# Scientia

## CELEBRATING DISCOVERY AND INNOVATION IN GENETIC SCIENCE

EXCLUSIVES:

- Alexander von Humboldt Foundation
- Worldwide Cancer Research

HIGHLIGHTS:

- The Evolution of Genetic Coding
- HTLV-1: The Forgotten Cousin of HIV
- Rewiring DNA: Gene Circuits in Synthetic Biology

Issue: #125

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# WELCOME...

This important issue of Scientia showcases the vital work of scientists in the field of genetics, the branch of biology concerned with the study of genes, genetic variation, and heredity. As a science, the study of genetics is thought to have commenced in the mid-19th century with Gregor Johann Mendel's experiments in pea plants and his discovery of the fundamental laws of inheritance. Incredibly, this work escaped the attention of other scientists for more than three decades. But from the start of the 20th century, Mendel's work triggered an explosion of further research, rapidly progressing our understanding of genes and how they function. Now, genetics is one of the fundamental cornerstones of modern biology, critical in its own right as a branch of science but also with far-reaching implications for other disciplines, including medicine and healthcare.

The first section in this edition is devoted to the essential study of genetics from a basic science perspective. Here we meet the researchers, who through the innovative development of techniques and methodologies, continue to advance our understanding of the function of genes and the specific mechanisms underlying key genetic processes. From revolutionising how we think about the origin of life itself to solving mysteries such as how our tiny human cells can hold our entire DNA, this section provides an enthralling account of contemporary and cutting-edge research in genetics.

Our second section explores the application of genetics to the improvement of healthcare and medicine. We meet the researchers who are using genetic science to make giant strides forward in tackling some of the biggest challenges of our age, from chronic obesity to cancer. We conclude this section and the overall issue with an exclusive interview with Dr Helen Rippon, the Chief Executive Officer of Worldwide Cancer Research. This provides a timely reminder of the importance of sustaining funding into the basic sciences – such as genetics – in order to continue to advance the development of effective treatment of disease in our ever expanding and ageing global population.

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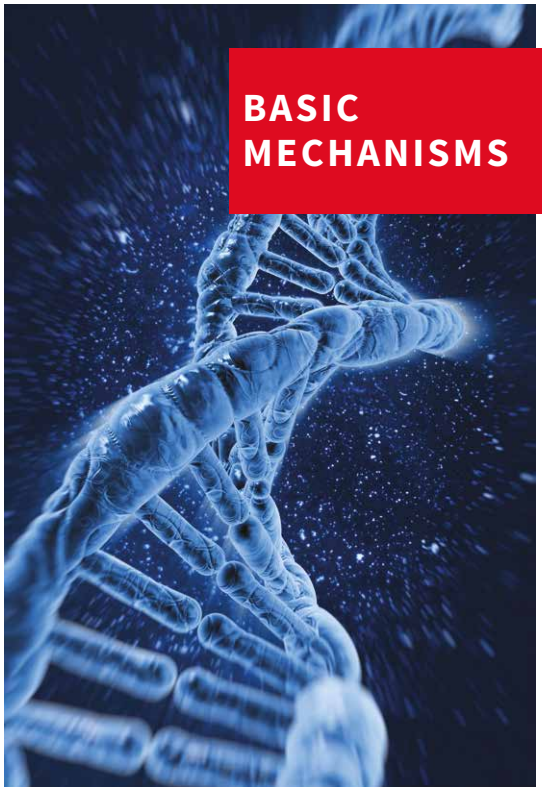


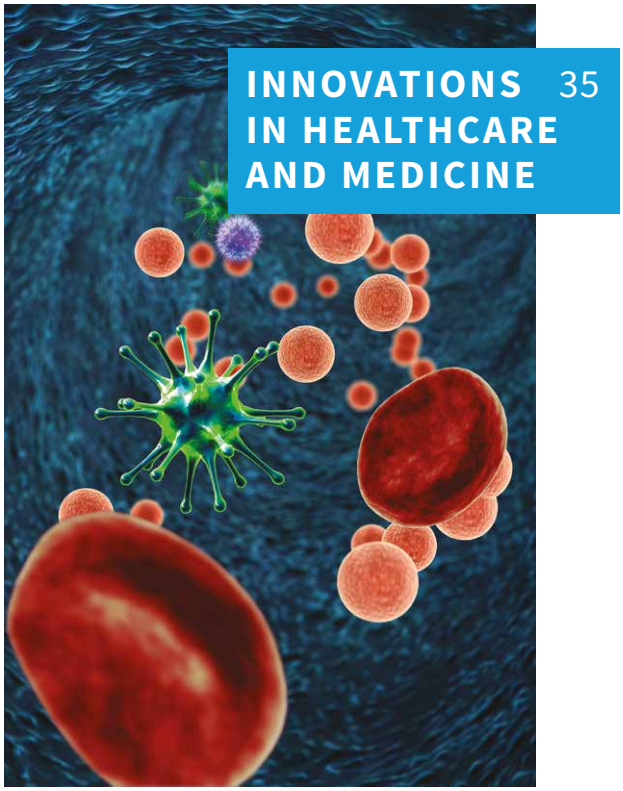


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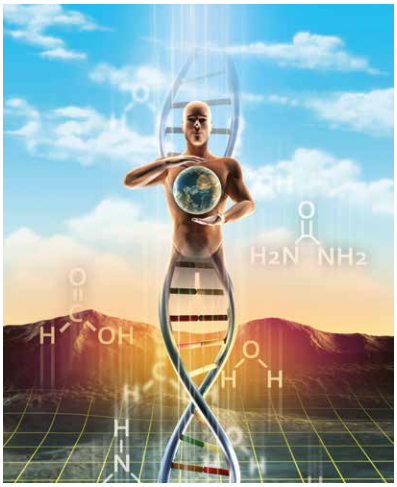
# BASIC MECHANISMS

# THE ORIGIN OF LIFE, STRANGE LOOPS, AND DOUGHNUTS



How we came to exist in the forms that we do is perhaps one of the most fundamental questions of our time. Our first section in this issue of Scientia showcases the exciting work of researchers seeking to resolve important mysteries in genetic science. We read of the cutting-edge research that is transforming our understanding of the origin of life, the novel and innovative approaches used to explore and explain key genetic processes, and even the significance of strange loops and doughnuts.

We begin with the work of Professor Charles Carter, of the University of North Carolina at Chapel Hill, who seeks to explain how the genetic code came to be, how genetic information is stored and recovered, and intriguingly, the role of strange loops in genetic coding. Using both experimental and computational approaches, we read how Professor Carter's groundbreaking research is challenging existing assumptions in evolutionary biology.

Continuing the exploration of underlying processes, we turn to the research of Professor Michael O'Donnell, at the Rockefeller University in New York. Professor O'Donnell examines the specific ways in which cells replicate their own genetic information to pass it on to the daughter cells. The question of how this DNA replication occurs with remarkably surprising speed and accuracy drives much of this work, and we read of Professor O'Donnell's pivotal role in identifying the structures of key components that are critical for DNA replication.

This includes the identification of sliding clamp proteins, which Professor O'Donnell recalls being likened to doughnuts in a light-hearted moment shared with colleagues upon their initial discovery.

We then move on to the question of how it is that human cells, despite their tiny size, contain the unfathomable quantity of information required to allow the generation of an entire human being. Professor Andrea Duina at Hendrix College, Arkansas, uses budding yeast as a model study the key mechanisms underlying DNA functioning. We also read of Professor Duina's ongoing commitment to the development of the next generation of scientists through providing mentoring in his laboratory.

We then turn to the work of Dr Zhihua Jiang at Washington State University, who is conducting innovative research leading to the development of new next-generation sequencing techniques. We read how these novel techniques can be used by scientists to better understand how RNA diversity occurs at the cell, tissue, and whole-body levels. Furthermore, we read how Dr Jiang's new methods allow further investigation into how RNA diversity plays a role in how animals grow and develop into their different shapes and forms.

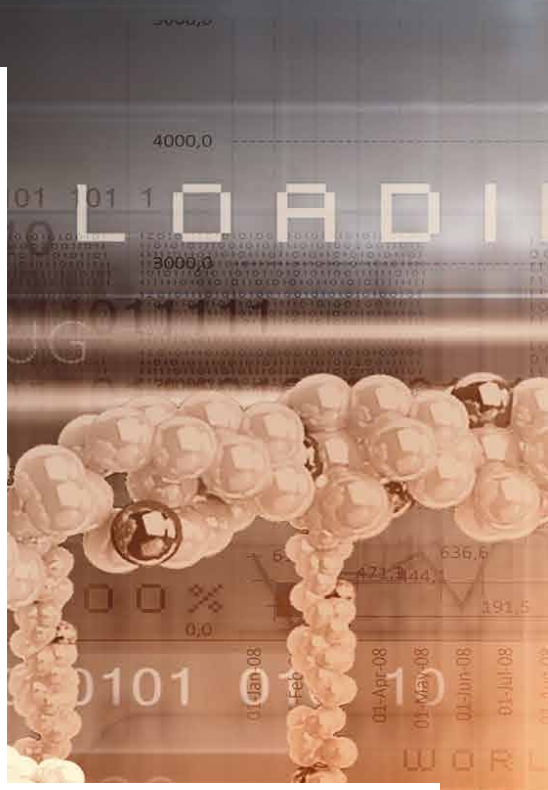
Taking a slightly different tack, we then read of Professor Pauline Schaap's work at the University of Dundee. Professor Schaap progresses our understanding of genetics through her work on social amoebas. Amoebas are organisms that are particularly useful for studying evolution and communication, not least because of their genetic diversity. We read how Professor Schaap has detailed the full family tree of the multicellular social amoeba, providing a fascinating account of its ancestral heritage and evolutionary history. By using a variety of experimental techniques, her work addresses critical questions in evolutionary biology.

Our final researcher, Dr Gábor Balázsi of Stony Brook University, New York, conducts research in synthetic biology, an interdisciplinary branch of biology and engineering. We read how he has pioneered network and systems approaches that allow the manipulation of biological systems –more specifically, synthetic gene circuits. This futuristic work explores how random genetic and protein-level changes occur over time, and Dr Balázsi embraces the power of prediction as a key component in his scientific endeavours.

It is clear from this array of recent discoveries and developments that scientists are using increasingly innovative and varied methods to investigate fundamental questions in science – with far-reaching implications. It is therefore fitting to conclude this section with an exclusive interview with Hans-Christian Pape, the President of the Alexander von Humboldt Foundation. The late Alexander von Humboldt (1769–1859) believed that progress is maximised when many different perspectives and approaches are considered. We read of the foundation's aim to promote the internationalisation and appeal of Germany as a research location, and their overreaching commitment to the attainment and advancement of knowledge across all academic disciplines.

# THE EVOLUTION OF GENETIC CODING

The research of **Professor Charles Carter**, University of North Carolina at Chapel Hill, USA, unravels some of the biggest mysteries of molecular evolution. His research is dedicated to investigating how information flows from genes to proteins found in living organisms via genetic coding. Much of this work centres around the structural origins of genetics and ultimately, how chemistry created biology.



**The Origins of Genetics**

One of the biggest evolutionary questions is 'how did we come to exist in the forms that we do?' The most significant contribution towards answering this question was the famous discovery of the DNA double helix by Watson and Crick in 1952. Due to their work, we now know that the four nucleotide bases, the building blocks of DNA, always line up in a characteristic manner along the double helix backbone; and that adenine and thymine always bind to each other, as do guanine and cytosine, ensuring that genes are passed correctly from one generation to the next. Genes are blueprints for making proteins. In order to access the information hidden in genes, the nucleotide sequence must be read and transcribed into a new version of the blueprint – messenger RNA, or mRNA.

Once transcribed, the RNA blueprint must be interpreted so that amino acids can be correctly strung together. This translation process depends on a code to assign one of the 20 amino acids to each triplet of bases (codons). Translation ultimately produces a unique, coded sequence of amino acids that can fold into 3D structures called proteins. However, exactly how the genetic code itself first arose and how information is stored and recovered remain unclear. These are the mysteries that Professor Charles Carter and his team at the Department of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill, USA, aim to solve.

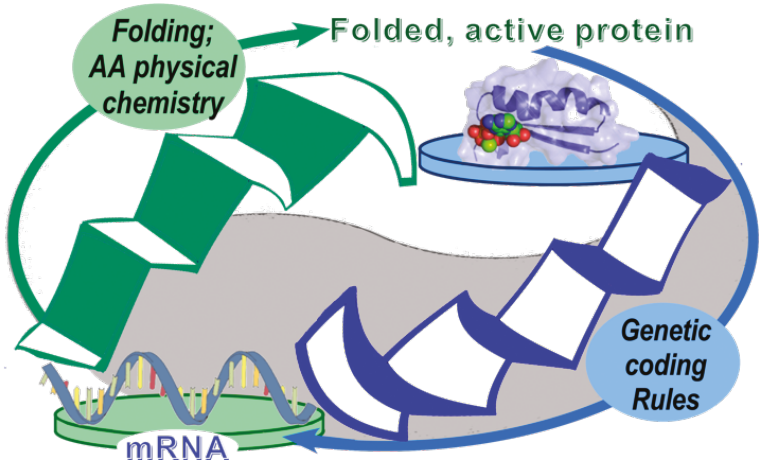
Two distinct kinds of information have been embedded in nucleic acids for as long as they have served as genes. First, we need to consider the process by which triplet codons consisting of the four bases are turned into the twenty amino acids that make up all possible proteins. This is facilitated by an adaptor molecule called transfer RNA (tRNA). Enzymes called aminoacyl-tRNA sythetases (aaRS) recognise tRNA, forming cognate pairs. These cognate pairs recognise each other very specifically and together, form the molecular dictionary. Each amino acid is associated with one or more cognate pairs made up of one aaRS and tRNAs, whose recognition properties allow the synthetase enzyme to activate and attach the correct amino acid to one end of its cognate tRNA.

Cognate pairs are capable of translating the genetic code from nucleic acid to protein because the aaRS specifically recognise cognate amino acids, and

transfer them covalently to their cognate tRNAs. Non-cognate pairs, that is, incompletely matching aaRS-tRNA-amino acid combinations, may occur but at such low levels that they rarely participate in the assembly of proteins. The aaRS enzymes form the basis of much of Professor Carter's research. He believes that they may be the most important contributor to the translation of the genetic code to proteins.

The second source of information comes from the collection of messenger RNAs (mRNA) found within a cell. These mRNAs represent a database of the amino acid sequences that are able to successfully fold and function usefully in the cell.

**‘This discovery opened the door to a virtual flood of new experiments that have transformed the way we think about the origin of life itself.’**




*Translation of aaRS genes is a paradoxical level-crossing strange loop.* mRNA operates at one level, furnishing blueprints for making proteins, including aaRSs. Translated proteins fold into functional 3D structures in accordance with amino acid physical chemistry (green ellipse) taking them to another level. However, folded aaRS proteins actually translate genes by enforcing the genetic coding rules by which they were originally assembled (blue ellipse). Professor Carter’s work elucidated many details of the Escher-like stairways by studying the rules connecting the two levels.

**Making Sense (or Antisense) of Bidirectional Coding**

A major surprise came with the discovery in 1990 that there are two distinct and apparently unrelated superfamilies of aaRS. The Rodin-Ohno hypothesis offered a rationale by suggesting that as DNA has two strands, perhaps each of the two strands acts as an individual coding strand, rather than one coding strand and one template (for copying) strand. The existence of bidirectional coding in this way has previously been considered controversial.

That controversy prompted Professor Carter’s group to develop new biochemical, bioinformatic, and phylogenetic approaches to test the critical Rodin-Ohno hypothesis that the two unique synthetase classes had evolved from ancestors that, while present on the same gene, were located on opposite sense and antisense strands. They designed and tested a bidirectional gene that produced gene products from both strands (compared to the norm of using just one strand). That gene coded for two specific peptides (amino acid chains which give rise to proteins). When they measured the levels of amino acid activation using enzymatic assays, both gene products accelerated amino acid activation by one million-fold. Professor Carter and his group believe that such a gene could have given rise to the first two enzymes capable of activating amino acids.

The complex process behind this discovery involved help from a colleague Professor Brian Kuhlman, whose computer program, called Rosetta, is able to design protein structures that obey bidirectional coding. This work showed that the peptides from opposite strands of the same gene had entirely different amino acid sequences. It also verified that it was indeed possible to encode two enzymes with very similar activities, and which were much smaller than modern enzymes. Professor Carter described the two enzymes as ‘a bone fide molecular Adam and Eve!’

Professor Carter has shown that one ancestral gene could code for two distinct, functional amino acid-coding enzymes, or protozymes. Thus, because two letters represent the simplest possible code, and because that initial code required both tRNA and protein, he suggests that biology did not replace an existing RNA-dependent system but instead arose directly from a very early partnership between RNA and peptides. Bidirectional coding thus limits the theory that life forms originally arose from an RNA-focused world, raising the question: should the focus should be on a peptide-RNA world instead?

**Tryptophan and the Chemistry of Amino Acid Side Chains**

Professor Carter and his group have worked extensively on the aaRS for one particular amino acid, tryptophan (the precursor of the neurotransmitter, serotonin). Their findings include analyses of how tryptophanyl-tRNA synthetase (the enzyme required for tryptophanyl-tRNA formation) carries out its function.

*Professor Carter and Research Group*

The ways in which polypeptide chains go on to form very complicated 3D protein structures are due to many variables. Work by the team verified several intricate links between the physical chemistry of amino acid side chains, which are the chemical groups attached to the main backbone of the molecule, and protein folding. The approach used by Professor Carter and his colleague Professor Richard Wolfenden to examine how amino acid side chains behave was multifaceted. First, they observed the polarities of the side chains, by measuring how they distribute between water and cyclohexane, a colourless, flammable liquid. The polarity of a molecule explains how positively or negatively charged it is. They also investigated the size of the side chains by measuring distributions between the vapour phase (a state when the molecule is free from interactions with other molecules) and cyclohexane.

Using these techniques, they also were able to demonstrate that one region of the tRNA crucial for recognition by the synthetases is its acceptor-stem. The code in the tRNA acceptor-stem is related to amino acid size, whereas the anticodon, a distant, alternative part of the molecule is related to amino acid polarity. They then realised that the two descendants of the ancestral bidirectional gene recognised two separate groups of amino acids that can be distinguished by whether they have large or small side chains. Thus, the inherent duality of the two aaRS Classes depends intimately on the physical chemistry of the amino acids that they recognise and is embedded into the tRNA structures.

**Strange Loops**

The symbolic transformation achieved by genetic coding of aaRS is a paradoxical, level-crossing feedback loop – a 'strange loop'. This is a hierarchy of levels, each of which is somehow linked to another. Remarkably, moving through the levels, one eventually returns to the starting point, i.e., the original level. How this works in genetic coding is illustrated in Figure 1. The feedback mechanism of the strange loop is perhaps best explained by a quote from another of Professor Carter's collaborators, Professor Peter Wills, who explains 'the enforcement of the relationship between genes and amino acids depends on aaRS, which are themselves encoded by genes and made of amino acids.' Professor Wills also notes that this process is essentially computational, and so transcends chemistry. Professors Carter and Wills propose that this feedback cycle was necessary for Nature to search rapidly for an optimal genetic code and for protein sequences that fold into functioning machines. In this sense, it represents an early emergence of 'symbolic meaning'.

**Next Steps in Understanding the Origin of Life**

Recent work by the group has established the basis of the mechanisms by which aaRS and tRNAs recognise each other (the underlying mechanisms of the blue Escher-like stairways in Figure 1) and how this duality was exploited to form the cognate pairs necessary to translate the genetic code.

Crucial for supporting their conclusions, was to understand how and why tRNAs recognised specifically by each aaRS Class obeyed the same duality. A major achievement for the team came from examining the crystal structures of aaRS-tRNA cognate pairs. This revealed that the main distinction between the two different types of cognate pairs (small or large side chain) was that the aaRS specific for large side chain amino acids bind to a different groove in the tRNA, in this case requiring formation of a hairpin loop. Only Class I aaRS can induce and recognise that hairpin only in Class I cognate tRNAs, whereas Class II aaRS prefer to bind the undistorted acceptor stems of Class II cognate tRNAs. Thus, architectural features of the two aaRS Classes dictate their interactions with their cognate tRNAs. The pattern of acceptor-stem bases that form the hairpin became hidden as the amino acid alphabet grew to its modern form. Importantly, this suggests that aspects of current day tRNAs may form a palimpsest, a molecule with a slightly altered structure, but which still bears resemblance to its original form as it would have been many, many years ago.

The methods involved in reaching this conclusion included both experimental and computational approaches, which together with other analyses such as protein engineering and phylogenetic analysis, advanced the toolset that will be required for future research in this field.

Professor Carter's team continue to discover new aspects of the genetic code that are intimately associated with the structures of the two synthetase classes. They have also provided new understanding about how the physical chemistry of amino acids drives 3D protein folding. This cutting-edge research, along with the exciting work that the group have planned for the future, provides new avenues to discovering how the very first biological life forms emerged from chemical reactions. This may ultimately challenge the status quo that RNA alone was behind the start of life.

# Meet the researcher

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Professor Charles Carter completed his PhD at the University of California, San Diego, in 1972. He then completed post-doctoral work at the Medical Research Laboratory of Molecular Biology, Cambridge, UK. His doctoral research led to his appointment as Professor of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill, USA. In recognition of his work focusing on macromolecular structure and function, Professor Carter received the Fulbright International Fellowship award in 2010 for work at the Pasteur Institute in Paris and was Elected Fellow in the American Association for the Advancement of Science in 2013 for his work on molecular evolution. Professor Carter was President of the American Crystallographic Association in 2002 and served as their representative to the American Institute of Physics (AIP) until 2016. He also sits on the Publishing Partners Advisory Committee for AIP Publishing. He remains a long-term supporter of the Biophysical Society, the American Society of Biological Chemists, and the Society for Molecular Biology and Evolution.

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Peter R. Wills, Auckland University, New Zealand

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## FURTHER READING

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# THE INCREDIBLE WAYS OF DNA REPLICATION

For over 30 years, **Professor Michael O'Donnell**, based at the Rockefeller University in New York, has focused on the mechanisms involved in the duplication of genetic material in cells, a process known as DNA replication. Professor O'Donnell's work spans from the early 1990s, when his team was the first to discover a ring-shaped protein that encircles DNA and clamps the replication machine to DNA. Most recently, the team has been studying the proteins involved in mammalian DNA replication.

The ability to reproduce is one of the most fundamental mechanisms needed by every cell. In order to reproduce, cells must be able to make a copy of their own genetic information and pass it on to the daughter cells. This is known as DNA replication.

The genetic material present in every cell – DNA – is made of two complementary strands, organised in the classic double helix. Each strand is a long chain with four different bases, adenine, cytosine, guanine and thymine, and the sequence of these bases forms a code needed to make every protein required for life. It is therefore essential that the DNA sequence is copied with great precision and accuracy.

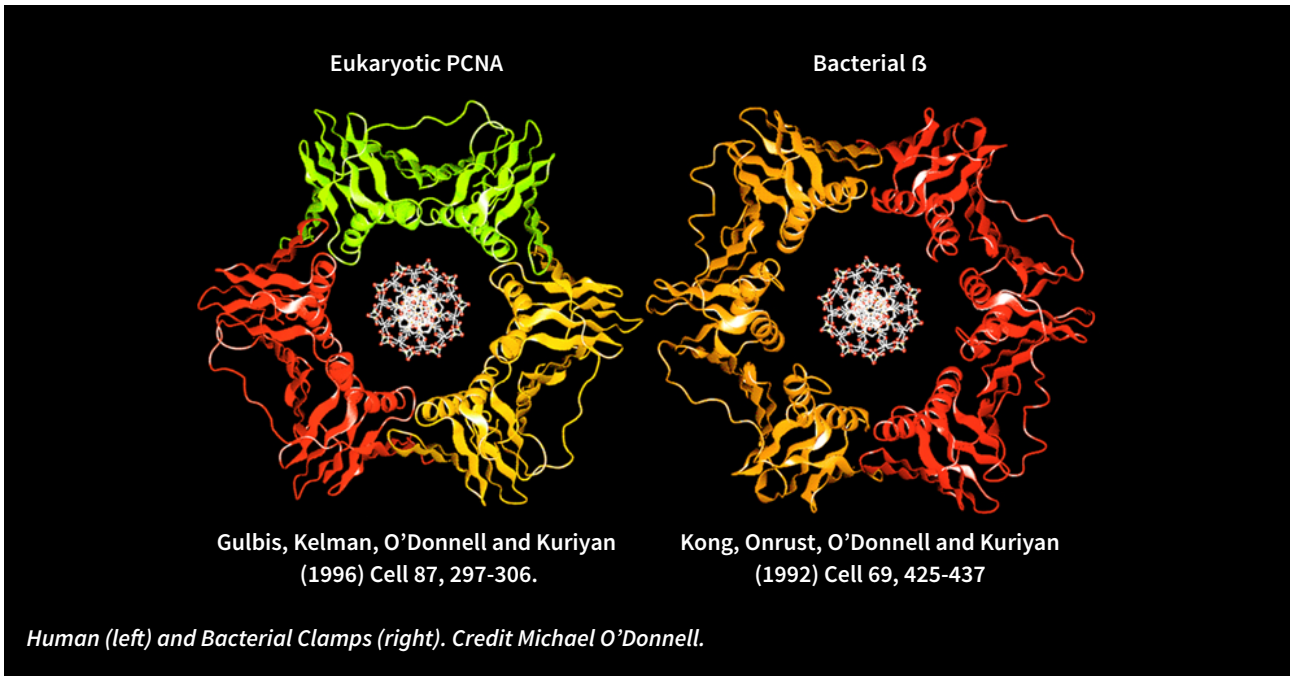
The DNA helix is duplicated by many proteins that work together as a machine. One protein, called a helicase, unzips the two strands of the double helix. This is where an enzyme called DNA polymerase comes in. DNA polymerase works by using each strand as a template to make a new strand, resulting in a new copy of the double helix.

