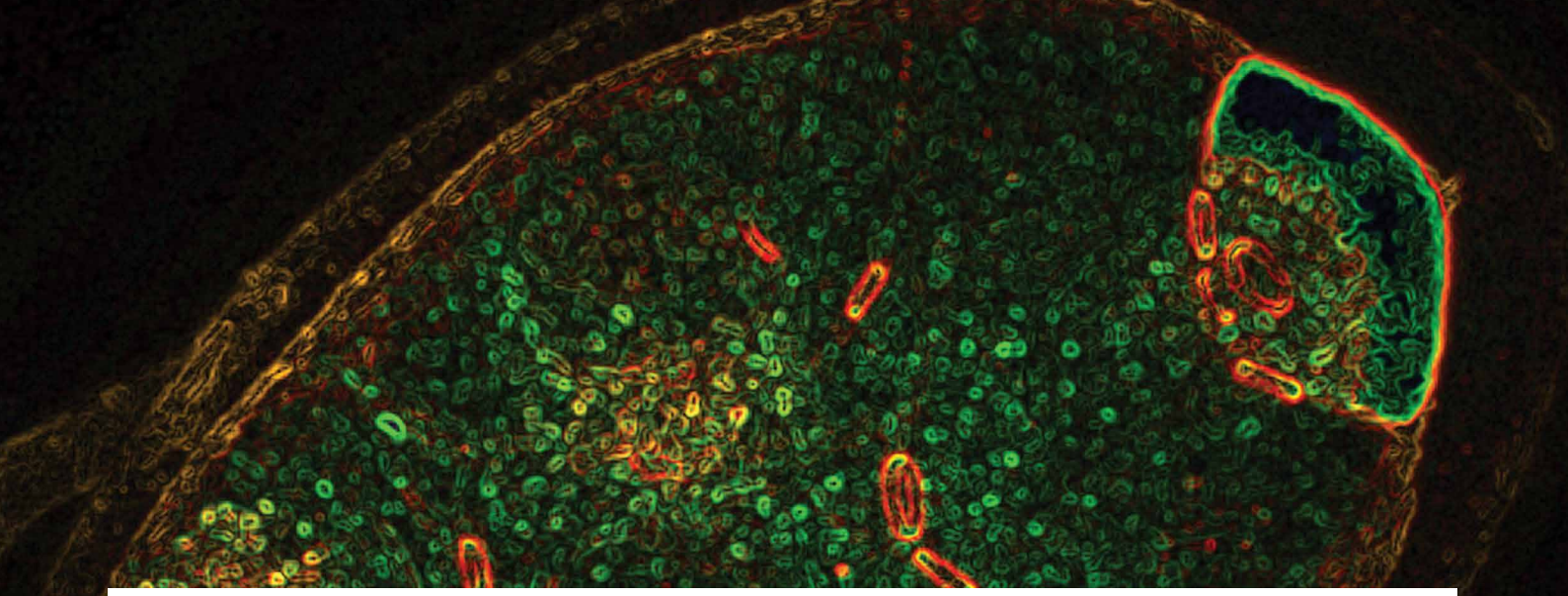
ZK Punja, R Rodriguez and S Chen, Assessing genetic diversity in *Cannabis sativa* using molecular markers, In: *Cannabis sativa* L. – Botany and Biotechnology, Edited by Suman Chandra, Hemant Lata and Mahmoud A ElSohly, Springer-Verlag Berlin Heidelberg, 2017 (in press).

M Feeney and ZK Punja, The role of *Agrobacterium*-mediated and other gene-transfer technologies in cannabis research and product development, In: *Cannabis sativa* L. – Botany and Biotechnology, Edited by Suman Chandra, Hemant Lata and Mahmoud A ElSohly, Springer-Verlag Berlin Heidelberg, 2017 (in press).

M Feeney and ZK Punja, Transformation of hemp (*Cannabis sativa* L.), In: Wang, K. (ed.) Methods in Molecular Biology, Second edition, Agrobacterium protocols, 2014, 344, 319–329.

G Rodriguez, A Kibler, P Campbell, ZK Punja, Fungal diseases of *Cannabis sativa* in British Columbia, Canada, Agrima Botanicals, Maple Ridge, BC, Canada; Simon Fraser Univ, Burnaby, BC, Canada. Phytopthology abstract annual meeting 2015.

SFU SIMON FRASER UNIVERSITY ENGAGING THE WORLD



# REAL-TIME VISUALISATION OF mRNA REGULATION AND TRANSPORT

**Dr Diana Bratu's** laboratory utilises biophotonics to image the movement of mRNA and the proteins it interacts with during the development of the fruit fly egg, informing research ranging from the basics of mRNA transport to therapeutic development.

**The Importance of mRNA Regulation**

Since graduate school, Dr Diana Bratu has been developing technologies to visualise the movement of RNA and proteins within the cell. When asked what attracted her to this particular field of research, she replies 'simply the curiosity of understanding the underlying mechanisms that drive development'. However, research on intracellular nucleic acid transport informs a much wider range of topics than growth and development – from the progression of Alzheimer's to the development of pharmaceuticals targeting proteins to treat HIV infection.

Eukaryotic messenger RNA (mRNA) is the critical intermediary molecule between DNA and protein as it is translated in the ribosome. The regulation of such mRNA by co-factor proteins is critical to controlling gene expression, having implications for research in embryonic development, neurodegenerative disorders, long-term memory, and learning processes.

As such, research efforts to better understand the regulation of mRNA are afoot worldwide, with one of the key challenges being the visualisation of mRNA and how it interfaces with these proteins to influence the expression of important genes. Dr Bratu and colleagues are pioneering this field through a gamut of unique biophotonic methods, from probe design to advanced imaging approaches, thus improving upon the detection and accuracy of mRNA visualisation and its co-localisation with trans-acting proteins important for the normal function of processes within a cell.

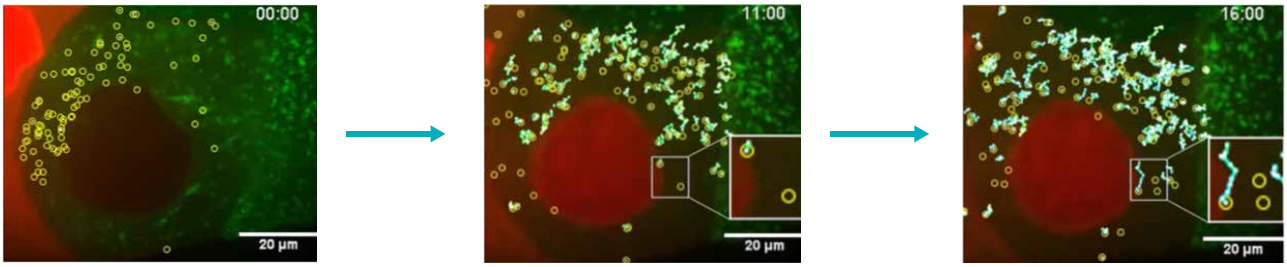
Using *Drosophila melanogaster* (the fruit fly) as a model organism, Dr Bratu's research group employs fluorescent probes and spinning disc confocal microscopy to track the movement of mRNA and proteins throughout the egg chamber. They employ genetically encoded fluorescent proteins and short molecular probes (i.e. molecular beacons) allowing for the detection of various endogenous proteins and mRNAs, thus enabling real-time tracking of these molecules as they are transported within the cell. While this has obvious implications for the research of oogenesis, these studies act as a biologic proof of principle to guide other researchers' studies examining mRNA transport in other systems. By combining molecular, genetic, biochemical, and advanced imaging approaches, Dr Bratu seeks to inform the scientific community on RNA transport machinery and metabolism while maintaining a strong mentorship program for undergraduate students interested in pursuing research in the biological sciences especially in biophotonics.

**Real-time Visualisation of mRNA-Protein Complex Movement**

The egg chamber of *D. melanogaster* is an ideal system to study mRNA transport, as it is a complex tissue yet easy to manipulate in the lab and stable enough to withstand long-term imaging. While there are countless types of mRNA whose localisation is important for the proper growth of the oocyte, Dr Bratu and colleagues initially selected *oskar* mRNA as the subject of their research. This mRNA is transcribed within the nurse cell nuclei in the egg chamber and then transported to the oocyte's posterior on a railway of microtubule tracks. It is not translationally active until it is successfully transported to the posterior. Upon translation, the Oskar protein is anchored at the posterior pole and plays a critical role in establishing the body axis of the developing embryo, as well as in determining the future germline.

During *oskar* mRNA transport to the posterior pole, it associates with several proteins, among which are Bruno and Cup. These two proteins work together to suppress translation of *oskar* mRNA throughout this process. Livia Bayer, a PhD student in Dr Bratu's lab is able to track these mRNA-

**‘It was only natural for me to follow the field of RNA biology and develop my own research group exploring the intricate processes that make up the life-cycle of an RNA molecule. Visualising these processes is the most attractive aspect of this field!’**




**Endogenously tagged Cup-YFP co-localise and travel together with *oskar* mRNA in live egg chambers.** Red: *oskar* mRNA, Green: Cup-YFP

protein (mRNP) complexes within the nurse cells and the oocyte, by employing a combination of techniques to visualise these proteins with fluorescent tags and the transcript when hybridised by fluorescent probes.

For visualising mRNAs, the team designs, synthesises and delivers into the egg chambers, fluorescently labelled, small, hairpin-shaped oligonucleotides called molecular beacons, which hybridise with high specificity to endogenously expressed mRNAs and emit a fluorescent signal. The team selects target regions within an mRNA utilising two computer algorithms, *mfold* and OligoWalk, which assist them in narrowing down sequence stretches for the highest affinity and accessibility results *in vivo*. The probes are then synthesised from modified phosphoramidites, which render the probes stable within the cellular environment, and labelled with bright fluorophores that provide a great signal for detection of the dynamic processes involved during an mRNA’s lifecycle. This allows for the visualisation of *oskar* mRNA transport to the posterior pole of the fruit fly oocyte and movement throughout the egg chamber over extended periods of time.

This technology will provide unique advancements in this field, as they are ideal for detecting very small targets, but also sensitive enough to identify them when they are in small quantities. Shorter probes have also been developed in Dr Bratu’s lab that can detect the smallest of targets, such as microRNAs.

**The Role of microRNAs**

MicroRNAs have received much attention in recent years, as their roles in both development and disease have become more evident. Because of their ability to downregulate, or silence, gene expression, they hold great potential for future drug development. MicroRNAs belong to the largest family of noncoding RNAs; they are not translated into protein, but rather directly influence the expression of other genes by binding to the mRNA and either repressing translation or destroying it.

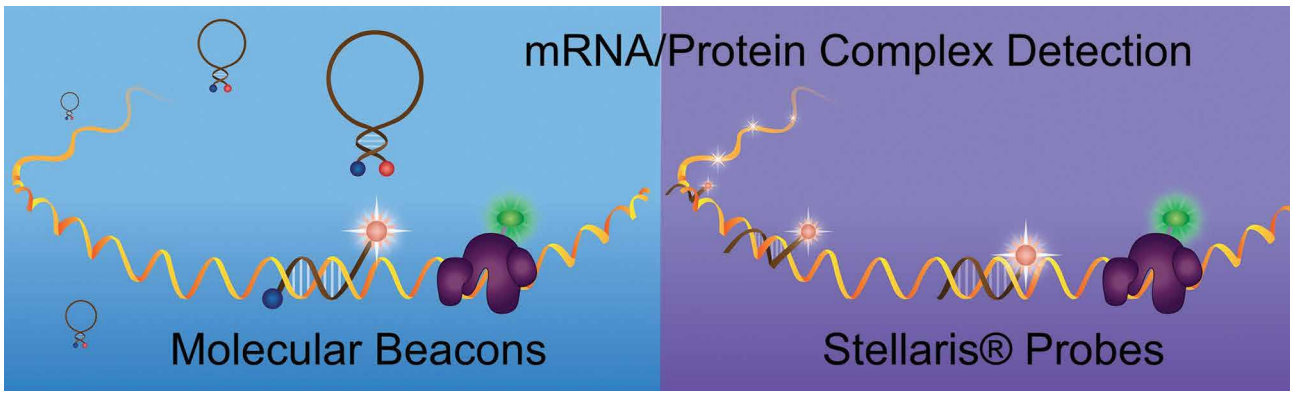
In the team’s most recent investigations into the role of microRNAs, via a study led by PhD student John McLaughlin, they have identified a conserved binding site for a particular microRNA, miR-305, on the maternal mRNA *bicoid*. They are currently investigating the in vivo function of miR-305 in the translational repression of this maternal mRNA during *D. melanogaster* oogenesis. Interestingly, the team’s cell culture experiments have so far shown that miR-305 can inhibit the translation of a *bicoid* mRNA reporter gene.

mRNA post-transcriptional regulation can also occur in organelles known as Processing Bodies (P-bodies), which have known roles in microRNA function, mRNA storage, decay, and translational repression. With this in mind, the team hypothesise that recruitment to P-bodies helps to stabilise *bicoid* mRNA during oogenesis, and this process may involve the microRNA machinery in mediating its translational repression.

**Untangling the Intracellular Vesicular Trafficking of RNA Viruses**

A project that Dr Bratu and her Senior Research Associate, Dr Irina Catrina, have undertaken as of late is elucidating the role of certain host proteins in viral RNA transport. The life cycles of RNA viruses, including HIV and Influenza A, rely heavily upon the transportation of genomic RNAs, from the nucleus into the cytoplasm, where translation or packaging occurs. Proteins involved in viral RNA transport during replication are of great interest as pharmacological intervention targets.

The host protein AGFG1 is necessary for HIV-1 and Influenza A replication, but not for cell viability, making it a promising candidate as an antiviral drug target. While it is understood that AGFG1 is involved in the movement of viral RNAs from the perinuclear region to the plasma membrane, elucidation of the cellular role(s) of AGFG1 and how it interacts with other proteins within the cell will allow for more efficient and accurate drug design. It has been shown that AGFG1 participates in the uptake of select cargo via endocytosis, however this does not fully account for the defects observed in viral RNA transport and localisation. Drs Bratu and Catrina set out to




**mRNP detection via fluorescence.** Co-visualisation of proteins and mRNAs via fluorescent protein tagging (GFP) and hybridisation with fluorescent probes in live (molecular beacons) or fixed (Stellaris® probes) cells.

identify the role(s) this protein plays in cytoplasmic transport.

Once again using *D. melanogaster* egg chambers, they study the fruit fly homolog of AGFG1 known as Drongo, in particular the effect of reduced Drongo protein levels on development of the egg. The results may then be extrapolated to highlight the actions of AGFG1 in human cells. Using fluorescence and real-time tracking during oogenesis, they determined that Drongo co-localises at the oocyte cortex with Clathrin, a major player in vesicle formation, and F-actin, a critical cytoskeletal component during vesicular transport. This association suggests that Drongo plays a role in endocytosis, much like its mammalian homolog, AGFG1. Dr Catrina determined that Drongo also co-localises with endosome markers Rab5 and Rab11, both of which play roles in endosome trafficking, and that proper localisation of Drongo requires functional Rab11 protein. By confirming the importance of Drongo/AGFG1 in intracellular vesicular trafficking, the team has provided valuable information that may guide the development of novel antiviral drugs that target conserved interactions between viruses and host co-factors.

**Creating the Next Generation of Scientists**

Dr Bratu has harnessed her knowledge in the use of molecular beacons and advanced live cell imaging to excite many cohorts of young scientists about visualising these processes that drive development and have such wide implications for the understanding of disease progression and pharmaceutical development. She pioneered a laboratory course for upper level undergraduates which has since been expanded to multiple affiliated institutions and is now offered to graduate students entitled, 'Laboratory in Fine Cell Structure: Fluorescence Imaging' as well as a seminar course entitled 'MicroRNAs and Development', both of which are always fully enrolled. The microscopy course introduces students directly to the many uses of light to monitor cellular events, ranging from confocal microscopy to high-resolution imaging (i.e. structural illumination microscopy). Students have the opportunity to spend time at the Bio-Imaging Facility at Hunter College which houses several advanced imaging set ups (fluorescence microscopes, laser scanning and spinning disc confocal microscopes, SIM/TIRF microscope). Under Dr Bratu's leadership as Scientific Director of this facility, several new microscopy set-ups have been acquired, thus improving the imaging capabilities available for her students and colleagues.

Using *D. melanogaster* and various fixation techniques, students learn the basics of fluorescent probe design and fly husbandry while generating images in which they are able to visualise cellular structures. The students then present their findings at local symposia, and many submit their images to various microscopy competitions, such as the annual 'Nikon's Small World: Photomicrography Competition'. Furthermore, these courses teach students to think critically as they analyse peer-reviewed journal articles, to learn scientific writing as they write reports, and to speak publicly as they prepare and give oral presentations. Many students in these classes reported them as being pivotal moments in their undergraduate career. One student's microscopy course feedback stated, 'This is the class that made me apply to graduate school'.

**The Next Steps for the Bratu Lab**

Dr Bratu and her students have many projects planned that will build upon recent findings. Now that they have successfully visualised real-time nucleic acid and protein transport within the oocyte, they hope to study this movement within other cell types – cell types more difficult to work with in a laboratory setting, such as neurons. As mRNA regulation is likely a key player in the development of neurodegenerative diseases, results of these studies could have vast implications for diseases like Alzheimer's, Parkinson's and Huntington's.

They will be expanding on their results on *oskar* and its association with Bruno and Cup by determining whether these translational repression mechanisms are coupled to the repression mechanisms of microRNA via recruitment into the P-bodies. By knocking down the expression of each translation repression factor in combination of other protein factors in a systematic fashion, Dr Bratu and colleagues will elucidate the translation control process of *oskar* mRNA and the many proteins it involves during oogenesis.

Building upon the discoveries of Drongo's co-localisation with Clathrin light chain and F-actin at the oocyte cortex, the team aims to dig even deeper, determining if Drongo participates in the endocytosis of VAMP7 (Vesicle Associated Membrane Protein 7) in fruit fly egg chambers or if it acts as a GAP effector for Arf6, a protein involved in biological membrane trafficking, using *in vitro* assays.

By deciphering these intricate biological processes, and gaining further insight into how mRNA-protein complexes influence gene expression and protein translation, Dr Bratu and others in her field pave the way for colleagues to unravel the complexities of developmental or neurologic disorders. This work may lead to the development novel therapeutics for deadly viruses like HIV or Influenza A.

# Meet the researcher

**Dr Diana P. Bratu**
Department of Biological Sciences
Hunter College, CUNY
New York
USA

Dr Diana P. Bratu is an Associate Professor in the Department of Biological Sciences and the Scientific Director of the Bio-imaging Facility at Hunter College, CUNY, US. She began her career at New York University, where she obtained her B.A. in Chemistry and Mathematics, and pursued a M.S. and Ph.D. in Molecular and Cell Biology. She then completed two postdoctoral fellowships, one in Molecular Genetics at the Public Health Research Institute, NJ and another in Cell Dynamics at the University of Massachusetts Medical School, MA. She became involved in cellular imaging during her doctoral training and has since been an avid contributor to the field of biophotonics.

## CONTACT

**E:** bratu@genectr.hunter.cuny.edu
**T:** (+1) 212 772 5235
**W:** http://bratu.bioweb.hunter.cuny.edu/
**W:** http://www.bratulab.com

## FUNDING

NSF
NIH
PSC-CUNY

## REFERENCES

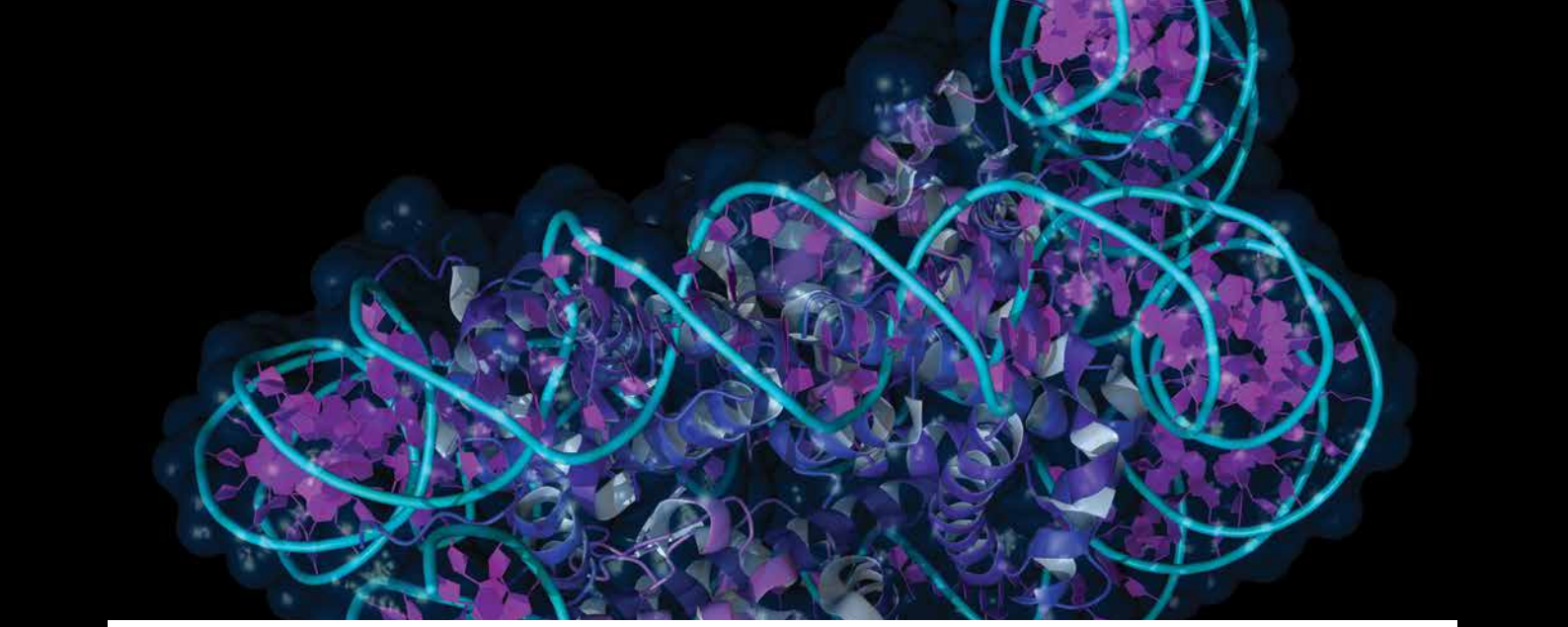
IE Catrina, LV Bayer, G Yanez, JM McLaughlin, K Malaczek, E Bagaeva, SAE Marras and DP Bratu, The temporally controlled expression of Drongo, the fruit fly homolog of AGFG1, is achieved in female germline cells via P-bodies and its localization requires functional Rab11, RNA Biology, 2016, RNA Biology, 2016, 11, 1117–1132.

LV Bayer, M Batish, SK Formel and DP Bratu, Single molecule RNA in situ hybridization (smFISH) and immunofluorescence (IF) in the *Drosophila* egg chamber, *Drosophila* Oogenesis: Methods and Protocols, Methods in Molecular Biology, Springer Protocols, Humana Press, 2015, 1328, 125–36.

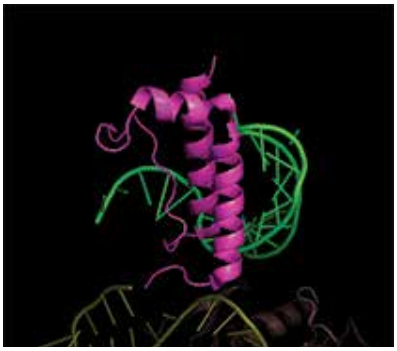
IE Catrina, SAE Marras and DP Bratu, Tiny molecular beacons: LNA/2'-Omethyl RNA chimeric probes for imaging dynamic mRNA processes in living cells, ACS Chemical Biology, 2012, 7, 1586–1595.

MM Mhlanga, DP Bratu, A Genovesio, A Rybarska, N Chenouard, U Nehrbass and JC Olivo-Marin, *In vivo* colocalisation of *oskar* mRNA and trans-acting proteins revealed by quantitative imaging of the Drosophila oocyte, PLoS One, 2009, 4, e6241.

DP Bratu, BJ Cha, MM Mhlanga, FR Kramer and S Tyagi, Visualizing the distribution and transport of mRNAs in living cells, Proceedings of the National Academy of Sciences USA, 2003, 100, 13308–13.



# HISTONES: TAILLESS mRNAs



**Professor William F. Marzluff** and his colleagues at the University of North Carolina study the regulation of gene activity in animal cells. Specifically, they are interested in the regulation of gene expression during the cell cycle by postranscriptional mechanisms. One system they study is the regulation of histone mRNA, both during the mammalian cell cycle and during early development in fruit flies (*drosophila*), frogs and sea urchins.

**Histone mRNAs are Unique**

Histones form the bulk of the protein component of chromatin – a complex of macromolecules found in cells, consisting of DNA, RNA and protein. Originally, histones were thought to be only involved in packing chromosomal DNA in eukaryotic cells, but now, they are clearly also important players in regulating gene expression. After histones are translated and incorporated into the chromosome, they can be considerably modified, and it is these modifications that play an important role in regulating gene expression. In metazoans – or animals – a family of replication-dependent histone genes encodes a majority of the histone proteins, which are classified as canonical histone proteins.

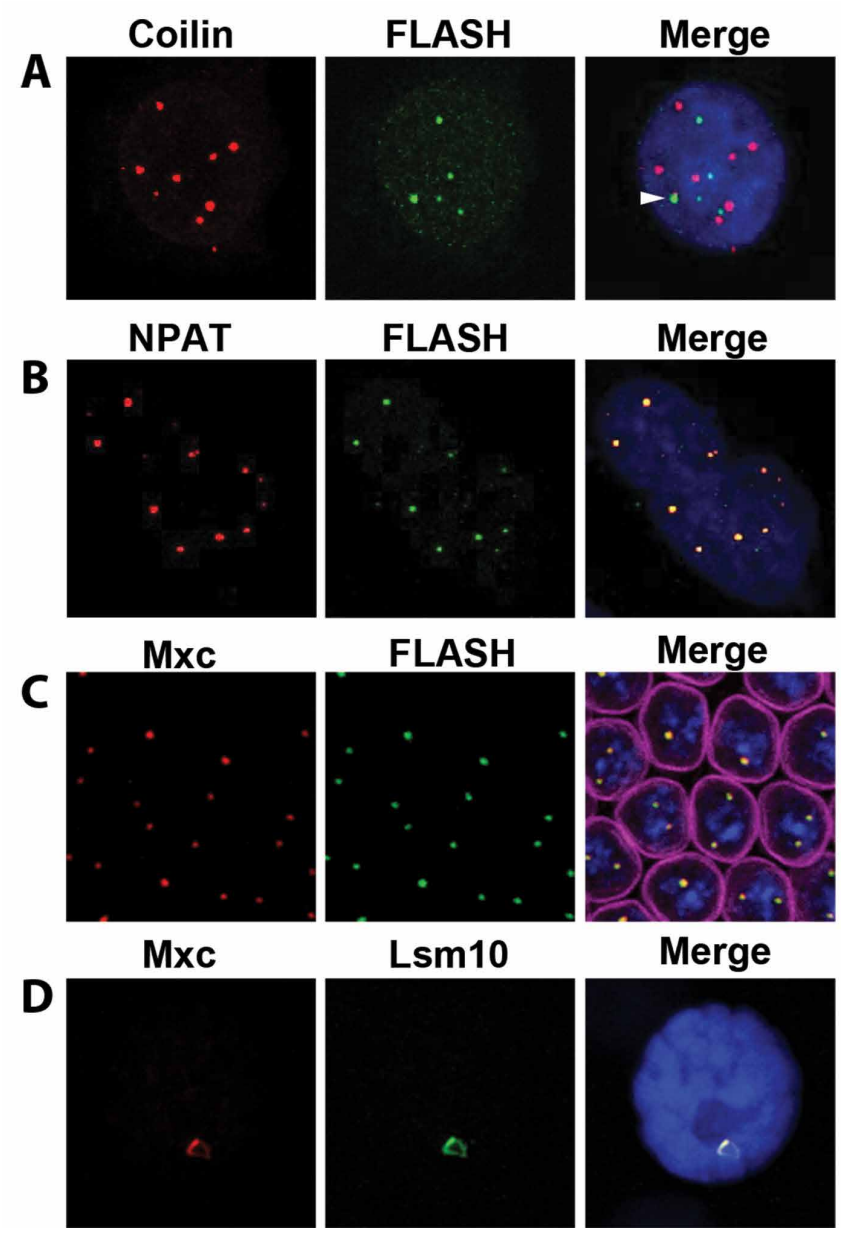
Histone proteins come together in a coordinated fashion to bind DNA to form chromatin. The genes H2A, H2B, H3 and H4 encode histone proteins that make up the nucleosome, while the H1 histones are bound to the linker DNA, found between the nucleosomes. Interestingly, the mRNAs of the canonical histone proteins do not have poly(A) tails – structures important for the nuclear export, translation and stability of all other cellular mRNAs.

Instead, these replication-dependent histone mRNAs have a 3′ stem-loop sequence at their end, which is crucial for their regulation. This 3’ stem-loop sequence is formed by the endonucleolytic cleavage of the pre-mRNA. The stem-loop consists of a 6-base stem and 4 nucleotide loop. Overall, the 3′ end of canonical histone mRNAs consists of a conserved 25–26 nucleotide sequence, which includes 5 nucleotides before the stem-loop, the 16-nucleotide stem-loop and 4–5 nucleotides after the stem-loop. This sequence is evolutionarily conserved in metazoans with a variety of features that are invariable, including the nucleotides in the stem, in the loop and before the stem-loop. Importantly, only one protein, the stem-loop binding protein (SLBP), binds to this 26-nucleotide sequence stem-loop and contributes to all aspects of histone mRNA metabolism. Within SLBP there is a small, 73 amino acid RNA-binding domain (RBD) that is not like the RBD of any other RNA-binding protein. Thus, given that these histone canonical mRNAs do not have the typical 3’ poly(A) tail they require a distinct set of factors for metabolism and regulation.

Histone mRNAs are only present in the S phase of the cell cycle – the part of the cell cycle in which DNA is replicated. Replication-dependent histone mRNAs need to be promptly expressed at the beginning of the S phase and must exist at high levels throughout the S phase in order to provide histones to package the newly synthesised DNA. They are destroyed when the S phase is complete or are rapidly degraded during the S phase if DNA replication is cut short. This type of regulation also requires specialised factors made specifically for these histone mRNAs.

As mentioned above, since they have a unique 3′ end, histone mRNAs require a different set of factors for their synthesis and translation, including the U7 small nuclear RNA (snRNA) and the Sm-like proteins LSm10, LSm11 (components of the U7 snRNP), SLBP and SLBP-interacting protein 1 (SLIP1). In addition, there are some factors of the metabolic machinery involved in histone mRNA metabolism that overlap with mRNAs with poly(A) tails. Thus, the regulation of histone mRNAs is quite complicated and must be conducted in a very efficient manner. There are still many aspects of these processes that have yet to be discovered.

Indeed, this is precisely the interest of Professor William F. Marzluff and his team at the University of North Carolina.




His laboratory, together with Professor Schümperli's lab at the University of Bern, was the first to clone the cDNA for SLBP, which, as mentioned above, binds the 3' end of histone mRNA and participates in all aspects of histone mRNA metabolism. Professor Marzluff's lab aims to understand how SLBP carries out its multiple functions, how SLBP itself is regulated and how this regulation is connected with other cell cycle regulators involved in the regulation of histone mRNA. Over the years, he and his colleagues have investigated these questions using mammalian and, together with Professor Robert Duronio, *drosophila* (fruit fly) models.

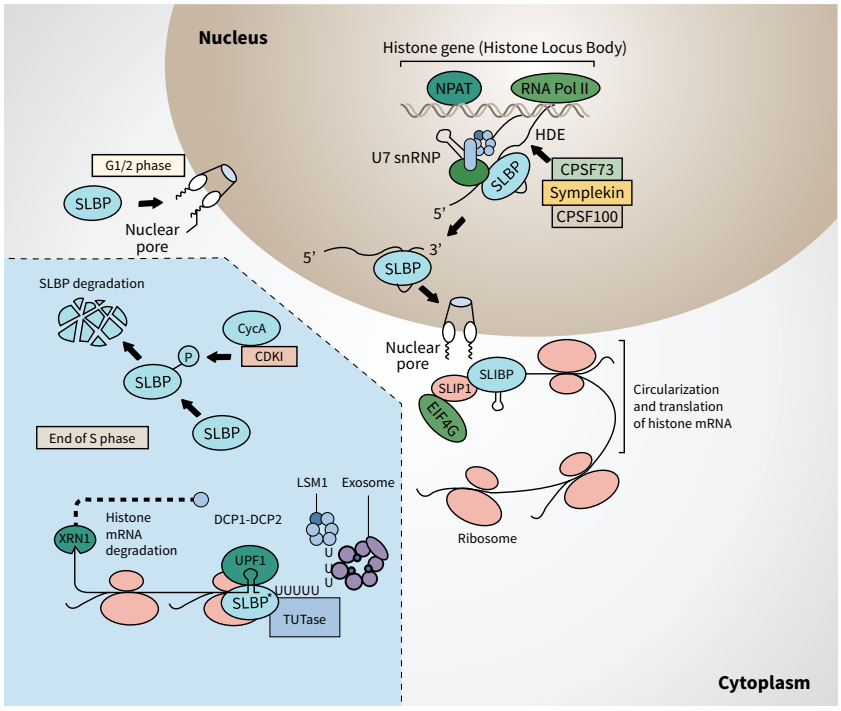
**SLBP in Mammals**

As we have discussed, histone mRNAs are unique in that they do not contain poly(A) tails. Given that the poly(A) tail is the main site for mRNA regulation, understanding how histone mRNAs which lack a poly(A) tail are regulated is a unique area of research. A key breakthrough was cloning SLBP. Professor Marzluff and his colleagues used a yeast three-hybrid selection system developed by Professor Marvin Wickens for cloning RNA-binding proteins. They cloned the cDNA for the SLBP from humans and frogs. The mammalian SLBP is a unique 31-kD protein that is unrelated to other proteins in the database and contains a novel 73-amino-acid RNA-binding domain. Professor Marzluff and his group developed antibodies specific for the cloned SLBP, which allowed them to study the function of SLBP. These advances by Professor Marzluff and his team have paved the way for much of the research performed in this field. They demonstrated that SLBP is the major protein that binds the 3' end of histone mRNA in both the nucleus and the cytoplasm and that it is required for histone pre-mRNA processing. They also showed that SLBP binds to the 3' stem-loop end of histone mRNA in polyribosomes. Thus, SLBP functions in both the nucleus and the cytoplasm. In addition, SLBP is tightly regulated like histone mRNA, and the SLBP protein is present only in S-phase, indicating that SLBP regulation is an important component of histone mRNA regulation. The discovery of SLBP was of huge significance, as, in Professor Marzluff's words: 'this protein is central to everything about histone mRNA metabolism. One of the most exciting days came 17 years after the discovery of SLBP when we first got to see the structure of SLBP bound to the stemloop RNA, as a result of the efforts of Dr Dhazi Wang and Professor Liang Tong (Columbia Univ.) who crystallised the SLBP-RNA complex.'