In the DNA helix, adenine bases only pair with thymine, and the cytosine bases only pair with guanine. The DNA polymerase recognises each base along the template sequence and finds the correct base pair match from a pool of free bases. The replicating machine contains two DNA polymerases, one for each unzipped strand, resulting in two new duplex copies and providing all the instructions needed for two new living cells. Indeed, Dr Arthur Kornberg (Stanford University), who was awarded a Nobel Prize in 1959 for the discovery of DNA polymerases, provided postdoctoral training to Dr (now Professor) Michael O'Donnell.

At first glance, matching two sets of bases may seem an easy process, but when you take into account that it needs to be repeated 6 billion times to make a new copy of DNA in a human cell, then it is inevitable that mistakes can happen and must be minimised. This susceptibility to error may suggest the need for a slow and careful enzyme, but surprisingly DNA polymerases are quite fast and in bacteria can go 1000 bases every single second.

For Professor O'Donnell, now at the Rockefeller University in New York, this creates an interesting conundrum: How can the DNA polymerase stay bound to DNA and accurately replicate DNA while at the same time move along the new strand at a relatively high speed? 'It seems a contradiction, that a DNA polymerase could achieve an extremely tight grip to DNA, yet move very fast during synthesis,' explains Professor O'Donnell. 'The finding came as a complete surprise, which is actually quite typical of most scientific discoveries. This puzzle has a beautiful solution.'

**‘The finding came as a complete surprise, which is actually quite typical of most scientific discoveries. This puzzle has a beautiful solution.’**




*Human (left) and Bacterial Clamps (right). Credit Michael O’Donnell.*

**It’s a Doughnut!**

The first clue came quite unexpectedly in the early 1990s. Professor O’Donnell had started a set of experiments with circular DNA (typical of bacteria) but ran out of this half-way through his experiments and decided to try linear DNA instead. This lucky decision would turn out to be key in solving the mystery. ‘I ran out of circular DNA, but there was a linear DNA in the freezer so I tried this instead,’ said Professor O’Donnell. ‘To my dismay, the protein did not bind the linear DNA. I tried it again, thinking I'd made a mistake, but still the protein did not bind the linear DNA.’

Professor O’Donnell realised that the simplest explanation was that the protein is also a circle, and encircles the DNA, and therefore slides off the end of linear DNA but stays on circular DNA because it has no ends from which to slide off. The researcher knew this was a crazy idea at first because there were no other proteins known to do this. Professor O’Donnell confirmed this provocative idea when linear DNA with blocks at the ends behaved the same way as circular DNA, but when the end-blocked DNA was cut in two pieces by a special DNA cutting enzyme, the protein did not stay on the cut DNA, implying it was sliding off the new and unblocked DNA end. Professor O’Donnell proposed the circular structure of the protein and named the protein a ‘sliding clamp’.

Many scientists did not accept the interpretation of a ring protein, so Professor O’Donnell enlisted the help of Professor John Kuriyan at Rockefeller University, a world-leading expert in X-ray crystallography (a method to determine the atomic structure of a protein) to determine the exact structure of this enigmatic protein. ‘When John called me up to take a look at the results, I'll never forget his words “Mike – you wanted a doughnut, and we give you a doughnut,”’ joked Professor O’Donnell. ‘Indeed, it was a ring, but never would I have expected it to look so very beautiful.’ It turned out the sliding clamp protein has a wonderful structure, built from two subunits, each having three identically shaped domains that together formed a beautiful six domain circle.

**Exploring Ways to Load the Clamp**

Professor O’Donnell very quickly realised that the clamp can’t get onto DNA by itself, but it needs a ‘clamp loader’ to open the clamp and then close the ring around DNA. In fact, their studies identified that a five-protein machine was needed to put the clamp onto DNA. But understanding the clamp loader mechanism proved more difficult to achieve. ‘I used to think that each of the five different clamp loader units must have one function, and therefore there must be five functional steps to the clamp loading process. This way of thinking did make progress in understanding clamp loader function, but…in the end, it was the wrong way to think about the clamp loader,’ says Professor O’Donnell.

Eventually, the team realised the clamp loader cannot be thought of as separate components with separate functions, but as a single multi-component machine that opens and closes the clamp. Professor O’Donnell’s biochemical studies, in collaboration with Professor Kuriyan’s structural work, revealed a beautifully orchestrated mechanism in which the clamp loader

binds to the enzyme, warping the clamp in such a way that the ring opens up, allowing DNA to enter into the centre. Once the DNA is inside, the clamp loader changes its conformation, allowing the clamp to close.

**Moving to Mammals**

At the same time as this work in bacteria was being carried out, several research groups were also working on a mammalian system to study replication. Among the proteins identified, there were two that caught Professor O'Donnell's attention: one called PCNA and one called RFC. 'John Kuriyan and I proposed that PCNA would be a ring of six domains arranged on three subunits and that RFC would be a clamp loader.'

In continued collaboration with Professor Kuriyan, Professor O'Donnell's team showed that the bacterial and mammalian clamp and clamp loader have a strikingly similar structure. 'The nearly identical structure of human and bacterial sliding clamps supports the hypothesis that humans and bacteria evolved from a common ancestor cell that lived billions of years ago. The structure of the sliding clamp from this ancient cell was passed down through the eons that followed, while different multicellular animals like us evolved. We have come a long way in evolution from a single cell bacterium, but deep down inside at a molecular level, we work in much the same way,' explained Professor O'Donnell.

**DNA in the Middle**

The last piece of the puzzle was to determine how the clamp and the clamp loader actually bind to DNA. Once again, Professor O'Donnell's team was stuck for a while, but after months of Professors O'Donnell and Kuriyan staring at the structure, Professor Kuriyan made a surprising revelation. He realised that DNA perfectly fits right in the middle of the clamp loader. 'In a complete surprise, the DNA fit nice and snug right into the centre of clamp loader! We just weren't thinking about the centre of the clamp loader as a place for DNA to be. But the fit was undeniable; the centre of the clamp loader was lined with residues for DNA binding, and these residues were even conserved in the bacterial clamp loader. On hindsight it seems obvious but when exploring in the dark of ignorance things are simply not that clear,' explained Professor O'Donnell.

**A Broader Research Focus**

After solving the clamp mystery, Professor O'Donnell's research interests expanded to include not only the clamp/clamp loader but also all the components involved in DNA replication, in both eukaryotic cells (like mammals) and bacteria. After a painstakingly slow and complex process, the team was the first to purify and reconstitute a functional eukaryotic replication machine involving 31 different proteins with each component individually cloned and purified.

The team knows this is only the first step and there remains a long road ahead of them, along with the work of many other laboratories, to fully understand DNA replication. They may not know exact details yet, but they have no doubts this will be a highly regulated and complex mechanism. After all, these proteins must be able to cope with errors during replications as well as orchestrate numerous encounters with multiple enzymes and unrelated proteins. Professor O'Donnell is very excited about this field, stating 'Many labs worldwide work on this complex replication machinery, and we look forward to all of our labs contributing pieces of the puzzle that finally, someday, understand the whole picture. It is important to human health and disease, because when gone awry, the process makes mistakes that can cause cancer. So, understanding exactly how it works will shed light on new medical applications to help people.'

Professor O'Donnell adds, 'the mammalian system is much more complicated than viral or bacterial systems and very little is presently known about how its functions. Many of its components are present to regulate the replication machinery, or simply known to be important for replication through genetic and cell biology studies, leaving the actual mechanistic understanding of function and regulation to biochemical, biophysical and structural approaches.'

**An Exciting Future**

For Professor O'Donnell, this field is at a turning point as different areas of DNA replication, repair, and recombination are now merging into one big field, providing new opportunities to understand how multiple proteins operate together to produce and maintain the genetic instructions that DNA provides for disease-free life to exist.

The excitement for Professor O'Donnell is unmistakable. 'It is exciting to obtain these puzzle pieces, put them in place, and watch the picture that emerges in your mind and before your eyes. The answers are almost always not the way one would have imagined. The way Nature works, and figuring it out with the limitations of our mind, is a very humbling experience. But each time that you figure out one of those puzzle pieces, the reward is amazing and deeper felt than almost anything else life has to deliver.'

# Meet the researcher

**Professor Michael O'Donnell**
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After completing a PhD in biochemistry at the University of Michigan and a post-doctoral position at Stanford University, Professor Michael O'Donnell started his research group at Cornell Medical College in New York City in 1986. Professor O'Donnell moved to the Rockefeller University in 1996, where he heads the Laboratory of DNA Replication. Professor O'Donnell has also been an Investigator at the Howard Hughes Medical Institute since 1990, and a member of the National Academy of Sciences since 2006. Professor O'Donnell is also a reviewer for several academic journals and funding organisations, including the National Institutes of Health, the Wellcome Trust, Medical Research Council, and the European Research Council. With over 250 publications spanning more than 30 years, Professor O'Donnell is renowned for his work in understanding DNA replication and its repair.

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# THE MANY COMPLEXITIES OF DNA PACKING AND GENE EXPRESSION

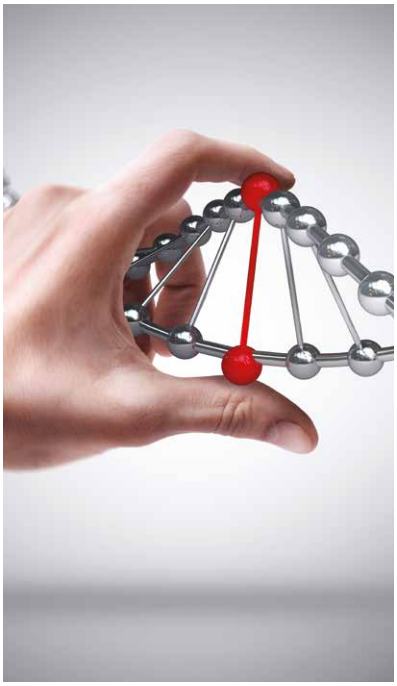
Although fundamental to life itself, many processes involving DNA and its packing inside cells still remain to be elucidated. Here, we follow the work of **Professor Andrea Duina** and his team at Hendrix College, Arkansas, USA, in their efforts to understand the dynamics of DNA packing and gene expression.

DNA is often described as the blueprint of life, with ~3.2 billion individual 'letters' of instructions scattered across 23 chromosome pairs providing enough information for a single cell to generate an entire human being. Within a single human cell, collectively all the DNA molecules measure an astonishing ~2 metres in length! Yet all of this DNA must somehow fit inside each and every nucleated cell in your body. Simply bundling everything together would never work, as anyone who has tried to untangle a set of cables can attest. Thankfully, our cells are more organised than our storage cupboards and use a series of packing steps to compress the DNA in an orderly and reversible fashion.

The basis of this packing is the nucleosome, a particle composed of a short stretch of DNA wound around the edges of a structure made up of pairs of four proteins known as histones. Nucleosomes are scattered along the DNA molecules like beads on a necklace. The nucleosome-beaded strands are then further packed into wound fibres to provide even more compression. Further packaging will occur when the cell is ready to divide, creating even more compact chromosomes. Nucleosomes are considered to be the fundamental unit of chromatin, a term used to describe the collection of DNA, histones, and other proteins found across different regions of chromosomes.

In addition to being necessary to fit the genetic information into the tight confines of the cell nucleus, packaging of DNA is also a target of regulation when genes are expressed by cells in a process called transcription. Packed DNA is so tightly wound around the histone proteins that the transcription machinery cannot easily access the DNA to read the underlying sequence. Human cells and other nucleated cells (collectively called eukaryotic cells) use this packing as a way to repress genes when they should not be expressed, but this also means that specific machinery must exist to unpack the DNA in order to allow for genes to be expressed at the appropriate time and place.

The processes of packing and unpacking of DNA are complex, and investigating them in humans can be especially challenging due to the inherent complexity of human cells. Thus, many scientists use simpler and more experimentally tractable model organisms to better understand the fundamental aspects of DNA packing and unpacking and how these are coordinated in order to ensure proper gene expression. Given the evolutionary



conservation between species, discoveries made in model organisms can be generally applied across the tree of life, including humans.

It is at this point that Professor Andrea Duina and his group at Hendrix College enter the picture. This group of geneticists and molecular biologists uses the budding yeast *Saccharomyces cerevisiae* model system to study the interplay between nucleosomes and one of the factors involved in packing and unpacking them, the FACT complex.

**Just the FACTs Ma'am**

FACT (FAcilitates Chromatin Transcription/Transactions) is an essential protein complex that interacts with nucleosomes and in so doing facilitates several chromosomal processes, including transcription. During transcription, FACT is thought to travel across genes along with RNA Polymerase II (Pol II), a protein complex that copies the DNA into 'working instructions', or RNA. The job of FACT in this process is to help disassemble, or unpack, the 'roadblocks' (in the form of nucleosomes) in front of Pol II so that the enzyme can read the DNA sequence. FACT is also required for reassembling, or repacking, the roadblocks in the wake of Pol II passage.

Although FACT's participation in this process appears simple at first, the devil is in the detail. How exactly does FACT interact with genes? How does it know when it is time to dissociate from a gene once the transcription job is completed? Are there other proteins that help FACT carry out its job? Answering these and other questions requires detailed and painstaking studies of a complex and dynamic molecular-scale process. This is the challenge undertaken by Professor Duina and his team.

**Getting Stuck Downstream**

As can be expected for such an important factor, conserved versions of FACT are found across all eukaryotic kingdoms (namely, fungi, plants, protists, and animals). The similarity of this complex and its associated functions across species means that research from one species (such as *S. cerevisiae*) can be applied to others, including our own.

Professor Duina's group focuses on the yeast complex yFACT. The yFACT complex comprises two separate proteins, Spt16 and Pob3. Assisted by another protein called Nhp6, yFACT physically interacts with nucleosomes and during transcription facilitates the packing and unpacking of nucleosomes as RNA molecules are synthesised by Pol II.

Using a combination of tools from the yeast toolbox, Professor Duina, while a postdoctoral fellow in the laboratory of Professor Fred Winston at Harvard Medical School, found that a tiny alteration in the nucleosome – a mutation that alters one amino acid in the histone H3 protein – causes a major change in how yFACT interacts across genes. Whereas yFACT normally associates across the entire body of genes and then dissociates at the end once the job of making an RNA is complete, the histone H3 mutation causes a dramatic build-up of yFACT specifically at the end of genes. This shift in yFACT distribution downstream, that is, towards the end of genes, suggests that the tiny alteration in histone H3 interferes with the normal dissociation of yFACT. This exciting discovery was the first demonstration that alterations in the structure of chromatin can have dramatic effects on how yFACT interacts with genes.

In subsequent work at Hendrix College, Professor Duina's group found that two other mutations, this time in histone H4, also cause a similar build-up of yFACT downstream of genes as seen with the original histone H3 mutant. In a eureka moment, Professor Duina realised that all three mutations were located very near each other on the structure of the nucleosome, therefore suggesting that a *specific region* of the nucleosome may play an important role in making sure that yFACT dissociates properly from genes following the transcription process.

In recent work, Professor Duina's group further characterised this region, which they named the Influences Spt16-Gene Interactions (ISGI) region, and found

that other mutations in it also cause yFACT dissociation defects. Examination of the nature of the mutations suggests that a specific electric charge landscape across the ISGI region may be important for ensuring that yFACT properly dissociates from genes. How does the ISGI region normally contribute to proper yFACT dissociation from genes? The answer to this question is unknown. One possibility is that a region of yFACT itself, potentially a region of the Spt16 subunit, needs to physically interact with the ISGI region in order for yFACT to properly disengage from a gene once transcription has been completed.

**What the Future Holds…and Why This Matters**

This is a fascinating glimpse into the interplay between nucleosomes and one of the factors that interacts with them during gene expression, but there are still many different facets to explore. Professor Duina, with the support of his group and colleagues, has a clear plan of action. In collaboration with Professor Alan Tackett's group at the University of Arkansas for Medical Sciences in Little Rock, Arkansas, USA, he is using a cutting-edge strategy involving an affinity purification step followed by mass spectrometry to assess and compare the chromatin environments present at the ends of genes in normal cells and cells harbouring ISGI mutations. The hope here is that any differences in these environments (for example, differences in the protein populations or in chemical modifications of histones – usually referred to as histone posttranslational modifications) may provide clues as to the mechanisms normally at play when yFACT disengages from genes.

In other experiments, Professor Duina's group is using more standard genetic and biochemical approaches to investigate whether other proteins, including other histones in addition to H3 and H4, as well as Pol II itself, play roles in ensuring normal dissociation of yFACT from genes upon completion of transcription. Finally, the group is also testing whether specific DNA sequences at the ends of genes contribute to normal yFACT dissociation.

A particularly enquiring reader may query the importance and impact of research on such a basic process in yeast cells. Professor Duina answers this question with an analogy: in order to fully understand how a car works, one needs to fully understand, at the most fundamental level, how all of the components work and interface with each other. Not only are the discoveries made in the process important in their own right, they are also essential for enabling us to fix cars when something goes wrong with them, whether it is a problem often experienced by cars or something completely novel and unexpected. Thus, Professor Duina continues, basic research (such as that carried out in his laboratory) on model organisms (such as *S. cerevisiae*) is essential for bringing us closer to a more complete understanding of how cells work. This work is not only exciting in itself but is critical for the improvement of human health. In the case of FACT, for example, recent studies have provided evidence that the FACT complex may facilitate the development of certain cancers – thus, basic research on FACT can also have implications for our understanding of cancer biology and could potentially lead to novel therapeutic strategies in the future.

**Investing in Our Future Scientists**

Professor Duina and his group are based at Hendrix College, a distinguished liberal arts college in Conway, Arkansas. Initially jump-started by funding from Hendrix and the Arkansas IDeA Network of Biomedical Research Excellence Program (Arkansas INBRE Program, formerly known as BRIN), Professor Duina's research has since been funded mostly through grants from the National Science Foundation. Hendrix is an undergraduate institution, and as a result research in Professor Duina's laboratory is carried out in large part by undergraduate students. For Professor Duina, the nurturing of young scientists is a critical component of his work and he feels privileged to be able to provide his students with invaluable first-hand experience in various aspects of scientific research through investigations of the fundamental processes related to DNA and chromatin function.

*Members of Professor Duina's group as of Spring 2018*

# Meet the researcher

**Professor Andrea Duina**
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Professor Andrea Duina completed his PhD in biochemistry, molecular biology, and cell biology at Northwestern University in Evanston, Illinois, in 1998 under the supervision of Professor Richard Gaber. He undertook postdoctoral research in the laboratory of Professor Fred Winston at Harvard Medical School in Boston, Massachusetts, before being appointed Assistant Professor of Biology at Hendrix College in Conway, Arkansas, in 2004. In 2016, he was promoted to the rank of Professor of Biology. Professor Duina's ongoing contribution to his research field includes a number of publications in prestigious journals, invited conference and seminar presentations, and participation in scientific outreach activities. He has also served as a Guest Associate Editor for *PLOS Genetics* and as a reviewer for several scientific journals. Professor Duina is currently a member of the External Advisory Committee for the Maine IDeA Network for Biomedical Research Excellence Program (Maine INBRE Program).

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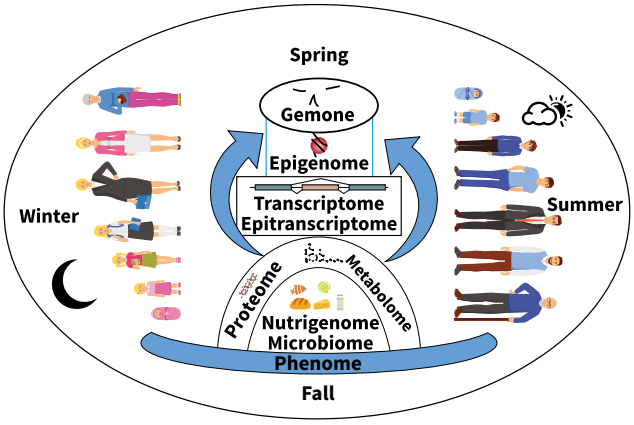
# RNA AND THE KEY TO THE DIVERSITY OF LIFE

Over 90% of a mammalian genome is transcribed into RNA molecules, including both protein-coding and non-coding transcripts. Genes often produce one or more expressed isoforms. **Dr Zhihua Jiang** at Washington State University has developed novel next-generation sequencing techniques that can be used to understand how RNA diversity occurs at the cell, tissue and whole-body levels and how this diversity contributes to the development and growth of animals into different forms or phenotypes.

**The Origin of Phenotypes**
*Pathological, Physical, Physiological and Psychological*




### The Origin of Phenotypes

The central dogma of molecular biology states that, 'DNA (the genome) makes RNA (the transcriptome) that makes protein (the proteome).' However, research has clearly indicated that many biological systems are far more complex. From the beginning of life with one fertilised egg to the end with $10^{12}$–$10^{16}$ cells in the human body, the genome remains relatively static. However, the shape and form of the body, or the 'phenome' (the collection of all an organism's phenotypes), changes dynamically in response to diverse functions.

How can a finite genome produce an infinite phenome during a life-span? The key to answering this question is understanding how alternative RNA transcripts are produced from genes.

We know that alternative transcripts coordinate information flow from genome to phenome in response to internal, external, universal and biotechnological environments. Indeed, a genome harbours a large number of DNA variants, most of which are silent. The only DNA mutations that may affect phenotypes include those located in regions that change protein expression or truncate proteins. In brief, functional DNA variants are those that potentially affect RNA transcription or structures. Recent research clearly points to the key role of RNA variants in the regulation of genome functions, but their contributions to the origin of phenotypes remain largely undetermined.

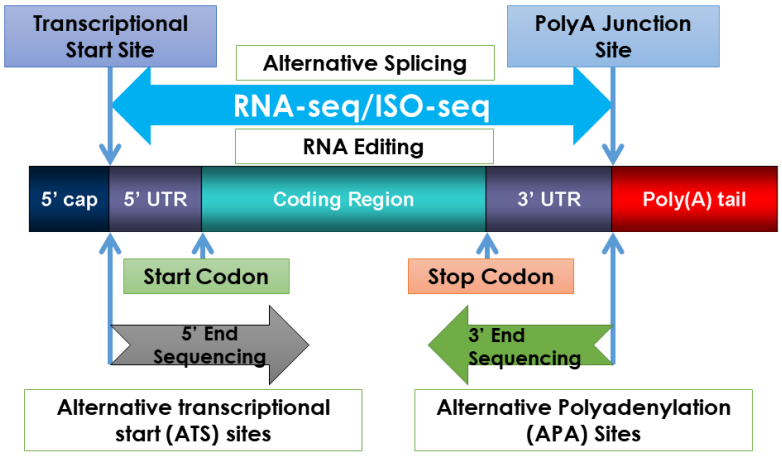
Generally speaking, RNA variants result from various mechanisms. Timely use of transcript variants is essential for cell maturation (differentiation) and development, and response to environmental challenges. The misuse of alternative transcripts often causes defects, diseases and disorders that affect nearly every body system. As such, alternative transcripts can be used as biomarkers to improve early diagnosis of diseases, conditions and even cancers. They can also be used as targets for novel treatment options.

### The Analysis of RNA Variants

There is a number of different types of RNA that are classified as coding and non-coding due to their diverse functions in molecular biology. Transitory mRNAs (messenger RNAs) aided by tRNA (transfer RNAs) and ribosomes containing ribosomal proteins and rRNA (ribosomal RNAs) are responsible for the synthesis of proteins. Small nuclear RNAs (snRNAs) and snoRNAs (small nucleolar RNAs) are often involved in either mRNA or rRNA processing, while miRNAs (microRNAs) degrade mRNAs and even silence gene function at the DNA level. Long non-coding RNAs (lncRNAs) are another type of RNA whose functions remain largely uncharacterised.

**‘The WTTS-seq assay serves as a powerful tool for the research community to investigate transcriptomes and reveal poly(A) site usages specific to complex phenotypes, disease stages, or biological processes in humans, animals, and plants.’**

## Transcriptome Profiling Strategies




Regardless of type, RNAs are usually converted in the laboratory into complementary DNA (cDNA) for sequencing. Nucleic acid sequencing determines the precise order of nucleic acids within a DNA or RNA molecule and establishes how amino acids are formed into proteins. DNA was first sequenced in the early 1970s and advances in technology have allowed scientists to decipher the entire genetic code of many different organisms faster and more efficiently. In the late 1990s, there were major advances in the speed at which machines could carry out sequencing reactions, as well as the length of the fragments that could be sequenced. In order to sequence RNAs from the transcriptional start site to transcriptional stop site, both next and third generation sequencing platforms have been developed. Basically, these methods were much quicker than previous sequencing methods.