**Beyond SLBP in Mammals**

The formation of mature mRNAs requires 3' end processing of nuclear pre-mRNAs. Most pre-mRNAs undergo a cleavage step that is coupled to the addition of the poly(A) tail. In contrast, as Professor Marzluff's group has shown, the cleavage of metazoan replication-dependent histone pre-mRNAs occurs by a different mechanism, which is not followed by the addition of a poly(A) tail. For histone pre-mRNAs, two sequence elements are required for processing, the stem-loop and the histone downstream element (HDE), and the cleavage occurs between these two elements. The team aimed to identify the mysterious factor directly responsible for cleaving histone pre-mRNAs. In studies led by Professor Zbig Dominski, they identified a protein known as CPSF-73, which also happens to be the enzyme that cleaves all the other pre-mRNAs before polyadenylation. This finding strongly suggested that histone pre-mRNAs, as well as the other pre-mRNAs that undergo cleavage/polyadenylation, utilise the same endonuclease during 3' end processing. 'This result meant that there likely were two complexes that contained CPSF-73, one for polyadenylated mRNAs and a second one for histone mRNAs,' Professor Marzluff explains.

However, the mechanism by which CPSF-73 is recruited to histone pre-mRNA was still unknown. One part of the processing complex they thought probably directly or indirectly participates in this process is




Lsm11, a component of U7 snRNP. To test this, Professor Marzluff and his colleagues investigated proteins that interact with Lsm11, and discovered that the protein FLASH interacts with the N-terminal region of Lsm11. Previously, FLASH was shown to localise in the vicinity of histone gene loci and had been shown to be required for S phase progression, suggesting that it might play a role in the expression of histone genes. During their investigations, the team, again led by Professor Dominski, revealed that FLASH is an essential factor for 3' end processing of histone pre-mRNAs. They also demonstrated that this role is conserved between vertebrates and invertebrates. FLASH is central in the processing complex because together with Lsm11 it recruits and/or activates CPSF-73. It may also play a vital role in integrating the expression of histone genes with other cellular events, including cell cycle progression and apoptosis. This finding was the breakthrough that allowed Professor Marzluff and his team to go on to identify the histone cleavage machinery.

Professor Marzluff and his colleagues defined a sub-complex of poly(A) factors that are necessary for histone pre-mRNA processing. These factors are present in a stable complex and interact with histone-specific processing factors. The results from this study suggest that there is a common core cleavage factor that is required for processing of histone and polyadenylated pre-mRNAs.

**Histone mRNA Regulation in *Drosophila***

At the same time that Professor Marzluff and his colleagues were investigating the role of SLBP and other histone mRNA regulators in mammalian cells, they also investigated the function of these proteins genetically over the past 15 years in collaboration with Professor Duronio, using *Drosophila* as a model system. To examine the function of SLBP genetically, Professor Marzluff and his team cloned the gene encoding *Drosophila* SLBP (dSLBP) and isolated flies with mutants in the SLBP gene. They found that each of the *Drosophila* histone genes possessed a poly(A) site just after the histone stem-loop, which allowed the flies to produce polyadenylated histone mRNA. These cells were viable, but the flies did not develop into adults. This finding allowed the team to undertake numerous genetic studies on histone gene expression.

While metazoan histone mRNAs are unique because their pre-mRNAs contain no introns, and the mRNAs possess a conserved stem-loop structure instead of poly(A) tails, in *Drosophila* there are canonical poly(A) signals located downstream of the normal cleavage site of each histone gene. These signals are employed when histone 3' end formation is inhibited.

Histone mRNAs are synthesised in a distinct subcompartment of the nucleus, termed the histone locus body (HLB), that concentrates many of the factors that are required for histone mRNA biosynthesis. As mentioned above, the protein FLASH and U7 snRNP are components of the HLB that participate in 3' processing of the nonpolyadenylated histone mRNAs by recruiting the endonuclease CPSF-73 to the histone pre-mRNA. Together with Professor Duronio they further examined this process in *Drosophila* using transgenes to complement a FLASH mutant. These studies revealed that unique domains of FLASH involved in U7 snRNP binding, histone pre-mRNA cleavage and HLB localisation are all required in vivo for proper FLASH function. In addition, the genetic manipulation of the HLB composition using mutations in FLASH revealed that mutations in the HLB assembly factor Mxc lead to the failure to concentrate FLASH and/or U7 snRNP in the HLB and impaired histone pre-mRNA processing. This malfunction leads to the accumulation of small amounts of polyadenylated histone mRNA and nascent read-through transcripts at the histone locus. Ultimately, these findings demonstrate that HLB concentrates FLASH and U7 snRNP to support efficient histone mRNA biosynthesis and unveil the coupling of 3' end processing with transcription termination.

**Persistence is Necessary**

Professor Marzluff and his colleagues have been investigating the regulation of histone mRNAs since the early 1980s. Through the years, as described above, they have made critical discoveries on how histone mRNAs are regulated throughout the cell cycle. Professor Marzluff alludes to the fact that these discoveries have not come easily: 'We were stuck on some things for 20 years before being able to figure it out – this encourages people to be persistent.' The large number of talented students and postdoctoral fellows who not only carried out the experiments, but often made the key insights that resulted in novel discoveries, deserve credit for much of the work. Indeed, their persistence continues, as his lab focuses on all aspects of histone mRNA metabolism using a combination of biochemical, molecular biological and genetic approaches.

# Meet the researcher

**Professor William F. Marzluff**
William Rand Kenan Distinguished Professor
Department of Biochemistry and Biophysics
University of North Carolina
Chapel Hill
USA

Professor William Marzluff first started his research into biochemistry and gene regulation during the final year of his undergraduate degree at Harvard University. After this, he went on to receive a Ph.D. in 1971 from Duke University for a thesis entitled 'Species Specificity of Histone Acetylation'. Following his postdoctoral training at Johns Hopkins University, in 1974 he took a position in the Biochemistry Division in the Department of Chemistry at Florida State University. Professor Marzluff then moved to the University of North Carolina in 1991, where he headed a Molecular Biology program that was a collaboration between the College of Arts and Sciences and the School of Medicine. Here, he also served 13 years as Associate Dean for Research in the School of Medicine.

During Professor Marzluff's undergraduate, graduate and postdoctoral training, he was also an avid rugby player, first for Harvard's team, then Duke's and finally for the City of Baltimore as a postdoctoral researcher.

## KEY COLLABORATORS

Professor Robert Duronio, University of North Carolina, USA
Dr Dhazi Wang, Colombia University, USA
Professor Liang Tong, Colombia University, USA
Professor Zbig Dominski, University of North Carolina, USA

## CONTACT

**E:** william_marzluff@med.unc.edu
**T:** (+1) 919 962 2141
**W:** http://www.unc.edu/depts/marzluff/Marzluff/Home.html

# RNA METABOLISM: HOW DIFFERENT BACTERIA REACHED THE SAME CONCLUSION IN THEIR OWN WAYS

**Dr Harald Putzer** at the Institut de Biologie Physico-Chimique, France, investigates how bacteria regulate the expression of genes by degrading their transcripts with RNase enzymes, and how organisms separated by billions of years of evolution, figured out how to do it with remarkably similar techniques.

It is reasonable to call it common knowledge that the blueprints for building a person are stored within our cells in the form of DNA. With slight variations, the basic mechanisms of how DNA is 'read' to produce the proteins which it codes for, are conserved in all of life's domains, from bacteria to complex multicellular organisms like animals and plants. One of DNA's most useful properties is its stability. DNA could be considered the master copy of the instructions, which are not read directly by the protein-building machinery (ribosomes). Instead, DNA is transcribed into a similar molecule called RNA, which is much less stable. RNA which is used in this way is called 'messenger RNA' or mRNA, to distinguish it from other types of RNA within the cell. If DNA is the master copies of the blueprints, then mRNA could be considered the photocopies taken to the building site.

It is the mRNA which is read by ribosomes, as instructions for putting together chains of specific amino-acids – the building blocks of proteins. Using mRNA in this way allows more finely tuned regulation of the amount of protein ultimately produced from a given gene. Each gene often exists as a single copy within the genome, and if a large amount of protein is required, which can change from moment to moment depending on the needs of the cell, then multiple copies of the gene can be produced in the form of mRNA. Each copy allows the production of multiple protein molecules as it is read by the ribosomes.

When the cell decides to stop producing a particular protein, and suspend further production of mRNA, the relevant mRNA molecules already present can be rapidly degraded and their constituent parts recycled. In bacteria this degradation can take place in a matter of seconds or in over an hour, depending on the specific mRNA molecule. 'In contrast to DNA which is a very stable, mRNA is a very fragile molecule that only survives a few minutes in a bacterial cell,' Dr Putzer explains. Bacteria are thought to regulate this process most efficiently by using an 'all-or-none' pattern, where control is implemented at the initiating step within the degradation process. Degradation of mRNA is carried out by a particular type of enzyme, called RNase. The many types of RNase vary in their specific functions, such as in their mRNA targets or whether they degrade mRNA from the terminal ends of the molecule (an exonuclease) or cleave the molecule somewhere between these termini (an endonuclease).

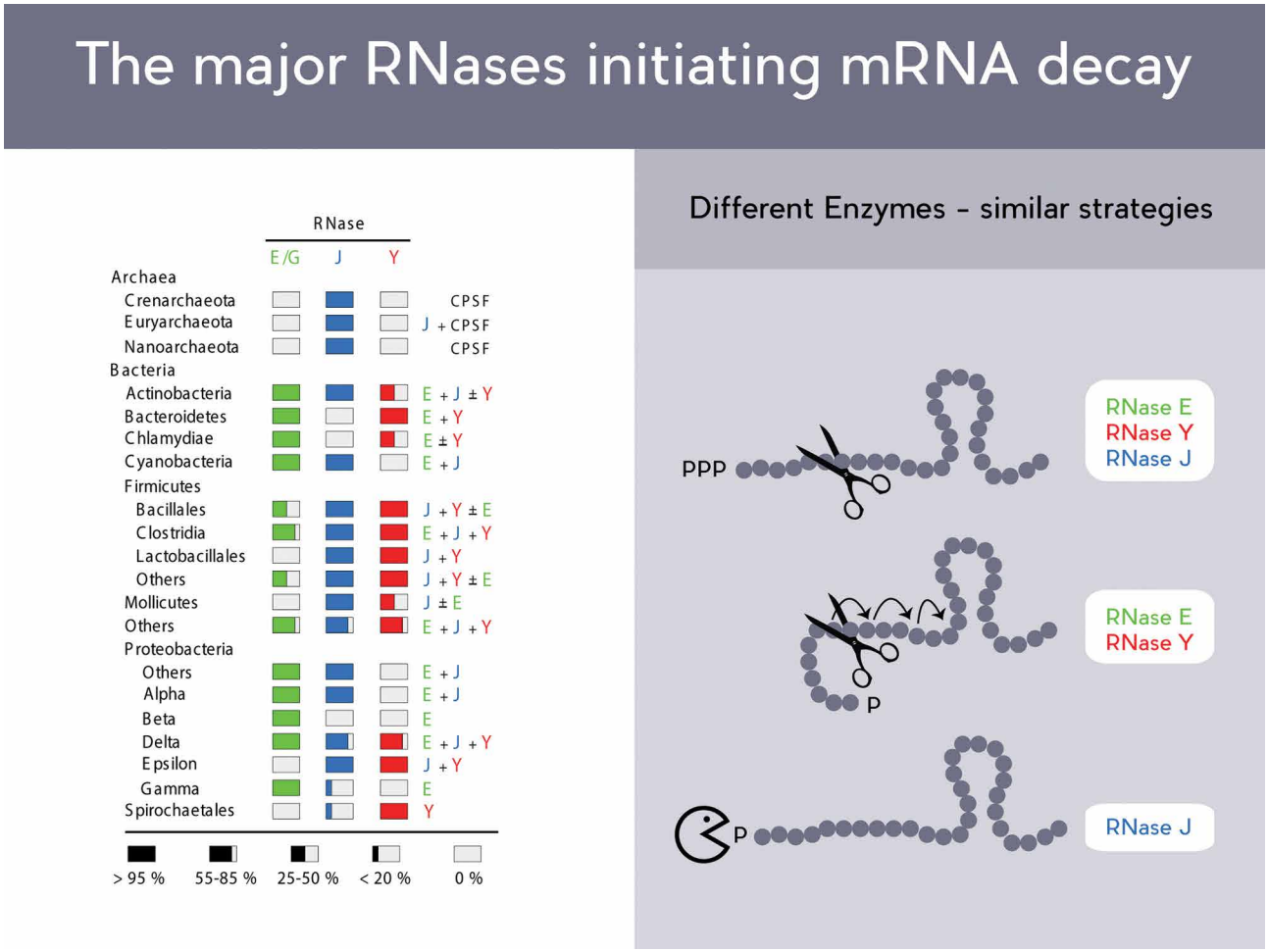
The interests of Dr Putzer and his group are concerned with the structure, function and evolution of this class of proteins and how they work to regulate gene expression and RNA metabolism.

**Evolutionary distance**

As humans, we might consider ourselves highly distinct from things such as plants, yeast, or single-celled amoebae, but we have a common biological feature which is absent in bacteria – a cell nucleus. The nucleus evolved around 2 billion years ago, but long before this (about 3 billion years ago) the evolutionary paths of the two major types of bacteria, gram negative and gram positive, diverged from each other. One of the defining differences between these bacteria is the structure of their cell walls, but the point is that in terms of evolutionary timescales, they are more distinct from each other than a human is from a mushroom. Two of the most highly studied representatives of these classes of bacteria are *Escherichia coli,* a gram negative and probably the most heavily studied organism in biology, and *Bacillus subtilis,* a gram positive. 'I started to work with *B. subtilis* because it became clear quite quickly that even among bacteria regulation of gene expression can involve very divergent mechanisms,' Dr Putzer tells us. 'The large evolutionary distance between *E. coli* and *B. subtilis* has been very rewarding in terms of exploring new mechanisms and enzymes involved in gene control.'

Both organisms contain RNase enzymes, but they are evolutionarily so distinct from each other that they could scarcely be said

**‘We focus on the structural and functional study of RNase Y, a key ribonuclease in *B. subtilis.* Together with RNase J and RNase E these three enzymes probably represent the major ribonucleases that determine the strategies used by bacteria to initiate mRNA decay.’**



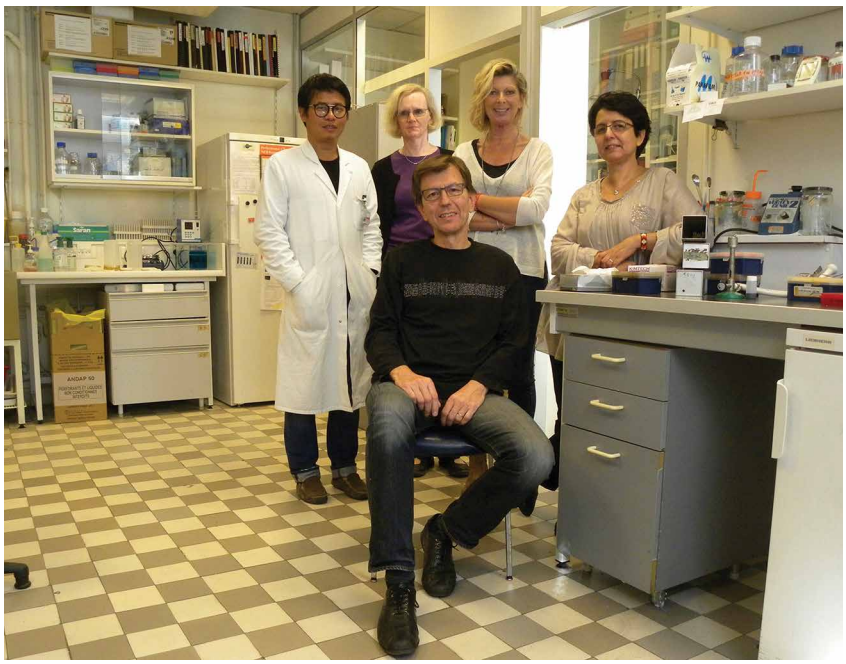

to be comparable at the DNA sequence level. In recent years, the work of Dr Putzer and his lab has shown that RNase E (from *E.coli*) and RNases Y and J, found in *B. subtilis,* have evolved – independently – highly similar structural properties and mechanistic features.

**Different enzymes – similar strategies**

In 2008, the Putzer lab elucidated the mechanism of function of the first RNase known to possess both endonuclease and exonuclease activity. The team has also demonstrated that these enzymatic activities are carried out by the same catalytic site within the protein. Though versions of this protein are present in throughout the bacterial kingdom, it is notably absent from *E. coli.* Structurally, RNase J and RNase E appear quite similar, despite a complete absence of similarity in the actual DNA sequences of the two genes which code for them.

A year later, the group published research into the role of another important RNase, called RNase Y. This enzyme is found in *B. subtilis*, as well as around 40% of other bacteria, and shows a similar effect to RNase E, on the total amount of mRNA within the cell. Rather than only degrading a small number of specific mRNA targets, these enzymes have wide ranging mRNA targets, having a major reductive effect on the total quantity of mRNA. Another similarity between these two enzymes lies in their propensity to interact with multiple, distinct enzymes, to form a complex of RNA and protein degrading enzymes, called a degradosome.

The discovery of RNase Y led to a re-evaluation of RNA decay models in *B. subtilis,* which until then had focussed predominantly on the exonuclease activity of RNase J, while RNase Y strongly affects the total mRNA of the cell through its endonuclease activity. The endonuclease specificity of RNases J and Y are remarkably similar to that of RNase E, with all three showing a strong preference for



targeting mRNA molecules which have been processed in a particular way, by the addition of a phosphate molecule at one end of the mRNA molecule.

The influence of a particular enzyme on a cell can also be modulated by its precise location inside the cell. Bacterial cells are not compartmentalised by the presence of internal membranes, unlike in eukaryotes. Regardless of this, some internal cellular components are still localised with some precision. RNase Y appears to show a similar pattern of localisation within the cell, as that seen for RNase E in *E. coli*. Both enzymes are found at the periphery of the cell, tethered to the cell membrane, though the reason for this pattern of localisation is not clear.

These shared properties make it likely that the way mRNA is processed and degraded are more similar between these two evolutionarily diverged classes of organisms – gram positive and negative – than previously thought.

**The effects of RNase Y**

As mentioned before, mRNA is only one of the types of RNA molecules present in the cell. RNA has numerous functions, often also involving regulation of gene expression, when it takes the form of 'non-coding RNA' – RNA that does not directly code for a protein. RNase Y is also thought to have a role in processing these non-coding RNA molecules. In experiments involving strains of bacteria where the amount of RNase Y is depleted, the specific mRNA and non-coding RNA molecules can be measured and compared to those found in a strain with normal levels of functional RNase Y. In these experiments, around 1600 mRNA transcripts are found in the RNase Y depleted strain, which are upregulated in comparison to when the enzyme in present, while several hundred non-coding RNA molecules are also found to be increased in its absence. Interestingly, across several studies, though the numbers of these molecules seem to be in agreement, the actual identity of the transcripts is highly variable.

The variability in the activity of RNase Y seems to be at least partly dependent on growth phase of the bacteria. Microorganisms show highly different patterns of gene expression based on whether they are rapidly dividing (exponential phase), such as they would do in ideal environmental and nutritional conditions, or if they are in a stationary phase, where their overall numbers are steady. Studies looking at the activity of RNase Y in pathogenic gram positives, such as *Staphylococcus aureus* (the 'SA' in MRSA) have shown that during exponential growth, the number of genes affected by depletion of RNase Y is only around 100 – far less than in *Bacillus subtilis* – but that this number is greatly increased during stationary phase.

Another experiment in *Staphylococcus aureus* artificially altered the localisation of the enzyme, from being on the membrane to floating around freely inside the cell, but found that this did not have much of an effect on the activity or specificity of the enzyme, at least during exponential growth. In *B. subtilis* however, Dr Putzer's group have found preliminary data suggesting that at least for certain transcripts, the enzyme's localisation at the cell membrane is essential for it to degrade them effectively.

**The future of RNase research**

Dr Putzer and his team plan to continue his laboratory's research into RNase Y and RNase E, by using a wide variety of approaches. Though the structure of RNase J is known to be similar to that of RNase E, the structure of RNase Y is still unknown, and the group is currently working towards determining it. He plans to sequence RNA across the whole genomes of these bacteria, in order to determine which ones are targeted specifically by these RNases. Another future aim of his laboratory is to study how interacting proteins of RNase Y, such as those involved in biofilm formation, modify the activity of the enzyme. His lab will also take advantage of recent advancements made in the field of super-resolution microscopy, which offers resolution beyond the theoretical limit of a light microscope, therefore allowing single RNA molecules to be imaged within the cell. Using super-resolution microscopy in live cells might help to answer questions about the patterns made by RNase E and RNase Y as they move along the inner cell membrane, as well as how they interact with other components of the degradosome as it assembles and disassembles. Dr Putzer hopes to use these techniques to learn more about the commonalities and differences between the mRNA degradation strategies in these two evolutionarily distinct organisms. 'Answers to these questions might help shed light on some fundamental aspects of mRNA metabolism that might be important to all bacteria,' he explains.

This research is fundamentally important to understand how essential biological processes are conserved between highly divergent bacterial species, and will have impacts on agricultural, industrial and biomedical research.

# Meet the researcher

**Dr Harald Putzer**
Head, Microbial Gene Expression Department
CNRS UMR8261 – Institut de Biologie Physico-Chimique
Université Paris Diderot
France

Dr Harald Putzer graduated from the University of Innsbruck, Austria in 1980, with a Master's degree in Natural Sciences. He then went on to complete a PhD at the Max Planck Institute of Biochemistry, in Germany, studying how light can regulate gene expression in slime molds. Dr Putzer then moved to Paris, where he performed research at the Institut de Biologie Physico-Chimique, largely focusing on the study of gene regulation in bacteria. Here, he currently holds the position of Head of the Microbial Gene Expression Department.

## CONTACT

**E:** harald.putzer@ibpc.fr
**T:** (+33) 1 58 41 51 27
**W:** http://www.ibpc.fr/UMR8261/equipePutzer/HomePutzer.htm

## KEY COLLABORATORS

Dr A. Carpousis, University Paul Sabatier, Toulouse, France
Dr FA Wollmann, Institut de Biologie Physico-Chimique, Paris, France

## FUNDING

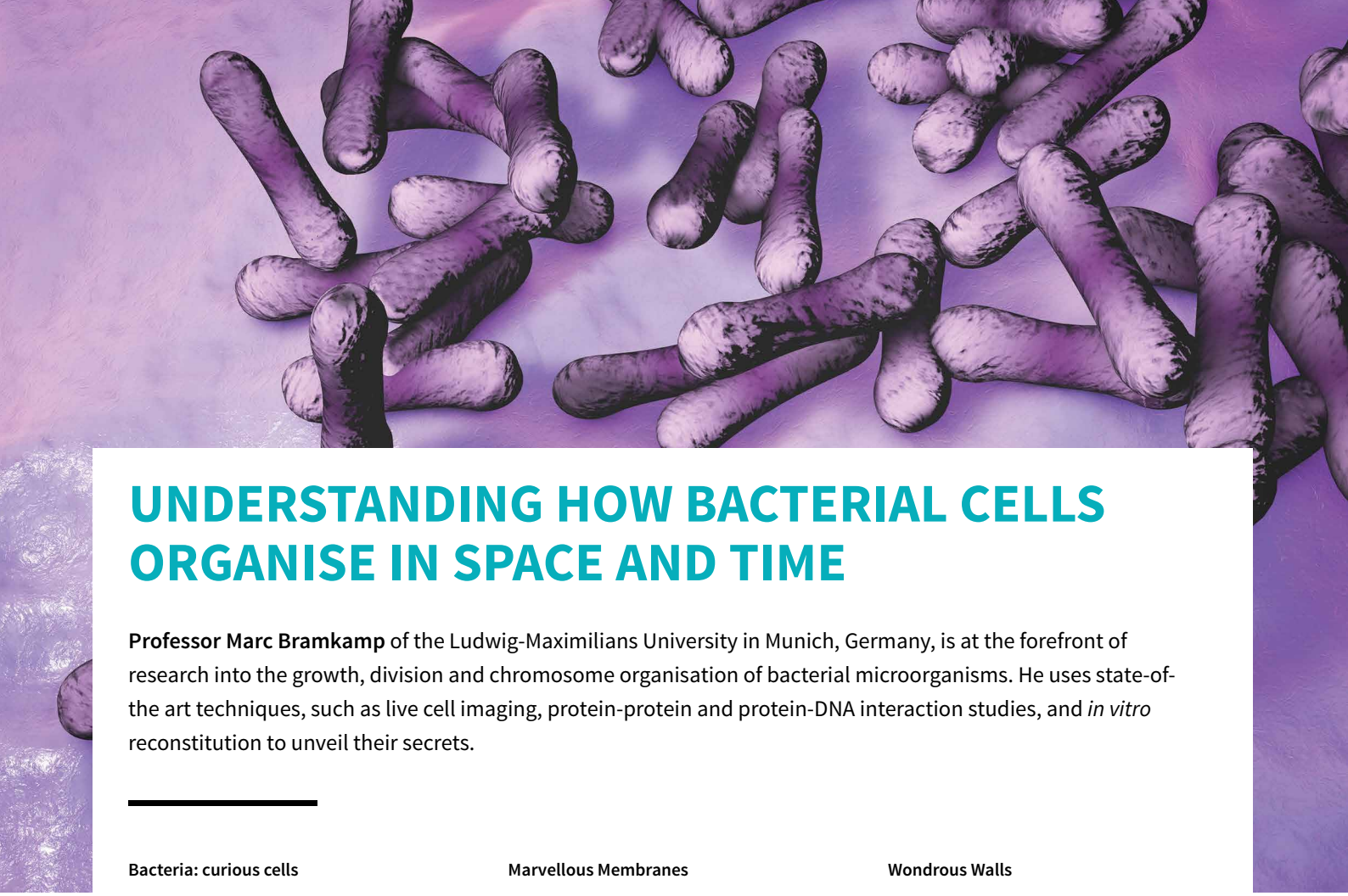
CNRS, Labex Dynamo and Agence Nationale de la Recherche (ANR)

## REFERENCES

S Laalami, L Zig and H Putzer, Initiation of mRNA decay in bacteria, Cell Mol. Life Sci., 2014, 71, 1799–1828

K Shahbabian, A Jamalli, L Zig and H Putzer, RNase Y, a novel endoribonuclease, initiates riboswitch turnover in Bacillus subtilis, The EMBO Journal, 2009, 28, 3523–3533.

IL de la Sierra-Gallay, L Zig, A Jamalli and H Putzer, Structural insights into the dual activity of RNase J, Nature Structural and Molecular Biology, 2008, 15, 206–212.



# UNDERSTANDING HOW BACTERIAL CELLS ORGANISE IN SPACE AND TIME

**Professor Marc Bramkamp** of the Ludwig-Maximilians University in Munich, Germany, is at the forefront of research into the growth, division and chromosome organisation of bacterial microorganisms. He uses state-of-the art techniques, such as live cell imaging, protein-protein and protein-DNA interaction studies, and *in vitro* reconstitution to unveil their secrets.

### Bacteria: curious cells

Bacteria are single celled prokaryotic microorganisms. Bacterial cells exhibit a wide range of shapes and differ in many ways to eukaryotic cells, such as those that make up the human body. Their cellular organisation, which is markedly different to ours, is one of the key focuses of Professor Marc Bramkamp and his team at the Ludwig-Maximilians University in Munich, Germany. He tells us about what drew him to the field of bacterial cell biology research: 'Bacteria were, for a long time, not considered to be interesting targets for cell biological research because their level of complexity seemed much lower than that of eukaryotic cells. They lack a sophisticated endomembrane system, have no organelles (with very few, but exciting exceptions, such as magnetosomes), and were actually not ideal models for imaging techniques because of their small size.' he explains. 'Our knowledge of subcellular organisation of bacterial cells was revolutionised in the early 2000s with the discovery that some bacterial cells contain an extended cytoskeleton, reminiscent to the eukaryotic actin cytoskeleton.' After completing his PhD in 2003, Professor Bramkamp wanted to be a part of this new field of bacterial cell biology.

### Marvellous Membranes

As Professor Bramkamp was particularly interested in bacterial cell organisation, this became the major focus of his work. 'Our work aims to understand how bacterial cells organise proteins in space and time. We focus largely on the organisation of the plasma membrane. This is the lipid membrane that surrounds the entire bacterial cell and defines the boundary between the inside and outside of the cell. Every molecule that needs to enter or leave the cell must pass through the membrane and hence, many transport systems are localised in the membrane to ensure correct exchange.' he explains. 'Furthermore, the cell needs to sense its environment and most of its sensors are integrated into the membrane'.

It has long been thought that these membrane embedded proteins are able to freely diffuse within the lipid building blocks of bacterial membranes. In their research, Professor Bramkamp and his team have shown that the membrane is highly organised and complex. They have also discovered that proteins are often clustered in defined domains. These domains are organised by flotillins, scaffolding proteins, previously known only from eukaryotic cells. Flotillins seem to recruit cargo proteins into defined membrane regions in which they function.

### Wondrous Walls

The team have also extensively investigated bacterial cell walls and have found that the proteins and enzyme complexes that synthesise bacterial cell walls are also highly organised. He gives a brief description of their structure and function: 'Most bacteria contain a cell wall made of sugar polymers that are crosslinked by peptide-bridges, called peptidoglycan. The cell wall acts as an exoskeleton and defines the shape of a cell and allows the cell to survive osmotic changes in the environment. Since the peptidoglycan cell wall is unique to bacteria, the enzymes making the cell wall are ideal targets for antibiotics, such as penicillin. The localisation of the cell wall synthetic complexes defines how a cell is shaped. Most rod-shaped bacteria such as *Escherichia coli* insert the new cell wall material at their lateral sites.'

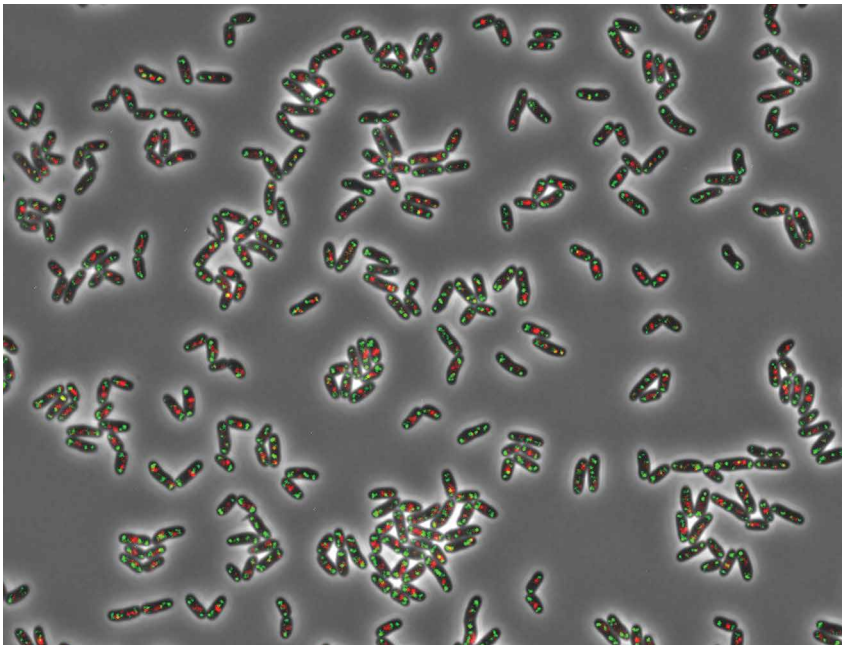
Such rod-shaped model organisms, which include *Escherichia coli* and *Bacillus subtilis*, have been used and studied extensively by a number of bacterial cell biologists, including Professor Bramkamp. A fundamental question that all cells need to deal with is the correct positioning of the cell division apparatus of which FtsZ, a bacterial tubulin, is the central structural element. Many bacteria, including *B. subtilis* and *E. coli* employ the so-called Min system for spatial

regulation of FtsZ assembly. The Min system is composed of an FtsZ polymerisation inhibitor, MinC, a Walker-type ATPase, MinD, and a spatial regulator. In 2008, he found a new member of the Min system that acts as a bridge to hook up MinCD to the sites of division. Against previous predictions, the group found that Min proteins are highly dynamic in *B. subtilis* and actively prevent re-initiation of cell division in vicinity to used division sites. However, Min proteins are not present in all bacteria, such as those that grow from their tips (cell poles). He and his team have therefore developed the *Corynebacterium glutamicum* model, that lacks the known Min system for cell division, to further investigate this. As he explains: 'Some species, such as the notorious pathogen *Mycobacterium tuberculosis,* grow from their cell poles and we know little about the organisation of this cell elongation machinery. We have developed a close relative to *M. tuberculosis,* the non-pathogenic bacterium *C. glutamicum*, into a model organism to study polar cell elongation, cell division and chromosome segregation.' The team hope that by gaining greater insight into the interactions and enzymatic properties of the proteins involved, they might be able to define new antibiotic targets.