RNA sequencing (RNA-seq) is a next-generation sequencing method that provides short reads, while isoform sequencing (Iso-seq) as a third-generation sequencing method that produces long reads for transcriptome analysis. Because research studies revealed that transcriptome diversity is largely due to the use of alternative transcription start and termination sites, Dr Zhihua Jiang and his group at Washington State University have focused on the development of methods to capture both ends of RNA transcripts.

### A New Way to Sequence

Dr Jiang and his team of scientists have developed two cutting-edge techniques, known as whole transcriptome start site sequencing (WTSS-seq) and whole transcriptome termini site sequencing (WTTS-seq). These techniques offer a number of improvements over existing RNA sequencing technologies such as RNA-seq, producing sequences that cover the entire transcriptome in cells, tissues, organs and the whole body.

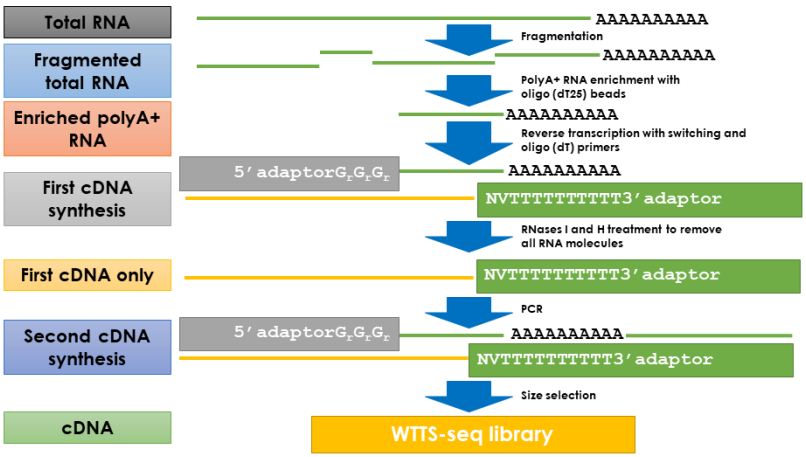
The techniques developed in Dr Jiang’s lab have a number of advantages over current RNA-seq techniques. For example, WTTS-seq can easily detect the alternative polyadenylation (APA) sites that mark the end of a transcript, which is difficult to determine with RNA-seq. The WTTS-seq method is also at least 67% cheaper than RNA-seq. Other challenges with RNA-seq include the under-representation of shorter fragments or RNA molecules in the final sample. Short fragments are easily lost, resulting in larger fragments dominating the sample. Bias can also occur when one or a few transcripts in a given sample are expressed at extremely high levels, thereby downplaying the number of times other mRNA molecules are identified or read.

RNA-seq often requires 10–20 times more reads than a typical sequencing method, thus creating challenges in analysis and computational methods due to the large amount of data that needs to be stored, retrieved and processed. In addition, RNA-seq cannot sufficiently detect genes or transcripts with low levels of expression. The process also remains inefficient due to the many steps involved in preparing the sequencing libraries. Dr Jiang stresses, however, that both WTTS-seq and RNA-seq can be used together to avoid further validation using other methods.

Requiring only a minute amount of RNA, the WTTS-seq method starts with fragmentation or separation of total RNA, followed by enrichment of regions of polyadenylation, called poly(A)tails, located at the end of RNA fragments and by PCR (polymerase chain reaction) to amplify products for size selection. After these steps, the fragments can be analysed by the Ion Torrent, a popular sequencing platform.

Recent examination of an online database revealed thousands of APA sites harboured in the genomes of humans, mice, rats and chickens that mark the end of transcripts. Using the whole transcriptome termini site sequencing (WTTS-seq) method, Dr Jiang first used a frog genome to demonstrate how the WTTS-seq method can unravel the APA patterns across diverse developmental stages.

# Whole Transcriptome Termini Site Sequencing (WTTS-seq)




## The Rules of Life via Alternative Polyadenylation

Using tissue collected from the frog species *Xenopus tropicalis*, Dr Jiang compared RNA transcripts between sexes in addition to their ages. The APAs were incorporated at different sites for males and females at similar ages. The researchers also observed striking differences in the use of APA sites on maternal and offspring RNA and between embryos and adults. Unravelling these differences in APA patterns generates a wealth of knowledge that can be used to predict how animals within the same species might express genes differently.

After the material was sequenced, statistical, visual and functional tools were used to study APA events. The team found striking differences in the use of APA sites between genes that went on to make protein and those that did not. Most coding genes had more than one APA site, with protein-coding genes containing an average of 5.87 APA sites per gene. Usage of APA sites was different between adult males and females, with young males using thousands fewer APA sites than young females. The researchers also examined the APA sites of older adult animals using their WTSS-seq technique. At younger ages, females had more APA sites that coded for protein than males, but the trend was reversed in the older age group.

In another study, the group used WTTS-seq technology to study APA events in mice with a gene that had been 'knocked out' or prevented from making protein, compared to mice that made the protein normally. These results indicated that both APA- and gene-based analyses produce very different results. In particular, the gene-based approach could not explain the changes in muscle development between the two animal models. The gene-based analysis had much less power than the APA-based analysis.

These results identified APA sites that were expressed differently in mice with the original gene intact. These differently-expressed APA (DE-APA) sites were located on the mRNA (that makes protein) in areas near the centre of the molecule and generated above-average protein levels. The researchers found that the DE-APA sites that generated lower levels of protein had APA sites located mostly in conventional locations, closer to the edges of the RNA molecule. They concluded that gene knockout may affect APA events, resulting in differences in the tissues of the mouse as it grows and develops, in this case, muscle.

Dr Jiang also recently published research in which his research group found distinct APA patterns associated with repair and inflammation of the uterus in healthy and unhealthy cows using WTTS-seq. Here, WTTS-seq also revealed essential microRNA genes that are involved in inflammation and anti-inflammation processes and dysregulated APA events in unhealthy cows in comparison to healthy cows.

## Moving Forward

Sequencing technology is rapidly advancing, and Dr Jiang believes the timing is right for the research community to assemble the whole transcriptomes of all species. 'This analysis is also important because it provides the first steps toward functional characterisation and annotation of genes/genomes previously revealed by DNA sequencing,' says Dr Jiang. 'The WTTS-seq assay serves as a powerful tool for the research community to investigate transcriptomes and reveal poly(A) site usages specific to complex phenotypes, disease stages, or biological processes in humans, animals, and plants.'

In addition to WTTS-seq, his novel WTSS-seq method is also a sensitive and powerful technology that can be used to show how information flows from DNA (genome) to RNA (transcriptome) to protein (proteome) and can be used to explain the variety of altered physical outcomes (or phenotypes) seen in animal development in a number of different environments. The group has filed a provisional patent for their WTSS-seq method and Dr Jiang hopes that the technology can trigger interest from the wider scientific community to use these techniques to pursue their own studies. With both WTSS-seq and WTTS-seq, the group also plans to establish their own online resources for recording both alternative transcriptional start sites and termination sites in cattle, chicken, mouse, rat and the *Xenopus tropicalis* species of frog in the near future.

# Meet the researcher

**Dr Zhihua Jiang**
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Dr Zhihua Jiang is a professor in the Department of Animal Sciences at Washington State University and a Hatch Program Chair in Animal Biology and Biomedicine. He completed his PhD at the University of Zagreb in Croatia and was a postdoctoral fellow at the Roslin Institute, University of Edinburgh, Scotland and the National Center for Scientific Research (CNRS), University of Rennes 1, France. Previously he was a research professor at the University of Guelph in Canada in the Department of Animal and Poultry Science and a professor in the Department of Animal Science at Nanjing Agriculture University in China. His current work focuses on studying the origin of phenotypes using state of the art genomics and transcriptomics approaches in combination with bioinformatics and cell/embryo biology.

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WASHINGTON STATE UNIVERSITY

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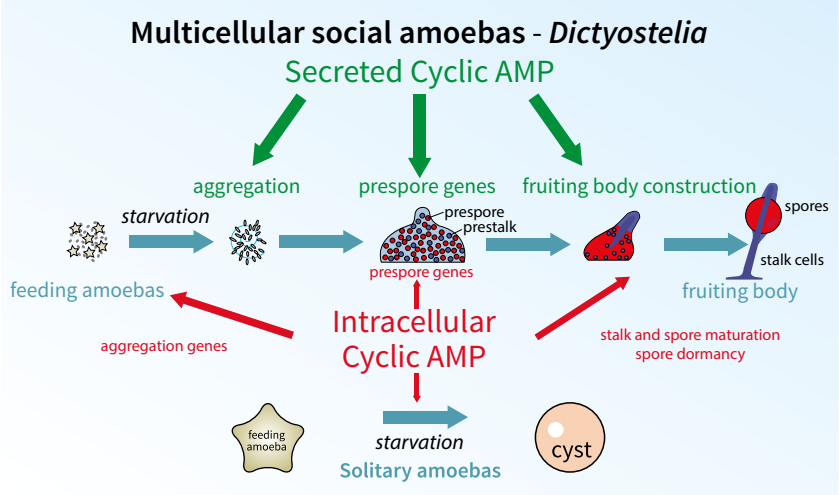
# FROM SO SIMPLE A BEGINNING – THE ORIGINS OF MULTICELLULARITY

**Professor Pauline Schaap** at the University of Dundee combines elegant yet powerful evolutionary reconstruction approaches with genetic and biochemical methods to unpick the evolution of multicellularity in the social amoebas. Her ground-breaking work has provided a deeper insight into the key ancestral processes that regulate the most basic aspects of the development of all organisms.




Biological processes are regulated by complex networks of interacting messages or signalling pathways. Exploring the evolution of these signalling pathways is just one of the research areas undertaken by Professor Pauline Schaap, Professor of Developmental Signalling at the University of Dundee. Professor Schaap has devoted much of her career to unravelling how these key biological processes have evolved in the ancient and enigmatic single-celled organism, the amoeba *Dictyostelium discoideum*.

**Understanding the Biological Processes**

Scientists use many diverse tools to investigate signalling pathways – genetic studies can identify the proteins that make up the signalling pathways, while biochemical and cell biological tools examine how these messengers work and interact with one another. Overarching mathematical models help to predict the regulation of whole networks.

But these tools cannot tell us about the underlying logic of a signalling network. This is because biological processes are not designed. They are the result of evolutionary processes – opportunistic recruitment of components that happened to be available at a particular point in time during the course of evolution and that made the organism more resilient to stressful conditions that it experienced at that time. To fully dissect and understand complex signalling networks Professor Schaap combines the use of more conventional biological methods with another powerful approach.

**Evolutionary Reconstruction**

The power of evolutionary reconstruction comes from its ability to foster integrative thinking about organisms as products of evolutionary processes. As Professor Schaap says: 'Evolutionary reconstruction is the only tool that can help us identify the deeply conserved core components of a network, to assign hierarchy in parallel processes and to understand why a particular pathway is built up the way it is.'

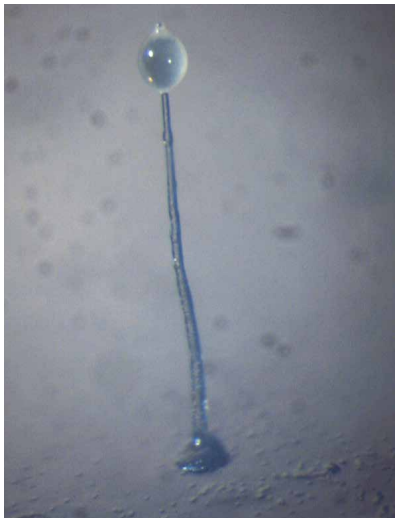
The beauty of this holistic approach is that it adds a depth of understanding to the underlying logic of current signalling complexity that would be impossible to unravel if the organism was studied in isolation. Using this approach, Professor Schaap has meticulously reconstructed the evolutionary origins of ancestral signalling pathways that control biological processes in the social amoeba – *Dictyostelium discoideum* – carefully tracing them back to an original role in the solitary, single-celled amoeba.

***Dictyostelium discoideum* – The Social Amoeba**

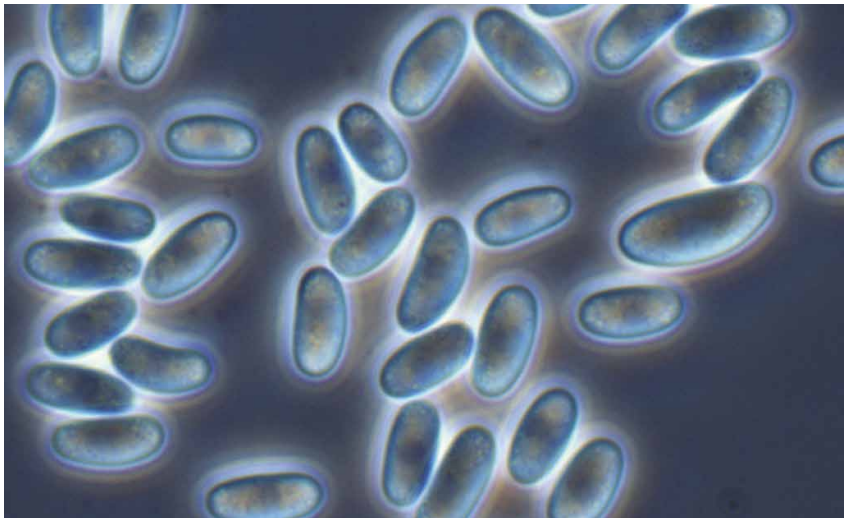
Amoebas are a fascinating group of organisms with a huge genetic diversity, and they offer an excellent experimental system for studying evolution and communication. All known *Dictyostelia* species are relatively easy and quick to grow in the laboratory. The cellular structures of *Dictyostelium discoideum* are transparent, enabling researchers to visualise their biological processes in action. Furthermore, the genomes of several Dictyostelium species have been sequenced, opening them up to sophisticated genetic studies.

Since amoebas are relatively closely related to animals, understanding how they work and evolve has implications for understanding how animals evolved. Importantly, *Dictyostelium discoideum* contains many genes that are defective

**'Our research aims to identify the genetic changes that cause the transition from uni- to multi-cellularity and enabled early multicellular organisms to specialise their cells for different functions.'**



*Dictyostelium discoideum fruiting body.*



*Dictyostelium discoideum spores.*

in human disease, making it a popular model for exploring how these genetic defects affect cell behaviour in health and disease.

**Developmental Signalling Pathways**

In a ground-breaking study, Professor Schaap and colleagues documented the full family tree of the social amoeba. The team compared highly conserved genes from all known species of social amoeba to construct the tree, illustrating how the complex social amoebas existing today can be traced back to their ancestor – the solitary single-celled amoeba.

Published in the prestigious international journal *Science*, this early work provided the necessary starting point to explore the evolution of the amoeba's multicellular lifestyle, allowing close examination of what happens at the molecular level as this species evolved and mutated from its single-celled ancestor.

Equipped with the family tree, Professor Schaap is now unravelling the fascinating evolutionary history of how these single-cell organisms developed to communicate and interact, creating multi-cellular structures in response to changing environmental conditions. One specific aim is to understand the evolutionary history of developmental signalling in the social amoebas, concentrating on an important signalling messenger called cyclic AMP.

The *Dictyostelium discoideum* species of social amoeba feed as single cells on soil bacteria, keeping to itself when food supplies are plentiful. However, when food supplies run low, they join with their comrades to form a multi-cellular mound. This mound first transforms into a slug-like organism that moves around to find an optimal place for fruiting body formation.

Once there, the slug projects upwards and becomes a fruiting body by first forming a stalk and moving all remaining amoebas to the top of the stalk, where they differentiate into encapsulated spores. The spores are then dispersed by insects or rain to new feeding grounds, where each spore spawns a single amoeba – and the cycle starts all over again.

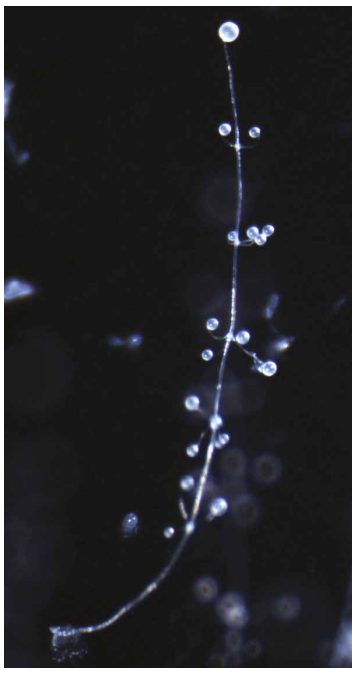
This ability to differentiate from a single-celled organism into a multicellular fruiting body offers an ideal model to examine how multicellularity evolved and how the organism has become more and more complex. Cyclic AMP has several functions in this developmental programme.

Firstly, it is secreted by the starving amoebas and acts to attract other amoebas to form the aggregate. Secondly, once aggregated, secreted cyclic AMP causes about 75% of the amoebas to prepare themselves for development into spores. Finally, an increase of cyclic AMP inside the cells causes these so-called prespore cells to mature into spores and the remaining 25% of the amoebas to develop into stalk cells.

In addition to forming a fruiting structure, the social amoeba has alternative survival tactics that can be deployed when food is scarce. The early forms of amoeba have the ability to encapsulate individually to form a dormant cyst, where it waits until conditions are more favourable for survival outside. The signalling pathways behind these processes have been the focus of Professor Schaap's research for over a decade.

**Important Signalling Factor has Ancient Roots**

In a series of elegant experiments, Professor Schaap investigated the presence, regulation and function of genes essential for the development of the amoeba's various forms.



*Polysphondylium pallidum fruiting body.*

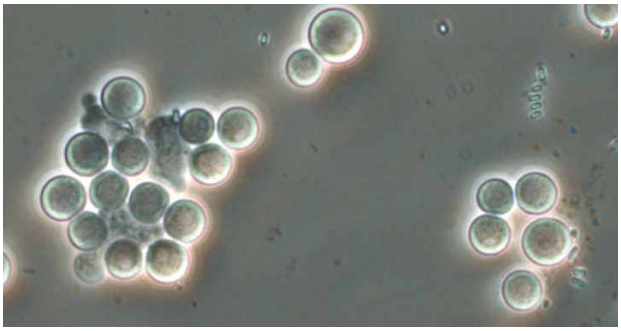
She meticulously examined different aspects of cyclic AMP signalling across the social amoeba species spanning the family tree. The *Dictyostelia* could be subdivided into two major branches. Closely comparing the amoeba groups within these two branches, Professor Schaap noted differences – one group, in particular, was distinctly different from the others. It had lost the ancestral survival strategy of forming single-celled cysts. Taking advantage of the subtle differences between amoebas in each group and using sophisticated genetic and biochemical studies, Professor Schaap and her team elucidated the exact signalling molecules involved in their biological processes.

Remarkably, they found that the role of cyclic AMP in prespore and spore differentiation was conserved across all *Dictyostelia*, but that its function as a secreted attractant to bring starving amoebas together was only present in the group that did not form cysts. Their work furthermore shows that the single-celled *Dictyostelia* ancestors increased cyclic AMP inside the cell when they were starving and that this increase triggered their development into the cyst.

Professor Schaap found that unlike their unicellular ancestors, the Dictyostelium amoebas secrete most of the cyclic AMP that they produce, and she proposes that the early *Dictyostelia* may have used accumulated extracellular levels of cyclic AMP in their aggregated 'slug-like' state as a signal to mature into prespore cells rather than cysts.

In one group of amoebas the secreted cAMP also started to function as an attractant for aggregation, with the other groups using a dipeptide, called glorin, for this role. The use of cyclic AMP as an attractant is correlated with the formation of larger aggregates and fruiting bodies and with the emergence of novel cell types to support the stalk and spore mass.

For the first time, Professor Schaap has provided compelling evidence to show how a complex cell communication system that controls the formation of large and complex fruiting structures can be retraced to a stress-induced pathway controlling the formation of survival cysts in the single-celled amoeba.



*Polysphondylium pallidum cysts.*

Her research highlights how an original repertoire of the genes involved in the production, detection and degradation of cyclic AMP gradually changed in the course of Dictyostelium evolution in order to perform an increasingly complex range of functions.

Unpicking this evolutionary story, Professor Schaap has revealed a hierarchical structure assigned to the many roles of cyclic AMP in modern Dictyostelia. Professor Schaap's research elegantly demonstrates the power of evolutionary reconstruction as an analytical tool – uncovering signalling pathways at the same time as identifying the origin of the many roles of cyclic AMP in today's *Dictyostelia.*

Professor Schaap's work has provided a framework that has pushed the frontiers of our understanding of the genetic changes that generated novel structures in the *Dictyostelia* as it evolved. She has recently received a large European Research Council grant to continue to expand on this evolutionary approach, integrating it with other methods of experimental biology.

She says that, 'this grant funds research that seeks to identify the genetic changes that cause the transition from uni- to multi-cellularity and enabled early multicellular organisms to specialise their cells for different functions.' This work will further unravel how the regulation and function of genes with important roles in development was altered and elaborated during the course of evolution to generate novel cell-types and structural features.

In addition, Professor Schaap and her team aim to examine the process by which amoebas form cysts during periods of environmental stress. As some disease-causing single-celled organisms form cysts that are resistant to antibiotic treatment and immune clearance, their work has important medical implications.

Professor Schaap's work tackles fascinating research questions in evolutionary biology and beautifully illustrates the power of evolutionary reconstruction, which in combination with other research tools deepens our understanding of why and how evolution has unfolded the way it has.

# Meet the researcher

**Professor Pauline Schaap**
School of Life Sciences
University of Dundee
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Professor Pauline Schaap completed her PhD in Natural Sciences with distinction at the University of Leiden in The Netherlands in 1987. She rose to Associate Professor level at the Department of Biology, the University of Leiden in 1993 before moving to the University of Dundee in 1999, where she is currently Professor of Developmental Signalling at the College of Life Sciences. Much of Professor Schaap's research is focused on the elucidation of the cell-cell communication systems that control gene expression and coordinated cell movement during the development of multicellular organisms. She is particularly interested in reconstructing how these communication systems evolved from environmental sensing in unicellular ancestors.

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University of Dundee

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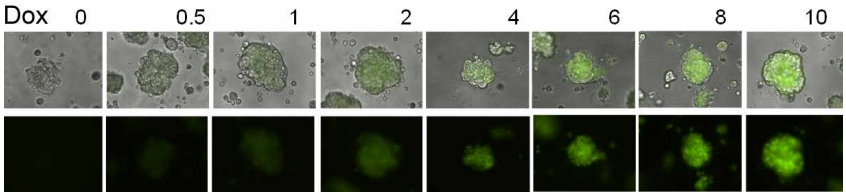
# REWIRING DNA: GENE CIRCUITS IN SYNTHETIC BIOLOGY

The futuristic field of synthetic biology has the potential to deliver exciting, innovative technologies – improved chemicals, materials, medicines, environmental solutions, and even smart biological devices – converging Mother Nature with human ingenuity. **Dr Gábor Balázsi**, of Stony Brook University, New York, is swapping silicon for DNA to create genetic circuits inside 'programmable' living cells. His research group is using synthetic gene circuits as research tools to study cell evolution, development, and cancer.




*An example of tuning the levels of the GFP protein with Doxycycline (Dox; concentrations in ng/mL) in breast cancer cells using the Linearizer synthetic gene circuit. Credit Gábor Balázsi.*

**Not All Circuits Are Electrical**

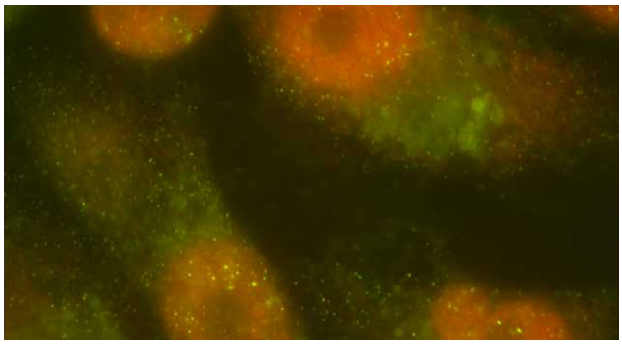
An organism's phenotype is determined by proteins, the cell's functional molecules. The production of proteins is determined by the genetic code – within the DNA genes. The genetic code consists of various combinations of four bases – A, T, C, and G – which encodes sequences of amino acids which join together to make proteins.

In gene expression, information flows from genes to RNA transcript molecules (a process known as transcription), and then from mRNA to protein production (translation). The ATCG genetic code is the biological equivalent to binary code in electronics – sequences of 1s and 0s. A major vision of synthetic biology is the ability to program the genetic code for a desired output, i.e., to create 'biological computers'. Synthetic biology is a multidisciplinary field, borrowing principles from electronics, computer science, engineering, and traditional biology. Nowhere is this more apparent than with gene circuits.

In nature, organisms have developed systems for regulating gene expression. Regulating the level of gene expression – 'turning the dial' for high or low production of proteins – enables cells to cope with stress. Certain proteins can induce or repress the transcription of specific genes – thus some genes can increase or decrease the expression of others – giving rise to gene regulatory networks.