**Creating *Corynebacterium glutamicum* into a cell biological model organism**

The subcellular organisation of *C. glutamicum* is significantly different to that of all previous model bacteria. In addition to lacking the Min division site selection systems, it elongates by polar peptidoglycan insertion and lacks an actin-like cytoskeleton. The Bramkamp group identified that chromosome segregation depends on a ParABS system (which is similar to *C. crescentus).* Its chromosome organisation also seems to be different to other model organisms; *B. subtilis* and *C. crescentus* encode SMC proteins, while *E. coli* utilises a homologous system, named MukBEF. In their preliminary studies, Professor Bramkamp and his team found that *C. glutamicum* actually encodes both of these systems. He adds: 'We have been the first to describe the molecular machinery that positions and segregates the chromosome in *C. glutamicum* and how this impacts on growth and division. We now want to take these initial descriptions to the next level and address the exact chromosomal organisation in *C. glutamicum*.'

**'Our knowledge of subcellular organisation of bacterial cells was revolutionised in the early 2000s with the discovery that some bacterial cells contain an extended cytoskeleton, reminiscent to the eukaryotic actin cytoskeleton'**



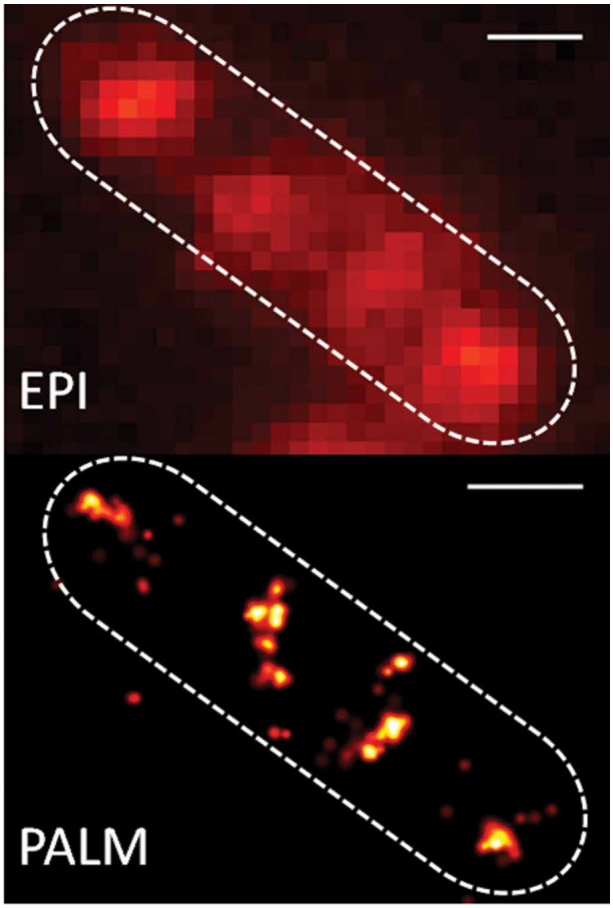
**Chromosomal Uncovering**

To produce viable offspring, bacterial cytokinesis must be regulated both spatially and temporally. This cell division is connected to other processes such as chromosome domain organisation, chromosome compaction and chromosome segregation. Professor Bramkamp plans to determine how these processes occur in *C. glutamicum*.

The analysis of chromosome organisation will be done in a few different steps. First, the team will use operator arrays to visualise chromosomal loci, then they will map the gene loci using chromosome conformation capture (3C/3C-seq) assays. Finally, they will localise and characterise the nucleoid associated proteins (mainly SMC/MksB).

Chromosomal compaction is an important process involved in the creation of a bacterial nucleoid. This is a submicron sized compartment in which the chromosome is highly compacted (approximately 1000 times), which has some loops of DNA that extend like bristles. *C. crescentus* has one circular chromosome, and the three-dimensional structure of the *C. crescentus* genome has recently been described as ellipsoidal with entangled arms.

Chromosome segregation is mediated by the ParA-ATPase complex, which Professor Bramkamp postulates could use the nucleoid spatial track. This is due to previous findings that have suggested that ParA proteins use the nucleoid as a template to gain positional information and as such, the structure of the chromatin also provides informational cues for segregation. He plans to address this theory in three ways using new experimental tools that have been developed in his lab: time lapse deconvolution microscopy, interference with chromosome organisation to proof the hypothesis of a nucleoid-track, and molecular and cellular analysis of Par protein mutants.




**Professor Bramkamps breakthroughs in *C. glutamicum* cell biology**

Thus far, Professor Bramkamp's lab has extensively investigated chromosome segregation in *C. glutamicum*, and in doing so has made some key discoveries. By introducing *tetO* arrays into an intergenic region at the *oriC*, the team has demonstrated that the *oriC* is located to the cell poles. They have also shown that the conventional ParB protein identifies consensus *parS* sites at the origin region and co-localises with the *oriC* to the cell poles. When replication is initiated, the newly formed origin migrates to the cell centre, were the cell is about to divide. By this, the segregated origin is placed close to the new developing pole. This process requires ParA, which localises across the nucleoid, with its focal point close to the cell poles, likely co-localising with ParB.

However, as of yet, they have been unable to determine the nature of ParA assembly through light microscopy. Professor Bramkamp postulates that ParA shows DNA-binding and ATPase activity in vitro, and the positioning of ParA proteins over the nucleoid may hint that the partitioning ATPase uses spatial information of the nucleoid for correct movement of the ParB-*parS* nucleoprotein complex. He continues to work towards identifying the molecular mechanism of the polar anchoring of the chromosome. And recently, he has identified that the *C. glutamicum* homologue of DivIVA is a landmark protein that interacts directly with an N-terminal motif in ParB and thereby tethers the ParB-*parS* complex to the cell poles.

In the same study, he and his team showed that other actinobacteria, such as *M. tuberculosis,* undergo a similar mechanism – DivIVA interacts with corresponding ParB proteins. In a follow up study, the interaction domains of ParB and DivIVA were mapped. However, Professor Bramkamp notes that, at present, it is not clear how the newly replicated origin with bound ParB escapes polar anchoring. Additional findings have led to the assumption that positional information of the nucleoid is a main regulator of division site selection, in accordance with the variable cell length of daughter cells in *C. glutamicum*. The team has also revealed the chromosome organisation in a number of processes related to cell growth and cytokinesis, through the use of microfluidic devices and time lapse analysis.

In his recent work, Professor Bramkamp and his team identified together with colleagues from Juelich around Professor Frunzke that the genome of *C. glutamicum* encodes for an actin-like protein. Corynebacteria are normally known for the absence of such a cytoskeleton, but close inspection revealed that this actin-like protein, termed AlpC, is encoded in a prophage region. Prophages are bacterial viruses that integrate into the host genome and replicate with the host DNA. Only under stress conditions, the phage 'revives' and multiplies its DNA. The two groups showed that AlpC builds a phage specific DNA segregation machinery in which AlpC connects to the viral DNA via an adapter protein, AlpA. AlpA recognises a DNA repeat within the phage DNA. The two research teams could show that viral DNA segregation is vital for correct DNA replication. These findings reveal that the relation between actin and viral DNA is evolutionary ancient and conserved from bacteria to humans.

**Trying New Technologies**

In the future, Professor Bramkamp and his team will be using new techniques to develop their studies of bacterial cell organisation. He notes that 'new microscopy methods are now available that allow single molecule detection. These techniques rely on the stochastic switching of fluorophores in a way that every fluorophore in a cell can be imaged separately and its precise position is than calculated individually.' This technique is called photoactivated localisation microscopy (PALM). Professor Bramkamp concludes: 'With PALM, we can address subcellular structures with unprecedented precision and can identify objects of about 20 nm – 10 times greater than previous resolution. This allows us to gain insights into the organisation of *Corynebacterium* cells. We are especially interested in understanding how the polar cell wall synthesis is coupled to chromosome organisation.'

# Meet the researcher

**Professor Marc Bramkamp**
Professor of Microbiology
Ludwig-Maximilians-Universität
Munich
Germany

Marc Bramkamp is Professor of Microbiology at the Ludwig-Maximilians-Universität, in Munich, Germany. He received his undergraduate degree in Biological Science at the University of Osnabrück in Germany and went on to complete his PhD at the same university in 2003. He worked under the supervision of Professor K. Altendorf throughout his PhD and subsequent postdoctoral studies until 2004 when he then moved to the Sir William Dunn School of Pathology at Oxford University in the UK for a further two years as a research fellow to work with Professor Jeff Errington. Prior to receiving his professorship in Munich in 2012, he worked at the University of Cologne in Germany (with Professor Reinhard Krämer) and was group leader of the Bacterial Cell Biology Lab.

## CONTACT

**E:** marc.bramkamp@lmu.de
**T:** (+49) 89 2180 74611
**W:** http://www.bacteriology.bio.lmu.de/

## FUNDING

DFG - Deutsche Forschungsgemeinschaft
BMBF (Bundesministerium für Bildung und Forschung)

## REFERENCES

Sawant P, Eissenberger K, Karier L, Mascher T, Bramkamp M, A dynamin-like protein involved in bacterial cell membrane surveillance under environmental stress, Environ Microbiol., 2016, 8, 2705–2720. doi: 10.1111/1462-2920.13110.

Donovan C, Heyer A, Pfeifer E, Polen T, Wittmann A, Krämer R, Frunzke J, Bramkamp M, A prophage-encoded actin-like protein required for efficient viral DNA replication in bacteria, Nucleic Acids Res., 2015, 43, 5002–5016. doi: 10.1093/nar/gkv374

Bramkamp M and Lopez D, Exploring the existence of lipid rafts in bacteria, Microbiol Mol Biol Rev., 2015, 79, 81–100. doi: 10.1128/MMBR.00036-14.

Gruber S, Veening JW, Bach J, Blettinger M, Bramkamp M, Errington J, Interlinked sister chromosomes arise in the absence of condensin during fast replication in *B. subtilis*, Curr Biol., 2014, 24, 293–298.

Donovan C, Sieger B, Krämer R, Bramkamp, M, A synthetic *Escherichia coli* system identifies a conserved origin tethering factor in Actinobacteria, Mol Microbiol, 2012, 84, 105–116.

# AWAKENING SLEEPING BACTERIA

**Professor Karl Forchhammer** and his colleagues analyse how cyanobacteria can survive and recover from long periods of starvation. They use the model strain *Synechocystis* PCC 6803, a non-diazotrophic, unicellular cyanobacterium. When deprived of a nitrogen source, the cells become chlorotic and survive in a dormant state. Upon re-addition of nitrate, a genetically determined program is initiated that brings back the cells to vegetative life.

**Dormant Cyanobacteria**

Cyanobacteria are prokaryotic cells that are also known as blue green algae. This name comes from that fact that cyanobacteria contain a blue pigment called phycocyanin, which along with green chlorophyll gives cyanobacteria a blue-green appearance. Cyanobacteria are essentially responsible for life as we know it. During the Archaean and Proterozoic Eras (2.5 billion years ago), it was cyanobacteria that were responsible for creating the Earth's oxygen atmosphere. Prior to this, the atmosphere had a very different chemistry, one that was unsuitable to sustain life in its present form.

Cyanobacteria reside in a wide range of habitats that include frozen lakes, acidic bogs, deserts and volcanoes, but they are most commonly found in aquatic environments. In addition, they are found in soil, on rocks and even in the atmosphere. In the light-exposed biosphere, cyanobacteria are ubiquitous. There they function as key players in global carbon and nitrogen cycles. Cyanobacteria are photoautotrophic organisms that absorb carbon dioxide and release oxygen. Several cyanobacteria can also absorb, or 'fix', elemental nitrogen present in the atmosphere, and thus, they do not have to rely on other combined nitrogen sources. In low nitrogen environments or in low carbon dioxide environments, cyanobacteria have gained to the ability to adapt and survive over the course of their evolution. These environmental challenges often limit growth in many terrestrial and aquatic ecosystems.

With respect to nitrogen use, cyanbacteria are divided into two physiological groups. The diazotrophic strains evade nitrogen starvation by expressing an enzyme called nitrogenase, which fixes all-pervading di-nitrogen ($N_2$) gas. In contrast, the non-diazotrophic strains halt their growth when a combined nitrogen source is absent and switch their metabolism from anabolism to maintenance. To make this switch, cyanobacteria first degrade their photosynthetic pigments. This process is known as chlorosis, and during its course, the cells noticeably change in colour from blue-green to yellow and they become dormant.

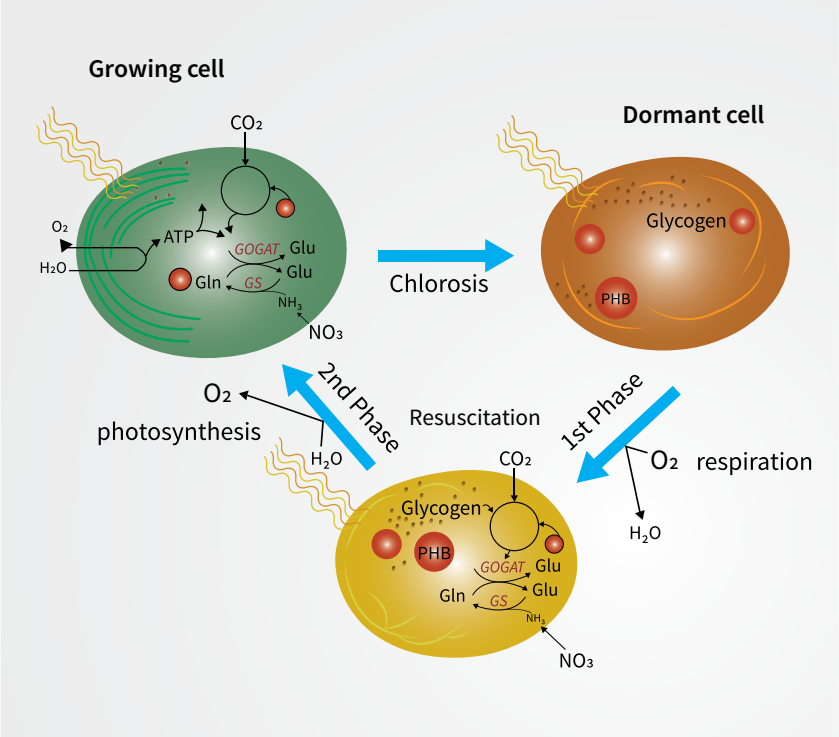
The ability of prokaryotes to become dormant is widespread, especially when nutrients in the environment are limited. There is a notion that dormant bacteria comprise a 'seed bank' – a reservoir of cells that can be resuscitated when favourable conditions are present again. This mechanism is largely responsible for the fitness of bacterial populations and contributes to the spreading of bacterial pathogens. In different bacterial taxa, growth limitation activates various molecular and cellular processes allowing the survival bacterial populations. The ways in which the cells enter dormancy and recover from the dormant stage are rather diverse. Some bacteria form endospores or exospores, others create encapsulated cysts, or even generate apparently non-differentiated persister cells, which is the case in *Mycobacterium tuberculosis*. In order to survive these extended periods of starvation,

the non-differentiated resting cells have the ability to maintain a residual metabolic activity. The ability of the bacteria to rapidly resuscitate from dormancy ensures the successful spread of bacterial populations, but this mechanism remains unclear.

To study the formation of dormant cyanobacterial cells, researchers have previously utilised the freshwater strain *S. elongatus* subjected to nitrogen starvation as a model. Based on these studies, researchers have determined that a protein called NblA, whose expression is controlled by the Nbl regulatory system, initiates the degradation of the major light-harvesting pigments. When the cells are moved to a nitrogen depleted medium, glycogen starts to build up. This accumulation of glycogen occurs very rapidly – within 12 hours – whereas it takes more time until the pigments are fully degraded – up to 5 days. These cells will only stop growing once they have completed a final cell division – which takes about 12 hours.

If the period of nitrogen deficiency is prolonged, the cells will degrade a majority of their cellular proteins and their photosynthetic apparatus until they reach a final chlorotic stage. When the cells enter into this stage, they sustain only residual levels of photosynthesis, which are approximately 0.1 % of their initial activity. By doing this, the cells are able to preserve full viability over at least six months.

Another unicellular cyanobacterium strain, *Synechocystis* sp. strain PCC 6803, has a similar response to nitrogen deprivation, and this species is widely used as a model organism to study underlying aspects of photosynthesis and cyanobacterial physiology. When these cells are in nitrogen deficient conditions, this strain produces a second carbon-storage polymer, polyhydroxybutyrate (PHB), in addition to the glycogen granules, that is presumed to contribute to the ability of the cell to handle chlorosis. In addition, some preliminary results suggested that *Synechocystis* can achieve long-term survival under nitrogen-deficient conditions, and these chlorotic cultures are particularly efficient at recovering from chlorosis. Given that this strain has such a rapid recovery process, it is optimal for studying the resuscitation of a dormant bacterium. By doing so, researchers can gain insights into a fundamental bacterial survival strategy. Indeed, this is precisely what Professor Karl Forchhammer and his team at the University of Tübingen have done. They have used cholortic *Synechocystis* cells to study the organisation of resuscitation at the cellular and molecular level as an example of bacterial awakening from dormancy.




**Two Phases of Resuscitation**

As part of a DFG-supported research training group, RTG 1708 (Molecular principles of bacterial survival strategies), PhD student Alexander Klotz tested the viability of *Synechocystis* cells and the accumulation of PHB (the carbon storage polymer) and glycogen during prolonged nitrogen depletion for up to 42 days. He demonstrated that, of the two carbon reserves, glycogen accumulated almost immediately after nitrogen was depleted. In contrast, the accumulation of PHB occurred much more slowly. In addition, almost all of the cells remained viable after being deprived of nitrogen for 42 days, and they rapidly recovered following the addition of a nitrogen source.
In addition, the research team used the long-term chlorotic cells to further understand the phases of resuscitation. When the cells were resuscitated by adding nitrate to the medium, they began to turn green again within 48 hours, and were completely pigmented and growing exponentially after 72 hours. They also found signs of photosynthetic activity after 24 hours.

As we have discussed, chlorotic cells have two types of carbon reserves – PHB and glycogen – that can fuel initial respiration. Given that previous studies suggest that PHB could be important for recovery, Professor Forchhammer and his group sought to determine whether this was true, as they had detected no significant decrease in PHB content during the first 24 hours of recovery. Additionally, they had observed that PHB degradation did not start before the cells had resumed photosynthetic activity.

In their experiments, the team actually found that PHB is not required for the recovery of chlorotic *Synechocystis* cells. In contrast, glycogen was immediately used after starting the resuscitation program by adding nitrate to the cultures. Based on their physiological studies, Professor Forchhammer and his group discovered two phases of the resuscitation process. In the first phase, which is within the first 16 hours after the reintroduction of nitrogen, respiration is activated, which is supported by glycogen consumption. As the cells transition to the second phase, photosynthetic activity increases at the same time that the cells start to become re-pigmented. In this second phase, despite reconstituting photosynthesis, which takes over the cellular energy supply, glycogen continues to provide cells with carbon skeletons for anabolic needs.

**Structural Changes During Resuscitation**

The structural changes that cells undergo when they experience different cellular and molecular processes often provide clues into the mechanisms behind such processes. Therefore, Professor Forchhammer and his team were interested in visualising the morphological changes of *Synechocystis* cells during their recovery. In collaboration with Dr Roman Sobotka (Trebon, Czech Republic) they found that the fully developed chlorotic cells, in contrast to the growing cells, were almost completely absent of a photosynthetic thylakoid membrane and instead were completely filled with glycogen granules. The granules disappeared during resuscitation, and 24 hours after recovery the thylakoid membranes started to reappear and were fully reconstituted by 36 hours. After 48 hours of recovery, the cells had almost completely re-established thylakoid membranes and a structure resembling lipid bodies was visible. By 66 hours the cells resembled the exponentially growing cells.

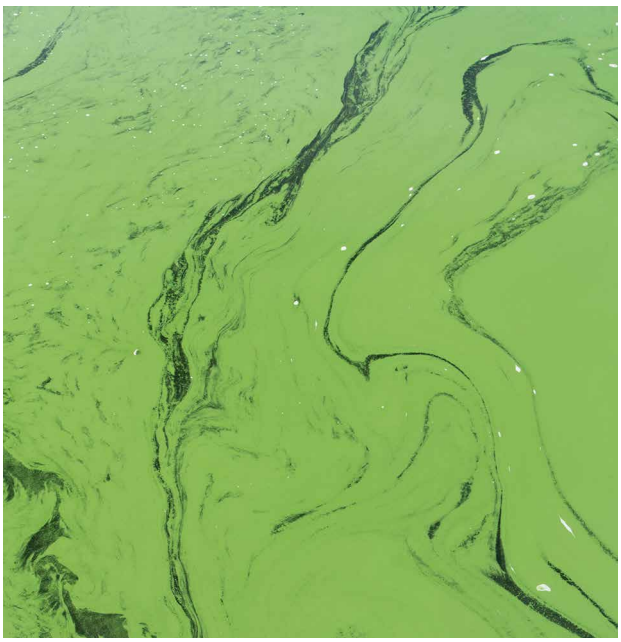
In addition, the team monitored the cell size and cellular DNA content during the recovery from chlorosis. Dr Watanabe found that in the healthy exponentially growing cells there was a narrow distribution of chromosome numbers per cell, centring at about 5–6 chromosomes per cell. In long-term chlorotic cells, the distribution was broader, with many cells having up to the double number of chromosomes per cell. Only when the cells started to divide again after having completely recovered, the chromosome number dropped again to initial values, indicating that the cell cycle was arrested in a pre-divisional stage during chlorosis.

**Gene Expression Changes During Resuscitation**

Microarray analyses are used to gain a deeper insight into complex processes, because they provide clues as to the genes that are regulating such processes. Professor Forchhammer's collaborator Professor Hess (University Freiburg) designed a high-density microarray to further understand the molecular mechanism behind resuscitation in recovering cells.

During the process of resuscitation, they found that the level of 1,572 transcripts, which corresponded to 17.6% of the entire transcriptome, was significantly changed during resuscitation. Among these transcripts, 781 were increased and 791 were decreased in abundance. Based on the time course of the changes, these positively and negatively responding transcripts were classified into three major groups. These groups reflected the chronological order of the transcriptome remodelling during resuscitation.

Interestingly, mRNA from the cells in chlorosis have an overall transcript abundance that is similar to that of growing cells based on the yield of the extracted mRNA. Therefore, the reduced transcriptional activity in the cells in chlorosis might be compensated by increased mRNA stability, which is a phenomenon that has been observed in various growth-arrested microorganisms. During the fully developed chlorotic state, the translational machinery is functioning at an absolute minimum level, and these transcripts make up part of the most strongly repressed of the entire transcriptome. This finding means that most of the transcripts in the chlorotic cells are translationally inactive. In addition, the microarray results revealed that there are highly abundant functionally characterised non-coding regulatory small RNAs, which are anti-correlated to their sense mRNAs. Thus, these RNAs likely play an important regulatory role in stabilising the dormant state. In contrast,



the transcript levels of the most classic global transcriptional regulators were only slightly changed compared to the levels in the growing cells. If fact, this rigid transcriptional repression of the protein biosynthesis machinery is a key feature of the starvation-induced stringent response in bacteria, and reversing this repression appear to be key to the resuscitation program.

By identifying these major cellular processes and the transcriptional dynamics during resuscitation, Professor Forchhammer and his team have revealed the existence of a highly sophisticated genetic program that guides the awakening of a dormant bacterium. There studies, ultimately, provide the framework for future exploration of targeting the regulatory mechanisms that are executing this resuscitation program.

**Conclusions**

Professor Forchhammer and his team present the first detailed analysis and demonstration of the series of events that regulate both the entry into bacterial dormancy and the recovery to an apparently normal, photosynthetically active, vegetative cell for the cyanobacterium *Synechocystis*. These findings have a wide-ranges of potential implications, which will contribute to the understanding of sporulation/germination, bacterial persistence in infection processes and may even influence our understanding of cellular aging. These studies by Professor Forchhammer shed light into the dramatic ultrastructural changes that occur in cells during chlorosis and their subsequent resuscitation. Remarkably, the recovery process is highly coordinated and does not involve the immediate regeneration of the photosynthetic apparatus, but rather it utilises a carefully orchestrated restoration. Ultimately, Professor Forchhammer and his colleagues have defined a perfect model system, which will enable researchers to dissect these processes in detail in the future.

This study was initiated and largely performed as part of the DFG-supported research training group, RTG 1708: 'molecular principles of bacterial survival strategies', headed by Professor Forchhammer at the University Tübingen. Under the framework of this RTG-funding, collaboration in the international team was facilitated, boosting the success of this project.

# Meet the researcher

**Professor Karl Forchhammer**
Chair of the Department for Microbiology
University of Tübingen
Tübingen
Germany

Professor Karl Forchhammer received his Ph.D. in Microbiology at the University Munch, where he was involved in the discovery of co-translational incorporation of the 21th amino acid selenocystein. Soon after, he went on to perform postdoctoral research at the Institut Pasteur in Paris, where he discovered the serine phosphorylation of the PII signalling protein in the cyanobacterium Synechococcus PCC 7942. Between 1994 and 1999, he was assistant professor at the University of Munich, and was then appointed as Professor of Microbiology at the Justus-Liebig-University, Giessen. Since 2007 he is the chair of the department for Microbiology/Organismic interactions at the University of Tübingen.

## CONTACT

**E:** karl.forchhammer@uni-tuebingen.de
**T:** (+49) 7071 29 72096
**W:** http://www.uni-tuebingen.de/fakultaeten/mathematisch-naturwissenschaftliche-fakultaet/fachbereiche/interfakultaere-institute-und-zentren/imit/beteiligte-einheiten/organismische-interaktionen/staff/forchhammer.html

## KEY COLLABORATORS

Elena Ermilova, St. Petersburg State University, Russia
Wolfgang Hess, University Freiburg, Germany
Luciano Huergo, Federal State University Curitiba, Brazil
Vicente Rubio, University Valencia, Spain
Roman Sobotka, Institute of Microbiology, Trebon, Academy of Sciences, Czech Republic

## FUNDING

Deutsche Forschungsgemeinschaft (DFG)

## REFERENCES

A Klotz, J Georg, L Bucinska, S Watanabe, V Reimann, W Januszewski, R Sobotka, D Jendrossek, WR Hess, K Forchhammer, Curr. Biol., 2016, 26, 1–11.

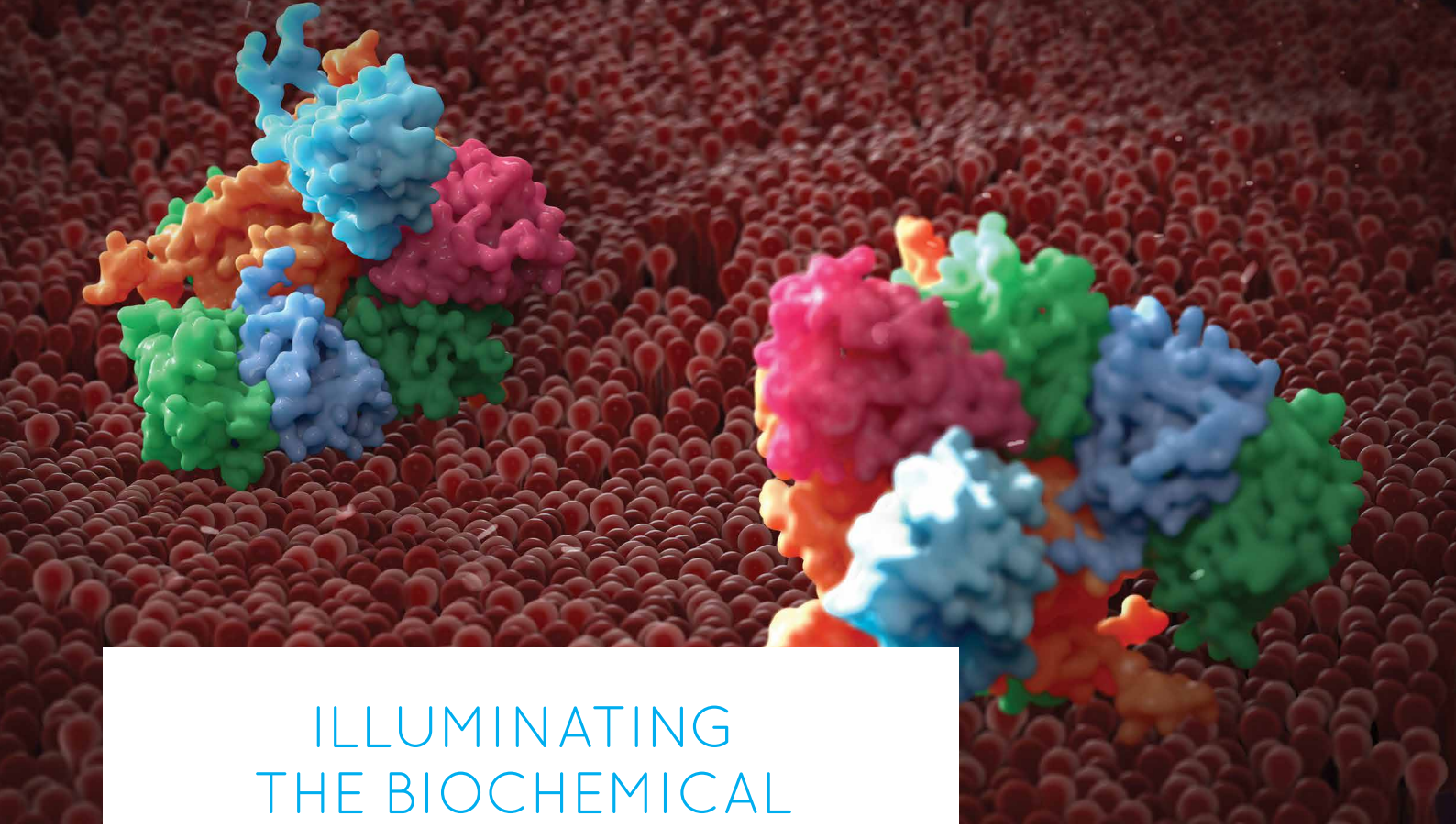
W Hauf, K Schmid, EC Gerhardt, LF Huergo, K Forchhammer, Front. Microbiol., 2016. DOI: 10.3389/fmicb.2016.01700

K Forchhammer, J Lüddecke, FEBS J., 2016. DOI: 10.1111/febs.13584

V-R Chellamuthu, E Ermilova, T Lapina, J Lüddecke, E Minaeva, C Herrmann, M Hartmann, K Forchhammer, Cell, 2014, 159, 1188–1199.

J Lehner, S Berendt, B Dörsam, R Pérez, K Forchhammer, I Maldener, 2013. FASEB J., 2013, 27, 2293–2300.