Synthetic biology allows us to build synthetic gene regulatory networks with novel functions that don't exist in the natural world. Synthetic gene circuits can be described as artificial gene networks assembled by humans. Complex functions can be engineered with such gene circuits – inducible expression, oscillators, logic gates, and even memory devices! The output in some synthetic gene circuits can become the input (creating feedback) – thus the network controls itself perpetually. Such self-control can be designed as positive feedback (PF – self-activation) or negative feedback (NF – self-repression).

Dr Gábor Balázsi, Henry Laufer Associate Professor of Physical and Quantitative Biology at Stony Brook University, New York, is a pioneer in using network and systems approaches in biology. Gene networks and synthetic gene circuits are at the heart of his group's research. A major part of this involves using synthetic gene circuits as perturbation tools, that is, applying precisely-controlled perturbations to cells in controlled environments and studying how the cells respond, or investigating how physical factors interact with known biological aspects of gene networks. Some of their most ambitious research has involved using



*Metastatic breast cancer cells. Red and green mark a protein promoting and suppressing metastasis, respectively. Credit Gábor Balázsi.*

synthetic gene circuits to create and evolve biological controller devices that mimic natural networks.

To have functionality, synthetic gene circuits must be inside a host organism – a 'chassis'. The group's first chassis of choice was that of yeast cells, known as *Saccharomyces cerevisiae*. The usefulness of yeast extends far beyond the making of bread and beer! It is a popular model organism for scientific research and biotechnology, and has several features that make it amenable to synthetic biology investigation. Yeast is easy to culture, easy to genetically manipulate, and its fully characterised genome resembles the human genome among others.

Gene circuits are assembled de novo. The individual genetic components are joined together to make a linear DNA construct and the construct is integrated into a circular DNA plasmid vector, allowing the transfer of genetic material into cells using standard molecular biology techniques. The artificial DNA is taken up by the yeast cells (or another host) in a process known as transfection. In this group's studies, the DNA construct is integrated into the host cell's chromosomal genome, whereas in studies by other groups, the synthetic circuit-containing plasmids can exist inside the cells independent of the chromosomes.

The cells are typically grown in liquid culture and subjected to various conditions. The output of the gene circuit is initially typically a fluorescent protein, to allow the researchers to test and troubleshoot circuit functionality. After culture, cells are normally subjected to flow cytometry and fluorescent microscopy. Flow cytometry is an ideal tool as it allows the circuit response in many individual cells to be measured quickly and automatically. It involves passing the cells single-file through a tube and measuring individual cell fluorescence.

'We are looking at single cells and at genetic systems that we can dissect and understand gene by gene with a high level of detail,' explains Dr Balázsi. The group has successfully transferred multiple synthetic gene circuits from yeast into mammalian cells – human cell lines. This is a major milestone, as it paves the way forward for medical applications of gene circuits.

**Gene Networks in the Evolution of Cancer and Infection**

Although we tend to think of evolution as occurring over millions of years, it can happen rapidly. Evolution plays a major role in rapidly augmenting the destructive cell populations that cause disease, including microbial infections and cancer. It is now recognised that evolution is not only driven by mutations in genes but also environment-dependent or random changes in protein levels. These changes happen largely by chance, stochastically or randomly, and genetically identical cells can have completely different phenotypes.

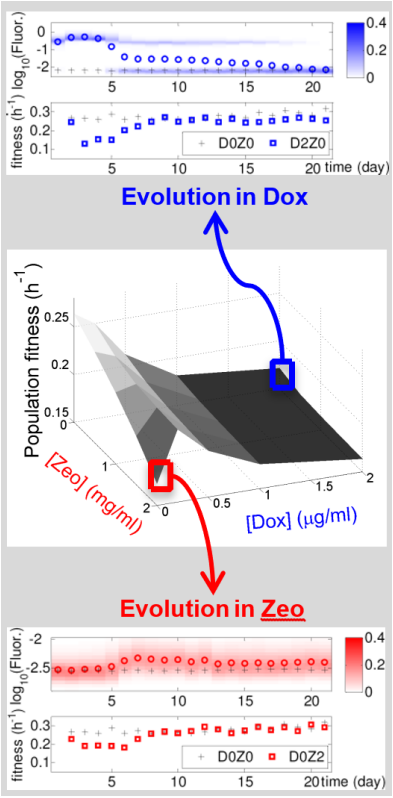
Disease-causing cells, including cancer cells and pathogens, start as a small group of genetically-identical cells, but then expand into a large phenotypically heterogeneous population. Some cells are stronger than others, and the strongest thrive. Surprisingly, cells that are strong in a given condition can be weak in another and vice versa. It is the various types of heterogeneity that are behind the disease's resilience and tolerance to treatments – often, cancer tumours stop responding to chemotherapy, and likewise, infectious microbes can develop resistance to anti-microbials.

Dr Balázsi's group has been investigating how cellular heterogeneity influences the evolution of cell populations, and the health implications of this. Dr Balázsi has recently been awarded a $1.8 million 5-year National Institutes of Health grant to search for the random triggers that turn benign cells into dangerous ones. 'Cancer cells often don't look the same in a matter of months, and drug-resistant microbes may not look the same in a matter of day,' notes Dr Balázsi, who would like to know what causes these changes, and how we can prevent them.

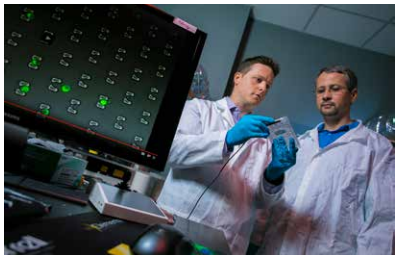
Dr Balázsi hopes to make great strides in decoding how random genetic and protein-level changes occur and interact. A synergistic collaboration with Professor Marsha Rosner at the University of Chicago has already yielded ground-breaking insights. With cancer, cells divide rapidly to form a tumour. When a tumour grows large enough, it becomes metastatic and cancerous cells spread to other parts of the body. Metastatic tumours typically consist of a heterogeneous cell population but how this emerges is not fully understood. The findings from Drs Balázsi and Rosner demonstrate that the development of heterogeneous tumours and metastasis involve random changes in protein levels besides mutations. They hope that these insights will lead to more effective, safer cancer treatment regimes.

**Developing Predictive Tools for Synthetic and Evolutionary Biology**

A major theme in Dr Balázsi's research is the power of prediction. In disease, as well as biotechnological applications, it is important to be able to predict a cell population's evolutionary path – but evolution's stochastic nature makes




*Fitness landscape and data from evolving the PF synthetic gene circuit. Evolution pushes cells towards the peaks on such landscapes. This means that expression has to drop in Dox (blue) and increase in Zeocin (red) for fitness to increase. Experimental measurements of gene expression (top timecourses) and fitness (bottom timecourses) confirm these expectations. Credit Gábor Balázsi.*



*Dr Gábor Balázsi and Dr Daniel Charlebois. Credit Gábor Balázsi*

this difficult. Gene circuits can be inserted into industrial or future therapeutic bacteria, but will the cell-circuit system be functional in the long-term?

Supported by a 2009 NIH Director's New Innovator Award, Dr Balázsi's group has built computational models using fitness landscapes to predict the outcome of evolving cell populations. They then tested these predictions with 'wet lab' experiments involving yeast cell cultures containing a PF gene circuit under various toxin dose conditions. In some cultures, inducer molecules were added to activate the circuit. The circuit, when active, expresses a protein that mitigates the effect of the toxin, but the circuit's activity is physiologically demanding on the yeast. When the toxin is present, the circuit's activity is beneficial, but when absent, the circuit is potentially damaging to the cells.

The group found that when the costs outweigh the benefits, mutations within the module will deactivate it. When the benefits outweigh the costs, mutations within the circuit and within the yeast's genome will jointly activate the module. Even in cultures lacking inducers, yeast still 'learns' to induce the circuit slightly through evolutionary selection in scenarios when this is beneficial – a remarkable finding! This work indicated that evolution can inadvertently switch genes and networks 'on' even when they are intended to be 'off' or vice versa – as can happen in disease. The group hopes to translate these findings into the development of more accurate predictive evolutionary models.

**Temperature Responses and DNA Dimming**

Synthetic gene circuits could be used in ecological applications, but they would have to stand up to the elements. For instance, they may be inserted into crops to create tolerance against pathogens or drought – but will they work in spring and summer temperatures? Microbes containing gene circuits could clean toxic spills – but will they work under fluctuating temperatures? Temperature is a major factor in gene circuit performance, and Dr Balázsi's group have investigated its influence.

In a recent study, they cultured yeast containing PF or NF circuits at cold, standard, and warm temperatures, and observed circuit responses with flow cytometry. They found that at non-optimal temperatures, cell-circuit systems make the choice between 'switching on' genes that confer stress resistance or stopping growth and protein synthesis. Moreover, temperature alterations influence the rates of biochemical reactions and protein structures. The findings suggest that at lower temperatures, achieving a certain gene expression level requires lower doses of inducer molecules – which could reduce the cost and environmental impact of reagents in industrial applications of synthetic biology.

Biotechnologists have long used 'genetic switches' to turn genes on or off, but a goal of synthetic biology is to intermediately adjust gene expression – much like a dimmer switch. In the late 2000s, in his previous post at University of Texas, Dr Balázsi led a group of researchers that achieved just that. They serendipitously engineered yeast with a 'DNA dimmer' and by adding varying doses of anhydrotetracycline (inducer) they found that they could precisely control production of green fluorescent protein. For example, doubling the anhydrotetracycline concentration made the cells glow twice as bright. Later they successfully transferred this dimmer gene circuit into human cancer cells. The ability to precisely control gene expression will be critical to future synthetic biology applications, such as therapies for genetic disorders.

**Looking to the Future**

Dr Balázsi is a pioneer in synthetic gene circuits – manipulating biological systems similarly to how an electrical engineer may rearrange or optimise the components of an electronic circuit, with promising insights for future applications and disease. He now hopes to interface natural gene networks with synthetic gene circuits to study how controlling network dynamics affects cell phenotypes in cancer and microbial drug resistance.

# Meet the researcher

**Dr Gábor Balázsi**
Henry Laufer Associate Professor
The Louis and Beatrice Laufer Center for Physical and Quantitative Biology
Associate Professor, Department of Biomedical Engineering
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Dr Gábor Balázsi is the Henry Laufer Associate Professor of Physical and Quantitative Biology at Stony Brook University, New York. He aims to develop a predictive, quantitative understanding of biological processes, including cellular decision making and the evolution of cell populations. Dr Balázsi is well-placed to lead his group with an impressive academic background in physics, biophysics, and systems biology. He graduated with BS Physics (1996) and MS Magnetism (1997), both from Babeș-Bolyai University of Cluj (Kolozsvár), the major center for Hungarian-language higher education in his native region of Transylvania, Romania. He then studied at the University of Missouri, St Louis, USA, where he gained his MS and PhD in Physics (1999 and 2001). In 2006, he took up an Assistant Professorship at the Systems Biology Department, University of Texas MD Anderson Cancer Center, before being promoted to Associate Professor in 2012. In 2014, he took up his current appointment.

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## KEY COLLABORATORS

Professor Marsha Rosner (University of Chicago)
Professor Lanying Zeng (Texas A&M University)

## FUNDING

2017: National Institutes of Health: Maximizing Investigators' Research Award ($1.8 million, 5 years)
2009: National Institutes of Health: NIH Director's New Innovator Award ($2.3 million, 5 years)

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Stony Brook University

# THE ALEXANDER VON HUMBOLDT FOUNDATION

*President Hans-Christian Pape. Credit Humboldt Foundation/Mario Wezel*

The Alexander von Humboldt Foundation promotes academic cooperation between excellent scientists and scholars from abroad and from Germany. To this end, it grants more than 700 research fellowships and research awards annually. These allow researchers from all over the world to come to Germany to work on a research question they have chosen themselves together with a host and collaborative partner. Researchers from Germany can also profit from the support and cooperate with a member of the Humboldt Network abroad.

The Humboldt Foundation's network embraces well over 29,000 Humboldtians from all disciplines in more than 140 countries worldwide, including 55 Nobel Prize winners – a community which shares values and experiences. The foundation's work helps to promote the internationalisation and appeal of Germany as a research location, enhances its visibility, and strengthens its position in global competition. In this exclusive interview, we speak to the President of the Alexander von Humboldt Foundation, Professor Dr Hans-Christian Pape, to hear more about the organisation's unique contribution to research.

**Who was Alexander von Humboldt? Can you tell us about his impact on science both during his lifetime and in more recent years?**

Alexander von Humboldt (1769–1859) was a nature researcher and a discoverer, a cosmopolitan and a fighter for the freedom of research, a humanist and a patron of excellent academic talent. His lengthy Latin American journey from 1799 to 1804 was celebrated as the second scientific discovery of South America. He was way ahead of his time and is thus still popular and relevant to this day. His thinking transcended the boundaries of disciplines and countries, and he shared his knowledge with others – a true networker. Humboldt is seen as a pioneer in many fields such as holistic ecological thought, the popularisation of science, and a world picture that is open to the views of other countries and peoples. He was convinced that progress is achieved when people work together to tackle problems and contribute their various points of view.

Mutual understanding coupled with academic freedom and excellence have remained the Humboldt Foundation's creed to this day. With Humboldt as a model, it maintains an international network of academic cooperation.

In 2019, the Humboldt Foundation is celebrating Alexander von Humboldt's 250th birthday with a campaign entitled humboldt-heute/humboldt-today. How modern, how relevant is Alexander von Humboldt 250 years after his birth? 'What does Humboldt today mean to you?' is the question the Foundation is putting not only to researchers in its global network but also to politicians, artists, and journalists.

**How is the Humboldt Foundation funded, and how, in turn, does the Humboldt Foundation support scientists?**

The Alexander von Humboldt Foundation is funded by the Federal Foreign Office, the Federal Ministry of Education and Research, the Federal Ministry for Economic Cooperation and Development, the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety as well as a number of national and international partners.

The Humboldt Foundation promotes outstanding researchers from Germany and abroad with its programmes. Sponsorship decisions are based on the applicant's achievements and qualifications to date. There are no quotas, neither for countries nor disciplines. The Foundation believes that even in the times of increasing team work the ability of the individual is

*The Humboldt Foundation in Bonn. Credit Humboldt Foundation/Michael Jordan*

*Fellows at the Annual Meeting in Berlin. Credit Humboldt Foundation/ David Ausserhofer*

the crucial factor for academic success. This is why it sponsors people not projects. Those chosen on this basis should be given as much freedom as possible to carry out their research projects. This includes allowing Humboldtians to choose their host institutions themselves and to conduct independent research without any stipulations from the Foundation.

Even after the first research stay in Germany has come to an end, the Humboldt Foundation maintains close links with their alumni and offers several alumni and networking sponsorship opportunities.

**There are almost 30,000 Humboldtians worldwide – What does it mean to be a Humboldtian? What are the benefits for individual researchers and what are the benefits for scientific progression more generally?**

'Once a Humboldtian – always a Humboldtian' – from the very beginning this was the hallmark of the Alexander von Humboldt Foundation. Humboldt sponsorship is enduring: the Foundation is a lifetime partner, maintaining the connections on a long-term basis through its alumni sponsorship programmes. As a result, an active knowledge network has been laid across the whole academic world. The alumni sponsorship measures provide flexible support for the individual life paths and development of Humboldtians. Moreover, the Foundation encourages its alumni to undertake their own initiatives and collaborations across disciplinary and national borders.

**Can you tell us about the Humboldt 'culture of welcome' in Germany?**

For more than a decade, the Alexander von Humboldt Foundation has been working with various partners to stablish and foster a 'culture of welcome' in Germany in order to attract researchers and their families, support them in their preparations and throughout their stay, and to maintain long-term relations after their stay has finished.

Between 2003 and 2005, the Alexander von Humboldt Foundation invited entries for the Award for Germany's Friendliest Immigration Office a total of three times. 'In these three years, the Award has made people aware that dedicated Immigration Offices are not only of huge importance to individual academics coming here; they also contribute to a positive image of Germany which helps to attract the best international researchers and students to Germany', the then President of the Humboldt Foundation commented. The Award was designated for infrastructure and further education measures as well as for networking both between authorities and universities/ research institutions and between other Immigration Offices.

Another Humboldt initiative was called 'Welcome Centres for internationally mobile researchers'. From 2006 to 2011, German universities were able to take part in this ideas competition to send a signal and generate models that would drive the creation of Welcome Centres as a central point at a university offering wide-ranging advisory and support services for visiting researchers and their families. Many universities were inspired by the competition and the strategies it generated to establish their own Welcome Centres.

*Fellows of the Humboldt Foundation. Credit Humboldt Foundation/Michael Jordan*

*Fellows at Poster Session. Credit Humboldt Foundation/ Michael Jordan*

From 2011-2016, the Humboldt Foundation ran an ideas competition for international alumni work, which sought exemplary concepts for permanently maintaining and making use of contacts to international alumni who have previously spent time researching at German universities and are now continuing their academic work abroad. The competition was part of the project 'International Research Marketing' which is a joint initiative by the Alexander von Humboldt Foundation, the German Academic Exchange Service, the German Research Foundation, and the Fraunhofer-Gesellschaft.

**The Humboldt Foundation is renowned for research cooperation with developing countries. What specific problems are faced by developing countries in terms of scientific competitiveness, and how does the Humboldt Foundation try to help overcome these?**

In a globalised world, a country's prospects for growth, affluence, and social harmony are inextricably bound up with scientific and technical progress. Innovative know-how is, however, a desired object and modern knowledge societies all over the world invest huge sums competing with each other to acquire it.

This contest makes it particularly difficult for developing and transition countries to develop functioning and internationally competitive science systems. How and where is the scientific training given to junior researchers really successful? How can academics be encouraged to address the special problems confronting the less developed countries in their research? At present, developing countries still face a serious imbalance in the distribution and focus of research activities which are largely oriented to the future issues occupying the industrialised nations worldwide.

But many problems can only be solved if developed and less developed countries cooperate with each more closely than they have so far. Climate change, future energy provision, and combating infectious disease are examples of some of the challenges no nation can meet alone. They require international cooperation, in science, too.

The Humboldt Foundation's answer to these issues and problems is targeted sponsorship for academic excellence in developing countries and the further consolidation of its transnational and transdisciplinary scientific networks in these regions. There is, for example, the Georg Forster Research Fellowship for researchers from developing or transition countries: The research outline should include aspects that are important for the continued development of the applicant's country or region of origin. The Foundation offers also Return Fellowships to sponsor reintegration into an institute abroad.

The Foundation also supports higher education and research in mathematics at the AIMS (African Institute for Mathematical Sciences) centres in Africa with the 'German Research Chair' programme for application-oriented mathematics. Already five new chairs at the AIMS centres in Senegal, South Africa, Ghana, Cameroon, and Tanzania have been successfully created.

**Finally, please can you tell us about how the Humboldt Foundation fosters the development of our future generation of scientists?**

The Sofja Kovalevskaja Award is one of the most valuable academic awards in Germany for outstanding research talents of all disciplines from abroad. With the Sofja Kovalevskaja Award, young researchers receive risk capital for innovative projects during an early stage in their careers. The Kovalevskaja Award allows them to conduct research at a German university or research institute for a period of up to five years and build up their own research groups – independently and largely untroubled by administrative constraints.

In addition to conferring the fellowships and awards, at all its conferences and events the Foundation attaches great importance to networking between experienced researchers and their junior colleagues. Colloquia, kollegs, award winners' forums and award ceremonies always seek to connect young researchers with established scientists and scholars.

**www.humboldt-foundation.de**
**(@AvHStiftung)**

Alexander von Humboldt
Stiftung/Foundation

# GENETICS

*key facts*

## GENETIC SIMILARITY

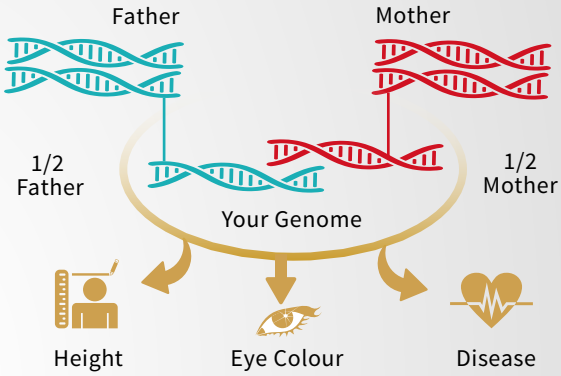
**99.9%**
Human and another human

**96%**
Human and Chimpanzee

**85%**
Human and mouse

**>60%**
Human and banana

## GENETIC DISEASES




**4,000** known **inherited conditions** are caused by a difference in a **single gene.**

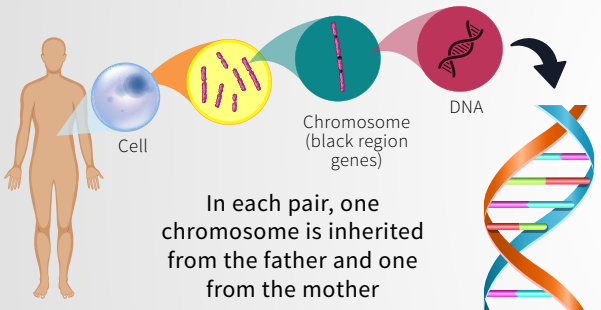
In the UK
The most common inherited disorder is **cystic fibrosis** (~1/2,500 babies born each year). Other examples include spinal muscular atrophy, sickle cell anaemia, Tay-Sachs disease and phenylketonuria

In the UK,

**3-10%**

of cancers are directly caused by inherited genes

## EACH CELL CONTAINS 23 PAIRS OF CHROMOSOMES




In each pair, one chromosome is inherited from the father and one from the mother

## GENETIC MAKEUP

- Adenine
- Guanine
- Thymine
- Cytosine

Our **genetic makeup** is constant throughout life - but our **environment** and **lifestyle** also determine our health

 YOUR GENES +  YOUR ENVIRONMENT & LIFESTYLE =  YOU

***Many common disorders***
*e.g. heart disease, disease, and most cancers are caused by these* ***complex interactions***

Sources: American Society of Human Genetics, Cancer Research UK, National Genome Research Institute

# INNOVATIONS IN HEALTHCARE AND MEDICINE


# TACKLING DISEASE AND IMPROVING HUMAN HEALTH

The second section of this important issue of Scientia showcases how advances in genetic science are driving forward innovations in healthcare and medicine. From understanding hereditary-based causes to informing novel treatments, we read here of the critical work conducted by researchers who are striding forward in their efforts to improve human health and tackle fatal diseases on a global level.

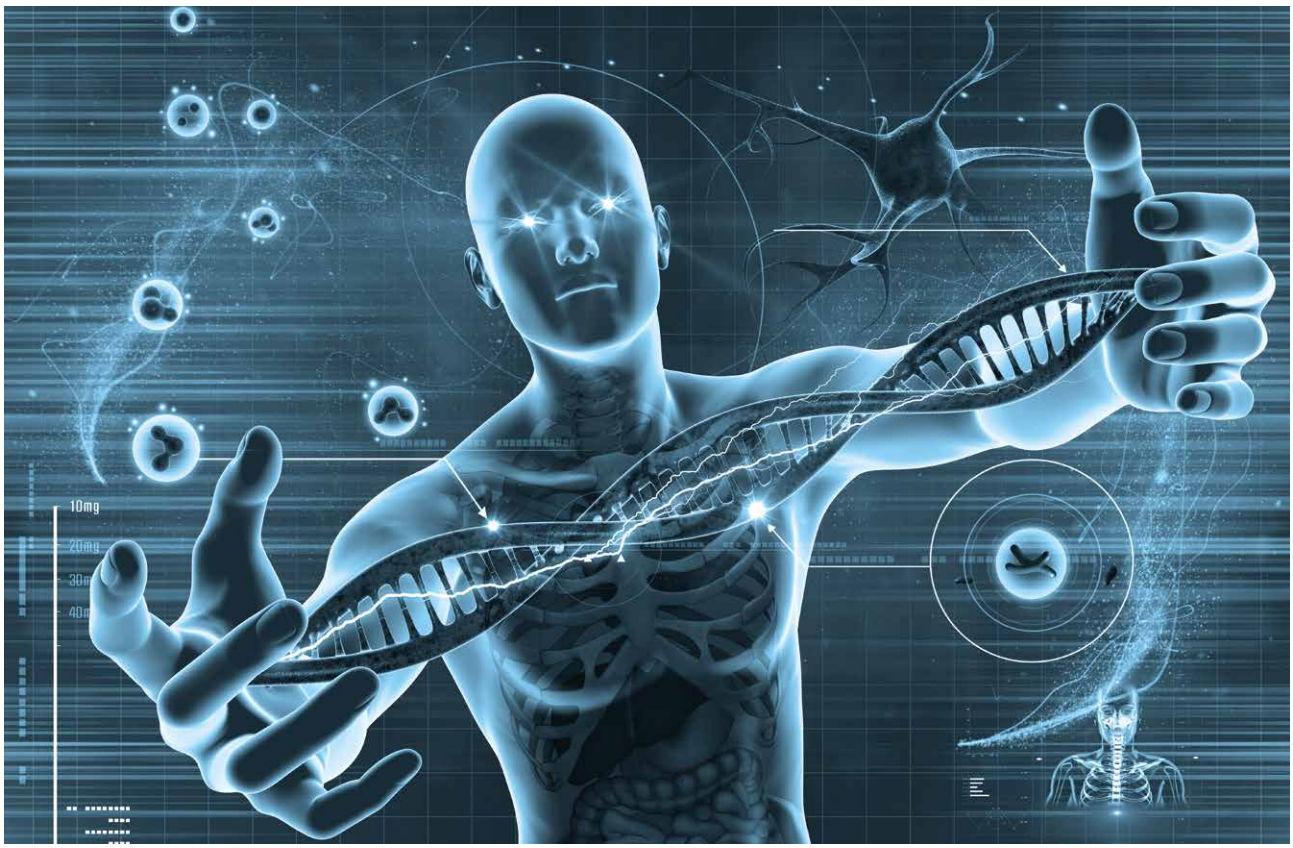
We open this section with Professor Charles Bangham at Imperial College, London, who focuses on human T cell leukaemia virus type 1 (HTLV-1). The HTLV-I virus is a distant cousin of the more commonly known human immunodeficiency virus (better known as HIV). It is also a retrovirus, functioning by incorporating its own DNA into the genetic code of the infected cells. We read how a key series of findings by Professor Bangham advance our understanding of how HTLV-1 influences the genetic code of infected individuals. By understanding how the virus leads to cancer and immune disorders, this work brings us closer to the much-needed development of effective treatments for HTLV-1.