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

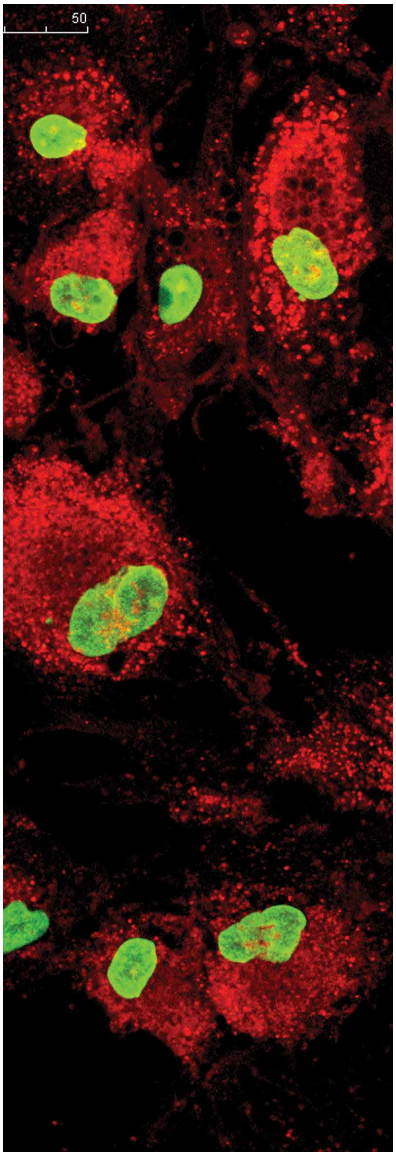


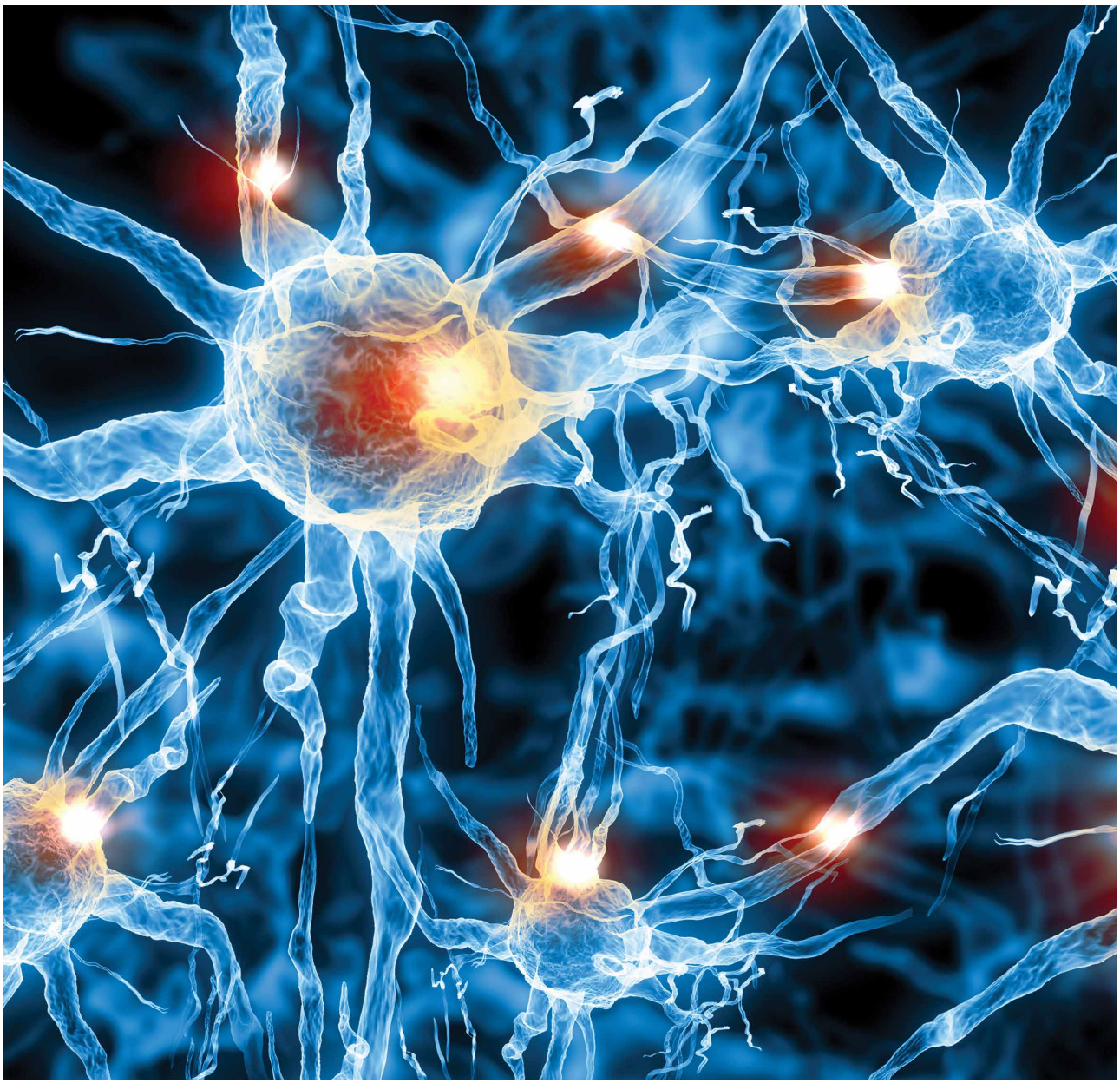
# ILLUMINATING THE BIOCHEMICAL MECHANISMS BEHIND AGEING

The vast majority of us will experience the detrimental health effects of ageing before we die. But biologically speaking, what actually causes this gradual decline in our cells' vitality? This question remains the topic of intense scientific debate, and currently, there are two schools of thought. The first of which is the damage concept, whereby the accumulation of damage over time – such as DNA damage caused by reactive oxygen species (ROS) – leads to the malfunctioning of our biological systems. Programmed ageing theory, however, says that predetermined internal processes are the main drivers behind age-related deterioration. One phenomenon that supports this concept is telomere shortening. Telomeres are regions of DNA found at the ends of each of our chromosomes, whose primary purpose is to protect the important genetic material contained within each chromosome. Because linear DNA is often incompletely replicated, when chromosomes are duplicated during cell division, the replicated telomeres are very slightly shorter. Over the course of our lifespans, these telomeres continue to become shorter and shorter in our tissues where cell turnover occurs. Ultimately, after around 40–60 human cell divisions, a critical telomere length is reached, known as the Hayflick limit, and cell death is initiated. In many studies, short telomeres have been implicated as a possible cause of a range of different degenerative age-related diseases.

However, this is just one of many possible biochemical factors that contribute to our inevitable decline. Illuminating the many complex molecular mechanisms behind ageing is today an extremely active area of research. Understanding these fundamental processes will not only help us to live longer, but more importantly, offer us increased health into old age. In this section, we highlight the work of three scientists, each dedicated to uncovering the biological basis of ageing, in the hope of finding new ways to treat age-related disease.

To introduce the section, we have had the pleasure of speaking with Professor Richard Faragher of the British Society for Research on Ageing (BSRA) – the oldest learned society in the world devoted to studying the fundamental biology of the ageing process. In this exclusive interview, Professor Faragher tells us all about the BSRA's activities in





promoting age-related research in the UK and further afield.

Next, we introduce the research of Dr Matthew Hirschey and his team at Duke University, who investigate how ageing processes can be initiated when enzymes in our bodies break down food into energy. In this fascinating work, Dr Hirschey and his team have made several new discoveries on the ways that metabolism contributes to ageing, and how molecules called sirtuins can offer protection.

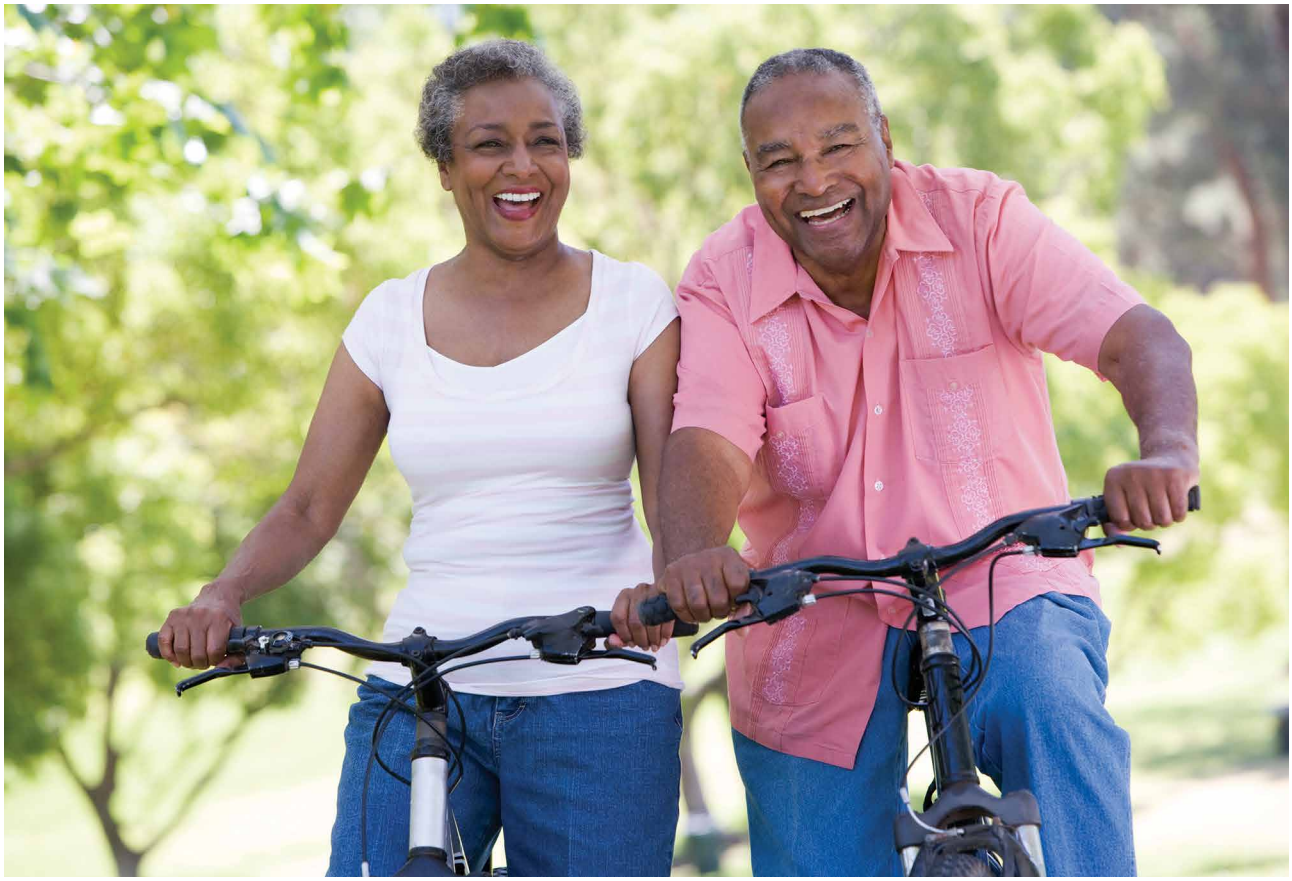
Often termed the powerhouses of the cell, mitochondria play a central role in our metabolism. Following on from Dr Hirschey's work, we move on to further investigate the mitochondrion's role in the ageing process. Although research is active in this area, the relationship between mitochondrial function, oxidative stress and ageing still remains somewhat of a mystery. One researcher who has been gaining new biological insight into this relationship is Dr David Marcinek and his team at the University of Washington. One aspect of Dr Marcinek's work focuses on how reactive oxygen species produced by mitochondria can actually lead to their damage, causing mitochondrial dysfunction over time. This mitochondrial dysfunction may then cause impaired muscle function in elderly individuals. Based on these findings, the research team have developed a novel pharmacological treatment that has already shown promising results in reversing age-related mitochondrial deficits, and restoring skeletal muscle function in elderly patients.

Also unravelling the mechanisms behind mitochondrial dysfunction and ageing is Professor Deborah Ferrington and her colleagues at the University of Minnesota. Over the last decade, Professor Ferrington has been uncovering evidence that mitochondrial dysfunction is associated with the development of age-related macular degeneration (AMD) – the leading cause of blindness amongst elderly people in the developed world. In their work, the team have delved deep into the mitochondrial genome, pinpointing exactly where AMD-associated damage occurs. In the pathogenesis of AMD, mitochondrial damage occurs long before vision loss, so Professor Ferrington hopes her team's work will lead to new treatments that restore mitochondrial health and prevent blindness from developing.

# THE BRITISH SOCIETY FOR RESEARCH ON AGEING

Founded sometime before 1939, The British Society for Research on Ageing (BSRA) is the oldest learned society in the world devoted to the study of the fundamental biology of the ageing process. The society's mission is to increase our scientific knowledge of the processes, causes and effects of ageing and to develop means for counteracting them. Here, we have had the opportunity to speak with **Professor Richard Faragher,** Chair of BSRA's Scientific Advisory board. Over the next few pages, Professor Faragher tells us all about the organisation's activities in promoting age-related research in the UK and further afield.

## ‘The most exciting scientific breakthroughs of the last ten years have been the discovery that the drug rapamycin improves multiple aspects of later life health leading to significant extensions in the lifespan of multiple species’



**Please tell us about the many ways that the BSRA supports age-related research?**

We are members of the Association of Medical Research Charites. Thus, we have an independent scientific advisory board which includes some of the most respected biological gerontologists in the world, but which also has an informed lay membership. We also have a development committee which includes representatives from academia and older people’s organisations together with philanthropists who give their time voluntarily to assist us.

For many years, we have awarded small travel grants to promising students as well as prizes for the best scientific presentations. Our Korenchevsky Award (named for our founder) allows a researcher to present at the Annual Scientific Meeting of our partner organisation, the American Aging Association. We also honour prominent gerontologists through the award of our Lord Cohen of Birkenhead Medal, which we believe is the longest standing award for gerontology in the UK.

Recently we have begun to award larger grants, notably PhD studentships. This has been occasioned by our recognition that there are limited routes by which a donor wishing to fund research in the biology of ageing could be assured of supporting the highest quality research, as well as the decline in support for charitable funding for biogerontology that has occurred in recent years.

**How exactly do you define ageing? Why is it that humans, and the vast majority of other animals, have evolved to have a finite life span, whilst others don’t, such as the Turritopsis dohrnii (the immortal jellyfish)?**

Ageing, in a population of organisms, is simply an exponential increase in death with increasing time. Ageing is not programmed, in the sense that no genes appear to have evolved purely to cause it – they are doing something else. It is the result of selection for early life fecundity. Non-ageing organisms can exist but they are relatively rare and have probably evolved as a result of evolutionary niches in which the advantages of early life fecundity are significantly reduced.

**As our knowledge of the biological basis behind ageing is ever increasing, do you think that someday it may be possible to prevent ageing from happening altogether? If so, what would the ethical ramifications be?**

This is an area marked by confusion and poor definitions. Journalists and some ethicists frequently worry about the ethical validity of

immortality. But immortality is not a state that humans can realistically be expected to achieve. Indeed, one of our Cohen Medalists has described speculation in this area as 'the ethics of never never land'.

A finite, but lengthened lifespan poses no ethical questions that do not already exist. The only ethical question that could meaningfully arise would occur if the extension of lifespan were coupled to poor health. There is no evidence that this situation has to occur.

Because of this confusion, biogerontologists prefer to keep the focus on their primary goal which is the improvement of health in later life.

**What are currently the most promising treatments being developed to increase vitality into old age and to increase longevity?**

The most exciting scientific breakthroughs of the last ten years have been the discovery that the drug rapamycin improves multiple aspects of later life health leading to significant extensions in the lifespan of multiple species. Pilot studies are already being conducted in older humans using drugs similar to rapamycin with the goal of improving immune function in later life.

In mammals, it has now been demonstrated that senescent cells are primary causal agents of ageing and attempts are being made to produce drugs that can remove them or block their effects.

The very diversity of ageing research means that there are multiple areas in which health, and thus longevity, can potentially be improved. For example, many of the genetic pathways that dictate physical fitness also overlap with cardiovascular health an ageing. Thus, the potential exists that existing drugs could be repurposed to improve the quality of life of older people.

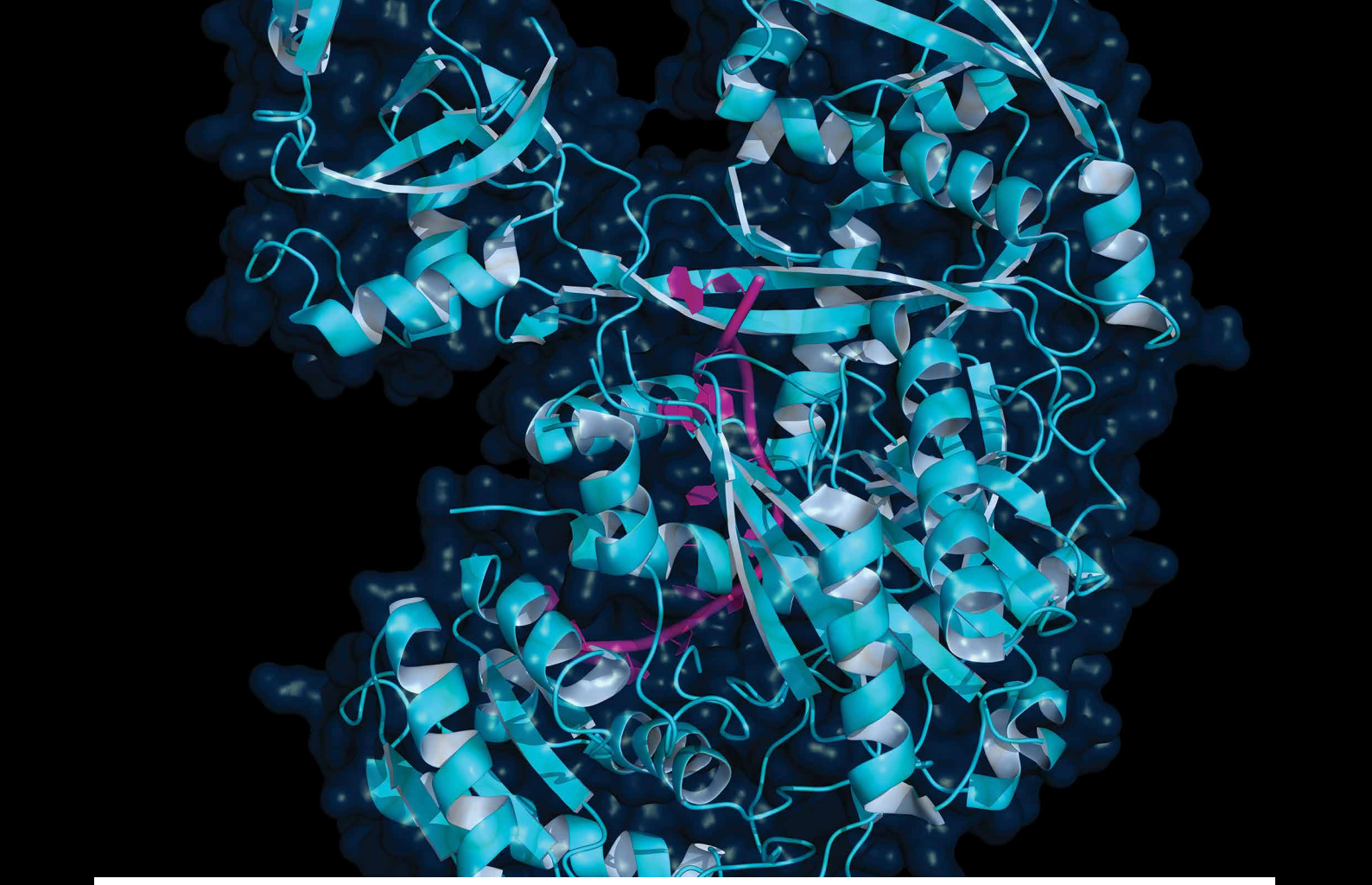
**'We are living in a time when small amounts of philanthropic funding, judiciously placed, could achieve significant things'**

**Finally, can you please share your thoughts on the future of ageing research in the UK, and the ongoing role of the BSRA in that future?**

Relative to the scale of the problem, funding for every aspect of ageing research falls far short of what is required in the UK today. This, ultimately, is a funding gap that the British government should seek to address, or at a minimum urgently review. However, we are living in a time when small amounts of philanthropic funding, judiciously placed, could achieve significant things. The BSRA will do today what it has always done, make the case for the science of ageing and the health of older people, both here and throughout the world.

British Society for Research on Ageing

**www.bsra.org.uk**



# COMBATTING CARBON STRESS TO KEEP CELLS HEALTHY

Aging is a complex process through which cumulative cellular wear and tear leaves us vulnerable to disease. One avenue for aging occurs when enzymes in our bodies break down food into energy. **Dr Matthew Hirschey** at Duke University aims to explain the biochemistry behind how metabolism contributes to aging and how the body defends itself.

**The History of Caloric Restriction**

In 1934, Mary Crowell and Dr Clive McCay of Cornell University published a paper in The Scientific Monthly describing how laboratory rats fed an extremely low calorie diet lived up to twice as long as normal. Fifty years later, at UCLA, Dr Roy Walford and his student Richard Weindruch went further to show that not only did caloric restriction cause mice to live longer, but they also maintained their youth. These mice looked younger, were more active, and showed delays in age-related diseases.

So far results in humans and nonhuman primates are less clear, so rather than advising people to simply reduce caloric intake, a more fruitful tactic lies in understanding the molecular mechanisms by which metabolism impacts aging.
One biochemical candidate implicated in aging is a class of enzymes called sirtuins. In 1999 Dr Matt Kaeberlein at the University of Washington showed that in yeast removing sirtuins reduced lifespan whereas adding extra copies of a sirtuin gene extended lifespan. Since then, the precise mechanism of sirtuin action remains unclear, and attempts to develop sirtuin-mimicking drugs that extend lifespan in healthy individuals have fallen flat. Clearly, the puzzle has missing pieces.

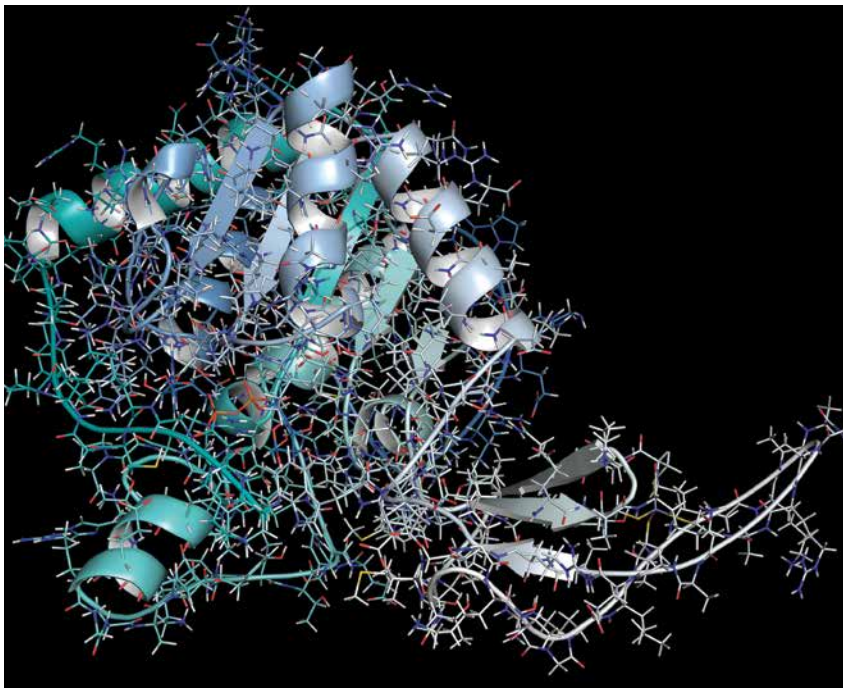
That's where Dr Hirschey steps in.

**Sticky SIRTuation**

Understanding how sirtuins might protect against aging requires a hypothesis about the metabolism-driven aging process that sirtuins combat. Dr Hirschey proposes that the very chemical reactions that break down food into energy could be wearing out metabolic enzymes.

Like a pilled sweater that has gone through the wash too many times, metabolic enzymes inside mammalian cells collect a lot of surface junk after repeatedly breaking chemical bonds to release stored energy. These biochemical hitchhikers take the form of acyl groups – molecules made up of carbon, hydrogen, and oxygen –

**‘Chronically, like oxidative stress, hyperacylation can be considered a type of carbon stress; protein damage would be expected to accelerate the development of age-associated diseases, including cancer, metabolic syndrome, cardiovascular disease and neurodegeneration’**



that are direct by-products of metabolic reactions. Left unchecked, the aggregation of acyl groups hampers metabolic enzyme performance, leading to age-related disease. Dr Hirschey refers to this process as ‘carbon stress’.

He theorises that healthy cells defend themselves against carbon stress by deploying sirtuins – numbering SIRT1 to SIRT7 – that scrub away acyl groups, analogous to the way remora fish clean parasites off sharks. By removing acyl groups at roughly the same pace that they are added, sirtuins maintain metabolic homeostasis.

Dr Hirschey laid out this general theoretical framework in a 2014 review paper in the journal Molecular Cell. Subsequently, he and his team have been working on models and experiments to flesh out the theory. These findings have recently been accepted for publication in Cell Metabolism.

**Carbon Stress, Disease and Aging**

Many of the diseases associated with carbon stress – diabetes, cardiovascular disease, neurodegeneration, and cancer – are also associated with aging. Perhaps by understanding the mechanisms underlying carbon stress, it will be possible to counteract age-related health decline in general.

In a wide array of animal models, loss of protein quality-control mechanisms accelerates the development of age-related diseases and shorten lifespan, and Dr Hirschey believes that sirtuins might play an important role in these mechanisms. Indeed, stimulating sirtuins extends lifespan. What remains to be shown – and what Hirschey’s group aims to uncover – are the precise mechanisms connecting carbon stress, sirtuin-mediated removal of acyl groups, and disease. Knowing the mechanism brings us one step closer to finding effective preventative therapies to counteract aging.

**Independence from Enzymes**

There is much agreement that acyl groups collect on proteins and regulate cellular metabolism, but it’s not clear why or how this happens. Furthermore, Dr Hirschey’s carbon stress model relies on acylation being independent of enzymes.

Dr Gregory Wagner – a former postdoctoral fellow in Dr Hirschey’s lab – and colleagues sought to demonstrate and characterise nonenzymatic acylation though a combination of modelling and biochemistry experiments. They discovered that a particular class of acyl metabolites are so highly reactive that they don’t require an enzyme to catalyse chemical bonding with proteins. As a result, metabolic reactions that produce this acyl metabolite need no further push for those acyl groups to turn around and attach themselves to the very enzyme that spawned them.

‘Beyond discovering new protein modifications and mechanisms of protein acylation, our study has implications for understanding the fundamental nature of protein acylation,’ Dr Hirschey told us.

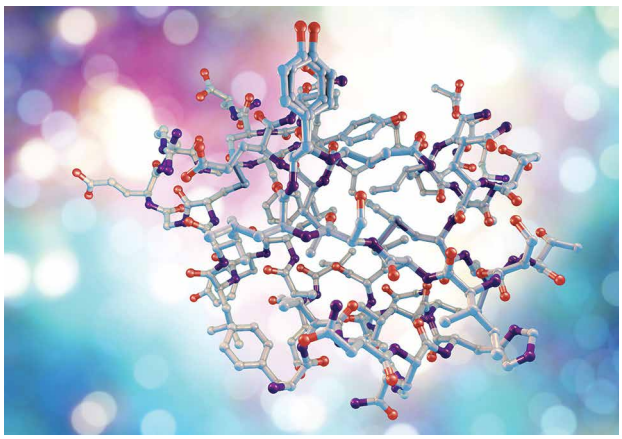
Taken alone, this mechanism regulates enzyme function through a negative feedback loop. As more acyl groups attach to the enzyme the more bogged down it gets and the fewer acyl metabolites it pumps out. Metabolic enzymes essentially shut themselves down in the absence of intervention.

Like barnacles on a boat, these acyl groups are so tenacious that they aggregate easily in the face of passivity. Counteracting acylation rests upon an active mechanism, which a companion paper from the lab finds in a particular class of sirtuins.

**From Models to Molecules**

SIRT4 has been weakly implicated in a host of different reactions, but Dr Hirschey’s group is the first to demonstrate a strong and consistent enzymatic pathway for this molecule.

The importance of SIRT4 for maintaining health has long been clear. Decreased SIRT4 is associated with the deadliest forms of cancer: lung, gastric and colorectal. Diabetes and obesity are also linked to SIRT4 depletion. The effects are there, but the mechanism is unknown.



At the helm of the project, research assistant professor Dr Kristin Anderson and postdoctroal fellow Dr Frank Huynh employed a host of methodologies – from computational simulations to biochemical assays to following SIRT4 knockout mice as they aged. Dr Hirschey called the project 'a technical tour de force'.

The researchers identified a structural region in the active site of SIRT4 that is highly conserved throughout evolution, so it must have been under strong selective pressure to solve a critical biological problem. Computational modelling revealed that this conserved region has an α-helix structure that facilitates removal of specific acyl groups, and bench experiments provided confirmation.

To explore the biochemical pathways involved, Dr Hirschey's lab set out to find SIRT4's molecular dance partners. Strikingly, they found that SIRT4 preferentially removes the very same acyl groups that Dr Wagner characterised in his companion paper.

**Insulin Resistance in Aging Mice**

So how does acylation and SIRT4 relate to aging? One major form of aging is insulin resistance. Insulin is a hormone released by the pancreas that helps control blood sugar. Sometimes – especially with age – the body begins to over-produce insulin, which leads to desensitisation, and ultimately insulin loses its effectiveness at regulating blood sugar. When the body enters this state of insulin resistance, blood sugar levels rise unchecked, which can ultimately lead to type 2 diabetes. The biochemical dominoes that set off this harmful disease were previously unclear, but Dr Hirschey's team has recently linked SIRT4 to insulin overproduction and subsequently insulin resistance in older animals.

Having shown that SIRT4 removes specific acyl groups that are known to impact metabolism, Drs Anderson, Huynh, and colleagues set out to explore the consequences of removing SIRT4 both at the level of pancreatic tissue and in intact, aging mice. When the researchers knocked out the SIRT4 gene in mice they found increased insulin production in isolated pancreatic tissue compared to genetically normal controls.

Even young SIRT4-knockout mice had elevated fasting blood insulin levels compared to control mice, but the knockouts were still able to regulate blood sugar at this point. As the age of the knockout animals increased, so did the fasting blood levels of both insulin and glucose. Control animals saw no such change.

Since SIRT4-knockout mice exhibited sustained high levels of insulin they developed insulin resistance by middle-age. In Dr Hirschey's words: 'pathway dysregulation in young animals leads to an accelerated aging phenotype.'

On the whole, these results suggest that SIRT4 protects against age-related insulin resistance by bolstering metabolic pathways from wear and tear.

**Push and Pull**

'Together, we believe that this pair of studies has uncovered a novel paradigm for how metabolic processes are regulated,' Dr Hirschey told us.

Dr Wagner's work showed that highly reactive acyl species generated from metabolism form never-before reported enzyme modifications that inhibit function. Drs Anderson and Huynh demonstrated the reversibility of this process by showing that SIRT4 removes these very same novel modifications in order to maintain metabolic functionality. Disrupting this delicate balance – as with the SIRT4 knockout mice – causes overproduction of insulin in the pancreas and accelerates the development of age-related insulin resistance.

Dr Hirschey's overall conceptual framework relates the push and pull of nonenzymatic acyl addition and SIRT4 removal as a coordinated regulatory mechanism whose breakdown contributes to the decline in health associated with aging.

**Pervasive**

As life originated in a primordial chemical goo – molecules randomly and promiscuously reacting – it is unsurprising that some lingering unforeseen reactions occur between enzymes and their metabolites. Therefore, the addition of toxic carbon appendages to metabolic enzymes is an inevitable and continuous consequence of metabolism itself. In this way, the natural consequence of using enzymes to metabolise food into energy is to cripple those very enzymes, aging the body, and increasing susceptibility to disease.

As this mechanism is at such a basic level of enzyme/metabolite interaction, it is also not surprising that these detrimental, unintended reactions are highly conserved across the evolutionary tree. A natural consequence of having a long phylogenetic timeline to counteract such a maladaptive process is the opportunity to evolve a variety of cellular quality-control programs.

Dr Hirschey's group is the first to propose a precise mechanism whereby sirtuin enzymes serve to remove suppressive and damaging nonenzymatic acyl modifications to metabolic proteins, thereby staving off senescence for one more day. Beyond the specific mechanisms tying SIRT4 to diabetes, Dr Hirschey's conceptual framework describes the push-pull between carbon stress and deacylation. This theory could explain the general role of sirtuins in stress resistance, healthspan, and even lifespan. Future work promises to uncover new roles of the sirtuin family in disease and aging as well as demonstrating how pervasive acylation and deacylation are as regulators of cellular function.

# Meet the researcher

**Dr Matthew D. Hirschey**
Associate Professor
Duke University Medical Center
Duke University
Durham, USA

Dr Matthew Hirschey completed a Ph.D. in Biochemistry and Chemistry in 2006 at the University of California, Santa Barbara. He went on to do a postdoc at the University of California, San Francisco until 2011 when he started his own lab at Duke University with the goal of studying metabolic control, mitochondrial signalling, and cellular processes regulating human health and disease. He has published in several high profile journals including Nature, Science and Cell. He has also received numerous awards including an Innovator Award from the American Heart Association, a New Scholar in Aging Award from the Ellison Medical Foundation, and the Helmholtz Young Investigator in Diabetes (HeIDi) Award.

## CONTACT

**E:** matthew.hirschey@duke.edu
**T:** (+1) 919 479 2315
**W:** http://lab.hirschey.org/

## KEY COLLABORATORS

Dr Christian Olsen, University of Copenhagen
Dr Donald S. Backos, University of Colorado Anschutz
Dr Deborah Muoio, Duke University
Dr Tony Capra, Vanderbilt University

## FUNDING

American Heart Association
the Ellison Medical Foundation
FARA
NIH

## REFERENCES

GR Wagner and MD Hirschey, Nonenzymatic Protein Acylation as a Carbon Stress Regulated by Sirtuin Deacylases, Molecular Cell, 2014, 54, 5–16.

GR Wagner, DP Bhatt, TM O'Connell, JW Thompson, LG Dubois, DS Backos, H Yang, GA Mitchell, OR Ilkayeva, RD Stevens, PA Grimsrud and MD Hirschey, A Class of Reactive Acyl-CoA Species Reveals the Nonenzymatic Origins of Protein Acylation, Cell Metabolism, 2017, In Press.

KA Anderson, FK Huynth, K Fisher-Wellman, JD Stuart, BS Peterson, JD Douros, GR Wagner, JW Thompson, AS Madsen, MF Green, RM Sivley, OR Ilkayeva, RD Stevens, DS Backos, JA Capra, CA Olsen, JE Campbell, DM Muoio, PA Grimsrud and MD Hirschey, SIRT4 Is a Lysine Deacylase that Controls Leucine Metabolism and Insulin Secretion, Cell Metabolism, 2017, In Press.

# GUIDING AGEING RESEARCH INTO MATURITY

Old age is a bigger part of life today than ever before, yet our ability to tackle age-related diseases is lagging behind our extraordinary leaps in lifespan. One of the many dedicated researchers delving into the ageing process is **Dr David Marcinek,** who is bringing fledgling therapies for mitochondrial dysfunction out of their infancy. Dr Marcinek is currently an associate professor in the Department of Radiology at the University of Washington Medical Center, where he focuses on the interaction between energetics, redox signalling, and muscle physiology in ageing and disease.

Some of the challenges predicted for an ageing population are already quite evident, and include not just the hardships endured by elderly individuals themselves, but also the collective strain on healthcare systems and the economy. There seems to be no sign of these problems resolving themselves: in the United Kingdom, for instance, one sixth of the population is over 65 years of age. Government forecasts predict that one quarter of the population will be in this category by 2050.

These statistics undoubtedly represent a triumph for modern standards of living, but fail to highlight the struggle of *quality* of life in keeping pace with *quantity* of life. Although we have formulated treatments for a vast range of age-related diseases, ageing still represents the single greatest risk factor for most chronic diseases. Thus, directly impacting the ageing process has the potential to significantly improve both quality and quantity of life. Despite these seemingly insurmountable and inevitable consequences of growing old, there is hope that age-related diseases can be dammed at their source through research into the ageing process itself. This is a huge area of study that is continually growing and uncovering the physiological factors that may contribute to ageing.

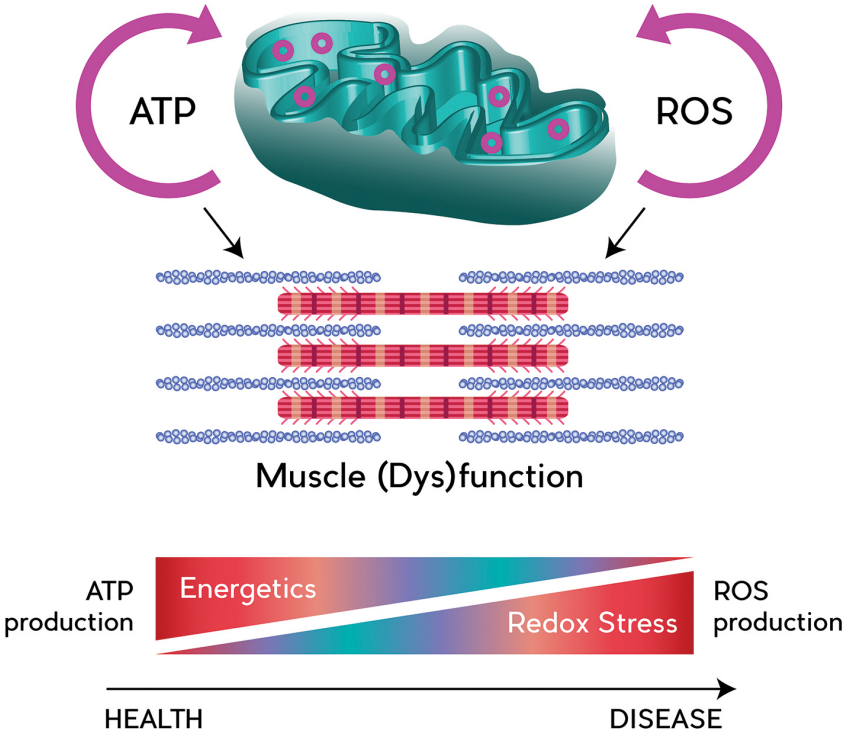
For decades, the mitochondrion has been implicated in the ageing process, as its role in producing adenosine triphosphate (ATP) to meet the energy demands of the cell naturally creates reactive oxygen species (ROS) as a by-product of oxidative phosphorylation. It used to be thought that cell-wide oxidative damage – caused by an imbalance between the production and scavenging of ROS such as $H_2O_2$ – played a principal role in the ageing process. However, recent investigations have challenged this simple version of the oxidative stress theory of ageing and suggest that the relationship between mitochondrial function, oxidative stress and ageing may be more complex. Such findings, as well as the observation that mitochondria become more dysfunctional with age, have only served to make them a more attractive target for ongoing research into ageing.

Despite the countless studies already dedicated to understanding mitochondria, every new discovery seems to uncover another layer of unexpected nuance to their function, or finds that there is more uncertainty than certainty along the road to potential therapies to counter mitochondrial dysfunction. Finally, however, mitochondria may be on the verge of succumbing to the weight of researchers' persistence and yielding their most promising secrets.

**Old Mitochondria and New Insights**

'My philosophy is to start with investigating the in vivo physiological effects of an intervention, manipulation, or model, and then work down to the cellular and molecular level,' explains Dr David Marcinek, who is currently an associate professor in the Department of Radiology at University of Washington Medical Center, where he focuses on the interaction between energetics, redox signalling, and muscle physiology in ageing and disease.

**‘Our research focuses on how changes in mitochondria energetics and oxidative stress affect the pathological and adaptive signalling in skeletal muscle. We have found that some pathological conditions associated with ageing may be more dynamic than previously thought and could be rapidly reversed by targeted intervention with agents such as elamipretide to restore redox or energetic balance.’**




Dr Marcinek traces his interest in bioenergetics back to his undergraduate physiology course, where he learned that tightly regulating and controlling energy flow was crucial to maintaining healthy systems. However, his specific focus on mitochondria and their role in muscle health only developed after completing his PhD in Physiology at Stanford University, and with the beginning of his postdoctoral work at the University of Washington at the turn of the century.

‘Most of the focus of mitochondrial research is on their role in ATP production,’ Dr Marcinek clarifies. ‘However, the mitochondria are also a main source of reactive oxygen species, or ROS, and thus provide a nexus between energy and redox homeostasis and cell signalling. This makes them key players in the ability of the cell to respond to stress.’

Mitochondria possess intrinsic antioxidant defence mechanisms to counter excess ROS and maintain redox homeostasis. A key aspect of mitochondrial function is the generation of a potential using the electron transport system to pump protons across the mitochondrial inner membrane (MIM; oxidation). This MIM potential is not only used to drive ATP synthesis (phosphorylation), but is also a key factor in the generation of ROS. One intrinsic antioxidant defence is the ROS-induced uncoupling of oxidative phosphorylation, which dissipates MIM potential without ATP generation. Since mitochondrial ROS generation is higher at higher MIM potential this uncoupling reduces mitochondrial oxidative stress. This uncoupling of oxidative phosphorylation naturally reduces the amount of ATP that mitochondria can produce by making them less efficient. The job of elucidating how ageing affects this uncoupling mechanism fell to Dr Marcinek and his colleagues in 2012.

The group induced acute oxidative stress in living mice before using a novel multi-modal spectroscopy system of their own design to assess its effects on intact hind-limb muscles. They carried out *in vivo* $^{31}$P magnetic resonance and near-infrared optical spectroscopy, allowing ATP production and $O_2$ consumption to be quantified, respectively. Through the success of this spectroscopy system, they found that aged mitochondria were already uncoupled and inducing a further oxidative stress led to further uncoupling beyond that observed in the young skeletal muscle.

This led to the conclusion that age-related oxidative stress may be inducing this uncoupling defence mechanism. As previously mentioned, this mechanism is something of a double-edged sword. Its presence in aged mice means that acute oxidative stress further reduces the already-diminished mitochondrial function and ATP production in aged mice. This finding would set the bearing for Dr Marcinek’s subsequent research direction, as it constituted an exaggerated response to normal physiological stress that might account for impaired muscle function in elderly individuals.

Explaining this finding further, Dr Marcinek further elucidated that ‘it was clear that disruption of mitochondria that caused elevated ROS generation could lead to further mitochondrial deficits and subsequent inhibition of skeletal muscle function, independent of the availability of ATP. This led our group to focus on the types of redox and energy stresses that lead to post-translational modifications to proteins that control both physiological performance and stress responses.’

**The Turn of the Peptide**

As the powerhouse of the cell, it may be unsurprising that mitochondrial dysfunction in humans is associated with exercise intolerance, fatigue and muscle atrophy. Muscle health, of course, represents a crucial factor in supporting the maintenance of independence and a high quality of life in the elderly – anyone who has suffered reduced function in a single muscle, through a strain or tear, can imagine the vulnerability of those with similarly limited function throughout their whole body.

Muscles are composed of bundles of myocytes, also known as muscle fibres, that house the actin and myosin filaments. According to the sliding filament theory, muscle contraction is the result of actin filaments sliding along the myosin filaments, causing local shortening and therefore an increase in tension within the muscle. This process relies heavily on ATP, and is therefore obviously dependent on mitochondrial function.

Despite the clarity of this link, a pharmacological treatment to reverse age-related mitochondrial deficits has been elusive, making it impossible to restore skeletal muscle function by this route. That is, until Dr Marcinek and his co-workers recently announced the discovery of such a treatment in an article published by the journal *Aging Cell.*

The group discovered that a member of the Szeto-Schiller (SS) family of peptides, which had previously been shown to elevate ATP production, reduce mitochondrial ROS production and decrease oxidative damage, amongst other beneficial effects, induced extraordinary changes in muscle energetics almost immediately after its injection in mice. Fascinatingly, both the efficiency and maximum capacity for ATP production in the old mice were restored within just one hour of administration of the drug to those levels observed in young mice. This treatment had no obvious effect on the muscle energetics of young mice, suggesting that it specifically reversed the age-related inhibition of mitochondrial energetics.

As well as looking at biochemical markers to validate the effect of the SS peptide, Dr Marcinek and his colleagues tested the fatigue resistance and exercise capacity of the mice after treatment. The distal tibialis anterior tendon of each mouse was surgically isolated to measure the force exerted by the muscle under electrical stimulation. This approach allowed the group to confirm that just one hour of treatment with the SS peptide dramatically increased the skeletal muscle fatigue resistance of the elderly mice. After eight daily injections, the whole-body endurance of the elderly mice was tested to failure using a treadmill, revealing that the SS peptide could boost the exercise tolerance of the mice substantially – albeit not to the levels reached by the young mice.

This finding had twin consequences. Not only did the group now recognise an exceptionally promising drug for rescuing muscle function in aged individuals, but the immediacy with which it took effect also indicated that the age-related mitochondrial disruption arose at least in part from regulatory modifications associated with elevated mitochondrial ROS production rather than from structural changes in the mitochondria. This is an exceedingly exciting finding, as influencing a signalling process is much more straightforward than mending cellular structural damage.

**Mitochondrial Therapies Coming of Age**

The next step after discovering that a drug has such astonishing activity in an animal model is to carry out clinical trials on humans. As a mark of its progress down the route to clinical application, the SS peptide has now been dubbed with a generic drug name: elamipretide (previously referred to as Bendavia, MTP-131, and SS-31). Stealth BioTherapeutics (Stealth), a clinical-stage biopharmaceutical company developing mitochondrial dysfunction-targeting drugs, recently carried out a phase 2 randomised, double-blind, placebo-controlled study with elamipretide. Elamipretide was administered to 40 patients, aged 60–85 years with demonstrated mitochondrial dysfunction, to establish whether or not the effects observed on the skeletal muscle energetics of mice could be reproduced in humans. Although the group at the University of Washington have yet to publish the findings in full, the results have been released by Stealth.

At the primary endpoint in the trial, elamipretide elicited an approximate 30% increase in mitochondrial energy production from baseline values, in comparison with an improvement of just approximately 10% in the placebo group. Patients treated with elamipretide demonstrated greater skeletal muscle function, with a treatment-emergent adverse events profile similar to placebo. 'This change is comparable to the improvement seen in my previous studies of endurance training, exercising three times a week for six months, providing hope for patients and their potential for improved muscle function,' commented Dr Kevin Conley, a professor at the University of Washington, and elamipretide trial investigator.

With such astonishing initial results, Dr Marcinek's research focus is naturally looking to build on the success of elamipretide. 'A main focus for ongoing research in the lab is to determine how changes in mitochondrial function alter energy and redox homeostasis, and thereby affect cell function,' he says, explaining that his team's finding that reducing redox stress can mitigate the effects of ageing in mitochondria has reinforced their efforts in this area. A key component of his future work will therefore be to identify the processes underlying cell–mitochondria communication, looking at redox thiol post-translational modifications and using metabolomics to identify the specific pathways involved. Dr Marcinek is also continuing to look for new acute and chronic interventions to improve skeletal muscle energy metabolism, contractile performance, and exercise tolerance. He says that 'one exciting result of this new perspective is that it indicates that some pathological conditions associated with ageing may be more dynamic than previously thought and could be rapidly reversed by targeted intervention to restore redox or energetic balance.'

# Meet the researcher

**Dr David Marcinek**
Department of Radiology
University of Washington Medical Center
Seattle
USA

David Marcinek obtained his PhD at Stanford University, California, in 2000. Afterwards, he pursued postdoctoral research at the University of Washington, where he was appointed as Research Assistant Professor in the Department of Radiology in 2005. He is currently Associate Professor in the Department of Radiology, Department of Pathology and Department of Bioengineering at the university. He is a member of the American Physiological Society, American Association for the Advancement of Science, Mitochondria Physiology Society, United Mitochondrial Disease Foundation, and Society for Free Radical Biology and Medicine. He studies the interaction between energetics, redox signalling, and skeletal muscle physiology in ageing and disease, with a particular focus on mitochondrial dysfunction.

## CONTACT

**E:** dmarc@uw.edu
**T:** (+1) 206 221 6785
**W:** http://blogs.uw.edu/marcinek/

## KEY COLLABORATORS

Peter Rabinovitch, University of Washington
Kevin Conley, University of Washington
Qei-Jun Qian, Pacific Northwest National Laboratory
Hazel Szeto

UW Medicine
UNIVERSITY OF WASHINGTON MEDICAL CENTER

## FUNDING

National Institutes of Health
American Federation for Aging Research
Glenn Foundation for Medical Research
UW Nathan Shock Center
Ellison Medical Foundation

## REFERENCES

MP Siegel, SE Kruse, G Knowels, A Salmon, R Beyer, H Xie, H Van Remmen, SR Smith and DJ Marcinek, Reduced coupling of oxidative phosphorylation in vivo precedes electron transport chain defects due to mild oxidative stress in mice, PLoS One, 2011, 6, e26963.

MP Siegel, SE Kruse, JM Percival, J Goh, CC White, HC Hopkins, TJ Kavanagh, HH Szeto, PS Rabinovitch and DJ Marcinek, Mitochondrial-targeted peptide rapidly improves mitochondrial energetics and skeletal muscle performance in aged mice, Aging Cell, 2013, 12, 763–771.

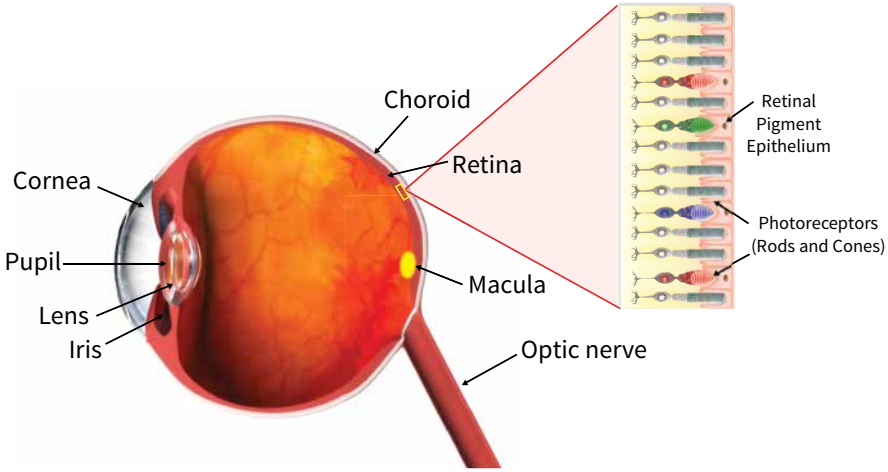
MP Siegel, T Wilbur, M Mathis, EG Shankland, A Trieu, ME Harper and DJ Marcinek, Impaired adaptability of in vivo mitochondrial energetics to acute oxidative insult in aged skeletal muscle, Mechanisms of Ageing and Development, 2012, 133, 620–628.

# UNCOVERING THE MYSTERIES OF AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration is the leading cause of blindness amongst the elderly population in the developed world. **Professor Deborah Ferrington** and her colleagues at the University of Minnesota are carrying out ground-breaking research on the cellular pathways underlying the pathology of this life altering disease.

**The Fight Against Blindness**

Age-related macular degeneration (AMD) is a life changing disease which robs people of central vision, limiting their ability to read, drive and even recognise faces. AMD affects over 10 million individuals in the United States and this number is expected to double by 2050 due to the Western world's rapid increase in the ageing population. With limited treatment options available, there is an urgent need to develop new methods for prevention and cure.




AMD leads to vision loss through the destruction of the macula, an oval shaped pigmented area at the centre of the retina. The macula contains a dense concentration of cone cells (which supply high acuity and colour vision). As these cones degenerate, central vision is lost. There are a number of factors which contribute to cone loss, including age, genetic profile and environmental insults such as smoking.

There are two primary forms of AMD. 'Wet' AMD manifests as the abnormal growth of blood vessels into the retina. There are successful treatment options available for individuals with this form of the disease, however only 10% of patients present with 'wet' AMD. The other type is 'dry' or atrophic AMD, which involves changes in and the eventual loss of cells from the retinal pigment epithelium (RPE). The RPE is a monolayer of cells located beneath the neural retina. The RPE maintains retinal health and homeostasis by supporting the function of rod and cone photoreceptors (structures in the eye that respond to light).

AMD leads to the formation of lipoproteinaceous deposits (known as drusen) that form between the RPE and choroid (the pigmented vascular area beneath the RPE). As the disease advances, drusen increases in quantity. Changes to the RPE become evident in the early stages of AMD and because these cells are post-mitotic, which means they are unable to divide, they cannot be replaced if damaged or lost. Without support from the RPE, photoreceptors die and vision loss occurs. There is currently no effective treatment for 'dry' AMD.

This is where the work of Professor Deborah Ferrington comes in. Her laboratory is focused on defining molecular changes that occur in the retina with AMD. 'The ultimate goal of my research is to identify therapeutic targets for treating AMD, which requires a thorough understanding of the disease mechanism,' she explains. Defining the molecular mechanism of AMD is no small task. Because the anatomical structure of the retina is unique to primates, no animal models are available which can faithfully replicate the retinal conditions associated with AMD. Therefore, Professor Ferrington and her team are using human donor tissues to study the disease. They aim to investigate AMD pathogenesis through studying changes in protein expression and in the mitochondria at progressive stages of AMD. 'It was previously thought that one simple

**‘If we can keep the mitochondria healthy, we may slow the progression to blindness… Because mitochondrial damage occurs before vision loss, early intervention would likely protect or rescue RPE mitochondrial function. This is the best place to start aiming therapies.’**

mechanism would cause AMD, but it’s not that way at all,’ Professor Ferrington says. ‘Genes implicated in AMD are clustered in several distinct biochemical pathways. You can develop what looks like the same disease many different ways, which is one of the biggest challenges in finding a treatment.’

**Deciphering Damage to Mitochondrial DNA**

Over the last decade, Professor Ferrington and her colleagues have been uncovering evidence that mitochondrial dysfunction is associated with AMD pathogenesis. The mitochondria are organelles found in the cell in which respiration and energy production occur. They also contain a small amount of DNA, separate from the DNA found in the nucleus. Mitochondrial DNA (mtDNA) contains 37 genes which are responsible for coding 13 proteins essential for producing energy and all of the machinery required to make those proteins. The remaining ~1800 proteins that reside in the mitochondria are encoded by nuclear DNA, produced outside of the mitochondria, and then transported into the mitochondria.

So, what evidence is there to suggest that mitochondrial damage may play a role in AMD? Mitochondria are a major source of superoxide anions in the cell. These anions generate highly toxic radicals and hydrogen peroxide that can damage the cell by reacting with proteins, DNA and lipids. This oxidative stress may play an important role in disease progression, as suggested by the increased levels of antioxidant enzymes that occur in response to the oxidative stress and protein adducts (generated from carbohydrate and lipid oxidation) found in AMD donor eyes. mtDNA is more susceptible to damage from oxidation than nuclear DNA and when mtDNA is damaged, this may interfere with production of key proteins involved in energy production.

Based on this evidence, the team analysed the sub-set of proteins present in the mitochondria isolated from the RPE. They found that a significant number of affected proteins were subunits of the mitochondrial ATP synthase complex (which is responsible for energy production). These results suggest specific pathological mechanisms involving altered translation of the ATPase subunits encoded by either nuclear DNA or mtDNA as well as defects in the transport of nuclear encoded proteins into the mitochondria. ‘Mitochondria are a really good target for therapy,’ says Professor Ferrington. ‘We see mitochondrial dysfunction in Parkinson’s and Alzheimer’s diseases, so mitochondria are like an Achilles’ heel for several age-related diseases.’

**Normal Ageing or AMD?**

A 2010 study provided further evidence that AMD development damages mtDNA inside RPE cells. Professor Ferrington and her team evaluated the extent of mtDNA damage in human RPE for donors of different ages but who did not have AMD to determine the effect of ageing and compared those results with damage in donors with AMD. The purpose was to distinguish damage associated with normal ageing from damage due solely to AMD.

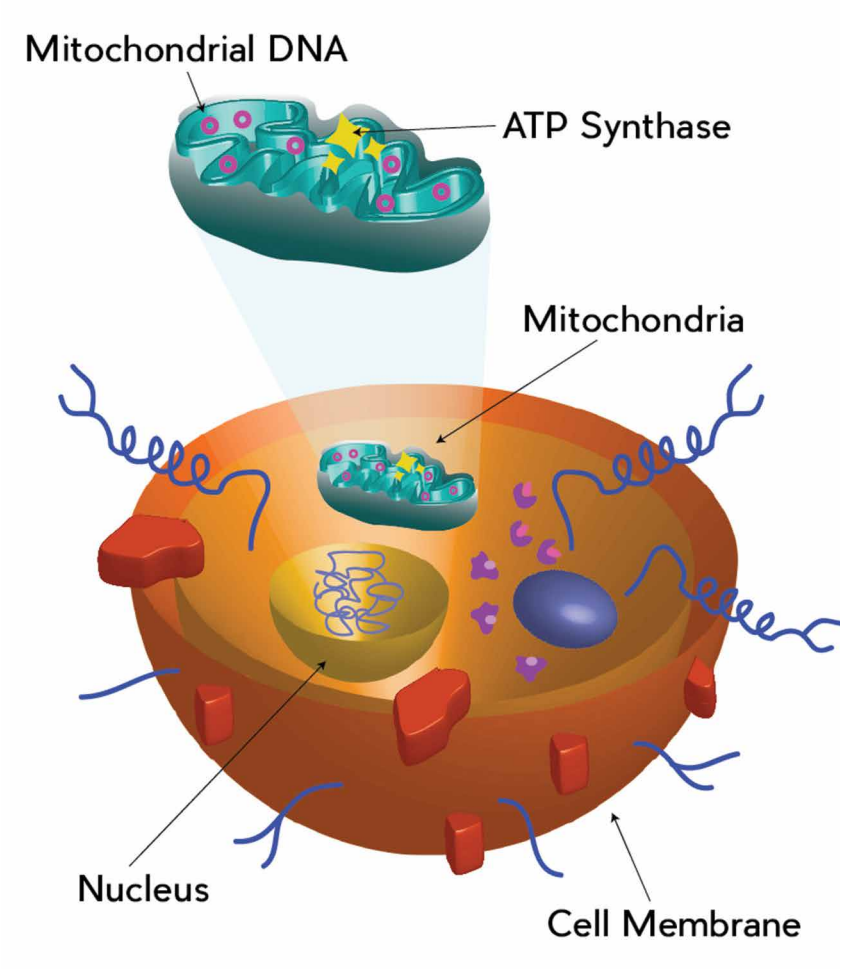
With normal ageing, increased mtDNA damage was limited to a specific region of the mitochondrial genome called the ‘common deletion’, which is an age-specific modification that had been reported to occur in other post-mitotic cells, such as neurons and skeletal muscle. Conversely, in RPE from donors with AMD, the team observed a significant increase in mtDNA damage throughout the mitochondrial genome. Lesion frequency also increased steadily with disease progression, suggesting a cumulative effect as the disease progressed.

The team also compared lesion frequencies in the mitochondrial and nuclear genomes to determine if mtDNA was more susceptible to damage. In these experiments, AMD tissue showed significant increases in lesion frequency in mtDNA but not in nuclear DNA. This indicates that mtDNA is preferentially damaged with AMD progression.

**Identifying Targets for Treatments that Stop Vision Loss**

An important consideration for treating AMD is to identify the retinal region (e.g., cell type or geographic area) that should be targeted. To address this point, Professor Ferrington and her colleagues measured the extent and distribution of mtDNA damage in the neural retina (containing glia and neurons, including the photoreceptors) and RPE. They also measured RPE mtDNA damage in the macula and peripheral sections, to test if the macula was selectively damaged in AMD. To gain insight into the potential functional effect of mtDNA damage, small segments of the entire mitochondrial genome were examined to determine whether specific regions are preferentially damaged.

The results showed that mtDNA was limited to the RPE and, as this group had previously shown, lesion frequency increased with disease severity. In contrast, disease stage had no effect on lesions content in the neural retina. ‘That was a complete surprise,’ Professor Ferrington tells us. ‘I had expected that with high damage in RPE the same result would be observed in the neural retina, but that was not so.’ The observation




that the retina does not accumulate mtDNA damage with disease progression suggests differences in how AMD manifests in specific tissues. Another important finding was that mtDNA damage was not limited to the macula but was equally abundant in the RPE cells in the peripheral region. This result dispelled a long-held belief that damage only occurs in the macula. Finally, measures of mtDNA damage in small segments of the mitochondrial genome shows that over half of the mitochondrial genome is not significantly damaged in AMD, refining the results of the team's previous reports of genome wide damage to more discrete regions.

These results are important clinically relevant findings – there is now a scientific basis for targeting RPE mitochondria as a treatment strategy. Because this damage occurs before vision loss, early intervention could prevent or at least slow down progression to blindness.

**Genetic Risk for Increased mtDNA Damage**

Previous genetic analysis of AMD had identified a number of high risk loci associated with the disease. The genes at these loci belong to diverse pathways, suggesting different pathogenic mechanisms lead to the clinical manifestation of the disease and implies that therapies targeting a single pathway will not be effective for all AMD patients. This idea provided the rationale for a 2016 study aimed to determine if individuals with a specific genetic background were at greater risk for mtDNA damage.

Donors were genotyped for several prominent high risk loci associated with AMD and the extent of mtDNA damage was determined in RPE cells. Professor Ferrington and her colleagues found that AMD donors carrying the high-risk allele for the gene complement factor H (CFH) had significantly more mtDNA damage than donors without the gene variant. This supports the hypothesis that the presence of the CFH risk allele makes mtDNA in the RPE more susceptible to damage. CFH is a key regulator of the alternative complement pathway (part of the immune system) that promotes the clearance of debris and dead cells and kills invading pathogens. The role of CFH is to protect host cells from inappropriate complement activation in order to avoid chronic inflammation or damage to healthy cells.

Another discovery was that a small number of healthy donors carrying the risk allele had lower mtDNA damage, suggesting that mitochondrial injury is not a direct consequence of the CFH variant. The team hypothesise that retinal changes associated with disease onset coupled with the presence the gene variant could create cellular conditions conducive for accelerated mitochondrial damage. Overall, this study provides a strong rational for a more personalised approach for treating AMD. Patients harbouring the high-risk allele for CFH may benefit from treatment that stabilise and protect the RPE mitochondria. The potential impact of finding an effective treatment for slowing down AMD progression in this patient subpopulation is immense when considering both their high risk for developing late stage AMD and the high percentage of patients (30–50% of all AMD patients) harbouring this variant.

**What Comes Next?**

Professor Ferrington and her lab have expanded their focus from discovery science to targeted treatments and are currently testing drugs which might boost mitochondrial function in human donor tissue. Pharmacological treatments such as N-acetyl cysteine are already proving effective in improving mitochondrial function in cultured RPE cells from AMD donors. Her lab has also been making RPE cells using induced pluripotent stem cells (iPSC). They envision using the RPE they create from stem cells to screen potential drug therapies for patients with early AMD or to restore lost RPE cells in patients with advanced disease. For more information, please see this video.

The team will continue to unravel the molecular mechanisms behind ageing. As Professor Ferrington explains, their research is focused on answering: 'What are the cellular changes that occur with ageing? What factors "tip the balance" to pathology? How does the cell respond to disease? How can we protect against pathologic changes? These are the questions that form the core of my research program.'

# Meet the researcher


**Professor Deborah Ferrington, PhD**

Department of Ophthalmology and Visual Neurosciences

University of Minnesota, Twin Cities, USA


Professor Deborah Ferrington is Professor and Elaine and Robert Larson Endowed Vision Research Chair in the Department of Ophthalmology and Visual Neurosciences at the University of Minnesota, Twin Cities. After completing her undergraduate degree in Biological Science and Scientific Illustration and a Masters in Education from the University of Pittsburgh, PA, Ferrington went on to receive her PhD in Biochemistry from the University of Kansas, where she also completed a postdoctoral fellowship. She has received several honours over the course of her career, including acting as Executive Board Member for the Ryan Initiative for Macular Research, the Fesler-Lampert Chair in Aging Research and the Elaine and Robert Larson Endowed Vision Research Chair at the University of Minnesota. Professor Ferrington's research aims to develop understanding of ageing at the cellular level witha particular focus on aging retina and age-related macular degeneration. She has successfully secured funding from organisations such as the Foundation Fighting Blindness, American Federation for Aging Research, Arnold and Mabel Beckman Foundation and National Institutes of Health. Professor Ferrington also acts as teacher, adviser and mentor to undergraduate, graduate and medical students and is the Program Director of an NIH-funded training grant that supports the training and education of doctoral and post-doctoral student in the field of aging research.

## CONTACT


**E:** ferri013@umn.edu
**T:** (+1) 612 624 8267
**W:** http://www.ophthalmology.umn.edu/bio/ophthalmology-department-facul/deborah-ferrington


## KEY COLLABORATORS

Dale Gregerson, PhD, University of Minnesota
Sandra Montezuma, MD, University of Minnesota
Erik van Kuijk, MD, PhD, University of Minnesota
James Dutton, PhD, University of Minnesota
Anand Swaroop, PhD, National Eye Institute
Rinki Ratnapriya, PhD, National Eye Institute
M. Christine Kenney, MD, PhD, University of California at Irvine
Robert Mullins, PhD, University of Iowa

## FUNDING


Minnesota Regenerative Medicine
National Institutes of Health – National Eye Institute, National Institute of Aging
American Federation for Aging
American Health Assistance Foundation
Arnold and Mabel Beckman Foundation
Foundation Fighting Blindness
Elaine and Robert Larson Endowed Vision Research Chair
Minnesota Lions Vision Foundation
Helen Lindsay Family Foundation
Anonymous Donor for AMD Research


## REFERENCES

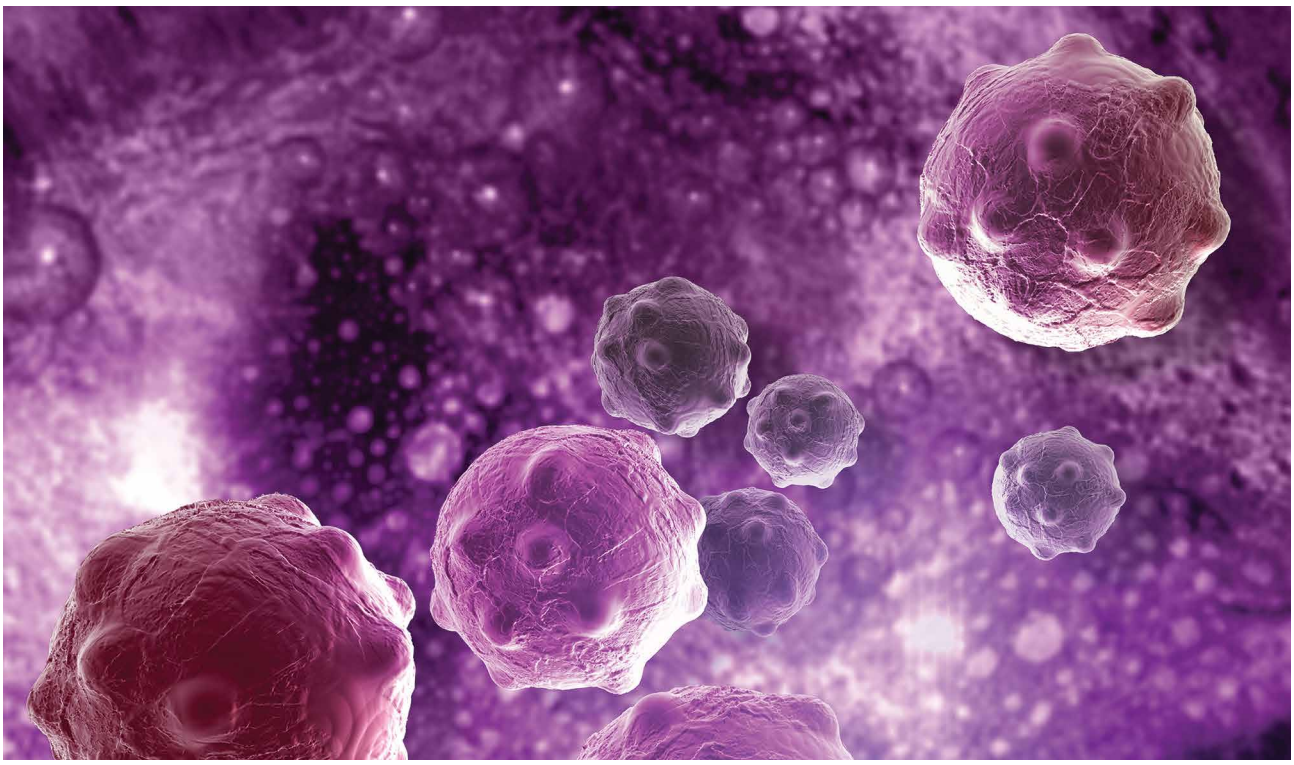

DA Ferrington, RJ Kapphahn, MM Leary, SR Atilano, MR Terluk, P Karunadharma, G Kuei-Jie Chen, R Ratnapriya, A Swaroop, SR Montezuma and MC Kenney, Increased retinal mtDNA damage in the CFH variant associated with age-related macular degeneration, Experimental Eye Research, 2016, 145, 269–277.

MR Terluk, RJ Kapphahn, LM Soukup, H Gong, C Gallardo, SR Montezuma and DA Ferrington, Investigating Mitochondria as a Target for Treating Age-Related Macular Degeneration, The Journal of Neuroscience, 2015, 35, 7304–7311.

PP Karunadharma, CL Nordgaard, TW Olsen and DA Ferrington, Mitochondrial DNA Damage as a Potential Mechanism for Age-Related Macular Degeneration, Investigative Ophthalmology & Visual Science, 2010, 51, 5470–5479.

CL Nordgaard, PP Karunadharma, X Feng, TW Olsen and DA Ferrington, Mitochondrial Proteomics of the Retinal Pigment Epithelium at Progressive Stages of Age-Related Macular Degeneration, Investigative Ophthalmology & Visual Science, 2008, 49, 2848–2855.