We then turn to the work of Dr Claudio Fiocchi of the Lerner College of Medicine, Cleveland Clinic, USA, who adopts a holistic approach to understanding Inflammatory Bowel Disease (IBD). Professor Fiocchi's 'IBD interactome' is a biological network that integrates information including the environmental, microbial, immune, and of course, genetic factors relating to the development of IBD. We read of the importance of considering all these factors and the interactions between them, allowing a fuller understanding of the onset, management, and critically, the treatment of this often difficult to manage disease.

The value of a multidisciplinary approach in understanding disease is further demonstrated by Dr Darren Higgins at Harvard Medical School. Dr Higgins investigates the mechanisms involved in the growth and spread of the bacteria that are known to invade human cells and cause disease. We read how Dr Higgins has pioneered the use of genetic manipulation techniques to inhibit the expression of specific genes.

This important work is informing the development of novel therapeutic approaches for diseases such as meningitis and other potentially fatal brain infections.

Turning then to diabetic retinopathy – one of the most severe complications of diabetes – we read of Dr Lalit Pukhrambam's work at Wayne State University. Dr Pukhrambam researches the molecular mechanisms that are responsible for diabetic retinopathy, and through the identification of these mechanisms, opens up the possibility of developing novel gene therapies to treat, and even prevent the onset of the disease. Dr Pukhambran now plans to investigate the potential of gene technology to help overcome the challenges not only of diabetic retinopathy, but also age-related neurological disorders such as Alzheimer's and Parkinson's disease. We then focus our attention on the challenges and benefits of harnessing the ability of haematopoietic stem cells (immature blood cells with the potential to develop into all other types of cell) to replicate and repair genetic damage. Professor Michael Rieger of the Goethe




University Hospital, Frankfurt, Germany is driving forward our understanding of stem cell replication and behaviour. His work is vital in developing new therapeutic options for patients suffering from various forms of cancer as well as other potentially fatal diseases.

The development of novel treatments across a range of diseases is also the focus of Dr Stuart C. Sealfon of the Icahn School of Medicine at Mount Sinai Hospital and School of Medicine in New York, USA. Dr Sealfon is at the fore in developing new research techniques that further our understanding of the human genome and the genes and biological processes underlying human diseases. Adopting an approach known as bioinformatics, Dr Sealfon combines mathematics and computer science with biology. We read how his work informs treatment development for common reproductive disorders, neurological disorders, as well as immune system-mediated diseases.

Dr Yi Li, of Texas A&M University – Kingsville, takes an alternative perspective by investigating the role of epigenetics in influencing chronic diseases such as obesity. The term epigenetics refers to the processes that control which genes are expressed (that is, provide instructions for a molecule to perform a function) and when. Many epigenetic processes are influenced by external and environmental factors, such as lifestyle and diet, as studied by Dr Li. We read how Dr Li's innovative research demonstrates how genetics alone will not provide all the health solutions we need, but that we need to take into account how our environment influences our health in order to develop interventions to prevent obesity and other metabolic conditions in our future generations.

Turning to our final featured researcher, we read of the pioneering work of Professor Yubin Zhou, from the Center for Translational Cancer Research at the Texas A&M University Institute of Biosciences & Technology, USA, in the field of optogenetics. By using light to control cellular function, Professor Zhou has discovered a way to remotely control immune cells. We read how light-based techniques may assist in the control of immune responses in cancer when added to immunotherapy treatment, and how these techniques may be used to influence the epigenetic processes known to be involved in a variety of other diseases, including developmental disorders and Alzheimer's disease.

In this issue of Scientia, we have shown how basic and applied research in genetics is transforming how we understand and treat diseases of many different kinds. Of these, cancer is one of the most serious threats we face. The World Health Organization have warned of an exponential rise in the incidence of cancer, suggesting that by 2035, around 24 million people worldwide will suffer from some form of the disease. We conclude this issue with an exclusive interview with Dr Helen Rippon, the Chief Executive Office of Worldwide Cancer Research. We read how the focus on funding highly exploratory early-stage research into all types of cancer aims to support the development of future treatments with the aim of combatting the impact of this disease across the globe.


DNA

# HTLV-1: THE FORGOTTEN COUSIN OF HIV

The catastrophic impact of HIV – human immunodeficiency virus – is well-known worldwide. Not so well known is its distant cousin HTLV-1, human T cell leukaemia virus type 1, which also has the potential to destroy lives. The study of HTLV-1 can shed light on HIV itself. A team at Imperial College London, led by **Professor Charles Bangham**, has been working to raise awareness of this under-researched virus and to find better treatments for the devastating illnesses that it causes.

### A Global Problem That Is on the Move

An estimated 10 million people worldwide are infected with the human T cell leukaemia virus type 1 (HTLV-1). About one in ten of them eventually develop adult T cell leukaemia, a cancer of the blood cells, or another condition called HTLV-associated myelopathy, characterised by progressive lower limb weakness and paralysis. These diseases typically emerge many years after initial infection. Neither disease is curable, both are debilitating, and the leukaemia is usually fatal despite treatment.

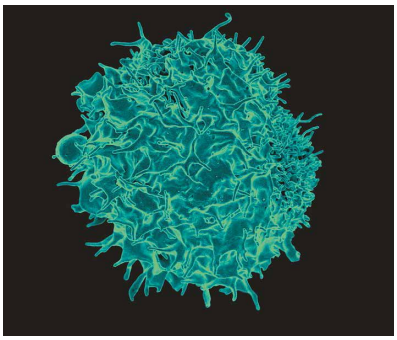
HTLV-1 infection is most prevalent in sub-Saharan Africa, South America and the Caribbean, Iran, Japan, and Australia. The virus is transmitted through breastfeeding, sexual intercourse, or infusion with contaminated blood products, and is therefore mainly transmitted within families and local communities. However, as travel becomes easier, and people move around more, it is becoming harder to identify those who carry the virus as they may no longer live in a high-risk area. For this reason, it is becoming increasingly difficult to prevent further spread of the virus.

Professor Charles Bangham, Head of the Division of Infectious Diseases at Imperial College London, argues that with HTLV-1 infection becoming more geographically spread there is an urgent need to raise awareness of it among both medical professionals and the public. Professor Bangham and his research team are committed to raising the profile of HTLV-1 and to finding more effective treatments for those coping with the long-term consequences of infection.

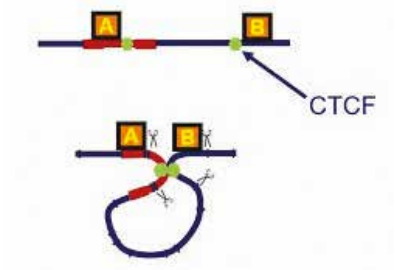
### HTLV-1 Latency: A Mysterious Balance

A person becomes infected with HTLV-1 when their cells come into contact with the cells of an infected person, for example, an infant during breastfeeding by an HTLV-1-infected woman. When this happens, the virus is able to cross from one cell to the other and enter the cells of the previously uninfected person. HTLV-1 is most likely to infect the T cell, a certain type of white blood cell.

Every cell in the human body holds a complete copy of an individual's DNA, the genetic code that contains the instructions for making all the proteins needed during life. HTLV-1 is a retrovirus,

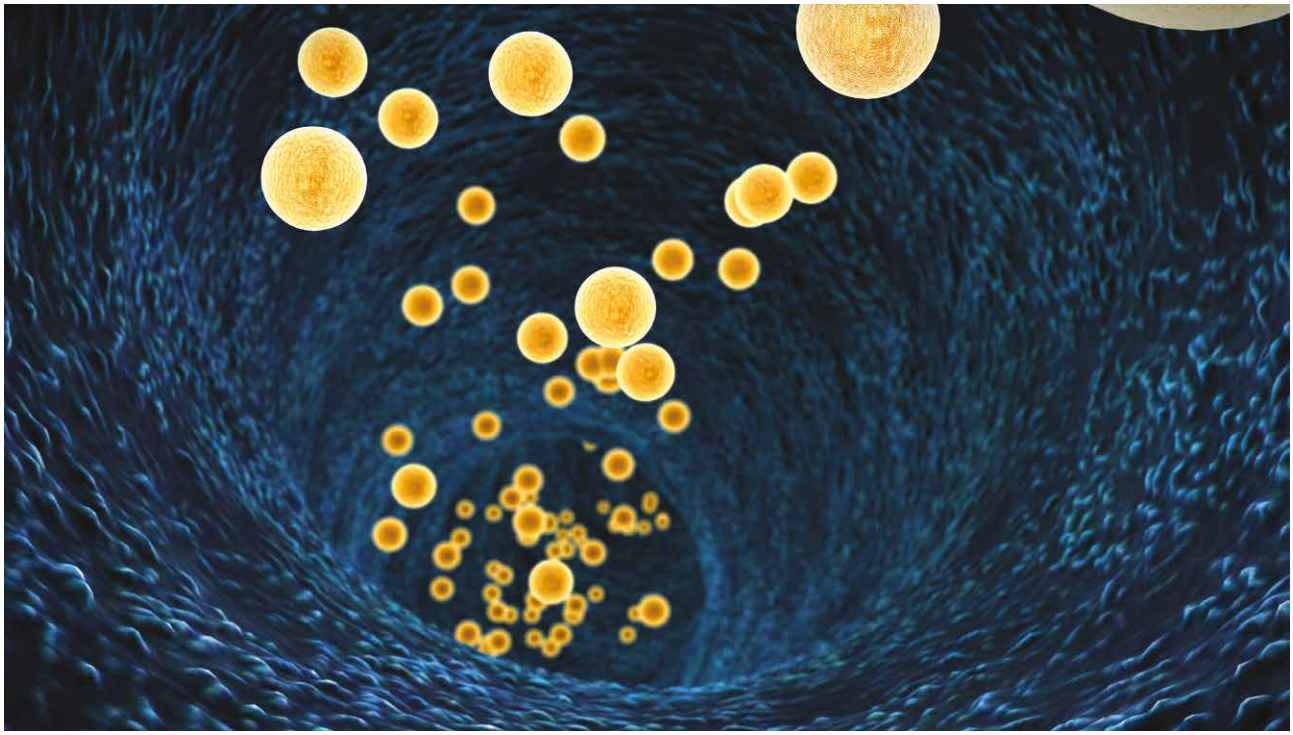


*Colorized scanning electron micrograph of a T lymphocyte. Credit: NIAID*




*The DNA of the HTLV-1 virus is able to bind to a protein known as CTCF, involved in forming the loops in DNA. Credit Charles Bangham.*

which means that it incorporates its own DNA into the genetic code of infected cells, causing them to make proteins wanted by the virus. These viral proteins cause the infected cells to appear different from their uninfected peers and thus make them targets for



destruction by immune-response cells. HTLV-1 infection also increases the rate in which T cells multiply, so the number of infected cells present at any one time is a balance between the production of new T cells and their destruction by the immune response.

Following infection with HTLV-1, it is usually decades before the symptoms of adult T cell leukaemia or HTLV-associated myelopathy develop, and up to 90% of those infected never become ill at all. One question that continues to challenge researchers is how the virus is able to maintain a population of infected cells in the body for so long, in the face of strong attacks from the immune system.

It was previously thought that the HTLV-1 virus lies dormant in T cells for the decades between infection and disease, during which time its DNA is present in the infected T cells but not used to make proteins. But Professor Bangham's research team found that the immune cells that attack infected T cells are continuously active during this time, so the infected cells must be making viral proteins in order for them to become a target.

What, then, do infected T cells spend their time doing? The team at Imperial College investigated the viral DNA in cells from HTLV-1-infected patients, to measure which genes were being expressed and when. Before a protein can be generated from a piece of DNA, a copy of that section of genetic code is produced, called messenger RNA, and this is then used by the cell's building machinery to generate the corresponding protein.

To measure which genes infected cells were producing, the research team generated fluorescently labelled probes for the messenger RNA of different HTLV-1 viral genes and used a microscope to visualise the production of this RNA in individual cells. This method provides much more information than those which only look at the average expression across a large number of cells. It has shown that the HTLV-1 gene that codes for a protein called Tax is expressed at very high levels but only intermittently in each infected cell. So only a small proportion of HTLV-1 positive cells, between 1 and 10 per cent, produce Tax at any one time. Critically, Tax marks infected T cells for destruction by the immune system.

This intermittent production of Tax results in an immune response that is continuous, but too weak to significantly reduce the overall number of infected cells. Only a small proportion of the population of cells are attacked at any one time, so the rest are allowed to multiply unhindered. The next step in understanding the virus will be to establish if the same process occurs in HTLV-1-infected cells in patients as in those grown in a dish. Professor Bangham plans to do this by rapidly analysing cells immediately after they have been taken from the patient's bloodstream.

This finding provides crucial new information on the interaction between HTLV-1 and the immune system. This increases our current understanding by helping identify the parts of the process that could be altered by therapeutics, perhaps, for example, to help the immune system fight infected T cells more effectively.

**From HTLV-1 Infection to Leukaemia: Filling in the Gaps**

Another unsolved question in the HTLV-1 puzzle is how infection with the virus can lead to cancer and a range of immune diseases. To try to answer it, Professor Bangham and his team have been investigating the effect HTLV-1 has on the genetic code of the infected person.

For a strand of DNA to make a protein it first needs to be unpackaged and made accessible. Viruses such as HTLV-1 and HIV enter cells and insert their own small segment of DNA into the genetic code of the person they have infected. The challenge for medical science is to understand the chain of events, from the insertion of this viral DNA into the infected person's genetic code onwards, that leads to the later development of symptoms.

The DNA that contains this code – the human genome – is not randomly scattered about the cell but is carefully coiled and folded into loops in a small compartment, called the nucleus. This DNA folding or looping is controlled by the binding of proteins to different sections of the DNA. The way in which this looping takes place is important, as it controls how easily different parts of the genome can be accessed and used by the cell to make new proteins.

Professor Bangham and his team recently found that the DNA of the HTLV-1 virus is able to bind to a protein known as CTCF, which is involved in forming the loops in DNA. This suggested to them that the addition of viral DNA into an infected person's genetic code might alter the way their DNA is arranged in the nucleus, therefore leading to later disease. To test this, they screened for contact points between the infected genetic code and the incorporated virus DNA. They looked for points that were close in 3D space but according to the sequence of the genetic code should be far apart, thus indicating that the DNA was forming a loop.

The researchers found that the viral DNA was indeed creating loops in the human genome that would not ordinarily be there. They then found that this abnormal DNA looping changes the expression of human genes near the viral DNA, and so can lead to abnormal production of proteins. If this results in the production of a cancer-causing protein, it could explain how HTLV-1 is able to cause adult T cell leukaemia.

The research team showed that, in addition to disrupting the genes in the immediate vicinity of its insertion, the viral DNA is able to disrupt the expression of much more distant stretches of the infected person's DNA. This is an important finding because it suggests that researchers may need to look further afield to identify the disrupted genes that cause adult T cell leukaemia in HTLV-1 positive patients, which is necessary to inform the ultimate goal of developing effective treatments.

**Knowledge Saves Lives**

Despite the large number of people infected with HTLV-1, and the likelihood that the number will continue to rise due to our increasingly mobile global population, little effort has been made by governments and health organisations to identify infected people and to limit transmission. There are currently no vaccines or effective therapies, and only limited methods are available for diagnosis and these are usually only used for research purposes.

Scientists working on HTLV-1 see themselves as having wider responsibilities in future beside continuing their research, to raise awareness of the virus both in at-risk populations and in the medical community. These kinds of initiatives have worked in the past. In Japan, for example, pregnant women have been tested for HTLV-1 infection since the 1990s. Women who test positive for HTLV-1 are given information on the virus and counselled to avoid breastfeeding. This has lowered the rate of transmission of HTLV-1 down the family line in this population. However, there remain many at-risk populations in other countries where no such services are offered.

**Moving Forward with the HTLV-1 Taskforce**

Professor Bangham is a member of the HTLV-1 Taskforce, which was set up in 2014 by the Global Virus Network to push forward the search for new methods of prevention and treatment of HTLV-1 associated diseases, and to promote basic research into the function of the virus. The work of the Taskforce and of Professor Bangham and his team is critical in addressing the societal and scientific challenges presented by HTLV-1.

# Meet the researcher

**Professor Charles Bangham**
Department of Medicine
Imperial College London
London
United Kingdom

Professor Charles Bangham qualified in Medical Sciences at the University of Cambridge and in Clinical Medicine at the University of Oxford, then spent several years working in hospital medicine. In 1987 he completed his PhD on the immune response to respiratory syncytial virus, based at the Medical Research Council National Institute for Medical Research (London) and the University of Oxford. Professor Bangham has continued his study of the immunology and virology of persistent virus infections, particularly HTLV-1, and has published 141 primary research papers in this area. Professor Bangham is now Head of the Division of Infectious Diseases at Imperial College, London. He is a Wellcome Trust Investigator, recipient of multiple Medical Research Council Project Grants, and is a Fellow of the UK Academy of Medical Sciences. He was awarded the BMC Retrovirology Prize in 2007 and the International Retrovirology Association Basic Science Prize in 2015.

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Wellcome Trust Investigator Award (2018–2022)
Medical Research Council Project Grant (2018–2020)

## FURTHER READING

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Imperial College London

# AN INTEGRATED APPROACH TO FIGHTING INFLAMMATORY BOWEL DISEASE

Inflammatory Bowel Disease (IBD) causes significant pain and discomfort to sufferers and severely affects quality of life. Research on IBD focusing on the individual contributing factors has failed to provide truly effective treatment options, and the broader picture has been somewhat neglected. **Dr Claudio Fiocchi** of the Lerner College of Medicine, Cleveland Clinic, USA, proposes an integrated, holistic approach which holds significant promise for improving both our understanding and treatment of IBD.

**The Challenge of Treating IBD**

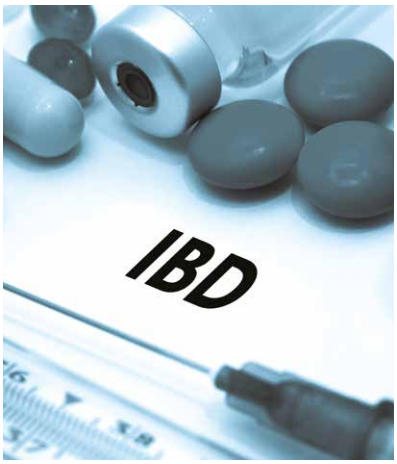
Inflammatory Bowel Disease (IBD) involves chronic inflammation of a patient's digestive tract. Ulcerative colitis and Crohn's disease are the two most common types of IBD. Symptoms include fatigue, severe diarrhoea, abdominal pain, rectal bleeding, weight loss and intestinal obstruction. According to the Centers for Disease Control and Prevention, in the United States alone, some three million people are reported as having IBD in 2015. According to the Crohn's and Colitis Foundation of America, as many as 70,000 new cases of IBD are diagnosed in the United States each year.

Despite advances in modern medicine, treating IBD is clearly proving to be a challenge. One of the main reasons for this is that IBD is characterised by an overwhelmingly complex set of pathogenic (disease-causing) mechanisms. The complexity of the disease is compounded by the diversity of each sufferer's symptoms, treatment experiences, and lifestyle choices. These factors make researching the disease and pinning down the specific causes all the more difficult.

Dr Claudio Fiocchi, Professor of Molecular Medicine at the Lerner College of Medicine, Cleveland Clinic, USA, is working towards a better understanding of IBD, and ultimately, improved treatment options. To achieve this, Dr Fiocchi firmly believes we have to embrace the idiom of 'out with the old and in with the new'.

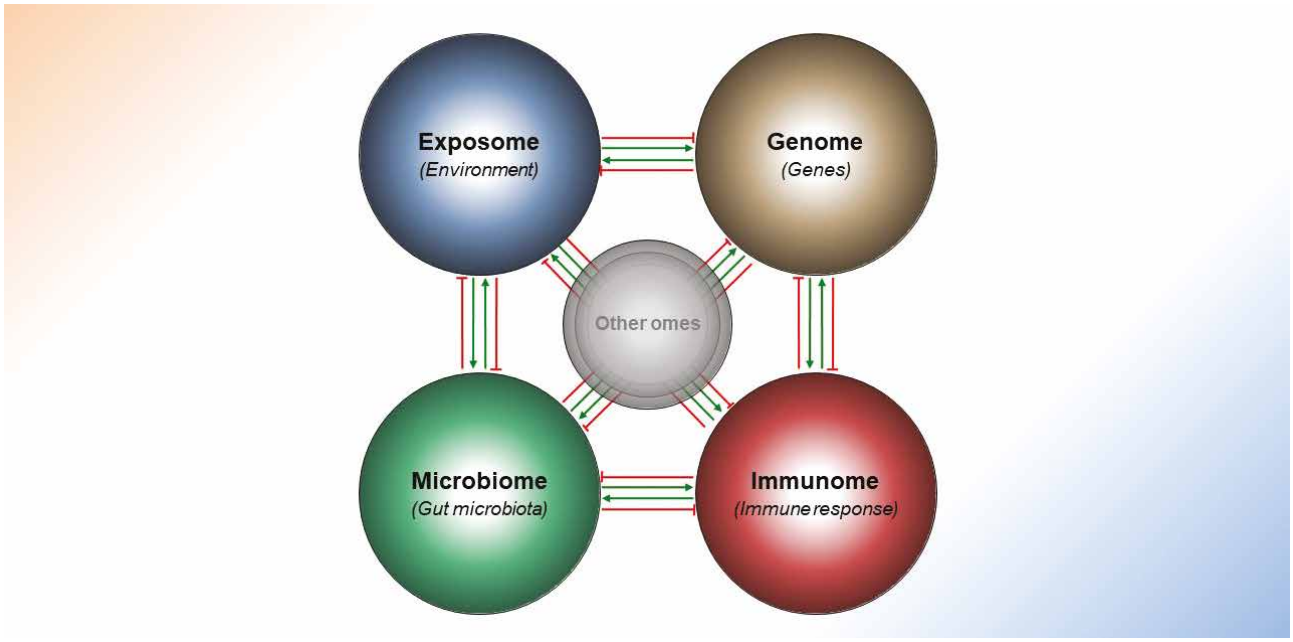
**The Need for a Holistic Approach to IBD Research**

Like in the case of other complex diseases, the causes of IBD are multifaceted and include environmental, genetic, microbial, and immune factors. However, as Dr Fiocchi points out, none of these factors alone is sufficient to trigger IBD. Rather, IBD is likely caused by complex interactions among them. Unfortunately, much of the research into IBD to date has focused on the individual contributing factors in isolation. As a result, progress has been limited. Dr Fiocchi argues that this blinkered approach




is a major obstacle to enhancing our understanding of IBD and identifying a cure.

**‘My current conviction is that the scientific and medical IBD community must accept new ways to look at IBD and realise that definitive answers, correct diagnosis and truly effective therapy can only be achieved if we adopt and integrate new methodologies that can evaluate the disease as a whole’.**




*‘The IBD Interactome’, credit Claudio Fiocchi*

If progress is to be made in treating IBD, then something needs to change. Dr Fiocchi explains, ‘my current conviction is that the scientific and medical IBD community must accept new ways to look at IBD’. He continues, ‘we must realise that definitive answers, correct diagnosis and truly effective therapy can only be achieved if we adopt and integrate new methodologies that can evaluate the disease as a whole’. With this premise in mind, Dr Fiocchi conceptualised the so-called ‘IBD interactome’.

**The IBD Interactome**

Dr Fiocchi wanted to develop a new approach to IBD research where all relevant information would be integrated into a comprehensive picture of the disease. In line with the terminology often used, he termed this integrated approach the IBD interactome. The interactome, as it pertains to IBD, is a biological network, in which Dr Fiocchi proposes that dysregulated interaction between ‘omes’ causes chronic intestinal inflammation.