UNIVERSITY OF MINNESOTA



# A BIOCHEMICAL APPROACH TO BEATING BREAST CANCER

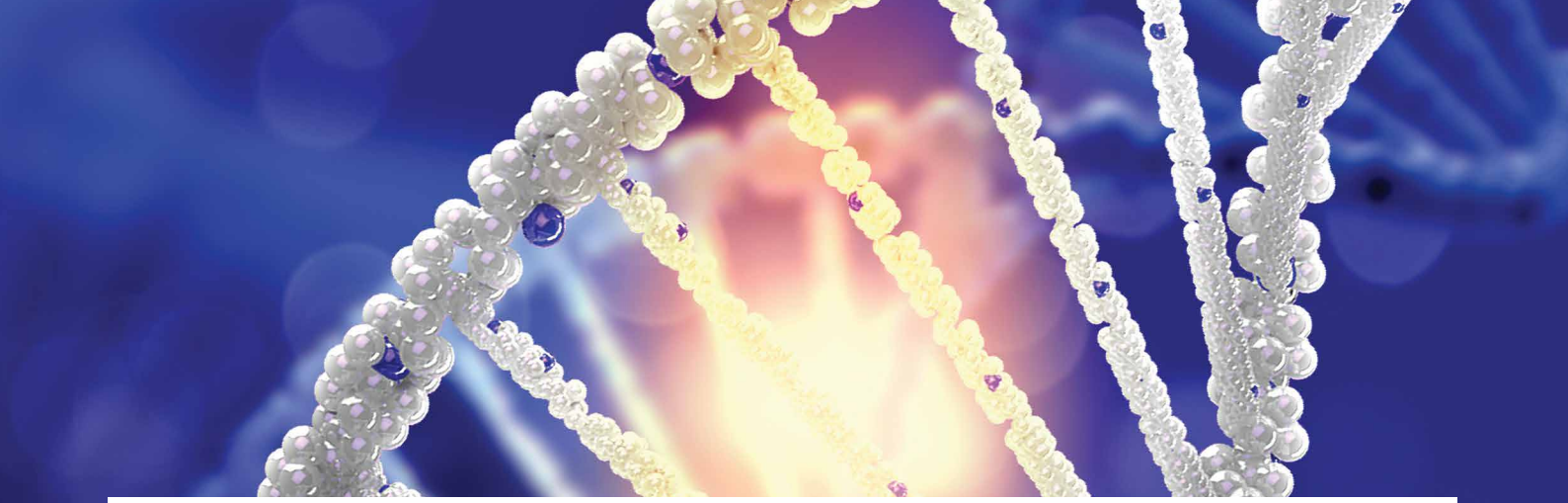
Breast cancer is by far the most common form of cancer in women across the globe. With high incidence in both developed and developing countries, the WHO estimates that 1.38 million people are diagnosed with breast cancer each year, while approximately 458,000 people die from the disease worldwide.

As cancer is an extremely complicated disease, researchers are still striving to understand the complex biochemical pathways that give rise to its development and progression. In this section of the magazine, we introduce two research teams who investigate breast cancer at the molecular level, in the hope that their new insight will lead to the development of novel therapies for this all too common disease.

In the first article of this section, we feature the work of Professor Tan Ince and his research group at the University of Miami, who grow different types of breast cancer cells in the lab in order to thoroughly investigate them at the molecular level. Furthermore, the team have developed a system of categorising the various types of epithelial cells found in breast tissue, by looking at different molecules expressed by the cells' receptors. More specifically knowing the type of breast epithelial tissue that has become cancerous will help doctors to offer much more targeted therapies, leading to greater survival rates.

Like most forms of cancer, detecting breast cancer early is key to reducing the risk of mortality. However, since many women experience only subtle or even no symptoms during the pathogenesis of breast cancer, the cancer can go unnoticed, giving it time to spread from the breast to other regions of the body in a process known as metastasis. Since survival rates for cancer that has metastasised are so low, researchers across the globe are striving to understand the biochemical factors that drive the metastasis of cancer, in the hope of finding ways to prevent it from happening.

Here's where the work of Dr Stefan Veltel and his team at the Universitätsklinikum Hamburg-Eppendorf in Germany comes in. Dr Veltel's group investigate the biochemical mechanisms that facilitate the progression of breast cancer, and its metastasis. In particular, the team focuses on a protein called Rab21, which helps to regulate the behaviour of integrins. Integrins are transmembrane receptors that help the cell adhere to the extracellular matrix. Because of their role in cellular adhesion, integrins can influence the ability of cells – such as cancer cells – to migrate and adhere to other organs in the body, implying key involvement in cancer metastasis. In their work on the integrin-regulator Rab21, Dr Veltel's group show how another protein that interacts with Rab21 could be a potential therapeutic target for the treatment of breast cancer.



# FIGHTING CANCER BY KEEPING CANCER CELLS ALIVE

Pathologist and cancer researcher **Professor Tan Ince** and his colleagues at the University of Miami investigate human cancers by developing cell culture media to grow cancer cells and normal cells to better study the relationship between the two.

**How Do You Study a Malignant Invader?**

As the U.S. President – played by Bill Pullman – found out unexpectedly in the midst of an alien invasion in the epic *Independence Day*, specimens of the alien force had been sequestered in the fabled Area 51 somewhere in the Southwest American desert. The aliens were poked and prodded; their capabilities were studied and reverse engineered. Scientists learned a lot from these captured aliens and this knowledge ultimately helped defeat the invaders. Study the invader and find ways to defeat it – discover its weaknesses, strengths and strategy. Perhaps this is all an allegory or an analogy for the life's work of Professor Tan Ince, cancer researcher and pathologist from University of Miami Miller School of Medicine in Miami.

For almost twenty years, Professor Ince and colleagues have been researching ways to efficiently grow cancer cells for examination and study, in the hopes of finding new and improved therapies for this most terrifying of diseases. Early in his career, Professor Ince developed a new cell culture nutrient medium that is now widely used to grow human breast and ovary cells. As a pathologist he can diagnose cancer cells taken from patients at surgery or at biopsy. As a cancer researcher, he tirelessly searches for ways to keep those cells alive to discover what they can tell him about the cancer growing inside his patient and find ways of defeating it.

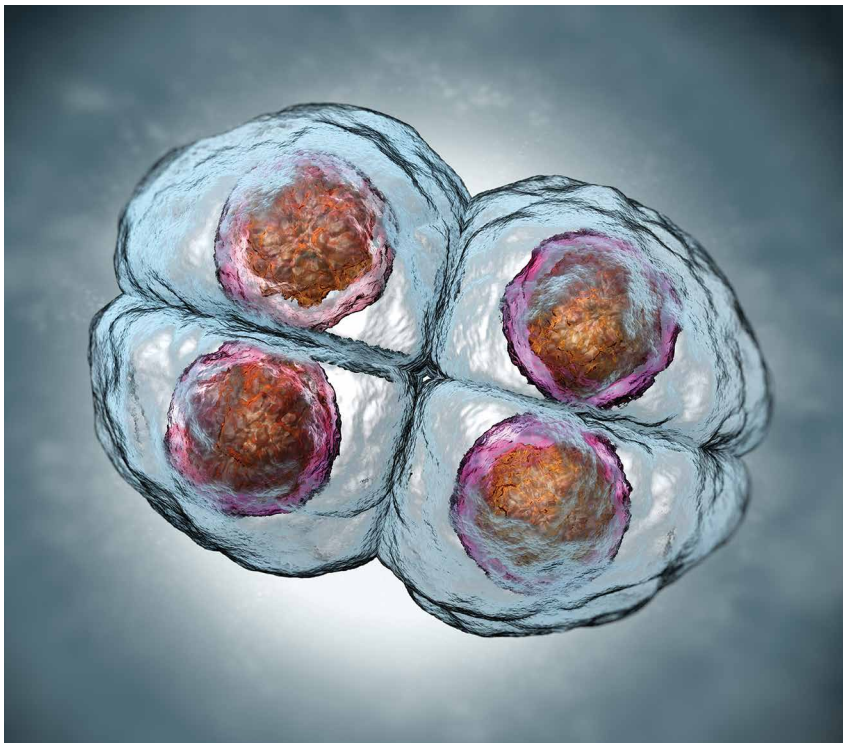
**Cancer is a Word – Cancers are a Diverse Population**

In 1951, a cancer patient, Henrietta Lacks, became immortal. Ms Lacks suffered from cervical cancer and unfortunately succumbed to her disease in October of 1951. However, cells taken from her tumour were successfully cultured, resulting in the so-called HeLa cell line – the first human cell line established in the laboratory – that has since been used extensively for scientific research. The cells from Ms Lacks' tumour had mutated so much – a fact later discovered to be due to infection with human papillomavirus B-18 – that the cells were immortal and could be used for experiments that were impossible with normal cells. To this day, HeLa cells are used in laboratories around the world. Perhaps ironically, over the decades that the HeLa cell line was used for various and important scientific research, including the testing of Jonas Salk's first human polio vaccine, the cells have contaminated other cell lines in laboratories worldwide, corrupting research done in laboratory after laboratory. Up to 20% of other cell lines may be contaminated by HeLa cells. Apparently cancers are not only invasive in their original human victims. But that isn't the only problem with cell lines like HeLa.

In the decades since the establishment of the HeLa line, human tumour cell lines have had a profound effect on cancer research and led to the development of a variety of human cancer therapies. Carcinomas grow uncontrollably in the body. But the malignant cells are often paradoxically difficult to grow in cell culture, unlike HeLa. A reliable cell line model that predicts patient response to various therapies would be of great value in the testing of new drugs for individualised therapy of tumour patients. But in spite of the many decades of improvements in methods for establishing cancer cell lines, it is quite difficult to routinely establish reliable, permanent cell lines from primary human tumours on a regular basis. This limits the number and diversity of cell lines scientists have at their disposal. As well, with many tumour types only very high-grade specimens have yielded cell lines. Thus, available cell lines do not accurately reflect the true spectrum of tumours encountered clinically. Patients with lower grade tumours are effectively out of luck if their tumour does not readily grow in cell culture.

Another problem is that the origins of many of the available tumour cell lines are unclear. What tumour or even what tissue did they come from? This is the result of a lack of 'fingerprinting' technology – such as DNA analysis – that was able to verify identity when the lines were developed. Also, the original tumour usually isn't available for analysis with modern DNA sequencing procedures. What scientists need is a more efficient method of establishing fresh human tumours in culture to indicate and reflect the heterogeneity of the human tumours from

**For almost twenty years, Professor Ince and his colleagues have been researching ways to efficiently grow cancer cells so they can be examined and studied, in the hopes of finding new and improved therapies for this most terrifying of diseases.**



which they are derived. If you are treating a patient with a certain type of breast cancer, you want to experiment on a cell culture from that type of cancer. This would give a more logical experimental model for drug testing, for example.

In fact, Professor Ince and fellow researchers wrote in *Nature Communications* about their development of a cell culture medium that allowed them to routinely establish cell lines from different subtypes of human ovarian cancers with over 95% efficiency. They described 25 new ovarian tumour cell lines that retained the genetics, histopathology and molecular features of the patient's original tumours. Importantly for therapeutic strategies, the molecular profile and drug response of these tumour cell lines correlated with distinct groups of primary tumours with different outcomes. In other words, patient outcomes were related to the different tumour types Ince could grow in cell culture. Using a tumour cell line that is not the same type – or even the same grade or malignancy – as the patient's tumour doesn't give the correct result.

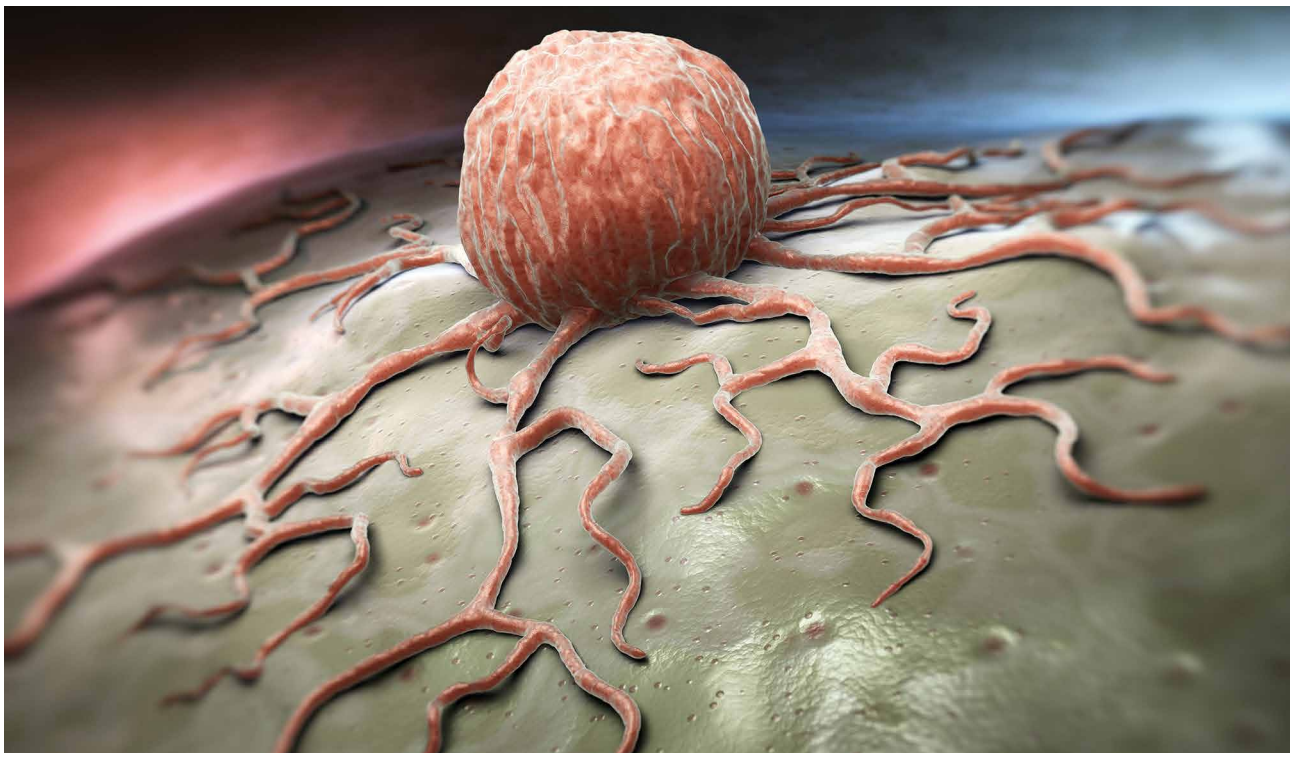
These tumour cell lines that Professor Ince derived represent a significantly improved experimental system to study human tumour pathophysiology and ultimately response to therapy. Using 'standard' cell culture, such as the historic HeLa line, just doesn't apply to all tumours. Patients need more specificity for a proper chance at some good result. Professor Ince and his team keep working toward that end in a variety of ways.

**Relating the Normal to the Abnormal**

To understand the pathophysiology of a disease and most appropriately determine treatment choices for a given cancer, the patient's doctor needs accurate classification of that cancer. For blood cell malignancies such as leukaemia, a classification scheme based on the phenotypic similarity between cancer cells and normal cells has been successfully used to define subtypes of the cancer. There is lymphocytic leukaemia, derived from lymphocyte cells, granulocytic leukaemia, derived from the other white cells, and so on. Beyond that, the use of normal cell types as a reference by which to classify solid tumours – such as carcinomas or sarcomas – has not been widely practiced, due, in part, to a more limited understanding of solid tumour cell differentiation. Professor Ince and his colleagues have tried to bridge this gap in understanding and basically develop a new paradigm in understanding of solid tumours. They looked at breast cancer – a very common cancer – and tried to get a number of breast cancer tumour types that make for a more diverse possibility for research into therapy of those types of tumours.

Breast cancer is usually a cancer of epithelial cells, the cell type that covers most of the internal and external surfaces of the body and its organs. To get a better handle on the subtypes of epithelial cells comprising the breast epithelium, Professor Ince performed a systematic analysis of a large group of breast epithelial markers in more than 15,000 normal breast cells. This exhaustive study identified eleven differentiation states for the normal breast cells. He used this information to classify breast tumours based on normal cell types into four major subtypes that were differentiated from each other by their metabolism of vitamin D, androgen (male hormone), and oestrogen (female hormone) receptor expression. In other words, some cells had receptors that bonded to vitamin D and some did not. Some had receptors that bonded to oestrogen or androgen and some did not. In this way he distinguished cells based on their receptor status for these three substances.

Professor Ince then looked at cells from 3,157 human breast tumours and found that these hormone receptor subtypes were distinct from the current classification scheme, which is based on oestrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. Importantly, he found that patient outcomes were better when tumours expressed all three hormone receptors (which he called subtype HR3) and worst when they expressed none of the receptors (called subtype HR0). Putting the whole picture together, these data provide a practical classification scheme associated



with actual differences in patient survival and provided doctors insights into possible treatment of these breast tumours. Basically, the more specific you can be regarding the identity of the cancer, the better the chance at treating it. Know your enemy is apparently good advice.

**Looking at What's Real**

Professor Ince's research started in the universe of what was already known, like the relatively unique HeLa line. But he has continued to search for what's real – tumours related to the real tissue from which they arose. It's not about a distant and unrelated tumour line from a patient who died half a century ago. It's about examining cells of the same type as the cells your patient is affected with. To that end, Professor Ince recently suggested a new approach to classification of breast cancer, based on his vast experience with specific cell lines developed from specific tumours and related to specific parent cell types.

While current classification systems for breast cancer are based on expression of prognostic and predictive biomarkers, Professor Ince proposes a hypothesis-based ontological breast cancer classification modelled upon the taxonomy of species as the evolutionary biologist sees it. His approach takes the normal breast epithelial cell types and differentiation lineages as the gold standard to classify tumours arising from breast tissue. In other words, relate the malignant cell type with the normal cell type that presumably parented it.

Professor Ince took his prior research – demonstrating at least eleven previously undefined normal cell types in human breast epithelium and that each breast carcinoma is related to one of these normal cell types – and found that triple negative breast cancers do not have a 'basal-like' phenotype. The breast cancer cells that were negative for receptors for vitamin D, oestrogen and androgen did not have a 'normal' parent cell. Cells from normal tissue never lack all of these receptors. The triple negative tumours must be pretty unique.

Normal breast epithelial cells exist in four novel hormonal differentiation states and almost all human breast tumours fall under one of these hormonal differentiation states. This can have significant survival differences, since you can tailor therapy to include, say, oestrogen receptor drugs or androgen receptor drugs. This real-life classification scheme can provide rational treatment guidance and an alternative approach for understanding tumour physiology and tumour classification. And Professor Ince means to follow his classification theory even to the deep molecular level.

Hot off the presses in the journal *Oncogene,* for example, Professor Ince's team reported a study of histone deacetylases (HDAC) in breast and ovarian cancer specimens. HDAC are enzymes that help regulate DNA expression by modifying histones, molecules that wrap around and control DNA activity. What he found was that two specific types, HDAC1 and HDAC7, are vital to the function of cancer stem cells, those cancer cells that allow tumours to grow and spread. This means that already existing drugs that inhibit HDAC can rationally be tried on these tumours. Again, classifying tumours by HDAC content can lead to more specific therapeutic choices. In this work Professor Ince has revealed another bit of information on the alien invaders to help in our fight against them!

The epitome of Professor Ince's research would be his proposal of a stepwise classification system that puts tumours into diagnostic categories based on their distinct tissue of origin, cell-of-origin and differentiation lineage – what he calls 'lineage based classifications'. After defining uniform lineage based classes, he proposes to use molecular and genetic classifiers – like the presence of HDAC1 and HDAC7 – to distinguish prognostic subsets within each lineage. In other words, in the future he plans to narrow the classifications down so much that if you have this or that cancer, there will be a classification relative to your tumour – we could have a therapy for you

# Meet the researcher

**Tan A. Ince, MD, PhD**
Associate Professor of Pathology
University of Miami Miller School of Medicine
Miami, Florida, USA

Professor Tan Ince received his MD from Hacettepe University School of Medicine in Ankara, Turkey, in 1988. In 1996 he received his PhD in Molecular Pharmacology from Cornell University in New York City. He then completed a residency in Anatomic Pathology at Massachusetts General Hospital and Harvard Medical School, followed by a fellowship in Women's and Perinatal Pathology at the Brigham and Women's Hospital there. Professor Ince was a visiting clinical scientist at the Massachusetts Institute of Technology from 2000 to 2007, where he developed a new cell culture nutrient medium that is now widely used to grow human breast and ovary cells. In 2010, he was recruited to the Braman Family Breast Cancer Institute at the University of Miami Miller School of Medicine, where he is now Associate Professor of Pathology.

Professor Ince's research interests include the role of normal cell-of-origin in determining tumour phenotype and development of culture systems for in vitro culture of primary human tissues and tumours. He has authored or co-authored over 80 articles published in peer-reviewed journals and other professional proceedings. He is also licensed by the states of Florida, Massachusetts and Indiana and certified in Anatomic Pathology by the American Board of Pathology.

## CONTACT

**T:** (+1) 305 243 1782
**E:** tince@med.miami.edu
**W:** http://sylvester.org/research/knowledgebase/scientist/t_ince

## KEY COLLABORATORS

Sandro Santagata, Harvard Medical School
Christopher S. Crum, Harvard Medical School
Joan S. Brugge, Harvard Medical School
Robert A. Weinberg, Massachusetts Institute of Technology
Gordon B. Mills, M.D. Anderson Cancer Center
Joyce Slingerland, University of Miami
Andrea L. Richardson, Harvard Medical School

## FUNDING

Breast Cancer Research Foundation
Women's Cancer Association
NIEHS
Department of Defence Ovarian Cancer Research Program
Bankhead-Coley Cancer Research Program
Woman's Cancer Association

## REFERENCES

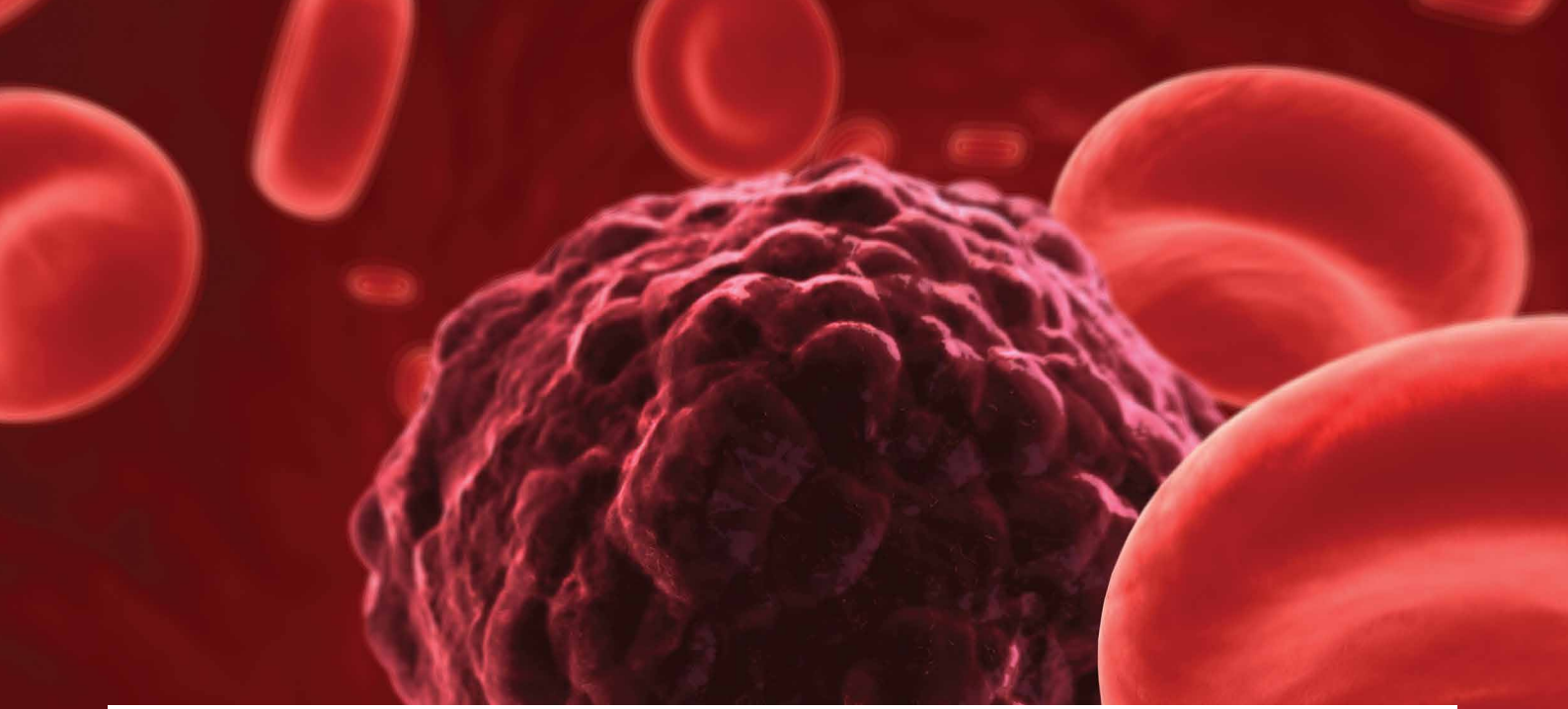
AE Witt, CW Lee, TI Lee, DI Azzam, B Wang, C Caslini, F Petrocca, J Grosso, M Jones, EB Cohick, AB Gropper, C Wahlestedt, AL Richardson, R Shiekhattar, RA Young and TA Ince, Identification of a cancer stem cell-specific function for the histone deacetylases, HDAC1 and HDAC7, in breast and ovarian cancer, Oncogene, 2016. DOI: 10.1038/onc.2016.337

A Thakkar, B Wang, M Picon-Ruiz, P Buchwald and TA Ince, Vitamin D and androgen receptor-targeted therapy for triple-negative breast cancer, Breast Cancer Res. Treat., 2016, 157, 77–90.

TA Ince, AD Sousa, MA Jones, JC Harrell, ES Agoston, M Krohn, LM Selfors, W Liu, K Chen, M Yong, P Buchwald, B Wang, KS Hale, E Cohick, P Sergent, A Witt, Z Kozhekbaeva, S Gao, AT Agoston, MA Merritt, R Foster, BR Rueda, CP Crum, JS Brugge, GB Mills, Characterization of twenty-five ovarian tumour cell lines that phenocopy primary tumours, Nat. Commun., 2015, 6, 7419.

S Santagata, A Thakkar, A Ergonul, B Wang, T Woo, R Hu, JC Harrell, G McNamara, M Schwede, AC Culhane, D Kindelberger, S Rodig, A Richardson, SJ Schnitt, RM Tamimi and TA Ince, Taxonomy of breast cancer based on normal cell phenotype predicts outcome, J. Clin. Invest., 2014, 124, 859–870.

UNIVERSITY OF MIAMI
MILLER SCHOOL
of MEDICINE



# DYSREGULATED CELLULAR MIGRATION: SEEING IS BELIEVING

**Dr Stefan Veltel** and his colleagues at the Universitätsklinikum Hamburg-Eppendorf in the Institute of Medical Microbiology, and Hygiene, study the molecular mechanisms of small GTPases in the context of various diseases. One protein in their focus is Rab21 and its role in integrin trafficking, cancer progression and metastasis. Another more fundamental mechanistic project involves the time-resolved crystallisation of an enzymatic GAP-GTPase complex.

**The Importance of Integrins in Cell Migration and Adhesion**

The process by which a cell moves or migrates involves a dynamic interaction between the cell and the extracellular matrix that it is attached to and over which it migrates. Cells can migrate in a variety of ways, and these range from the movement of single cells to the cooperative migration of groups of cells, where intercellular interactions are maintained and cells move together in a highly coordinated manner. Integrins are trans-membrane receptors that serve as the main and best-characterised mediators of the dynamic interactions between the extracellular matrix and the actin cytoskeleton during cell motility. Integrin-based adhesion provides a model for studying the fundamental roles of adhesion in migration.

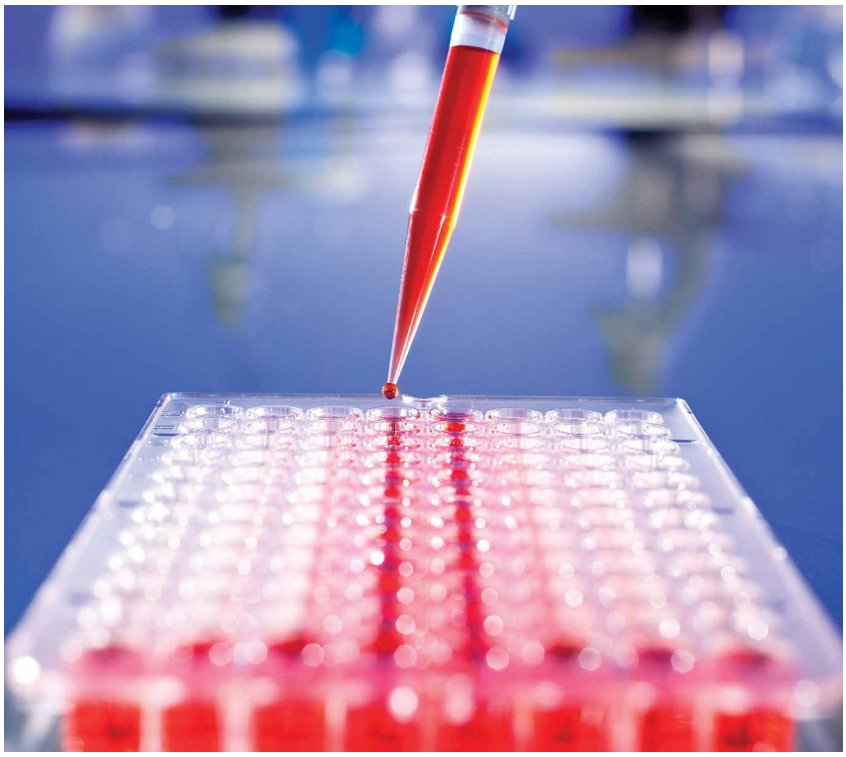
Integrins are implicated in variety of cellular migrations, including leukocyte trafficking during immune surveillance, the cellular movements that mediate tissue regeneration and repair and migration during embryonic morphogenesis. The importance of integrins is highlighted by the fact that the dysregulation of integrin-mediated adhesion and migration contributes to a variety of human diseases, including cancer. In fact, cancer metastasis starts with cancer cells first detaching from the tumour, migrating to different sites through blood or lymphatic vessels, invading new tissue and adhering to it. Thus, there has been a strong research interest in understanding and potentially targeting integrin-mediated migration to treat cancer, and other diseases. However, studies aimed at understanding cell migration are faced with challenges, because migration is the result of transient, localised adhesion and signalling. The key to really understanding integrin-mediated cell adhesion and migration involves uncovering how the formation and disassembly of integrin-mediated adhesions are regulated temporally and spatially during directed cell migration.

Indeed, Dr Stefan Veltel and his colleagues are interested in the notion that it is not only the activation and deactivation of integrins that is important, but also the endo-/exocytic cycle of integrins plays an important role in mediating integrin function by determining the localisation, the signalling and the fate of integrins. Dr Veltel's group focuses on the small Ras-family GTP-binding protein Rab21, which is a major regulator in the endocytic trafficking of integrins. At a molecular or structural level, the mode of action of Rab21 remains poorly understood mainly due to the lack of known Rab21-interacting proteins. One of the main goals of Dr Veltel's research is to gain insight into how Rab21 organises integrin trafficking on a molecular-mechanistic level.

**Identification of a Novel Interaction Partner of Rab21**

Small guanine nucleotide-binding (G) proteins (or GTPases) like Rab21 are ON-OFF switches of the cell. These molecules can be activated by guanine nucleotide exchange factors (GEFs), which reduce the nucleotide binding affinity of GTPases, allowing a fast exchange of GDP to the more abundant

**‘We are focusing on the discovery of novel proteins that assist Rab21 to regulate integrin trafficking and hope to both broaden our understanding in the mechanisms of integrin trafficking as well as to find novel targets for cancer therapy’**



GTP. GTPases are switched off again by hydrolysing GTP to GDP – a step that is catalysed by GTPase-activating proteins (GAPs). GTPases transduce the cellular signal to their downstream effector proteins. These effectors can only bind to the GTP-bound form of small GTPases ensuring the signal is transmitted only when the GTPase switch is in the ‘ON’ position. GTPases and their GEFs, GAPs and downstream effectors are potential therapeutic targets for developing drugs to treat various diseases, including cancer.

Dr Veltel and his team are interested in exploring the molecular mechanisms of early integrin trafficking that is regulated by Rab21. ‘We are focusing on the discovery of novel proteins that assist Rab21 to regulate integrin trafficking and hope to both broaden our understanding in the mechanisms of integrin trafficking as well as to find novel targets for cancer therapy,’ he explains. Currently, a limited number of Rab21 binding partners are known (both regulators and downstream effectors). Thus, the mechanistic details of this process are lacking. Dr Veltel aims to identify novel interacting partners of Rab21 during integrin trafficking.

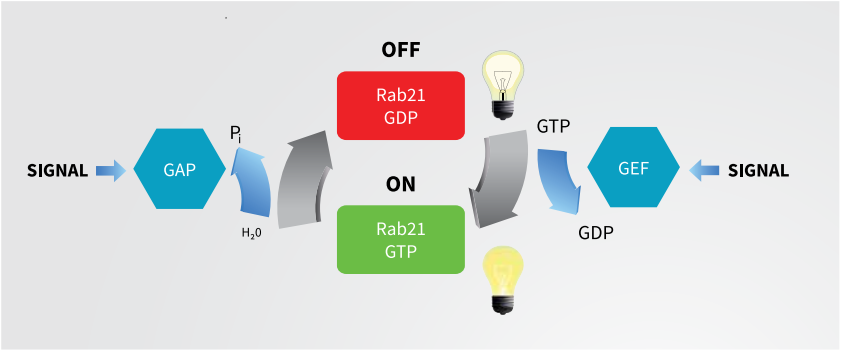
Interaction-based screens to identify regulators and downstream effectors of small GTPases are troublesome, because these interactions might occur with low affinities and abundancies in the cell. To increase the specificity of such a screen, Dr Veltel’s group combined the isolation of cellular protein complexes with quantitative mass spectrometry.

To conduct these screens, Dr Veltel’s team created GFP-tagged active and inactive versions of Rab21 and cloned them. In order to purify the interaction partner of Rab21, they isolated cellular GFP-Rab21 complexes, to capture Rab21. They used the MDA MB-231 breast cancer cell line, as this is an established model for aggressive and strongly metastasising cancer cells. From more than 10 experiments, the team repeatedly identified several potential interacting proteins of Rab21 by comparing their amounts in immunoprecipitations of GFP-Rab21 WT or the GFP-Rab21 active mutant (Q78L) to GFP alone or the GFP-Rab21 inactive mutants (T33N, D135N). The protein quantification was done by mass spectrometry using the SILAC method (stable isotope labelling by amino acids in cell culture) in strong collaboration with the group of Matthias Selbach from the MDC in Berlin.

Surprisingly, Dr Veltel and his colleagues identified a few actin-binding proteins to be among the best hits from the screen. Together with microtubules and intermediate filaments, actin filaments built up the cytoskeleton. Microtubules have been known for a long time to be the major cellular highway for vesicular transport inside the cell. In contrast, the role of actin filaments in vesicular transport processes are less established. Major parts of the actin cytoskeleton are localised close to the plasma membrane and are coupled to it via integrin and integrin-binding proteins. This peripheral actin ring stabilises the cellular cortex and its dynamic behaviour is very important for a cell to be able to migrate.

A direct physical interaction between Rab21 and actin-binding proteins as true GTP-dependent downstream effectors of Rab21 could link actin to vesicular trafficking processes. This might happen close to the plasma membrane after the initial endocytosis of integrins when integrins are subsequently transported to different destinations in the cell.

The current research in the Veltel lab concentrates on the function of one actin-binding, an actin-bundling protein. Recent data from the lab shows that this protein is strongly regulating early trafficking steps of integrin in alliance with Rab21. Mechanistically, the silencing of the actin-bundling protein effectively reduces the speed of Rab21 vesicles as shown by live cell imaging with TIRF microscopy methods. As a result of modulating integrin trafficking, the silencing of this protein also effects adhesion and migration of MDA-MB-231 breast cancer cells. This could have a direct clinical impact. The collaboration partner of Dr Veltel, Dr Leticia Oliveira-Ferrer from the UKE, found out that patient samples from triple-negative breast cancers (TNBCs) – a very aggressive and metastasising form of breast cancer – show a higher expression of the actin-bundling protein than milder forms of breast cancer. This puts the Rab21 interaction partner into the place of a potential therapeutic target for the treatment of breast cancer.




**A Deeper Understanding of Signal Transduction – 'Seeing is Believing'**

In the studies mentioned above, Dr Veltel's group focused their efforts on understanding the signal transduction related to cell migration and adhesion using biochemical and cell biological methods to identify an important regulator of Rab21. In another aspect of his studies, Dr Veltel's team takes their investigation on signal transduction to a deeper level.

To really understand how signalling proteins function, it is necessary to uncover the time-ordered sequence of events that lead to the signalling state. Specialised, but widely used techniques to obtain information of signalling proteins on an atomic level are methods in structural biology like X-ray crystallography, NMR or electron microscopy. For highest atomic resolution, X-ray crystallography is still the method of choice. However, Dr Veltel tells us that 'a pitfall of this classical method is that we can only see static structural snapshots, but not the dynamics of the reaction so far.' Progress has been made in the field of time-resolved structure biology in the description of electron transport reactions in the photosystem II of plants. In contrast, information regarding time resolved structural studies of enzymatic reactions is extremely limited. When the messenger is a chemical molecule, the time required to diffuse though a crystal and bind in the active site of a signalling protein is typically far longer than the timescale that occurs when protein conformational changes occur. Therefore, chemical-based signalling is not readily compatible with structural studies that use fast time-scales.

Dr Veltel's group aims to understand the structural determination of the kinetics of the enzymatic reactions of small GTPases and their co-enzymes. As mentioned above, this system is important in cell biology and particularly impacts disease processes, such as cancer. Given that small GTPases of the Ras superfamily (like Rab21) serve as molecular switches that cycle between a GTP-bound active and a GDP-bound inactive, and this switch is catalysed by GEFs and GAPs, it is important to understand how these reactions occur temporally. The intrinsic GTP hydrolysis of small GTPases is typically very slow, occurring in minutes to hours. However, in the cell, GAP proteins accelerate the GTP-hydrolysis reaction to faster than 1/sec in order to quickly deactivate the GTPase. Studies using spectroscopic FTIR measurements show that the GAP-assisted GTP-hydrolysis can be divided into different reaction steps with different time-frames. However, up until now, there are no high-resolution X-ray structures available that reflect the single kinetic steps of this reaction, and the enzymatic influence of certain residues in the GAP and GTPase is only deduced from static protein structures in combination with biochemical and spectroscopic experiments. Dr Veltel's group has set forth experiments to determine the time resolved structural characterisation of a GAP-catalysed GTPase reaction. This challenging project requires expertise from different scientific disciplines. Dr Veltel collaborates with structural biochemists, physicists and electrical engineers from the DESY (Deutsches Elektronen Synchrotron) to combine knowledge of the biological process with protein crystallography and laser physics. This interdisciplinary research project is funded by PIER – a strategic partnership between DESY and Universität Hamburg, which aims to support cutting edge science in Hamburg.

**Structural 'Movie' of an Enzymatic Reaction**

To characterise a GAP-catalysed GTPase reaction, Dr Veltel's group has chosen the Arl3-RP2 complex as the GTPase-GAP pair. The gene encoding the GAP protein RP2 is highly mutated in patients with X-linked Retinitis pigmentosa, with mutational hotspots in residues catalysing the GAP reaction on Arl3. In addition to its clinical relevance, they also chose this system as a model system for time-resolved structure determination because Dr Veltel had previously crystallised the complex of Arl3 and RP2 in the presence of a non-hydrolysable GTP-analogue and solved the structure using classical X-ray structure determination methods.

In order to not hydrolyse the Arl3-bound GTP in its complex with RP2, a hydrolysis-protected GTP – caged-GTP – has to be used in crystallisation setups. In the crystal, the reaction can be started by de-caging the GTP with high intensity UV-laser light. Dr Veltel's team is able to capture millisecond time-resolved snapshots of the Arl3/GAP RP2 structure using the pump-probe method. This method involves a laser pulse (pump) that photoactivates a caged-GTP molecule that is complexed to a protein, after which a suitably delayed X-ray pulse (probe) passes through the crystal and records its diffraction pattern on a 2D detector.

The central step in the time-resolved crystallography experiments of enzymatic reactions is the simultaneous initiation of the reaction start for all of the molecules in the crystal. Therefore, Dr Veltel's group is focussing on optimising the crystal size in correlation to the required energy supplied by a high intensity pump laser. By elucidating the experimental parameters that are necessary to trigger and to synchronise the reaction, they will record the diffraction data and process the data with polychromatic or monochromatic X-ray pulses using a serial crystallography approach. The time difference between the UV-laser inducing the start of the reaction and the X-ray laser determining the structure reflects the structural snapshot at one certain reaction time of the enzymatic reaction. A sequence of the snapshots after (for example) 10 ms, 50 ms, 100 ms, 500 ms, 1 sec creates the molecular movie of the enzymatic GAP-GTPase reaction. Overall, this project is a novel 'proof-of principle' methodological approach that promises a high scientific impact on the structural determination of enzyme kinetics.

# Meet the researcher

**Dr Stefan Veltel**
Institute of Medical Microbiology, Virology and Hygiene
AG Medical Biochemistry
Universitätsklinikum Hamburg-Eppendorf
Hamburg, Germany

Dr Stefan Veltel is the head of a research group at the Universitätsklinikum Hamburg-Eppendorf in the Center for Diagnostics at the Institute of Medical Microbiology, Virology and Hygiene. Dr Veltel received his Ph.D. from the Max-Planck-Institute in Dortmund, Germany and did his postdoctoral research at the VTT (State Technical Research Center – Technology for business) in Turku, Finland. Over the course of his career, he has been presented with many awards, including the Feodor Lynen Stipendium (Alexander von Humboldt-Stiftung) and an EMBO Long-Term-Fellowship.

## CONTACT

**E:** stefan.veltel@gmail.com
**T:** (+49) 40 7410 58184
**W:** https://www.uke.de/english/departments-institutes/institutes/medical-microbiology-virology-and-hygiene/research/working-groups/ag-veltel.html

## KEY COLLABORATORS

Professor Matthias Selbach, Max Delbrück Zentrum, Berlin
Professor Johanna Ivaska, University of Turku, Finnland
Professor Martin Aepfelbacher, University Medical Center, Hamburg
Dr Sabine Windhorst, University Medical Center, Hamburg
Dr Leticia Oliveira-Ferrer, University Medical Center, Hamburg
Dr Markus Perbandt, DESY (Deutsches Elektronen Synchrotron), Hamburg
Dr Guoqing Chang, CFEL, Hamburg

## FUNDING

DFG (VE 750/2-1)
PIER Seed Projects

## REFERENCES

A Arjonen, J Alanko, S Veltel and J Ivaska, Distinct recycling of active and inactive beta1 integrins, Traffic, 2012, 13, 610–625.

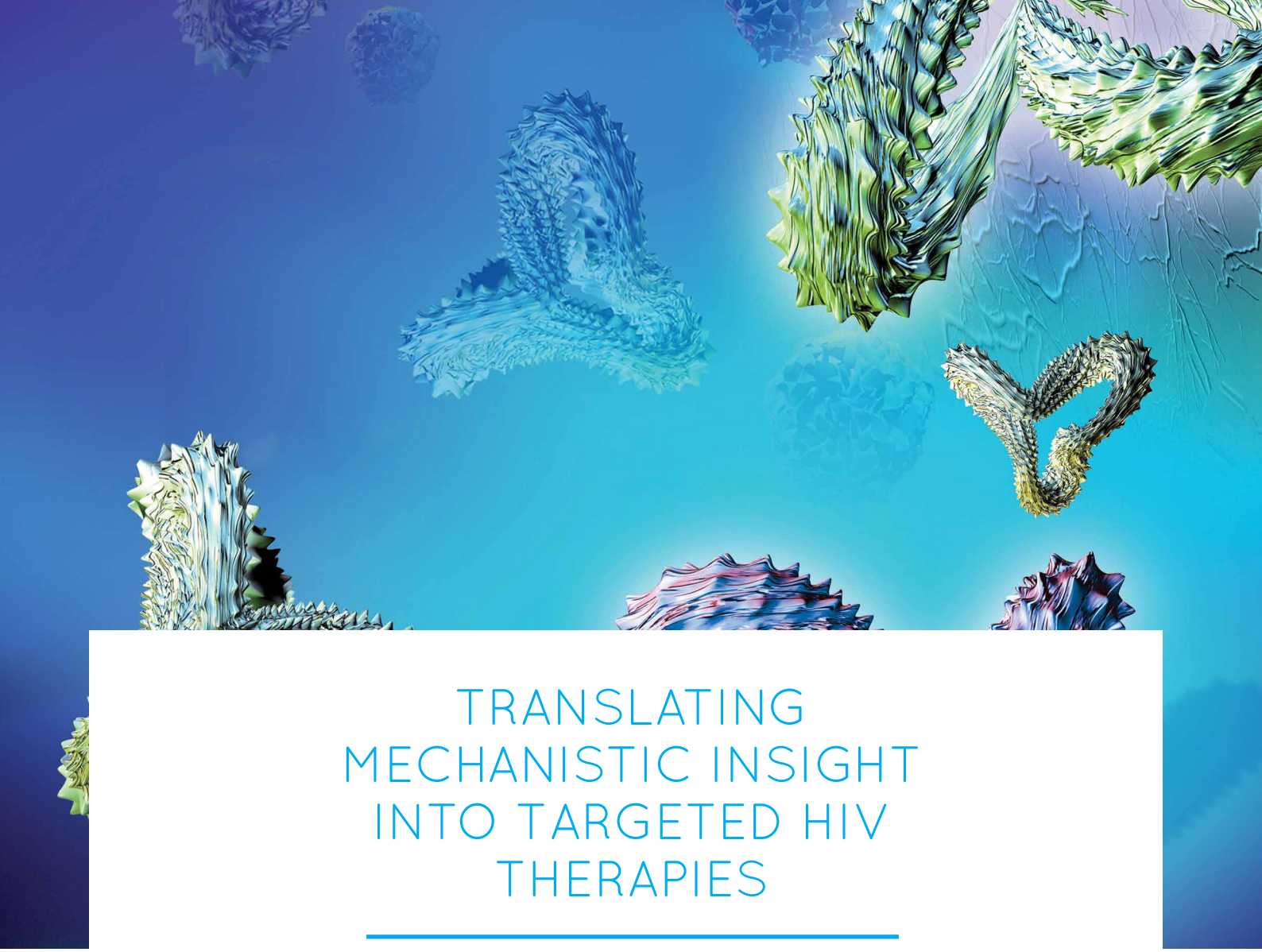
JK Rantala, J Pouwels, T Pellinen, S Veltel, P Laasola, et al., SHARPIN is an endogenous inhibitor of beta1-integrin activation, Nature Cell Biology, 2011, 13, 1315–1324.

A Mai, S Veltel, T Pellinen, A Padzik, E Coffey, et al., Competitive binding of Rab21 and p120RasGAP to integrins regulates receptor traffic and migration, Journal of Cell Biology, 2011, 194, 291–306.

S Veltel, R Gasper, E Eisenacher and A Wittinghofer, The retinitis pigmentosa 2 gene product is a GTPase-activating protein for Arf-like 3, Nature Structural & Molecular Biology, 2008, 15, 373–380.

S Veltel, A Kravchenko, S Ismail and A Wittinghofer, Specificity of Arl2/Arl3 signaling is mediated by a ternary Arl3-effector-GAP complex, FEBS Letters, 2008, 582, 2501–2507.

Universitätsklinikum Hamburg-Eppendorf



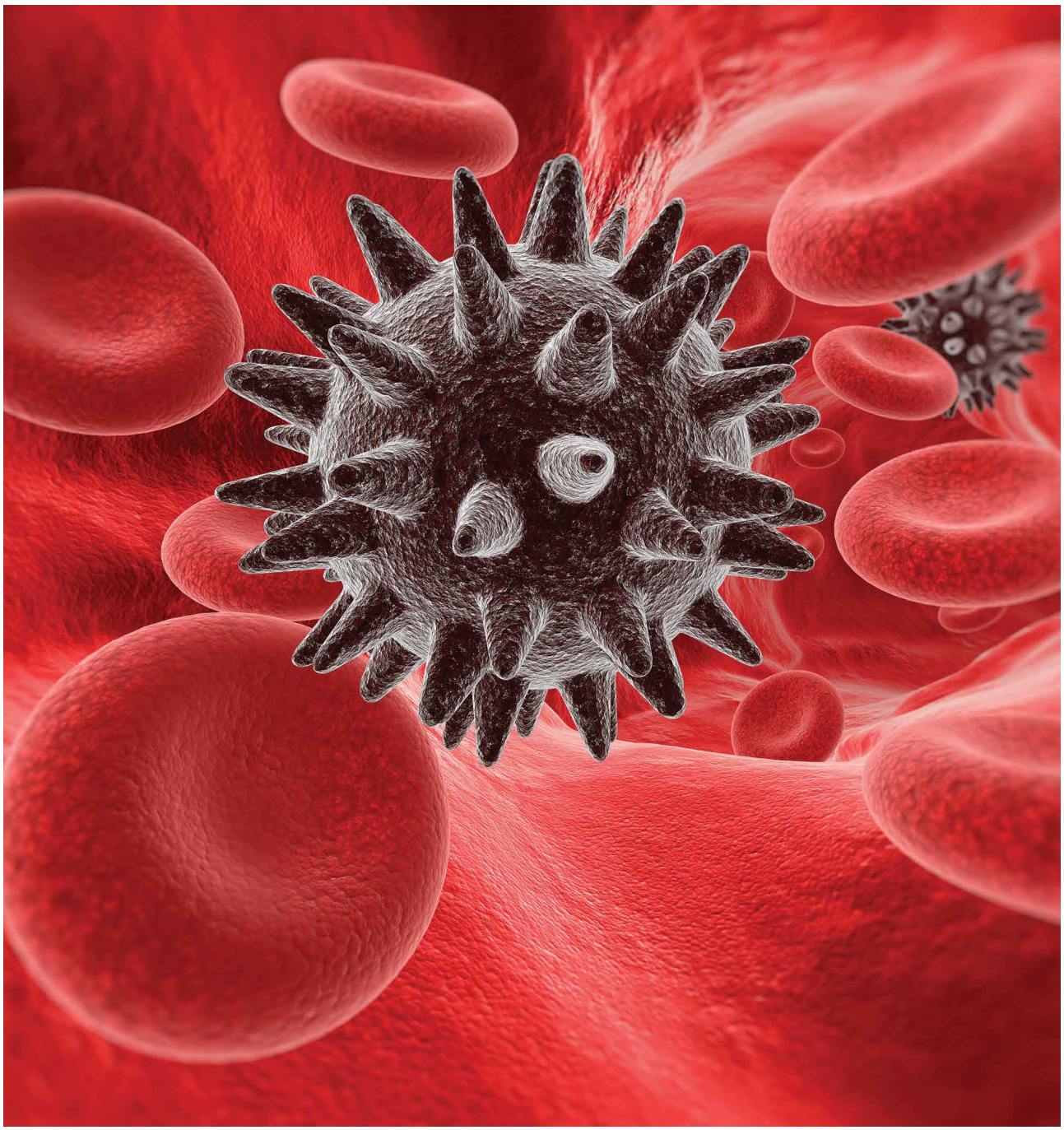
# TRANSLATING MECHANISTIC INSIGHT INTO TARGETED HIV THERAPIES

According to the World Health Organisation, more than 70 million people have been infected with the human immunodeficiency virus (HIV) and about 35 million people have died, since the start of the HIV epidemic in the 1980s. The virus is believed to be a mutated form of the simian immunodeficiency virus (SIV) that infects non-human primates in many African countries. On more than one occasion, different strains of SIV are thought to have transferred to humans though hunting and consumption of undercooked meat, which subsequently mutated into the various strains of HIV affecting humans today.

With the ability to infect a variety of immune cells such as CD4+ T cells, macrophages, and microglial cells, the HIV virus harms patients by impairing their immune systems, causing the development of acquired immunodeficiency syndrome (AIDS). AIDS leaves patients vulnerable to a range of life threatening opportunistic infections and cancers, which ordinarily would be quelled by healthy immune cells.

When first discovered in the 1980s, individuals infected with HIV had an average life expectancy of just 9–11 years following infection without treatment. However, through gaining an understanding of how HIV functions at the biochemical level, researchers developed a wide range of anti-retroviral therapies. Now, through effective pharmaceutical management, HIV infection has become a chronic condition, where patients can expect to live almost as long as the general population. Due to the widespread use of anti-retrovirals, the number of people dying from HIV infection and AIDS continues to decline each year, being just 1.1 million worldwide in 2015 – about 45% fewer than in 2005. Furthermore, because effective HIV treatment lowers the amount of virus in body fluids, anti-retroviral therapy greatly reduces the chance of transmission. According to the latest WHO HIV progress report, fewer people became newly infected with HIV in 2015 than in any of the 25 years prior.

However, through viral recombination processes, the HIV genome can evolve to acquire drug resistance, rendering current anti-retroviral therapies ineffective. Indeed, many drug resistant forms of HIV exist today, which are not amenable to effective treatment with conventional anti-retroviral drugs. This is where the work of Professor Paul Dent and his research team at Virginia Commonwealth University comes in. In this final section of the magazine, we detail Professor Dent's work on AR-12, a small molecule drug, that acts by inhibiting chaperone proteins. Chaperones are found in all cells in the body, as they are required to regulate the folding of other proteins. However, they are particularly ubiquitous in cells that synthesise significant amounts of protein, such as cancer cells or those infected with a virus, making chaperones a promising target to disrupt pathological functionality in these cells. After discovering the anti-cancer activity of AR-12 in combination with another drug, Professor Dent and his team recently demonstrated that this chaperone inhibitor also acts as a versatile antiviral agent, for



many viral infections such as Ebola, Rubella, Influenza, Mumps and HIV. Perhaps most excitingly, the team showed that this drug also inhibits forms of HIV that are resistant to conventional drugs, suggesting that it may be effective for tackling HIV in treatment-resistant patients.

In our final article of this edition of Scientia, we explore the research of Professor David Guidot and his group at Emory University, who are seeking new ways to treat lung disorders in people living with HIV. Although lung disease remains the highest cause of mortality amongst individuals with chronic HIV infection, many of the biochemical mechanisms that underlie their development remain unknown. In his research, Professor Guidot has discovered why the lungs are so vulnerable to disease in HIV patients, by noticing that while many components of the immune system respond very well to anti-retroviral therapy, immune functioning in lung tissue remains severely impaired. In particular, his research team found that the small airways of the lungs are deficient in antioxidants and zinc in HIV patients, and these deficiencies impair local immune function. This leaves HIV infected lungs vulnerable to not only infections and cancer, but also asthma and COPD.

To explore this further, Professor Guidot investigated the importance of zinc and an antioxidant known as SAMe (S-adenosylmethionine), in maintaining the immune function of alveolar macrophages – immune cells found in the alveoli of the lungs. After obtaining promising results in a rat model of HIV, the team then carried out a pilot clinical trial investigating the efficacy of this simple dietary supplement to improve alveolar macrophage function in humans. Amazingly, the treatment appeared to increase the patients' immune cell count within just two months. The team are now currently working hard to further investigate the efficacy of this treatment, and the future looks bright!

# HIV AND AIDS WORLDWIDE

**36.7 million**
Number of people living with HIV in 2015

**2.1 million**
Number of people that became newly infected in 2015

**1.1 million**
AIDS related deaths in 2015 – a ~45% reduction compared with 2005

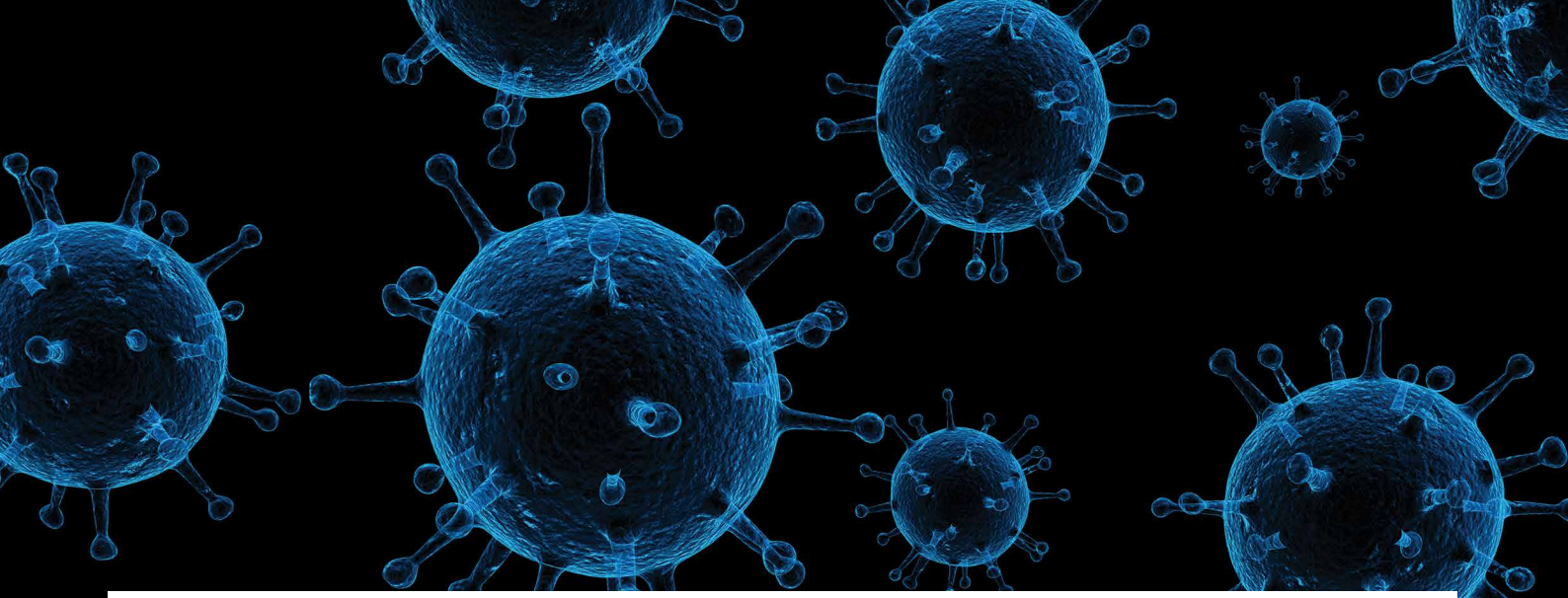
**1 in 4**
new cases are in youths aged 13–24

**390,000**
AIDS-related deaths in 2015 were caused by Tuberculosis – the most common life-threatening opportunistic infection affecting people with HIV

**1 in 6**
People living with HIV are unaware of their illness

**There has been a huge Increase in the number of people on antiretroviral treatment for HIV in the last decade**

| 2005 | 2010 | 2015 |
|---|---|---|
| 2 million people | 7.5 million people | 17 million people |



# A VERSATILE ANTIVIRAL DRUG WITH POTENTIAL FROM EBOLA TO HIV

**Professor Paul Dent,** of Virginia Commonwealth University, has conducted extensive research into the potential of AR-12, a small molecule drug, to act as a versatile antiviral agent. The drug exploits clever targeting of ubiquitous chaperone proteins, leading to inhibition of a wide range of viruses, including HIV, Ebola, Mumps, Influenza, Measles, Junín and Rubella.

**Viral diseases – a human cost**

Viral disease remains a huge source of morbidity and death. Historically, viral infections such as smallpox and influenza have ravaged human populations. The routine use of effective anti-viral medications is relatively rare, compared with the plethora of antibiotics commonly prescribed for bacterial infections. For many viral infections such as influenza, few effective treatments exist and most cases are left to run their course and are treated simply with rest and fluids. While some vaccinations against viruses exist (perhaps most notably contributing to the eradication of smallpox), for many viruses these have not yet been developed, or are not completely effective. The 1918 flu pandemic resulted in the infection of 500 million and the deaths of up to 100 million people globally. While influenza vaccines exist, they are not always completely effective given the rapid and unpredictable nature of viral mutation, and the consequent need to develop new vaccines every year. Despite significant advances in healthcare since 1918, another flu pandemic, with serious consequences, is entirely possible.

When first discovered, human immunodeficiency virus (HIV) infection was typically an acutely fatal condition, with an average life expectancy of 9–11 years after infection without treatment, prior to the development of effective anti-retroviral therapies. Although HIV infection has now progressed to a chronic condition through effective pharmaceutical management, drug resistant forms of the virus exist, which are not amenable to effective treatment with conventional anti-retroviral drugs. The recent Ebola outbreak in West Africa, which is the largest yet recorded, resulted in almost 30,000 cases and over 11,000 deaths. Despite the high mortality, no specific treatments have been developed or approved and treatment is largely supportive, in the form of rehydration and management of pain.

There is a clear need for effective antiviral therapeutics that can counteract viral propagation and action, for the benefit of the many patients affected by viral infections. The research of Professor Dent has produced some very promising candidates in this regard. In this article, we discuss the background to his ground-breaking research into anti-viral drugs and some of his key results to date.

**Chaperone proteins and their role in viral replication**

Molecular chaperones are a family of proteins, primarily involved in regulating the folding of other proteins. In order for proteins to function correctly, they are required to be folded in the correct manner, and to maintain their resulting three-dimensional shape when performing particular functions. Chaperones help proteins to achieve a specific three-dimensional shape during the folding process and maintain it thereafter, and thus are a vital component of many protein-mediated processes in the cell. However, chaperones are not just necessary for the correct functioning of proteins present in healthy cells, but also play a vital role in protein processing in cancerous cells or cells that have been infected by viruses.

Indeed, cancerous cells and virally infected cells synthesise significant amounts of protein, requiring the up-regulation of chaperone expression, and so chaperones are a promising target to disrupt pathological functionality in such cells. Under normal circumstances, this increased protein synthesis would trigger a stress response and result in the cell dying, through a process

called autophagy, but viruses are able to suppress these cell responses and keep the cell alive long enough to achieve their own ends. In virally-infected cells, this increased protein load largely goes into producing viral components such as the viral capsid. When a cell becomes infected with a virus, it becomes 'hijacked', whereby its own cellular machinery is used to produce new viral particles, which are eventually released when the cells dies. The capsid is a protein coat, used to package and protect all the other viral components. Preventing the correct folding of viral components such as the capsid, by inhibiting chaperone proteins is an effective way to reduce or negate viral action and disease.

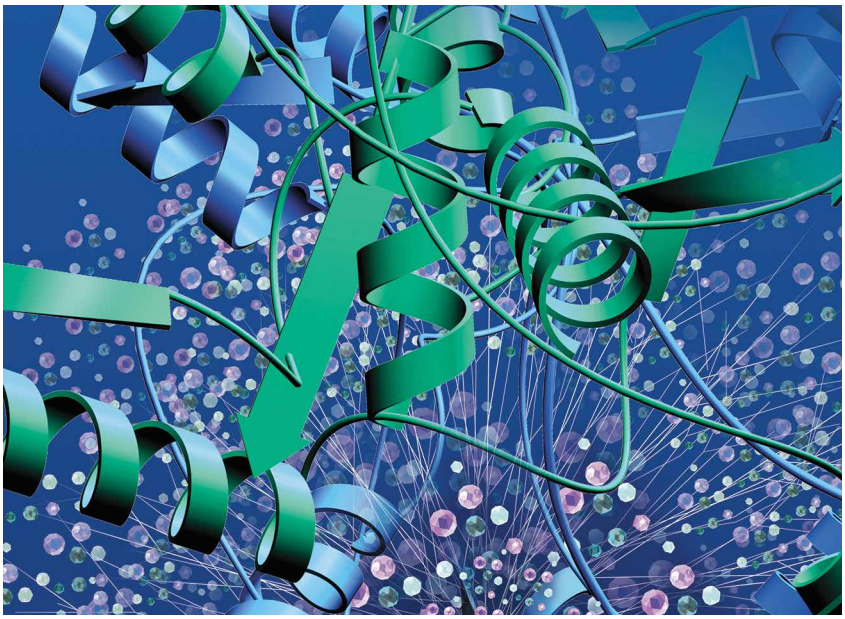
There are a variety of chaperones that function to regulate protein folding under normal circumstances. However, there are several that have been noted as particularly important during the replication of specific viruses. For example, a chaperone protein called GRP78 is thought to be essential for the reproduction of the Ebola virus. Knock down of GRP78, which means preventing it from being expressed for the purposes of experimentation, has been shown to protect mice from the Ebola virus. Professor Dent, along with his research team and collaborators, have demonstrated the potent and versatile antiviral action of AR-12, a small molecule chaperone inhibitor. However, initially, the team were examining the potential of AR-12 for the treatment of cancer, and came to investigate its potential as an anti-viral agent by rationally identifying AR-12 targets that are also implicated in viral disease.

Interestingly, GRP78 has even been linked to neuro-degenerative disorders such as Alzheimer's Disease. Along with other chaperones, GRP78 is over-expressed in the neurons of Alzheimer's patients, in an effort to maintain cell viability by influencing tau protein and preventing the formation of insoluble aggregates. Thus the possibility of using AR-12 or its analogues as treatments for Alzheimer's is currently being developed by several research groups.