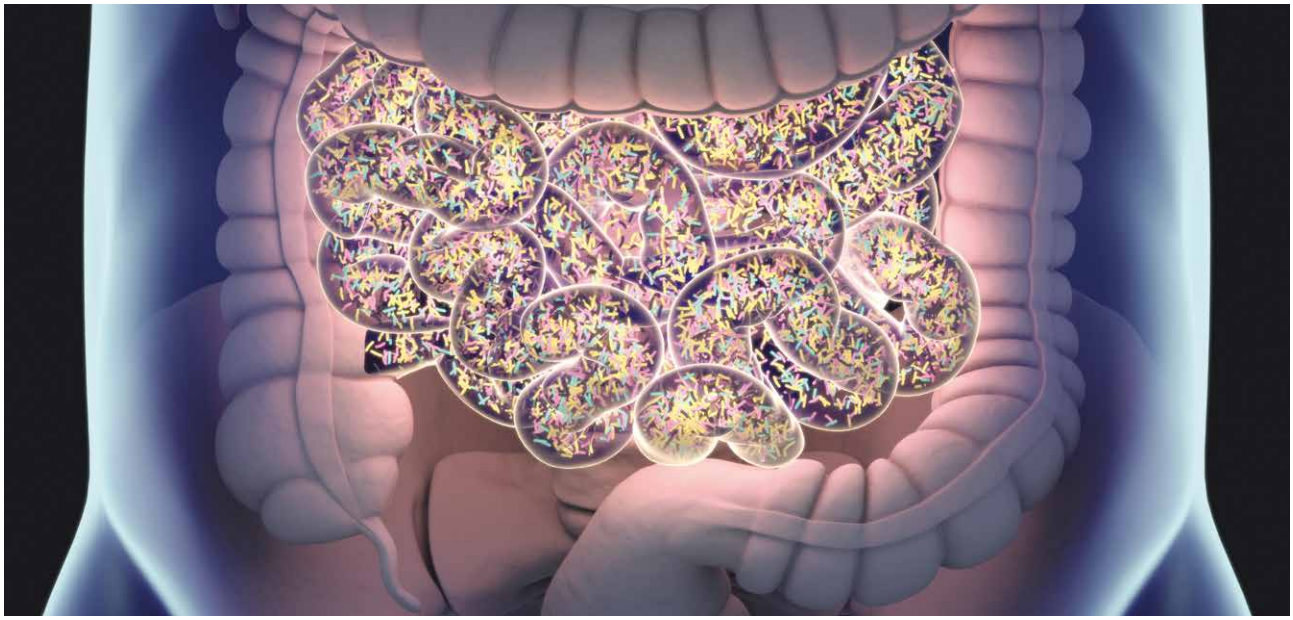
To understand this definition, we need to understand what is meant by omes. The term originates from terminology that conveys a sense of the totality of any particular complex system, wholeness, or completion. For example, contributing factors related to the environment are referred to as the patient’s exposome. Genetic, microbial, and immune contributing factors are referred to as the patient’s genome, microbiome, and immunome, respectively. These are among those most often researched but there are, of course, other potential contributing factors, or other omes.

The overarching purpose of the IBD interactome concept is to move away from studying single omes that contribute to IBD in mere isolation. By adopting a holistic, integrative approach, Dr Fiocchi hopes to gain deeper, more detailed insights into the disease-causing mechanisms. By facilitating an unbiased investigational approach, the IBD interactome is capable of detecting relevant pathogenic interactions at a molecular level. Such an in-depth approach, however, requires methodological innovations to existing procedures and introduces a whole new set of challenges.

**Methodologies for Studying the IBD Interactome: Systems Biology**

According to the National Institutes of Health, systems biology is an approach to biomedical research that aims to understand the larger picture – be it at the level of the organism, tissue, or cell. Systems biology is the opposite of reductionist biology, which, in contrast, involves taking the pieces apart to further our understanding. In light of the theoretical premise of the IBD interactome, a systems biology approach is ideal for studying IBD. However, it also introduces further methodological complexity.

For example, a systems biology approach to researching IBD requires one to collect a large range of bio-samples including blood, serum, endoscopic biopsy samples, and stool.



Researchers working in different fields of expertise need to analyse these samples for a whole range of different aspects. They then need to collect and integrate the data accumulated from these tests and deduce it into something biologically meaningful. It becomes immediately apparent that application of this approach in IBD would entail an enormous amount of work, as well as some serious information processing power. The good news is that there is a way in which this may feasibly be achieved – and it lies in the science of bioinformatics.

**Bioinformatics and Computational Biologists to the Rescue**

Bioinformatics refers to the use of statistical modelling and algorithmic processing to understand complex biological data. In the case of IBD research, Dr Fiocchi proposes that a large body of data from analyses covering vast research areas would be fed into an appropriate bioinformatics tool. These tools would then help to construct meaning from the results. However, this would not be a mindless process in which a computer spits out some sort of magic number. Rather, the use of bioinformatics in the study of IBD needs to be supported by computational biologists who are knowledgeable about the disease processes. With this expertise on hand, data from an extremely large body of analyses can be translated into something meaningful to IBD researchers.

However, it is important to remember that bioinformatics, at present, is not a 'silver bullet' for IBD research. There are still limitations to just how much data can be processed. Although there are a number of omes of interest, we do not currently have bioinformatics tools and algorithms capable of integrating all the analyses required. Thus, work to date focuses on only the most significant omes known to contribute to IBD.

In summary, evaluating the large amount of information compiled using a systems biology approach to IBD research requires state-of-the-art bioinformatics tools to synthesise this complex and varied data. As well as simultaneously bringing together all the relevant data, this approach will also allow researchers to study the disease at a more detailed, molecular level. Dr Fiocchi believes that future research using a bioinformatics systems biology approach will be critical in identifying the key molecular components in IBD. In this way, we will be able to understand how these molecular components regulate their networks, as well as how these various networks interact with one other. This work will contribute to a much better understanding of the mechanisms underlying IBD.

**Moving Away from the 'Parallel Separate Approach'**

In conclusion, the work of Dr Fiocchi represents a significant departure from the 'parallel separate approach' to IBD research. In this approach, the potential causes of disease have been treated as isolated entities by researchers operating in their own field of expertise in their study of a sole contributing factor. Although knowledge and expertise grow in these fields of research, they continue to run parallel to one another and the potential connections between them remain elusive.

Dr Fiocchi has pioneered a new approach to the study of IBD by conceptualising the IBD interactome in which all the potential causes of IBD and associated fields of research are brought together into one entity. This integrated and convergent approach will ensure that as knowledge increases, the connections between fields become apparent, thus addressing the inherent complexity of IBD diseases. Future research into the IBD interactome will be facilitated by a systems biology methodology and powerful bioinformatics tools that will analyse and construct meaning from the large bodies of data. This holistic and integrated approach to studying IBD holds considerable promise for the development of much-needed treatments for IBD.

# Meet the researcher

**Dr Claudio Fiocchi**
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Dr Claudio Fiocchi received his M.D. from the Santa Casa de Misericordia de São Paulo School of Medicine, São Paulo, Brazil in 1969. Between 1970 and 1980, Dr Fiocchi undertook further training in internal medicine, immunology, and gastroenterology and has since held several staff positions and prestigious professorships at the Cleveland Clinic and Case Western Reserve University, USA. Having published prolifically on inflammatory bowel disease, Dr Fiocchi has been an invited speaker at many international conferences and served on a number of grant and journal review boards. As a result of Dr Fiocchi's longstanding commitment to research and education in the field of inflammatory bowel diseases, he has received numerous accolades, including the MERIT Award, National Institutes of Health, USA (1999), the Honorary Foreign Membership, Brazilian National Academy of Medicine (2010), the Henry Janowitz Lifetime Achievement Award from the Crohn's & Colitis Foundation of America (2010), the International Herbert Falk Award, Falk Foundation, Germany (2013), and most recently, the Research Mentor Award from the American Gastroenterological Association (2018).

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## FUNDING

National Institute of Diabetes and Digestive and Kidney Diseases (National Institutes of Health)
European Crohn's & Colitis Organization

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# UNDERSTANDING THE PATHOGENIC MECHANISMS OF INTRACELLULAR BACTERIA

**Dr Darren Higgins** and his team at Harvard Medical School use a multidisciplinary approach to investigate the mechanisms involved in the growth and spread of disease causing bacteria that invade human cells. The team is developing new therapies to combat these life-threatening diseases.

### A Cunning Pathogen

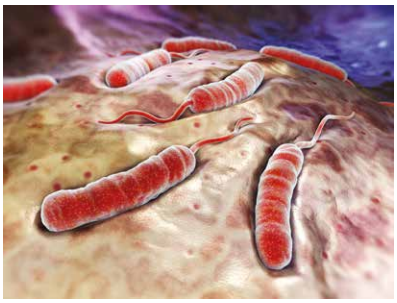
The human body is a very restrictive host – with help from its immune system and protective barriers, it fends off attacks from an array of pathogenic or disease-causing bacteria. However, in some circumstances, certain bacteria are able to evade these defences and enter host tissues to cause serious illnesses such as meningitis, encephalitis, respiratory and diarrheal diseases. With millions of people affected worldwide, there is a clear necessity to further understand the interactions between these bacteria and their human hosts.

Since completing his graduate studies into the disease processes of the bacterium that causes cholera, *Vibrio cholerae*, Dr Higgins has spent over two decades pursuing research in intracellular bacterial pathogenesis, specifically how disease-causing bacteria invade cells, replicate and spread. He describes that, 'understanding the mechanisms that these bacteria use to get inside of cells, replicate and spread is leading directly to the development of new therapeutic treatments and the development of new preventative measures such as vaccines.'

With this in mind, Dr Higgins and his colleagues are not only attempting to characterise the host-specific factors involved in the development of infections within cells but are also searching for proteins within bacteria that determine their virulence – how well the pathogen infects its host. They also hope to identify how bacteria control the process of infection and how the immune system recognises bacteria to stimulate an immune response.

Dr Higgins is a Professor of Microbiology and Immunobiology at Harvard Medical School, where his research team has focused on understanding the mechanisms these disease-causing bacteria use to survive, grow and spread inside the human cell. 'Building upon extensive expertise in the areas of microbial pathogenesis, immunology, and the cell biology of infection, our group has pioneered multiple approaches and made significant contributions to understanding the mechanisms of intracellular bacterial pathogenesis and the host immune response to infection,' he explains.

The team decided to focus some of their studies on the well-characterised bacterium *Listeria monocytogenes*, hoping that the information gleaned

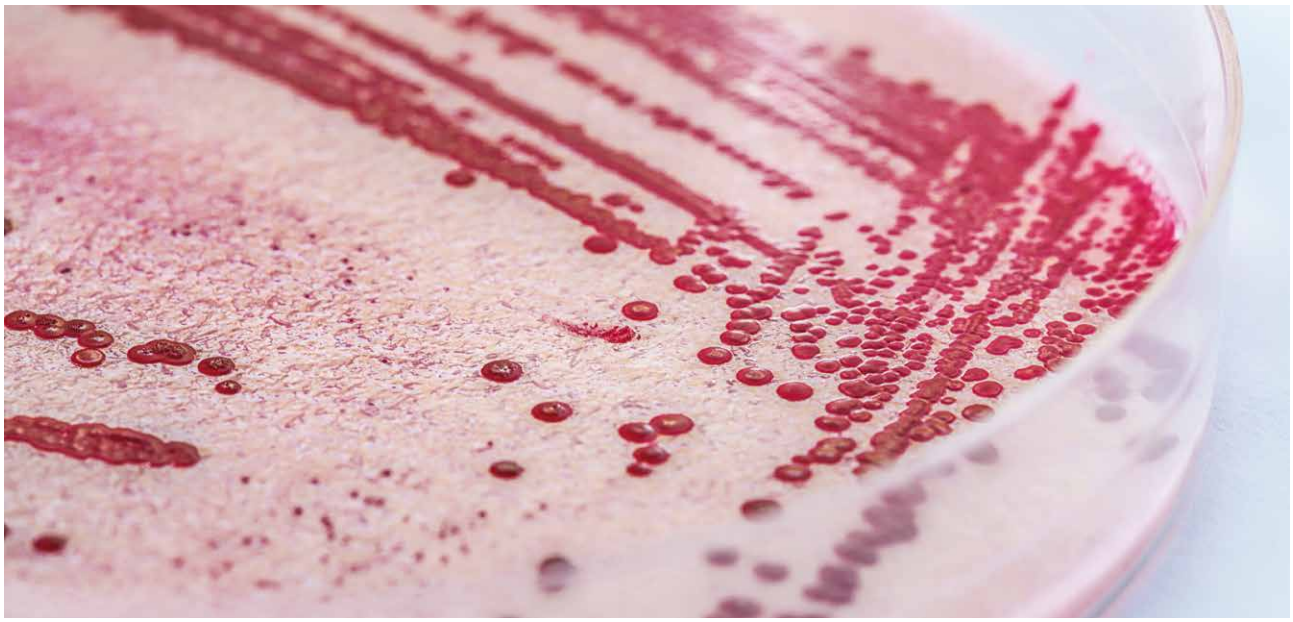


could be translated to other species. *Listeria monocytogenes* is a food-borne, intracellular bacterial pathogen capable of invading numerous host cell types and causing severe diseases in people, particularly affecting those whose immune systems are compromised, such as the elderly and pregnant women. Of greatest concern is its ability to invade the brain and cause life-threatening meningitis and encephalitis. Dr Higgins' laboratory is currently focused on attempting to ascertain how these bacteria enter the brain with potentially fatal consequences.

### Internalisation of the Bacteria

When a bacterial pathogen enters the body, phagocytic cells, a type of cell that forms part of the immune system, engulfs the foreign pathogen and traps it in a vacuole – a specialised compartment within the cell. The phagocytic cell then destroys it ensuring

**‘Understanding the mechanisms that these bacteria use to get inside of cells, replicate and spread is leading directly to the development of new therapeutic treatments and the development of new preventative measures such as vaccines.’**



the bacteria do not have access to the host’s internal tissues.

However, pathogenic bacteria such as *Listeria monocytogenes* have found clever ways to escape the vacuole to access the interior of the host cell, where the environment is optimal for its replication and the infection of neighbouring cells. This escape from the vacuole of the phagocytic cell is paramount to ensure survival and spread of the bacteria.

This vacuolar escape has been accredited to the secretion, by the bacteria, of the protein listeriolysin O (LLO) and also two others called phospholipases C, a broad-range phospholipase C (PC-PLC) and a phosphatidylinositol-specific phospholipase C (PI-PLC). LLO is a pore-forming toxin, which creates holes in the phagocytic vacuole to promote bacterial escape. It is suggested that PC-PLC and PI-PLC assist in this by either promoting LLO activity and/or inhibiting the repair of the holes created by LLO.

Dr Higgins and his team have pioneered the use of genetic manipulation techniques such as RNA interference (RNAi) – a technique that inhibits expression of a specific gene – to determine what in the host is required for intracellular infections. Knowing that LLO is responsible for slowing down vacuole maturation, they used this RNAi technique to show a correlation between reduced phagocytic vacuole development with the escape of *Listeria monocytogenes* from this compartment in the cell.

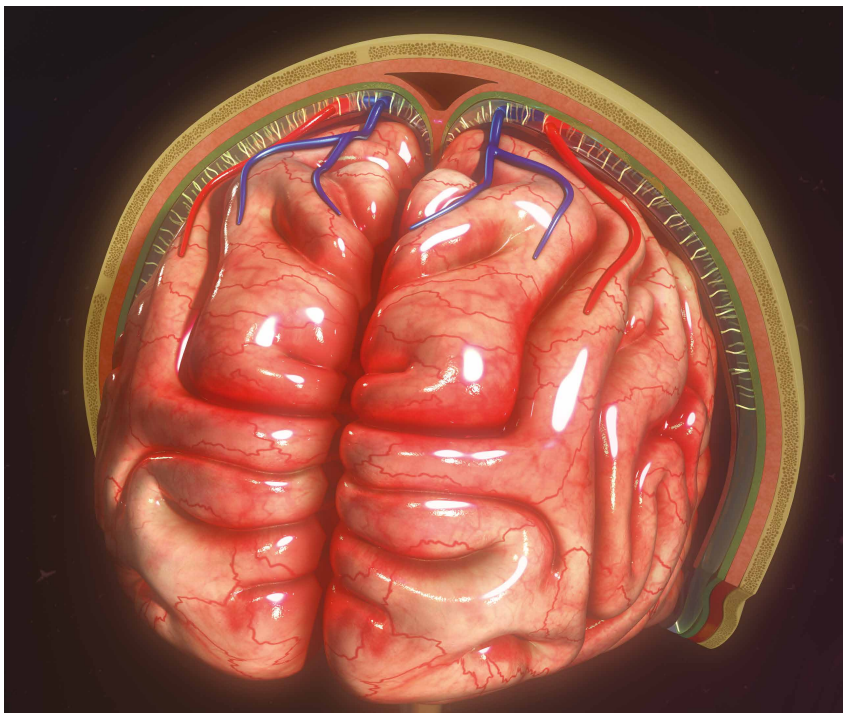
With their findings on LLO activities, Dr Higgins and his colleagues have identified the pathogen-specific proteins that induce a protective immune response. These proteins and the method by which they were determined are being further developed for the generation of novel vaccines by Genocea Biosciences, a vaccine development company co-founded by Dr Higgins in late 2006.

**Cell-to-Cell Movement**

Once the pathogenic bacteria enter the interior of the host cell and replicate, the next goal is to infect neighbouring cells. Each bacterium recruits to its cell surface host cytoskeletal proteins – protein structures that help cells maintain their shape and mechanical support – to assemble a tail. This continuous lengthening of the tail pushes the bacteria out against the host cell membrane, forming an asymmetrical protrusion.

As well as engulfing and destroying pathogenic bacteria, phagocytic cells also engulf damaged or dead cells within host tissue. They do this by binding to a phospholipid called exofacial phosphatidylserine (PS), which is found on cells or cellular debris after their external membranes have been compromised.

In collaboration with Dr John Brumell, Dr Higgins’ team have shown that *Listeria monocytogenes* takes advantage of this clearance system to promote cell-to-cell spread during infection. It does this by presenting the asymmetrical bacteria-containing protrusion from the primary infected cell to a second phagocytic cell, tricking it, so it too is engulfed.



This process also ensures that the bacteria avoid the space outside of the cell and any immune molecules, such as antibodies, within it. Using this finding, the team believes that, 'PS-targeted therapeutics may be useful in the fight against infections by *Listeria monocytogenes* and other bacteria that use similar strategies of cell-to-cell spread during infection.'

Once the second cell engulfs the protrusion filled with the microbe, a second vacuole is formed around it. The pore-forming toxin LLO again plays a key role by damaging this second vacuole to allow the bacteria to escape.

**The Genetics of Replication**

To ascertain the bacterial and host factors necessary for efficient replication within the host cell, Dr Higgins and his colleague Dr Perry adapted a screening method called fluorescence-activated cell sorting (FACS) that uses mutagenized fluorescently labelled bacteria to identify the genes involved in the replication of *Listeria monocytogenes* within the cell.

Their work revealed that deletion of the genes *menD* or *pepP* from *Listeria monocytogenes* stopped the bacteria from efficiently growing within host cells cultured in the laboratory. They also showed that when *menD* or *pepP* were deleted *Listeria monocytogenes* had a reduced ability to infect mice. This validated the team's screening method to identify genes that control the intracellular growth rates of bacteria.

**Infection of the Brain**

The blood-brain barrier, as the name suggests, is a highly restrictive barrier protecting the brain from foreign objects in the blood. *Listeria monocytogenes* is amongst a select group of bacterial pathogens, which after effectively invading the host body, is able to cross the blood-brain barrier to infect the brain, causing life-threatening meningitis and encephalitis. 'The long-term goal of our current work is to provide further insights into how intracellular pathogens invade the brain and to identify novel approaches to prevent bacterial infections of the brain,' says Dr Higgins.

It is well known that *Listeria monocytogenes* is capable of invading other non-phagocytic host cells, through interactions between the proteins found on the bacterial cell surface and the host cell. Two proteins called InIA and InIB interact with the host cell surface proteins E-cadherin and the Met receptor, respectively.

This interaction ensures that *Listeria monocytogenes* is internalised into the host cell, although studies have shown InIA and InIB do not play a role in the bacterial internalisation within the brain, suggesting E-cadherin and the Met receptor may not be responsible for allowing these bacteria to cross the blood-brain barrier. With that in mind and with funding from the National Institutes of Health, Dr Higgins and his colleague's Dr Halvorsen and Dr Ghosh went on to investigate the protein interactions involved in blood-brain barrier penetration.

They discovered the interaction between vimentin, a protein found on the host cell surface and InIF, another protein found on the cell surface of *Listeria monocytogenes*, is required to facilitate efficient bacterial infection of the brain. This finding may indicate that vimentin is a central factor used by many other bacteria to cross the blood-brain barrier to colonise the brain and cause meningitis. The team hopes that a greater understanding of this vimentin-InIF interaction could provide possible targets for the development of novel therapeutics for meningitis infections.

**Looking Ahead**

The research team recognises the lack of knowledge about the exact mechanism of the bacterial invasion and the progression of brain infections. Their future goal is to determine if *Listeria monocytogenes* infects specific brain cell types and to assess cell-to-cell spread between different host cell types.

This is important, as it will provide valuable knowledge of the mechanisms by which *Listeria monocytogenes* invades the brain and causes potentially fatal meningitis. They also hope to find additional factors that facilitate *Listeria monocytogenes* invasion of the brain and identify novel therapeutic targets to prevent brain infections by this and other pathogenic bacteria.

# Meet the researcher

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Dr Higgins is a professor at Harvard Medical School in Boston, within the department of Microbiology and Immunobiology. Dr Higgins received his undergraduate degree in Microbiology from Texas A&M University, then went on to complete his PhD in Microbiology and Immunology at the University of Michigan Medical School in 1995, followed by postdoctoral training at the University of Pennsylvania School of Medicine and the University of California, Berkeley. Initially joining Harvard Medical School as an Assistant Professor in 1999, Dr Higgins was promoted to Associate Professor in 2005 and to Professor in 2009. His laboratory at Harvard is currently focused on understanding fundamental host-pathogen interactions that lead to virulence and the development of protective immunity to intracellular bacterial pathogens. He has served as an editorial board member on several prominent journals in the field. Dr Higgins also co-founded and serves on the scientific advisory board of Genocea Biosciences Inc., a company created to commercialise key breakthroughs in vaccine discovery for intracellular pathogens.

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# TARGETING THIOREDOXIN-INTERACTING PROTEIN: THE FUTURE FOR TREATING DIABETIC RETINOPATHY?

Diabetic retinopathy is one of the main causes of blindness in developed countries. Currently, there is no known cure. **Dr Lalit Pukhrambam** is working to change that. Along with his group at Wayne State University, he is investigating the influence that a molecule, known as thioredoxin-interacting protein, has on the development of diabetic retinopathy and whether targeting this molecule will provide novel therapies in the future.

**Diabetic Retinopathy**

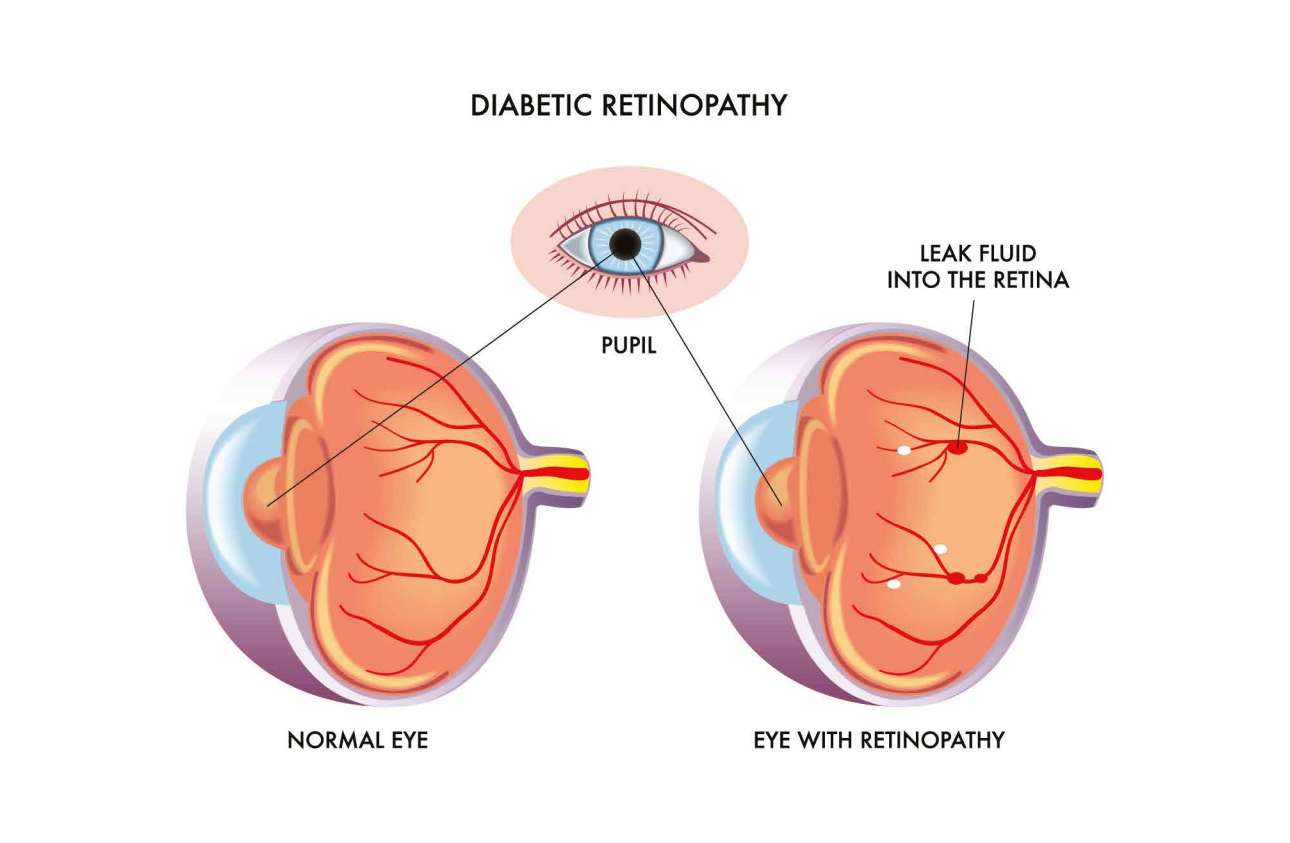
There are two main types of diabetes – Type 1, in which insulin production is deficient and Type 2, in which the body is resistant to the insulin produced. The majority (around 90%) of diabetics are Type 2 and this form of the disease is known to be associated with lifestyle choices. There are many complications associated with diabetes, including diabetic retinopathy.

Diabetic retinopathy is one of the most severe complications of diabetes. Diabetes can lead to problems with the smallest blood vessels in the body; persistently high sugar levels can damage the tiny blood vessels that deliver a constant supply of blood to the back of the eye, the retina, and ultimately lead to blindness. This was originally thought to be a consequence of problems associated with the blood vessels in the eye, but it is now known to be more complex. The neurons in the eye are also affected by diabetic retinopathy, with neuronal injury occurring early on in the development of the disease.

Given the high rate at which the retina must use oxygen to produce energy, reactive oxygen species (ROS), by-products of oxygen metabolism, are also considered to play an important role in the development of diabetic retinopathy, along with low-level inflammation caused by chronic hyperglycaemia, or high sugar levels.

It was recently demonstrated that the level of one particular molecule, thioredoxin-interacting protein (TXNIP), is increased in rodent models of diabetic retinopathy and hyperglycaemia. TXNIP binds to thioredoxin, a protein that normally prevents the accumulation of ROS and consequent damage to cells caused by oxidative stress. Therefore, an increase in TXNIP leads to a decrease in the scavenging activities of thioredoxin and an increase in ROS stress. Normally, there are antioxidant systems in retinal cells to deal with ROS, but under acute or chronic stress the ROS are still able to accumulate and damage mitochondria (the energy producing 'powerhouses' of the cell), DNA, and cell membranes.

TXNIP is important in the link between glucose toxicity and the death of insulin producing beta-cells in the pancreas. Indeed, mice without TXNIP are resistant to some models of diabetes. Although known to be complex, the relationship between TXNIP and diabetic retinopathy remains to be clearly elucidated. It is this relationship that Dr Lalit Pukhrambam, of Wayne State University, is trying to understand.




**TXNIP and the Development of Diabetic Retinopathy**

Dr Pukhrambam and his group are interested in the molecular mechanisms responsible for diabetic retinopathy because identification of these opens up the possibility of developing novel gene therapies to treat, or prevent the onset, of diabetic retinopathy.

The intricacies of the underlying mechanisms linking TXNIP to diabetic retinopathy remain unclear. It has previously been suggested that several metabolic pathways may be involved, as the retina consumes large amounts of glucose and oxygen in order to function correctly. Therefore, any damage to the energy producing pathways in the retina has severe effects and potentially contributes to the development of diabetic retinopathy.

Early work by Dr Pukhrambam investigated one aspect of this relationship by focusing on a protein called RAGE (short for receptor of advanced glycation end products).

A sustained increase in the number of molecules that bind to RAGE is an important factor in the development of diabetic complications. An increase in RAGE binding leads to a subsequent increase in RAGE expression, which can lead to inflammation. The study, which was collaboration between Dr Pukhrambam, Dr Lorena Perrone, Ms Takhellambam Devi, Professor Ken-ichi Hosoya, and Professor Tetsuya Terasaki, investigated whether RAGE induced inflammation in rat retinal cells kept under diabetic conditions. The ultimate aim of the study was to investigate whether high glucose and RAGE induce inflammation through TXNIP expression or changes in other genes.

Dr Pukhrambam and the team concluded that RAGE does mediate TXNIP and hyperglycaemia-induced overexpression of molecules associated with inflammation. They also observed that TXNIP changed the structure of chromatin. As DNA is made up of chromatin, changing the structure of chromatin allows regions of the genome to be 'opened' so that certain genes can be expressed.

Following on from this previous work, Dr Pukhrambam further investigated the role of TXNIP in diabetic retinopathy. Using a diabetic rat model, his team showed that TXNIP expression was elevated in the retina and correlated with an increase in markers of inflammation. Furthermore, after blocking the expression of TXNIP in diabetic rat retinas, they abolished diabetes-induced damage of the nerve cells in the retina. These findings support those of the previous study by showing that TXNIP has a critical role in inflammation and, furthermore, may present potential therapeutic approaches that could be used to block onset and progression of diabetic retinopathy.

Another role for TXNIP in diabetic retinopathy involves the NLRP3 inflammasome, which usually acts as a defence against invading viruses and bacteria. The inflammasome is a signalling complex inside the cell, made up of many proteins. Its job is to recognise threats and mobilise the necessary troops from the immune army to deal with the intruder.

Dr Pukhrambam's research has shown that high glucose levels increase TXNIP, which is also known to be involved with the NLRP3 inflammasome under oxidative stress. This presents another potential therapeutic target – reducing or blocking TXNIP and the inflammasome, in combination or singly, may reduce the chronic low-level inflammation observed with diabetes and related diseases.

**TXNIP and Energy Imbalance**

More recent work by Dr Pukhrambam's group has looked at the type of cells that TXNIP acts on. Retinal neurons are known to be injured during the early stages of diabetes and retinal Müller cells are a type of cell that act as support cells for neurons. Dr Pukhrambam proposes that retinal Müller cells react to prolonged high glucose levels by upregulating TXNIP, as well as other cell survival and defence mechanisms.

However, under chronic cell stress situations, these mechanisms may lead to premature cell death and increased progression of diabetic retinopathy. One example of this is mitophagy, where defective mitochondria in cells are removed via lysosomes following chronic damage or stress. Disturbed mitophagy leads to accumulation of non-functional mitochondria, oxidative stress, and low ATP production. This fits in with the idea that mitochondrial dysregulation, and the resulting energy imbalance, are critically important in diabetes-associated diseases. Therefore, therapies targeting TXNIP could break this chain and prevent excessive oxidative stress arising from sustained responses to high glucose levels.

Leading on from the idea that energy imbalance plays a role in the development of diabetic retinopathy, further work by Dr Pukhrambam's group aims to investigate the link between TXNIP and mitochondrial dysfunction. To do this, they manipulate the TXNIP levels in rodent models of diabetes using a cutting-edge gene editing method known as CRISPR/Cas9. They also use in vitro methods in the laboratory, including the use of retinal Muller glia and other cells from the eye, such as endothelial cells, pigment epithelial cells, and neuronal cells, to try and dissect the ways in which TXNIP induces mitochondrial dysfunction.

Understanding the extent to which mitochondrial flux, that is, the movement of molecules through the membrane of the cell via the lysosomal exocytosis is beneficial or detrimental remains a priority for diseases such as diabetes. Dr Pukhrambam has hypothesised that manipulation of mitochondrial flux may be able to track the development of diabetic retinopathy. Future studies by his group will aim to answer this question.

**Manipulating TXNIP to Prevent Diabetic Retinopathy**

Given the close relationship between TXNIP and diabetic retinopathy, using gene therapy methods to decrease TXNIP expression presents a potential therapeutic approach.

If it is possible to link the TXNIP gene promotor with the insulin gene, or another gene of interest, this would allow an adult stem cell population to be induced to insulin producing pancreatic beta-like cells. The TXNIP gene promoter is the region of the genome that sits very close to the TXNIP gene and it is responsible for the TXNIP gene being switched on and off, thus controlling the amount of TXNIP being produced. These new insulin producing cells may subsequently alleviate some of the complications associated with diabetes, as they could be put back into patients after they have been manipulated outside the body.

Dr Pukhrambam and his colleagues have recently applied for a patent to do just this, and plan to investigate the possibility of using this gene therapy technology in in vivo and ex vivo settings. However, for this to eventually become a viable treatment option, there are many hurdles to overcome. These include the problem of getting the gene system into the retina, as it would probably have to be injected directly into the eye as the retina has a blood-retinal barrier and to minimise the immune response from the rest of the body. Gene incorporation in the retina may be achieved by using adeno-associated virus or lentivirus delivery systems.

**Applications of TXNIP Beyond Diabetic Retinopathy**

In addition to diabetic retinopathy, TXNIP is known to be upregulated in ageing and age-related disorders such as Alzheimer's and Parkinson's diseases. In these neurodegenerative diseases, mitochondrial dysfunction, protein misfolding, and mitophagy dysregulation are known to be linked to disease development and progression. Manufacturing a TXNIP promoter linked inhibitory RNA targeted to TXNIP messenger RNA itself with the ability to decrease its own expression could also have an immeasurable impact on patients suffering from other diseases associated with mitochondrial dysfunction. Dr Pukhrambam makes the exciting proposal that these TXNIP-promotors could be engineered in such a way that gene expression may be activated by a physiological trigger, such as the high glucose levels encountered after a meal.

# Meet the researcher

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Dr Lalit Pukhrambam obtained his PhD in Biochemistry from the Indian Institute of Science, and is currently an Associate Professor in the Department of Ophthalmology, Visual and Anatomical Sciences at the School of Medicine, Wayne State University. His research focuses on many facets of diabetes, including epigenetics, gene and cell therapies, and the cellular and molecular mechanisms of the disease. In particular, his group investigates the critical role of thioredoxin-interacting protein in diabetic retinopathy. With a prolific publication record in his field, Dr Pukhrambam is an active editor, as well as an experienced journal and grant reviewer. In addition to his research responsibilities, Dr Pukhrambam is engaged in teaching at both undergraduate and postgraduate levels.

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Juvenile Diabetes Research Foundation
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Research to Prevent Blindness (RPB)

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WAYNE STATE UNIVERSITY

# ALWAYS FORWARD, NEVER BACK

Stem cells are the therapeutics of the future, but to use them to their full potential we first need to understand how they function within the body. **Professor Michael Rieger** of the Goethe University Hospital, Frankfurt, Germany is working to understand the complex intricacies of stem cell replication and behaviour.

Blood is quite an amazing substance. Packed full of cells with a multitude of roles, be it carrying oxygen, clotting wounds, or gobbling up unwanted invaders, this mere five litres of fluid manages to keep us alive and healthy all day, every day. There is a large number of different types of blood cells – erythrocytes, neutrophils, lymphocytes, platelets, and many more. All with widely differing roles and attributes and all coming from the same point of origin.

Every one of this multitude of cell types currently floating around your bloodstream developed from a single type of precursor, known as a haematopoietic stem cell (or HSC for short). These cells can be thought of as the raw material that can be formed into any one of the blood cell types, a process of irreversible specialisation and development – differentiation – known as haematopoiesis. The act of differentiation occurs in steps, with cells converting into ever-more specialised forms based on the cellular signals that they receive.

The ability of HSCs to form any blood cell has not gone unnoticed by the medical community. Indeed, haematopoietic stem cell transplantation, the act of providing patients with HSCs from a donor source, has the potential to treat diseases ranging from cancers to immune-system diseases and has become clinical practice.

Yet there are significant hurdles to be overcome, not least of which is the problem of getting enough HSCs in the first place. Donors are limited in the samples they can provide, even exceptionally rich sources of stem cells such as umbilical cord blood do not provide enough cells to treat an adult patient. But cells replicate, dividing to produce two daughter cells, bringing up a simple question – if there are too few stem cells, then can't we just grow some more?

**From One, Many**

Blood cell production in the human body is a frantic process, with around 500 billion blood cells produced each day. To keep up with this demand stem cells need to constantly renew their numbers and their progeny need to replicate frantically to keep up with the required pool of blood cells. This is actually quite a rare ability – the majority of cells can only divide a certain number of times before steadily accumulating DNA damage causes them to self-destruct. Stem cells such as HSCs, by contrast, have an extended ability to replicate and repair genetic damage. A single HSC could, in theory, eventually produce all the blood a human could require.

Going from theory to practice, however, can be challenging. HSCs taken outside the body will rapidly begin to differentiate, losing their stem cell abilities within three to five days. This means that every transplant is a race against time to harvest cells, clean them, genetically modify them as needed, and provide them to the patient. Many groups have tried to extend this time frame, mostly without success. The sheer complexity of the differentiation process makes it difficult

**‘We found a new class of molecules, so-called microRNAs, which are misregulated in acute leukaemia. We are now using this knowledge to develop clinical protocols for the diagnosis and treatment of leukaemia, using these advanced therapeutics.’**

for scientists to stop or prolong it at precise points.

This is where the work of Professor Rieger of the Goethe University Hospital in Frankfurt am Main comes in. An expert in stem cell research, he and his team have long been interested in the way stem cells specialise and differentiate into their final forms. ‘We want to understand what makes blood stem cells so special,’ he says, ‘and which molecules in blood stem cells are responsible for their action. How can undifferentiated blood stem cells differentiate into very-specialised blood cells with distinct functions, which are very different from the functions of the originators?’

Innovative single cell technologies applied in his laboratory will uncover the function and molecular composition of individual HSCs and resolve cellular complexity.

**Balanced Multiplication**

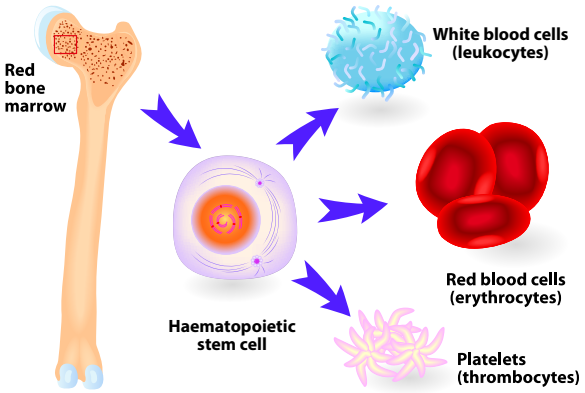
Stem cell replication and differentiation is a multi-step process. A dividing HSC will leave daughter HSCs (to renew the population) and progenitor cells, known as multipotent progenitors. These cells have irreversibly started upon the differentiation process and they follow a path of maturation that steadily decreases the number of available options until the final form is reached – the mature blood cell. The maturation process is controlled by a number of molecular signals such as colony-stimulating factors and growth factors. These trigger changes in the gene expression profile of the cell, driving the cellular modifications that cause progression along the differentiation pathway.

Although they can replicate to produce new stem cells, HSCs need to carefully balance their numbers – too few and they cannot produce enough blood cells for demand, too many and they begin to overrun the body leading to haematologic disorders. Although the basic effects of growth factors on HSC replication and differentiation have been known for many years, it is only recently that scientists are beginning to understand the many other factors that contribute to, balance, and fine-tune the process. One of these contributing factors, and indeed a major focus of Professor Rieger’s team, is that of microRNAs.

**A Microscopic Stop Signal**

RNA is formed from DNA and acts as a template for protein production. MicroRNA is essentially a very small piece of the normally single-stranded

## Haematopoiesis & Stem cells




RNA that has been bent back upon itself to form a hairpin of double-stranded RNA. Double-stranded RNA is commonly used by viruses and so many species are capable of detecting it and using the molecule as a template for the destruction of any further RNA containing the same sequence.

Over the millennia this cellular defence process was co-opted by evolution to provide deliberate destruction of the cell's own RNA. The microRNA thus acts to control the expression of genes into proteins within plant and animal cells, essentially providing tightly targeted decreases in the production of specific proteins.

There are many different microRNA variants, with the human genome alone appearing to code for more than 1000 of them. Taken together, these fragments of genetic information can target almost 60% of the genes in the human body. Given this, it is perhaps no surprise that microRNAs also play a role in HSC replication and haematopoiesis. Indeed, one of the most important microRNA factors involved in this process was discovered by Professor Rieger and his group and is known by the name of miR-193b.

The researchers showed that miR-193b acts as a negative regulator of the stem cell replication process. An HSC that has received a signal to replicate also produces increased amounts of miR-193b. This microRNA targets a protein known as c-KIT, one of a family of proteins known as tyrosine kinases that transmit and amplify signal information throughout the cell. Expression of miR-193b leads to degradation of the RNA coding for c-KIT, reducing the amount of protein present and the potential for signal amplification. The disruption to cellular signalling then acts to counter the replication signal and returns the stem cells to their base state, creating a negative feedback loop.

Expanding on these initial observations, the researchers were able to show that artificially modifying the level of miR-193b controlled HSC replication and differentiation both in cell culture and in mice. Removing miR-193b reduced the rate of differentiation and therefore led to rapid expansion of HSC numbers, while increasing expression of the gene led to reduced stem cell renewal.

Interestingly this effect, the negative feedback that prevents haematopoietic stem cell growth, can also be seen in cells that are descended from HSCs. One of the most important examples of this comes from studies on acute myeloid leukaemia, a particularly dangerous form of blood cancer in which rapidly-replicating myeloid progenitor cells take over the bloodstream – this can often lead to death within months.

Work from Professor Rieger's group identified the role the HSC-regulating microRNA miR-193b plays during acute myeloid leukaemia. They found that, much like in HSCs, the presence of the microRNA had a suppressive effect on cell growth. Indeed, patients whose cancer cells did not contain miR-193b had significantly poorer outcomes for their disease. Subsequent laboratory work showed that introducing this microRNA back into leukemic tumour cells was able to significantly reduce tumour growth.

This fact, plus the role miR-193b plays in stem cell differentiation, makes it a potential target for new anti-cancer drugs. 'We found a new class of molecules, so-called microRNAs, which are misregulated in acute leukaemia,' confirms Professor Rieger, 'we are now using this knowledge to develop clinical protocols for the diagnosis and treatment of leukaemia, using these advanced therapeutics.'

### Tuning Transplants

The work so far is ground-breaking, but perhaps most exciting is the future potential of controlling these differentiation and replication factors. Control of miR-193b could open up the possibility of further improving stem cell transplants. A transient decrease in miR-193b levels in the donor stem cells could help expand the culture (removing the limitation of cell quantity) or increase the fitness of stem cells to bring the culture past the typical three to five-day limit – immensely improving the efficiency and logistics of transplantation.

Similarly, the potential to affect cancer outcomes by modifying miR-193b levels may have incredible consequences for the treatment of acute myeloid leukaemia. This is not the only goal – many different types of cancer have similar controls over cell replication and differentiation. Indeed, as Professor Rieger commented, 'we believe that we may transfer common mechanisms in blood stem cells and cancer stem cells in leukaemia to other cancer stem cells of different tissue entities. Some of the new target molecules may even be used beyond blood cancer – currently, we are also working on colon cancer, one of the most prevalent cancers in humans.'

This could lead to new therapeutic options for patients suffering from these life-threatening conditions. For the moment, at least, the future of HSCs seems brighter than ever before.

# Meet the researcher

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Michael Rieger is Professor of Basic Mechanisms in Stem Cell Biology at the Goethe University Hospital of Frankfurt am Main in Germany. Professor Rieger first began leading an independent research group in 2009, before rapidly being picked for an Associate Professor role at Goethe University Frankfurt in 2012. His successful research career (with over sixty publications and counting) soon led him to his current role as a tenured professor. His research focuses on the molecular mechanisms which underlie the development of somatic stem cells into their eventual final cell types, in healthy humans and cancer patients.

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German Cancer Consortium (DKTK)

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# USING MATHEMATICAL MODELLING TO PREDICT BIOLOGY

With unprecedented advances in scientific research comes a growing body of data. Accurately interpreting these data is a significant obstacle to an improved understanding of biological systems and their behaviour during disease. To overcome this challenge, **Dr Stuart C. Sealfon** of the Icahn School of Medicine at Mount Sinai is pioneering innovative approaches that incorporate mathematical modelling and computational prediction to further our understanding of human diseases.

### A Multifaceted Approach to Research

Researchers today are going to great lengths to improve the acquisition of scientific data and to enhance its accurate analysis and interpretation. A growing number of novel techniques are being developed and utilised to achieve this.

Dr Stuart C. Sealfon, MD, at Mount Sinai Hospital and School of Medicine in New York, USA, is one such intrepid researcher. His ongoing endeavour is to shed more light on the complex physiological functions of the body using a combination of exploratory techniques. He explains: 'My research is directed at using single cell biology, systems biology, mathematical modelling and bioinformatics to understand emergent cell responses in normal physiology and disease.'

Dr Sealfon, who also directs the multi-institutional Programme for Research on Immune Modelling and Experimentation (PRIME) – a National Institutes of Health (NIH)-funded Modelling Immunity for Biodefense Center – has pioneered numerous research approaches. These include experimental techniques used to measure the expression of various genes and the use of single-cell assays to reveal the intricacies of cellular signalling. His laboratory employs multifaceted methodologies that primarily involve the integration of mathematics, computer science and experimentation to make predictions about the responses of these complex biological systems to further understand disease processes.

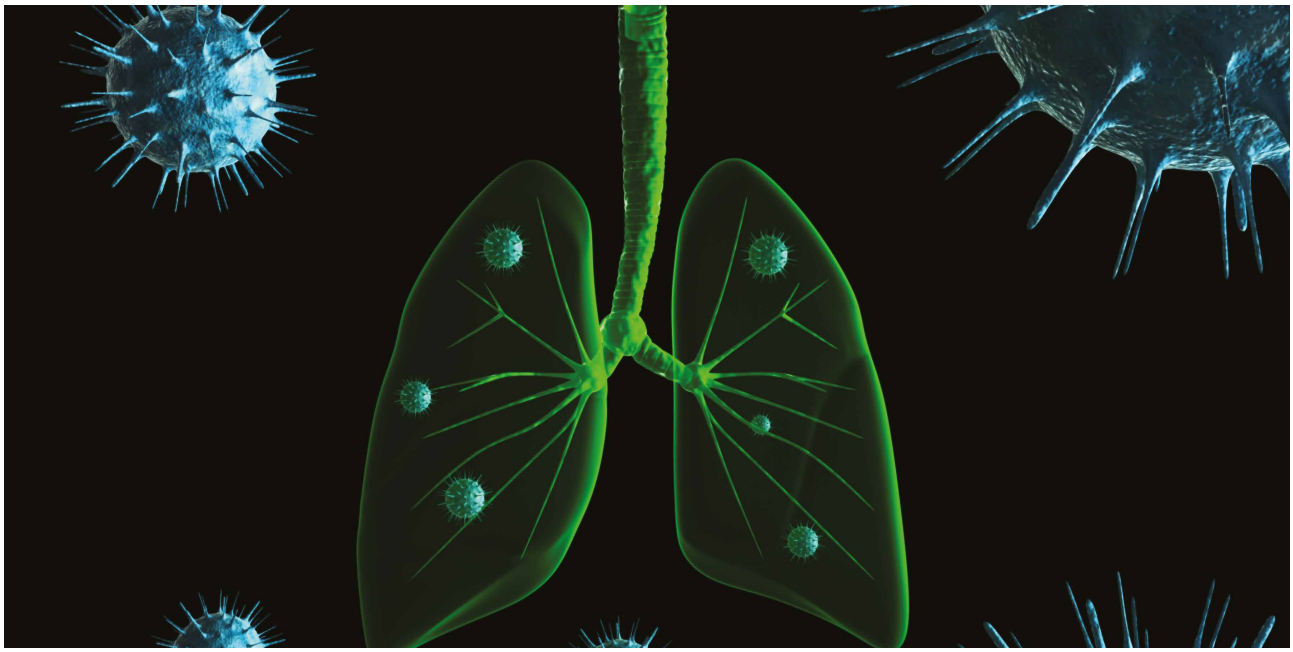
At present, Dr Sealfon and his team are undertaking five major research programmes that encompass an impressive range of scientific goals. These include the development of experimental data-validated mathematical models of the response of the human lung to influenza infection, the identification of mechanisms underlying reproductive-hormone sensitive diseases, and the improvement of blood-based diagnosis of disease and different disease subtypes.

### Pushing the Boundaries of Experimental Immunology

Over the past decade, there have been remarkable advances in the use of 'bioinformatics' – a relatively new field of research that combines mathematics and computer science with biology. Under this umbrella term exists a multitude of research approaches that have allowed significant scientific breakthroughs in immunology, particularly in our understanding of the human genome and the identification of genes and biological processes underlying human diseases.

However, the extension and interpretation of this rapidly growing collection of data are presenting

**‘My research is directed at using single cell biology, systems biology, mathematical modelling and bioinformatics to understand emergent cell responses in normal physiology and disease.’**



fundamental challenges for experimental and computational immunology researchers. Questions are emerging from our increasing understanding of the interactions between the different components of the immune system and the pathogens or disease-causing organisms that trigger immune responses.