**AR-12 anti-cancer and anti-bacterial action through chaperone inhibition**

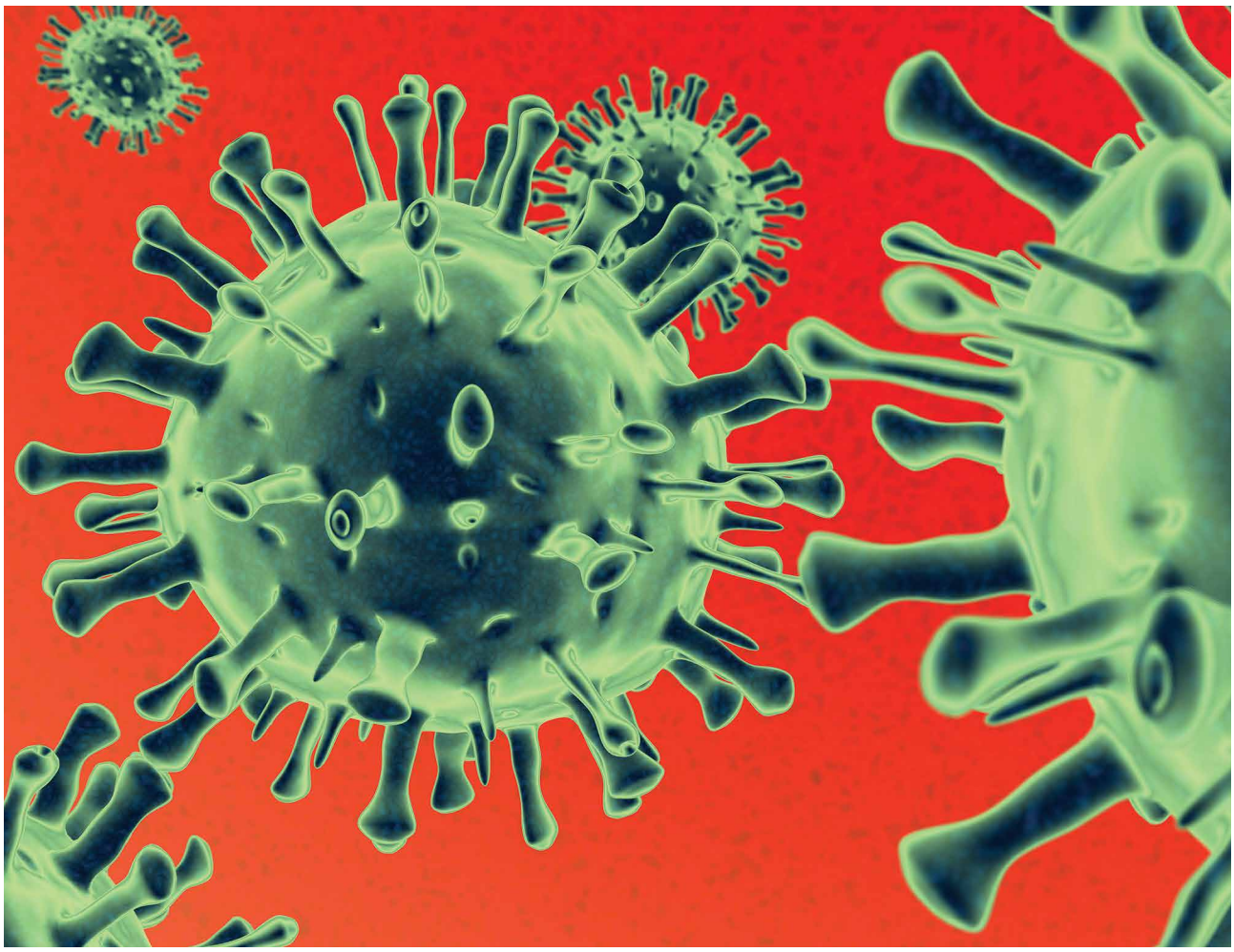
Professor Dent explained to Scientia how the team came to investigate the potential of AR-12 in viruses: 'Our virus work with AR-12 grew out of a cancer based project. We had discovered that AR-12 inhibited chaperones, which explained how it killed cancer cells. Stopping chaperones also stops viruses.' Previous work by the team has shown that AR-12, in combination with Viagra, is an effective anti-cancer agent, producing cytotoxic effects in a variety of cancer cells, including difficult to treat brain cancer stem cells. Viagra, most commonly known as a treatment for erectile dysfunction, is an inhibitor of an enzyme called phosphodiesterase type 5 (PDE5). In penile tissue, this acts to increase blood flow, an effect that can be used to treat certain types of erectile dysfunction. However, PDE5 is not just expressed in the penis, but is also present and active in other tissues, including in certain cancer cells. The team have previously shown that PDE5 inhibitors, such as Viagra, can enhance the effects of AR-12. The team determined that the AR-12/Viagra combination resulted in inhibition of the GRP78 chaperone protein, causing the death of cancer cells. The treatment works by significantly reducing the half-life of the GRP78 protein, meaning that the protein becomes degraded much more quickly than it would normally, causing levels to fall by over 90% in a wide array of assayed cancer cell lines. After learning of the potent chaperone inhibition produced by this drug combination, the team began thinking about applying it to other disease states in which chaperone action is heavily involved. These included bacterial and viral infections. Bacteria possess a prokaryotic version of the GRP78 chaperone, called Dna K, which is important in normal bacterial function by chaperoning proteins like Rec A, which performs an essential role in the replication of bacterial DNA and crucially, in bacterial resistance mechanisms to antibiotics. The team discovered that AR-12 in combination with PDE5 inhibitors, could reduce Dna K levels in antibiotic resistant bacteria, including N. gonorrhoeae, E. coli, MRSA and MRSE. This resulted in either outright cell killing effects, or increased sensitivity to antibiotics.

**'Our virus work with AR-12 grew out of a cancer based project. We had discovered that AR-12 inhibited chaperones, which explained how it killed cancer cells. Stopping chaperones also stops viruses.'**



**Results to date – AR-12 anti-viral action**

After determining the potential of AR-12 and PDE5 inhibitors in cancer and bacterial infections, the team turned their attention to viral infections, where the increased protein synthesis and chaperone expression



also make this drug combination a promising option. The researchers investigated the effects of the drug combination on the replication of an array of major viruses, in infected cells. Excitingly, they found that the drugs could prevent the replication of a huge number of seemingly diverse viruses, including Chikungunya, Mumps, Measles, Rubella, Junín, Ebola and Influenza viruses. Perhaps most excitingly, forms of HIV, that are resistant to conventional drugs, were also shown to be inhibited, suggesting that this drug combination might be of use in treatment-resistant patients. The team also examined if these antiviral effects were present in living animals, as opposed to isolated cells. To do this, they set up a rabbit model of hemorrhagic fever virus. Some of the infected rabbits were treated with AR-12, and they found that this treatment increased rabbit survival from approximately 30% to 60%. The team also examined liver damage in the rabbits caused by the virus and found that AR-12 reduced this, as measured using several indicators of liver damage. In their most recent work, the team identified that AR-12 can inhibit a variety of chaperone proteins, from both the main classes of chaperones: the HSP90 and HSP70 families. This gives some indication as to how it is able to interfere in the replication of so many types of virus.

The team have invested significant effort in determining how the drug works. They think that by inhibiting the chaperone proteins with AR-12 they are both causing viral proteins to become misfolded, thereby rendering them non-functional and prone to degradation by the cell, and also triggering a stress response in the cell, which results in cell death by autophagy. This double effect prevents viral replication both by disruption of viral processes in the cell and death of infected cells themselves, preventing the spread of the virus elsewhere. It is also thought that when these cells die via autophagy, they may release a host of viral components and debris into the surrounding environment, which are highly immunogenic, potentially causing an activation of the host immune system against the virus and enabling the host to begin to destroy other viral particles elsewhere.

**Future work**

Professor Dent feels that their promising anti-viral results indicate that a clinical trial is warranted for this drug treatment, and would like to pursue one in future, when funding is available. In fact, a clinical trial for AR-12 has been carried out previously in cancer patients, several years ago. The drug was well tolerated by the patients, some of whom were on the trial for up to 33 weeks, and who had been heavily pre-treated with multiple other anti-cancer drugs, and at much higher doses than that used to produce anti-viral effects in these studies (Professor Dent estimates that the team used 25% of the achievable plasma levels of AR-12 in their lab-based studies). Consequently, AR-12 has significant potential as an antiviral therapeutic, for a wide range of viral infections, including treatment-resistant HIV and Ebola. This work would not have been possible without collaboration between researchers at such varied locations as the USA, Argentina, Australia and Spain.

# Meet the researcher


**Professor Paul Dent**

Professor and Universal Chair in Signal Transduction
Department of Biochemistry & Molecular Biology
Virginia Commonwealth University


Professor Paul Dent is Professor and Universal Chair in Signal Transduction in the Department of Biochemistry and Molecular Biology in Virginia Commonwealth University. After receiving a 1st for his BSc degree in Biochemistry from Newcastle University, he went on to do a PhD at the University of Dundee in Scotland. After graduation, Professor Dent became a Postdoctoral Fellow in the University of Virginia, US, and later set up his own laboratory. Through securing funding from the National Institutes of Health and the Department of Defence, Professor Dent and his colleagues have explored the ways in which drugs can be combined to kill tumour cells. In addition to his cancer research, Professor Dent has also conducted extensive studies into the potential of AR-12, a small molecule drug, to act as a versatile antiviral agent. Through targeting chaperone proteins, this drug can inhibit a wide range of viruses, including HIV, Ebola, Mumps, Influenza, Measles, Junín and Rubella.

## CONTACT


**E:** Paul.Dent@vcuhealth.org
**T:** (+1) 804 628 0861
**W:** http://www.biochemistry.vcu.edu/Faculty/Dent.html


## KEY COLLABORATORS

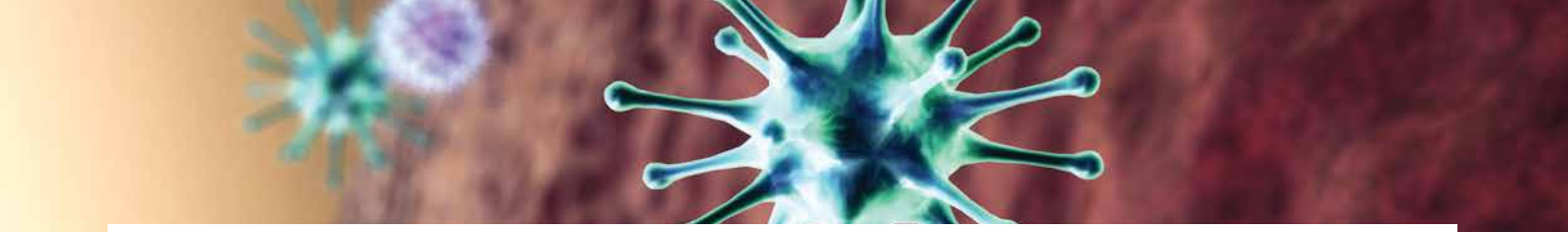
Laurence Booth, Virginia Commonwealth University
Jane L. Roberts, Virginia Commonwealth University
Heath Ecroyd, University of Newcastle
Sarah R. Trisch, United States Army Medical Research Institute of Infectious Diseases
Sina Bavari, United States Army Medical Research Institute of Infectious Diseases
St. Patrick Reid, United States Army Medical Research Institute of Infectious Diseases
Stefan Proniuk, Arno Therapeutics
Alexander Zukiwski, Arno Therapeutics
Abraham Jacob, University of Arizona Ear Institute
Claudia S. Sepúlveda, Ciudad Universitaria
Federico Giovannoni, Ciudad Universitaria
Cybele C. García, Ciudad Universitaria
Elsa Damonte, Ciudad Universitaria
Javier González-Gallego, University of Leon
María J. Tuñón, University of Leon

## FUNDING


NIH-NCI
NIH-NIAID
Department of Defence


## REFERENCES


L Booth, JL Roberts, H Ecroyd, SR Tritsch, S Bavari, SP Reid, S Proniuk, A Zukiwski, A Jacob, CS Sepúlveda, F Giovannoni, CC García, E Damonte, J González-Gallego, MJ Tuñón and P Dent, AR-12 Inhibits Multiple Chaperones Concomitant With Stimulating Autophagosome Formation Collectively Preventing Virus Replication, Journal of Cellular Physiology, 2016, 231, 2286–2302.



# EXPLORING ALVEOLAR MACROPHAGES AS HIV RESERVOIRS

Although pulmonary disorders remain the highest cause of mortality amongst individuals living with chronic infection by the human immunodeficiency virus (HIV), many of the mechanisms underlying their development remain unknown. **Professor David Guidot** and his team at Emory University are exploring these mechanisms in order to develop novel treatments targeting the alveolar spaces in the lung.

**HIV Influence on Pulmonary Disease**

Human Immunodeficiency Virus (HIV) is the retrovirus responsible for the development of Acquired Immune Deficiency Syndrome (AIDS). AIDS leaves the host vulnerable to many opportunistic infections that can be fatal. Thanks to the widespread use of anti-retroviral therapies (ART), many individuals infected with HIV can live well for decades with the illness. In spite of this, lung diseases continue to be the primary cause of death amongst individuals living with HIV. As well as being vulnerable to infections, HIV promotes the risk of non-infectious lung diseases such as asthma, chronic obstructive pulmonary disorder, pulmonary hypertension and lung cancer. New therapies for HIV also bring new complications such as sarcoidosis.

So why is lung disease so high in this population? As Professor David Guidot explains: 'We have identified that the localised immune functions within the lung remain severely impaired even when other components of the immune system appear to respond very well to anti-viral drugs. In particular, the small airways are relatively deficient in antioxidants as well as being deficient in zinc, and these deficiencies impair local immune function in the airways.' Professor Guidot has brought together a multidisciplinary team to identify previously unrecognised factors that determine an individual's risk for HIV-related lung disease. 'The goal of our studies is to determine if we can improve the immune function in the lungs of people living with HIV, using dietary supplements of zinc and an antioxidant known as SAMe (S-adenosylmethionine), with the hope that this will make them even healthier and significantly decrease their risk of pneumonia and other lung diseases,' he tells us.

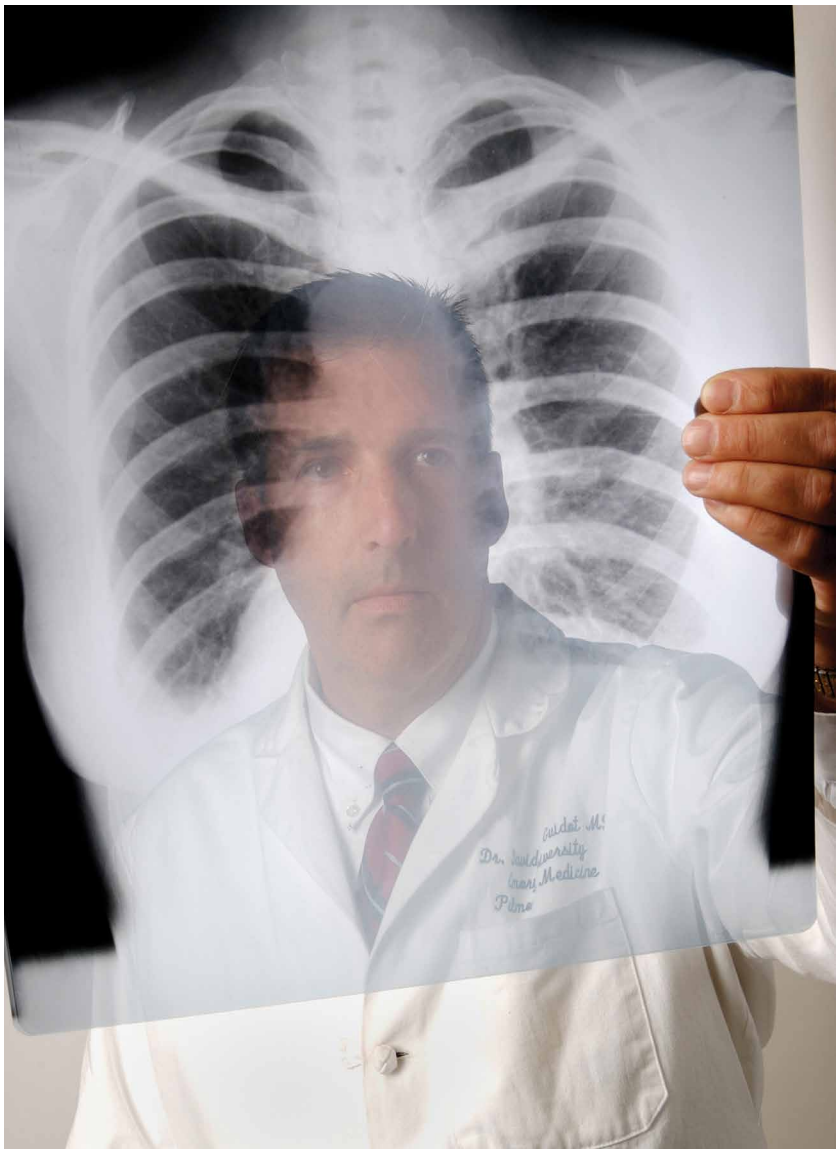
Although the CD4+ T-lymphocytes are the primary target of HIV, the virus can also infect the innate immune cells in the airways known as alveolar macrophages. Macrophages are large white blood cells that ingest foreign particles and microorganisms. The team hypothesises that infected macrophages may be acting as reservoirs of HIV infection within the alveolar macrophage pool (alveoli are tiny air sacs in the lung that allow for rapid gaseous exchange). This is a problem because these reservoirs are relatively long lived, less susceptible to ART and stored in membrane-bound compartments that are inaccessible to antibodies and small compounds. Therefore, therapies that enhance macrophage function could potentially decrease both lung infections and chronic airway diseases in individuals living with HIV.

**Potential Mechanisms of Impaired Immune Response**

Professor Guidot and his team have two primary questions to answer. Firstly, does the alveolar macrophage pool serve as a reservoir of HIV even when peripheral viral suppression has been achieved by ART? Secondly, how does this reservoir alter the environment within the alveolar space and impair alveolar macrophage immune function? To find the answers to these questions, it was necessary to first determine the mechanisms by which HIV infection damages the lungs. Experimental evidence suggests that HIV inhibits antioxidant defences within the alveolar space and causes severe oxidative stress. This occurs through the induction of zinc deficiency in the microenvironment, which prevents the alveolar epithelium and macrophages from generating glutathione and other antioxidants that are critical to maintaining a healthy redox potential within the alveolar space. By this process, HIV promotes its own ability to infect macrophages and accumulate a large pool of intracellular proviruses, thereby producing a large HIV reservoir. The resulting impaired innate immunity leads to both the increased risk of infections and resistance to clearing the viral reservoir. Research has also shown that the relatively high concentrations of HIV-related viral proteins in the airway lead to lung epithelial barrier dysfunction and are also toxic to alveolar macrophages independently of direct viral infection, which further impairs the immune system.

Professor Guidot explains how he came to work in this field: 'We had been studying the mechanisms by which alcohol abuse renders individuals susceptible to pneumonia and lung injury. We used animal models to identify several novel such mechanisms, including profound depletion of the antioxidant glutathione in the airways and a block in zinc transport into the airways. We translated these findings to clinical studies and have a clinical trial underway in which we are testing if dietary supplementation with zinc and/or S-adenosylmethionine – also known as "SAMe", which is converted in the body to glutathione – can improve immune function in the lungs of alcoholics. About eight or nine years ago we started examining HIV transgenic rodents and found that chronic HIV expression causes remarkably similar defects that we had identified in the "alcoholic lung". We published several research papers showing that chronic HIV expression also causes glutathione and zinc deficiency in the airways, and that dietary zinc supplementation can improve lung health in these animals.'

**‘People living with HIV have a much better prognosis and with current medications can now live for many decades. However, they remain much more susceptible to pneumonia and other lung diseases than people without HIV’**



**HIV Transgene Expression in Rat Models**

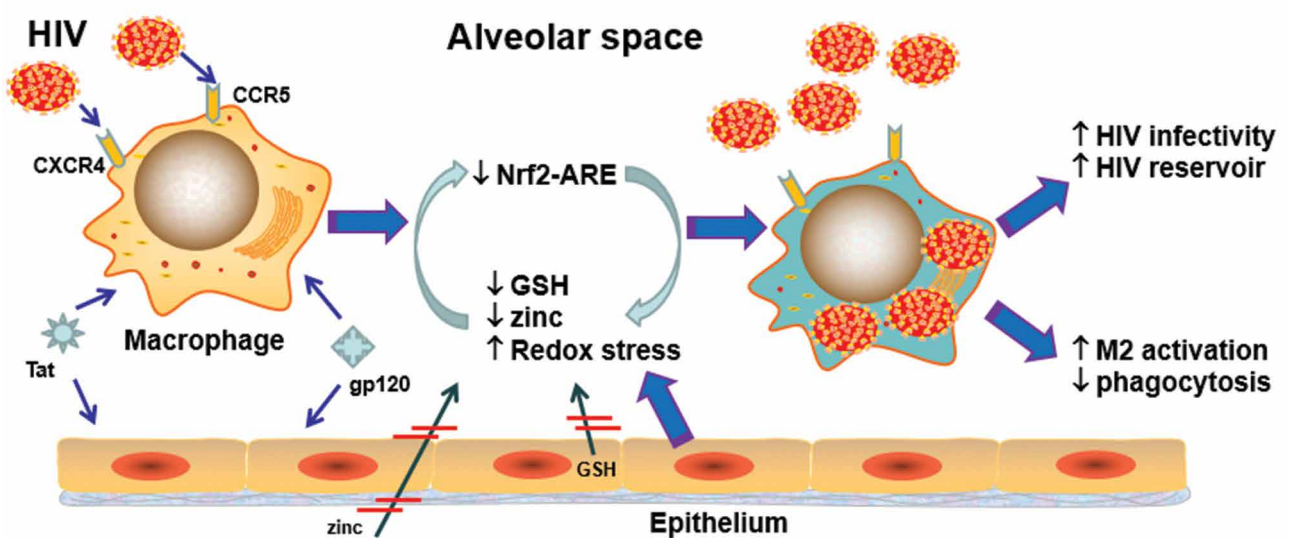
A HIV transgene rat model was used to investigate the mechanisms by which HIV infection of the alveolar macrophage alters macrophage activation and impairs immune function. In these HIV rat constructs, the gag and pol genes have been deleted resulting in a pro-virus insertion in which there is no viral replication or infection. However, other HIV related proteins are expressed with efficient viral gene expression in many organs. The rats develop muscle wasting, cataracts, nephropathies and immune deficiencies, all of which are consistent with an AIDS-like phenotype.

The first study aimed to determine the effects of HIV transgene expression on alveolar epithelial barrier function in rats. The researchers found that although inflammatory markers were similar in both wild type rats and HIV transgenic rats, oxidation was three times higher in HIV transgenic rats. This oxidative stress, combined with impaired epithelial barrier function and altered expression of critical tight junction proteins, suggests novel mechanisms by which HIV infection renders individuals susceptible to acute and chronic forms of lung disease.

The second study investigated the importance of pulmonary zinc status in maintaining alveolar macrophage immune function. Alveolar macrophage maturation and function depend on a pathway involving granulocyte-macrophage colony-stimulating factor (GM-CSF). Macrophages respond to this through the GM-CSF receptor, which has a binding subunit and signalling subunit. Alveolar macrophages from alcohol-fed rats have significantly fewer receptors and decreased signalling, resulting in decreased immune function. It is hypothesised that a similar process occurs with HIV infection. Professor Guidot and his colleagues found that HIV transgenic expression selectively decreased alveolar macrophage expression of the GM-CSF signalling subunit and impaired bacterial phagocytosis (the ingestion of bacteria by specialised cells). In vitro studies also showed similar findings. This suggests that the capacity of alveolar macrophages to maintain a robust signalling response to GM-CSF is dampened by chronic HIV related protein expression.

This mechanism appears to involve zinc deficiency. Therefore, the study also examined the role of zinc deficiency as a potential mechanism for these detrimental effects. The team found that HIV transgenic rats have significantly lower levels of zinc in the alveolar space and macrophages when compared with wild type rats. In addition, treatment of cell lines with a zinc chelator (which lowers zinc levels) decreased signalling receptor expression and phagocytosis, whereas treatment with zinc restored phagocytic function and zinc levels in alveolar macrophages. This indicates that zinc plays a role in the regulation of cellular glutathione and protects cell membranes from oxidative damage.

They also observed age-related effects on zinc levels, noting a progressive decrease in intracellular zinc levels of alveolar macrophages in rats as they aged. No corresponding decrease was observed in wild




type rats. The increase of AIDS-like pathologies in ageing rats implies a progressive burden of HIV related protein expression and consequent tissue injury over time.

**Translating Animal Models into Clinical Trials**

In the experimental model, zinc supplementation restored alveolar macrophage innate immune function in HIV transgenic rats. The research team hypothesised that treatment with zinc and a glutathione precursor could improve macrophage function in immunological non-responders (individuals who display a suboptimal response to ART). In order to determine if adjuvant therapy with dietary zinc and SAMe decreases the HIV reservoir pool within the alveolar macrophage pool, Professor Guidot and his colleagues first designed a prospective cross-sectional study to quantify the HIV viral load within the alveolar macrophages in a cohort of healthy HIV-infected individuals. Evidence supports the compartmentalisation of HIV between lungs and peripheral blood, suggesting that alveolar macrophages could be a reservoir for HIV. They also wanted to determine if alveolar macrophage pro-viral DNA was associated with immune dysfunction. In this study, alveolar macrophages were found to harbour HIV even in otherwise healthy subjects with undetectable plasma viral loads, supporting the hypothesis of a potential reservoir for the virus. They also found that subjects with positive pro-viral DNA had a significantly lower phagocytic response compared with other subjects. This suggests that supplemental therapies to ART may be necessary to target alveolar macrophage reservoirs and improve lung function.

Professor Guidot and his team followed this study with a pilot clinical trial that treated immunological non-responders with zinc and SAMe dietary supplements in order to improve alveolar macrophage function. The treatment appeared to increase peripheral CD4 T-cell count (immune cell count) in infected individuals within just two months. After twelve months, the researchers hope that treatment will continue to increase peripheral CD4 counts in non-responders and decrease the HIV reservoir in the alveolar macrophage pool. They anticipate that this will correspond with improvement in both alveolar and systemic biomarkers of HIV-induced stress. Currently they are following up this promising pilot study with a larger clinical trial in which individuals living with HIV are treated with these dietary supplements for two years to determine if prolonged therapy will restore overall airway health, including zinc bioavailability, anti-oxidant defences, and innate immune capacity.

**‘The goal of our studies is to determine if we can improve the immune function in the lungs of people living with HIV, using dietary supplements such as zinc and an antioxidant known as SAMe, with the hope that this will make them even healthier and significantly decrease their risk of pneumonia and other lung diseases’**

**Moving Towards the Future**

Guidot and his team are not finished yet: ‘The next steps include analysing the results of our clinical trial to determine if this dietary strategy is effective. In parallel, we are testing other potential therapies including naturally occurring compounds found in plants that can activate multiple anti-oxidant defences simultaneously. Although we are confident that zinc plus SAMe will have beneficial effects, there will likely be room for even greater improvement in lung immune function in these individuals.’ At the same time, the team have evidence that HIV affects the functions of the cells lining the airways, and people living with HIV are also at increased risk of emphysema, lung cancer and fibrosis (scarring) of the lung. Therefore, they ‘plan to eventually determine if these strategies that are focused on improving lung immunity will also have benefits for the structural health of the lung.’

# Meet the researcher


**Professor David M. Guidot**
Director and Professor of Medicine
Division of Pulmonary, Allergy, Critical Care and Sleep Medicine
Emory University Department of Medicine
Atlanta, USA


Professor David M. Guidot, MD, is Director and Professor of Medicine in the Division of Pulmonary, Allergy, Critical Care and Sleep Medicine in the Emory University Department of Medicine. After obtaining his MD from the University of Michigan, Professor Guidot trained in Internal Medicine at the University of Minnesota and later completed clinical and research fellowship training in Pulmonary and Critical Care Medicine at the University of Colorado. He was recruited to Emory University in 1995 and has served in numerous leadership positions at that institution including Chair of the University Research Committee, Director of the Emory Alcohol and Lung Biology Center and Training Program, and Section Chief at the Atlanta Veterans Affairs (VA) Medical Center. Since starting an independent research laboratory at the Atlanta VA Medical Center in 1995, which later moved to the Emory University campus in 2009 when he assumed his current position, he has been funded by the National Institutes of Health (NIH), the Department of Defense, the American Lung Association and the VA for his research that focuses on the mechanisms by which chronic insults such as alcohol abuse and HIV infection cause oxidative stress and render individuals more susceptible to pneumonia and acute lung injury. In 2010, Professor Guidot was named the Jeffery R. Pine Endowed Chaired Professor of Medicine at Emory University. He continues to lecture medical students, residents, fellows and other faculty as well as mentoring post-doctoral researchers and physicians. He currently chairs the Institutional Training Grants (T32) Review Panel for the National Heart, Lung and Blood Institute (NHLBI) at the NIH.

## CONTACT


**E:** dguidot@emory.edu
**T:** (+1) 404 712 2970
**W:** http://medicine.emory.edu/pulmonary-allergy-critical-care/faculty-directory/guidot-david.html


EMORY UNIVERSITY

## KEY COLLABORATORS

Dr Lou Ann Brown, Emory University
Dr Sushma Cribbs, Emory University
Dr Paul Spearman, Cincinnati Children's Hospital
Dr Mike Koval, Emory University
Dr Xian Fan, Emory University
Elena Morales, Clinical Research Coordinator, Emory University

## CURRENT EXTRAMURAL FUNDING


R01 HL125042 - National Heart, Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) – Principal Investigator

R01 AA025857 – National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the National Institutes of Health (NIH) – Principal Investigator

R01 HL116958 – National Heart, Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) – Collaborator

T32 HL116271 – National Heart, Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) – Program Co-Director


## REFERENCES


SK Cribbs, J Lennox, AM Caliendo, LA Brown and DM Guidot, Healthy HIV-1-infected individuals on highly active antiretroviral therapy harbor HIV-1 in their alveolar macrophages, AIDS Res. Hum. Retroviruses, 2015, 31, 64-70.

C Lassiter, X Fan, PC Joshi, BA Jacob, RL Sutliff, DP Jones, M Koval and DM Guidot, HIV-1 transgene expression in rats causes oxidant stress and alveolar epithelial barrier dysfunction, AIDS Res. Ther., 2009, 6, 1.

PC Joshi, R Raynor, X Fan and DM Guidot, HIV-1–Transgene Expression in Rats Decreases Alveolar Macrophage Zinc Levels and Phagocytosis, Am. J. Respir. Cell. Mol. Biol., 2008, 39, 218–226.

# Do you want to become a regular **READER OF SCIENTIA?**

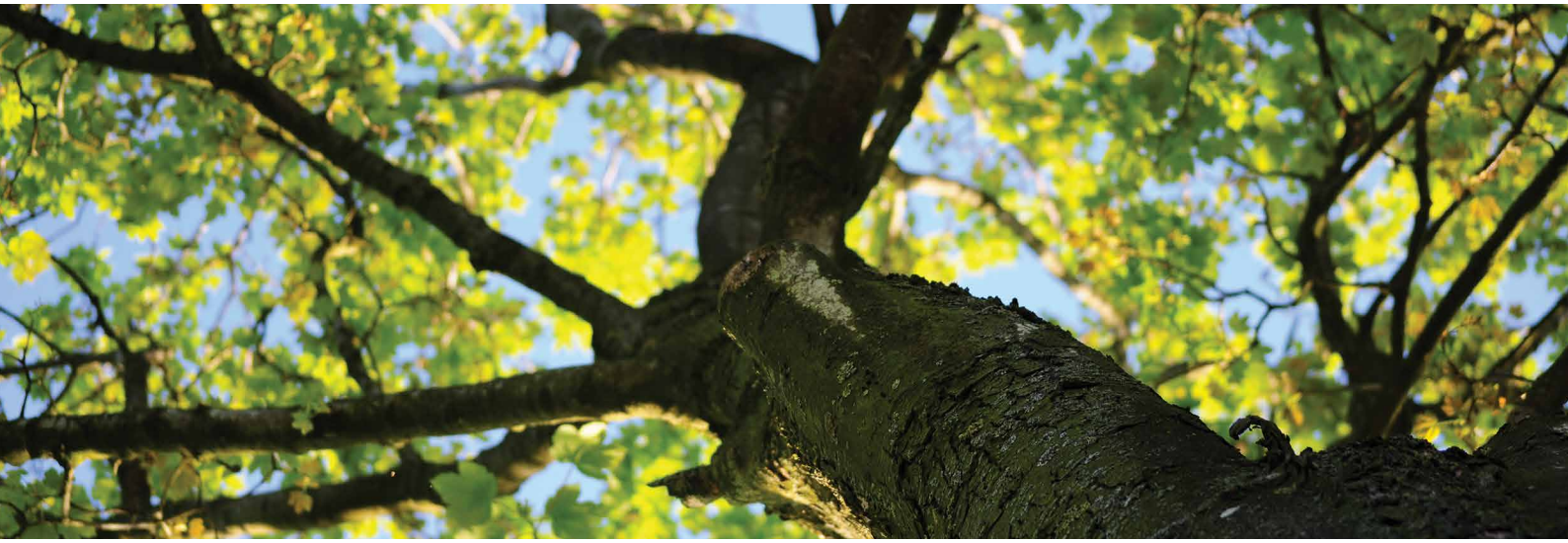
Scientia's mission is to connect people: scientists and educators, policy-makers and researchers, and the public and private sectors. We communicate science into the wider world, providing **high-quality, engaging,** and **relevant information** to our audience of thinkers and explorers.


Register today for your **FREE** subscription at:

**www.scientia.global/subscribe/**

# Trees for Cities

Breathing life into your neighbourhood



## WHO WE ARE

Trees for Cities is the only charity working on an international scale to create greener cities. Since 1993, we have engaged over 70,000 people to plant over 650,000 urban trees in parks, streets, schools and housing estates across the UK, as well as internationally, revitalising these areas and improving the lives of the people who live in them. We strengthen communities through volunteering opportunities and inspire children to grow and eat good food and to connect with nature.

## WHAT WE DO AND WHY WE DO IT

We focus on planting trees and greening community spaces where the social and environmental impact on local people is greatest. In London this might mean planting trees to clean the air or transforming unused community spaces into vibrant green areas, making our communities happier and healthier places to live, whilst in Nairobi it's planting fruit trees for food and sustainable livelihoods.

## HELP US PLANT A MILLION URBAN TREES BY 2020

To date we have planted over 650,000 trees in cities. We have now set ourselves an ambitious new target to strive to plant 1 million urban trees by 2020. Help us meet this exciting new milestone...

**CORPORATE PARTNERSHIPS AT**
**www.treesforcities.org/corporate-services/**

**DONATE ONLINE AT**
www.treesforcities.org/donate/

**FIND OUT HOW TO GET INVOLVED AT**
**www.treesforcities.org/get-involved/**

## MISSION

- Planting trees and greening cities worldwide.

## VALUES

- **People-led:** Although our reach is global, we value the importance of a local focus. We always work through and within local communities to strengthen them and empower their members.
- **Quality-driven:** Both the quantity and quality of the trees we plant are at the forefront of our planning so that we constantly strive to maximise the impact of our projects to the environment and society.
- **Delivery-focused:** We are an organisation that gets things done. What we talk about, we do – effectively, efficiently and on-time.

## WHY TREES MATTER

- Trees help our environment and the impact of climate change:
- They remove 4m tonnes of carbon from the UK atmosphere each year (Forestry Commission 2010)
- They can cool the air by 2 - 8 degrees C
- Trees absorb water , lowering stress on storm water drains and mitigating flood risk
- A single mature oak tree can host up to 423 different species of invertebrates that support birds and mammals
- Each year Trees for Cities plant around 65,000 trees in cities worldwide, revitalising cities and enhancing the lives of the people that live in them.