Bioinformatic methods of scientific experimentation – such as multidimensional and high-throughput technologies that involve the screening of biological compounds for activity against defined biological targets through the use of automation or robotic systems, miniaturised assays, and large-scale data analysis – have led to the development of sophisticated mathematical models designed to address a wide range of questions that have been unanswerable using conventional scientific methods.

By applying these techniques to several immunological diseases, in conjunction with more traditional data-driven approaches, Dr Sealfon and his team are examining complex immune system pathways and identifying the relationships between their components at both the cellular and molecular level, in both healthy individuals and those with infections or immune system-mediated diseases. Ultimately, Dr Sealfon is aiming to determine how the behaviour of the immune system in health and disease occurs as a result of molecular, genetic, cellular, and environmental influences.

**Mathematical Modelling of Influenza**

At the forefront of his current research, Dr Sealfon and his colleagues, with the aid of a grant from the NIH, are investigating the early immunological responses to influenza A virus – a significant cause of annual global illness and mortality.

In the face of attack by the influenza A virus, the human immune system is able to prevent the establishment of chronic infection. However, many aspects of the development of this protective immunity and the maintenance of immune homeostasis or balance and an appropriate response in the human lung are largely unknown. Predictive immunological modelling – the use of mathematical modelling to predict immunological activity – is needed to understand this complex system.

Using a range of techniques comprising mathematical modelling, single-cell assays, the study of native and laboratory isolated H1N1 influenza A viruses, and analysis of public human gene expression data, Dr Sealfon and his team have gathered detailed immunological data on the activity of human cells in lung tissue over a relevant time course.

In particular, they have discovered more about the function of dendritic cells – cells that during infection, process and present pathogenic disease-causing materials or antigens to activate T cells, a type of white blood cell that plays a critical role in the immune response. Dendritic cells in the human lung play an important role in cellular immunity to the influenza A virus.

Notably, Dr Sealfon and his colleagues have investigated the differences between the immunological responses of dendritic cells and T cells to pandemic viral strains that have the

ability to spread widely compared to seasonal strains of influenza A viruses and have identified a novel mechanism that allows pandemic influenza A virus strains to evade the immune response. This work has far-reaching consequences, as enhanced insight into the integrated immunity to influenza A virus initiated in the lung may assist the development of viable treatments of influenza infections and improve influenza vaccines.

**Mapping Hormone-sensitive Reproductive Diseases**

In addition to his work on influenza viruses and the immune system, Dr Sealfon is also currently using an integrated research approach to uncover the underlying regulatory mechanisms behind common reproductive disorders. His work in this area is renowned, with earlier research being key in the discovery of Orilissa™ (elagolix), the first oral non-opiate therapy for endometriosis – a widespread reproductive illness affecting women that often involves long-term pelvic pain.

Similarly to the immune system, the endocrine signalling system in the body depends on complex intracellular and extracellular relationships. A key relationship exists between the nervous system and the endocrine system to control the reproductive system through the release of gonadotropin-releasing hormone (GnRH). This occurs through the hypothalamus – the portion of the brain that links the nervous system to the endocrine system via the pituitary gland.

During puberty and throughout the female menstrual cycle, pulsatile release of GnRH from specialised hypothalamic cells coordinates the biosynthesis and secretion of the important female reproductive hormones – follicle stimulating hormone (FSH) and luteinising hormone (LH). Notably, higher GnRH pulse frequencies result in greater LH secretion from gonadotropes, specialised endocrine cells in the pituitary gland, whereas lower GnRH pulse frequencies result in greater FSH production.

Although this action is relatively well known, a complete understanding of the signalling mechanisms supporting this regulatory system is lacking. To overcome this shortfall of knowledge, Dr Sealfon and his team developed three biologically anchored mathematical models to help identify the genes responsible for varying GnRH pulse frequencies. This task was aided by the employment of a high-throughput experimental system that permitted the team to analyse over 4,000 gonadotrope cell samples.

The ongoing progress of this research has high potential. 'We aim to determine the mechanisms underlying the complex response to hormonal stimulation patterns of the pituitary gonadotrope cell that underlies reproduction and the alteration of this system in reproductive-hormone sensitive disease,' says Dr Sealfon. Indeed, the successful development of a predictive model of gonadotropin function that incorporates both intracellular and extracellular regulatory activity and is validated in human patients has the potential to advance the treatment of many hormone-sensitive diseases.

**Expanding the Horizon of Scientific Research**

In short, Dr Sealfon's research accomplishments are wide-ranging and have addressed a variety of research topics, such as finding new signalling pathways that are activated by drugs for Parkinson's disease and finding a new brain receptor complex implicated in schizophrenia as a potential target for novel antipsychotic medication.

In addition to disease mapping, his projects also include collaborating on the development of a gene map of the molecular responses to acute exercise and exercise training. Additionally, to encourage collaboration across scientific fields, particularly between modellers and experimentalists, Dr Sealfon and his team have contributed to the development of a range of computational tools and resources for interpreting large-scale experiments and the mining of public data.

These efforts have significant value, as greater success in predictive analysis will benefit from the additional data and the subsequent release of more experimental and clinical trials data to the public. This could prove invaluable for new scientific discoveries or for predicting why drugs might succeed or fail in the clinic.

# Meet the researcher

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Dr Stuart C. Sealfon, MD, is Glickenhaus Family Professor and Chairman Emeritus of the Department of Neurology, the Director of the Center for Advanced Research on Diagnostic Assays (CARDA), and Professor of Neurobiology and Pharmacology and Systems Therapeutics at the Icahn School of Medicine at Mount Sinai in New York. Dr Sealfon joined the Icahn School of Medicine in 1986, after completing his medical training at Columbia University and his post-doctoral internship and residency at Massachusetts General Hospital in Boston. He is the author of multiple book chapters and is currently the Review Editor of *Molecular and Structural Endocrinology* at *Frontiers in Endocrinology*. He has contributed to more than 100 original research articles and holds various patents for his work including work on the Gonadotropin Releasing Hormone Receptor which led to the new drug to treat endometriosis elagolix (Orillisa™).

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## FUNDING

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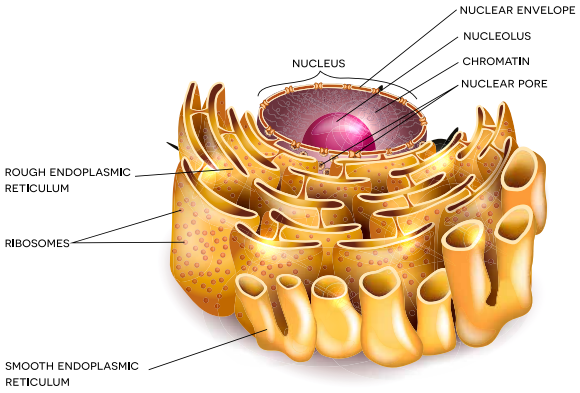
Icahn School of Medicine at Mount Sinai

# HEALTH STARTS IN THE CELL: APPROACHING OBESITY FROM THE INSIDE OUT

Over the last few decades, obesity has become substantial public health concern. Obesity is associated with a myriad of other ailments and is on the rise in most developed countries. As with many chronic diseases, the development of an obese body type is often more complex than expected and involves a combination of environmental factors, genetic predisposition, and lifestyle choices. **Dr Yi Li**, of Texas A&M University – Kingsville, studies interactions between our environment and the inner workings of our cells to generate novel insights about chronic diseases such as obesity.




*Nucleus and endoplasmic reticulum*

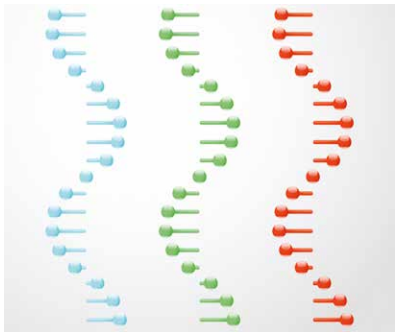
**On Top of Your Genes**

Two decades ago, scientists were eagerly looking to genetics to unlock all of the mysteries about chronic diseases, hoping for a silver bullet to cure all human ails. Researchers were on the hunt for *the* gene for obesity and *the* gene for diabetes and *the* gene for male pattern baldness. As with many things in life and in science, it turned out that the closer they looked, the more complicated it got. While science has identified gene variations that appear to play a role in some chronic diseases, no obvious villains emerged. In fact, it became even more obvious that regardless of genetics, factors such as nutrition and lifestyle played important roles in health outcomes.

It makes sense that scientists were looking to genes for answers – genes influence our health by providing instructions for a cell to make proteins. These proteins go on to shape the behaviour of the cell, which shapes the behaviour of the tissue that cell resides in, ultimately shaping every aspect of our overall health and behaviour. The type of proteins that a given cell makes influences the type of cell it becomes during development. However, every cell in your body, from the neurons in your brain, to the light receptors in your eye, to the cells that form your skin, has the exact same set of genes but is only expressing the genes it needs to perform its duty. Alterations in the rates that a certain cell type expresses different genes are often associated with disease conditions, such as obesity, diabetes, and cancer.

So, what determines which genes a cell expresses and how often it expresses them? A suite of processes collectively known as epigenetics. Epigenetics literally means 'on top of genetics' – these processes control which genes are expressed and when, but are not controlled by genes themselves. The technology to study many epigenetic processes has only become widely available in the past decade, so we still have a great deal to learn about the role they play in health. What has become clear is that unlike genes, many epigenetic features can be readily influenced by factors such as diet and lifestyle. Epigenetics represents the link that ties our life experiences to how our genes are expressed.

**‘I have been trying to address how endoplasmic reticulum stress and epigenetic modifications induced by nutrition factors, including elevated blood lipid and glucose levels, are involved in regulation of genes related with development of obesity and type 2 diabetes’**



Dr Yi Li, an Assistant Professor of Nutrition at Texas A&M University-Kingsville, is working to understand how these external factors shift the activity inside our cells in hopes of identifying targets for treatments that could someday help people struggling with chronic metabolic diseases. He explains, ‘I have been trying to address how endoplasmic reticulum stress and epigenetic modifications induced by nutrition factors, including elevated blood lipid and glucose levels, are involved in regulation of genes related with development of obesity and type 2 diabetes.’

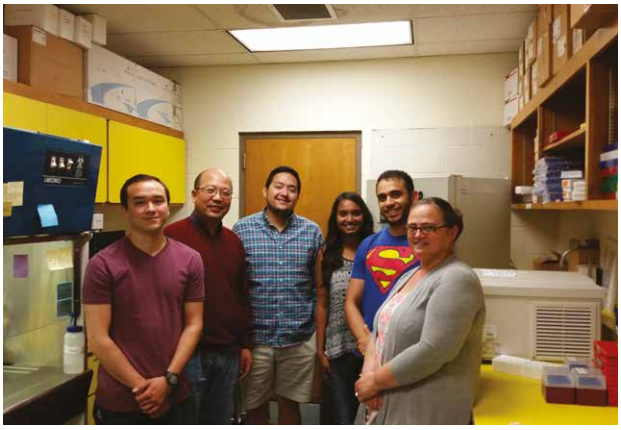
**Managing Stress**

Dr Li’s fascination with the inner workings of the cell and its role in chronic disease began during his graduate work on the endoplasmic reticulum (ER). The ER is a cellular organelle that resides in every cell in your body. Along the curved walls of the ER, your cells build the proteins and fats that influence nearly every activity that occurs in your body. The majority of your hormones, neurotransmitters, and the building blocks for your cells are manufactured in the ER. Proper function of the ER is critical for cellular health, so when the organelle becomes distressed it can cause the cell to shut down and even die.

Transcription factors are a type of proteins with the ability to start the process of translating genes into new proteins. Dr Li’s ER research focused on a group of transcription factors that are often involved in metabolism and stress responses. He found during stressful events, such as cellular starvation, one of these factors, known as C/EBPß, is synthesised using the genetic information from one single gene as two isoforms: LIP, a transcription repressor and LAP, a transcription activator. Early during a stress event levels of the two factors remained similar, but as stress wore on, the transcription repressing factor became more prevalent under some situations. Ultimately this combination of LAP and LIP began to repress the expression of factors that are protective against the effects of stress, while allowing genes that induce cell death to be translated. In adipose tissue, the levels of LAP and LIP isoforms of C/EBPß are regulated by ER stress and they are subsequently involved in regulation of other genes functioning in adipogenesis in development of obesity.

Dr Li’s results demonstrate how responses to metabolic stress are complex and can damage cells over time, as well as identify potential targets, such as LIP, for stress preventative treatments.

**‘In recent years, we have revealed that saturated fatty acids and polyunsaturated fatty acids differentially regulate genes involved in adipogenesis via epigenetic modifications.’**



**Epigenetics and Obesity**

As Dr Li’s career has progressed, he has become interested in other cellular mechanisms that modulate short term cellular responses to changing conditions, particularly those that are involved in obesity. He has shifted his focus to epigenetics in hopes of understanding how these processes that alter gene expression are influencing the cell. Dr Li’s work focuses on two key epigenetic processes: DNA methylation and microRNAs (miRNA).

DNA methylation occurs when a methyl compound attaches to a cytosine – one of the four nucleotides that compose DNA. Methylated DNA is often expressed less frequently than its unmethylated counterparts. Numerous studies have demonstrated that methylation is influenced by changes in nutrition and exercise, along with other environmental factors.

Messenger RNA (mRNA) is intermediary between DNA and the cellular components that build proteins. During protein synthesis, mRNA copies of a gene are made and then transported to the ER for translation into a protein. miRNA are tiny snippets of RNA that alter gene expression by interfering with this process, usually by binding with the RNA copy of the gene and rendering it useless. As with DNA methylation, miRNA usually serves to decrease the expression of a gene.

While epigenetic processes such as DNA methylation and miRNA production can be influenced by events that occur during your life, they can also potentially be passed on to future generations. Not all epigenetic marks can be passed to children, but those that can have a strong chance of influencing childhood, and subsequent adult, health. Dr Li is interested in determining which epigenetic factors could potentially pass to an infant and place the child at higher risk for obesity. His current work is identifying methylation and miRNA markers that are associated with obesity in adults, determining their function in the cell, and finding if it is possible for these markers to be passed on to the next generation.

**Shaping the Future of Preventative Medicine**

In a five-year project plan, Dr Li and his team are planning to track the health of obese parents and their new born children to identify how these factors come together to shape childhood risk of obesity. In preliminary studies, Dr Li has made some critical discoveries that could shape how we approach childhood health. He describes, ‘In recent years, we have revealed that saturated fatty acids and polyunsaturated fatty acids differentially regulate genes involved in adipogenesis via epigenetic modifications.’

Put simply, Dr Li has demonstrated that exposure to saturated and polyunsaturated fats shift epigenetic mechanisms in the cell that play a role in the formation of new fat cells. He has found evidence that changes in both DNA methylation and miRNA activity take place in response to these nutritional events, influencing both metabolism and fat cell growth. Interestingly, many of the same factors involved in ER stress are influenced by these diet-induced epigenetic changes. If it is found that these changes can be passed on to future generations, it could hold the key to preventative interventions for children at high risk of developing obesity.

**Building a Healthier Future**

As our understanding of the relationship between our life experiences and the intricate workings of our cells deepens, it has become clear that genetics alone will not solve the world’s health problems. Innovative research in epigenetics and cell biology will reveal clues about how our environments shape our health and how that influences the health of our children. Dr Li’s research program will provide a foundation for future treatments to prevent obesity and other metabolic conditions in the next generation.

# Meet the researcher

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Dr Yi Li obtained his PhD in Nutritional Biochemistry from Case Western Reserve University (Cleveland, Ohio, USA) in 2009. The focus of his doctoral work was regulation of gene expression by endoplasmic reticulum stress. Following postdoctoral positions at Yale School of Medicine (New Haven, Connecticut, USA) and Duke University (Durham, North Carolina, USA), Dr Li joined the faculty of Human Nutrition program at Texas A&M University-Kingsville, where he currently serves as an Assistant Professor of Human Nutrition. Dr Li's current work focuses on nutrition induced epigenetic modifications that regulate gene expression in obesity and type 2 diabetes. Dr Li is an active research supervisor and serves on the editorial boards of several journals in his field of expertise as part of his ongoing contribution to science.

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# LET THERE BE LIGHT!

**Professor Yubin Zhou**, from the Center for Translational Cancer Research at the Texas A&M University Institute of Biosciences & Technology, USA, is developing ways to use light to control cellular function. The researcher and his team are responsible for a series of breakthroughs in this field – known as optogenetics – including the exciting concept of using this method to develop novel ways to treat cancer.

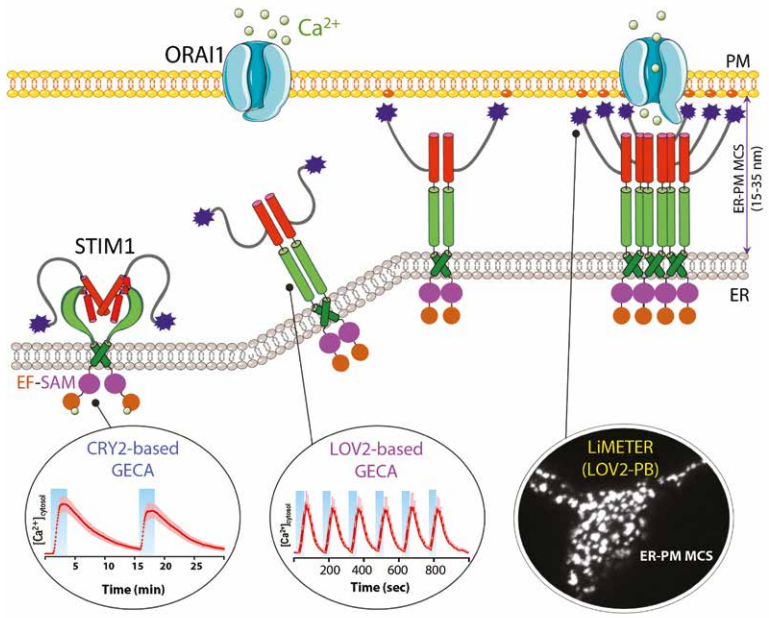



*Figure 1. Optical control of calcium signalling at membrane contact sites. Credit Yubin Zhou.*

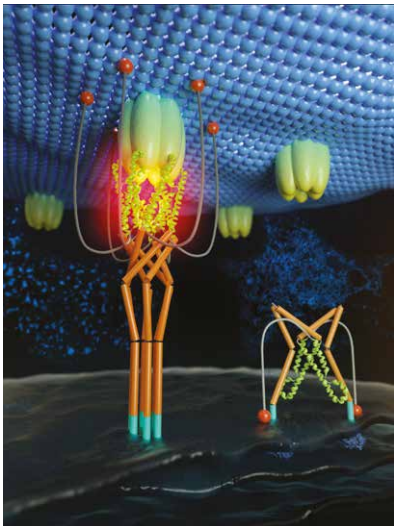


*Figure 2. Transporting Calcium. Credit Yubin Zhou.*

The ability to control what cells do is vital to understand cellular mechanisms. It may not be the most obvious option, but control with light has become a popular approach in a field known as optogenetics (Figure 1). Early work involved neurons genetically modified to express channels in the cellular membrane that were sensitive to light. This represented a simple level of control with light on to open the channel and light off to close it.

In the past few years, there's been a boom in this field and many researchers are now starting to consider whether this can be applied in other areas. One of them is Professor Yubin Zhou, from the Center for Translational Cancer Research at the Texas A&M Institute of Biosciences & Technology. 'My group is working on developing innovative tools by combining optogenetics and cell signalling,' explains Professor Zhou. 'These light-sensitive tools allow us to control the flow or localisation of signalling molecules, proteins, and enzymes, so that we can manipulate biological pathways and gene expression in a non-invasive and reversible manner.'

The idea is sound, but with immune cells instead of nerve cells as their main target, the team had a major challenge to overcome first. Stationary nerve cells are easy to reach, but what about fast-moving immune cells? How to develop a way to shoot accurately at a moving target?

**Calcium Signalling**

Even before the optogenetics idea, one of the early interests for Professor Zhou's laboratory has been a deeper understanding of cell signalling with a focus on calcium signals. This ion may be present only in small amounts in the cell, but it is crucial in controlling a variety of processes, from muscle contraction and communication between neurons, to gene expression and even cell death. Not surprisingly, abnormal activity has been linked to many different conditions, including cancer and cardiovascular problems.

As the signal relies on sharp and effective releases of calcium from its cellular stores (the endoplasmic

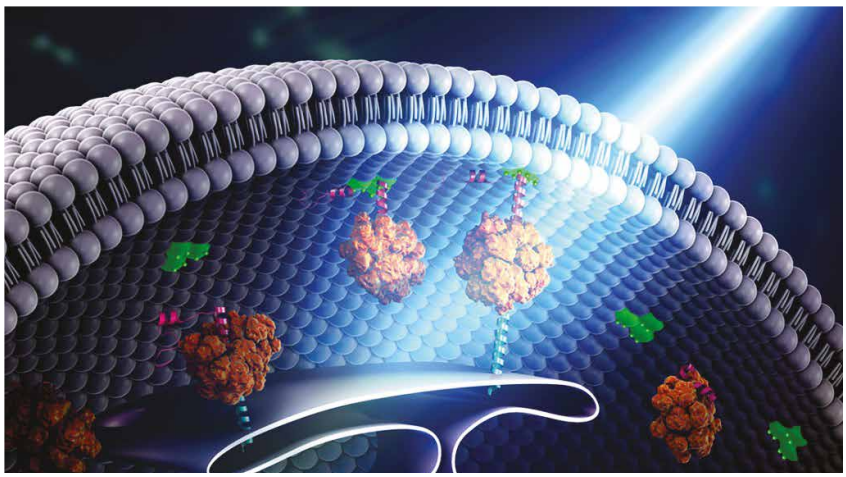


*Figure 3. Engineered photosensitive proteins serve as the building blocks of the 'bridge' between organelles in the cell. Credit Yubin Zhou.*

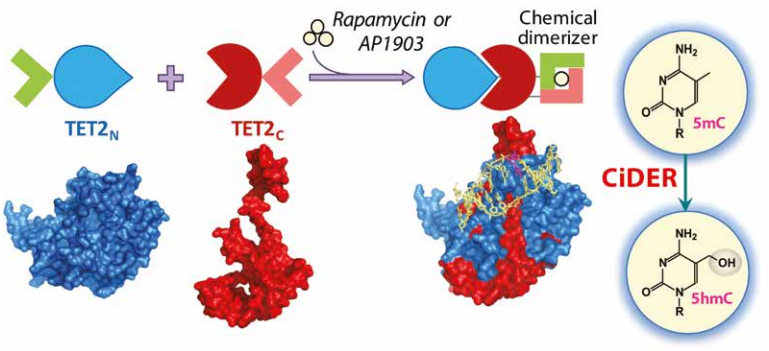



*Figure 4. Design of a Chemical-Inducible Epigenome Remodeling (CiDER) Tool Based on a Split TET2 Enzyme. Credit Yubin Zhou.*

reticulum being the most important) or sudden calcium influx from the extracellular space, it is crucial to maintain the correct calcium levels. To achieve this, cells resort to specialised sections linking the cellular membrane and the endoplasmic reticulum. Curiously, these structures don't even touch, but recently researchers are beginning to understand their importance in regulating calcium signals.

Understanding these signals has been on Professor Zhou's agenda since 2015. The first step included the identification of all the proteins involved in transporting calcium, which when put all together form a multi-protein structure spreading across the membrane (Figure 2). Further studies unravelled conformational changes in this complex to regulate calcium influx. During rest, everything stays closed and inactive. When there's a need for a burst of calcium, however, there's a quick change with two arms extending to open the gates.

Surprisingly, Professor Zhou also found different sensitivities to calcium in these structures. Some can be fast but need large calcium fluctuations to work, whereas others have a weaker binding affinity to calcium and are more responsive to small fluctuations. Professor Zhou and his team believe this combination makes for efficient regulation of calcium levels in the cell.

**The Power of Light**

It turns out this early work studying calcium channels was essential in solving Professor Zhou's big challenge: that is, to develop a new system to remotely control immune cells using light. Eventually, the team managed to design a way to mimic the conformational changes in calcium signalling described earlier. In essence, Professor Zhou's team created a form to trigger calcium release at will in immune cells following light stimulation.

As a test, the team genetically engineered immune cells with a specific protein involved in calcium signalling becoming light sensitive (designated 'Opto-CRAC'). Through collaboration with a nanotechnologist, Dr Gang Han, the team then used a laser to penetrate deep (in this case, deep means about a centimetre or two) into the mouse's thigh, where nanoparticles converted the laser light into blue light. Exposing immune cells to blue light from the nanoparticle opened the calcium gates to release calcium, whereas darkness stopped the flow. The system even has a built-in fine-tuning mechanism, where more light increases calcium release and less light means a gentle trickle.

It's not difficult to imagine that these light-based techniques could allow 'remote control' of immune responses within the body, in particular in combination with immunotherapy to fight cancer at a specific time and location. 'My group is among the first to advocate the concept of optogenetic immunomodulation to advance next-generation anti-cancer immunotherapies with reduced off-target cytotoxicity,' notes Professor Zhou.

The fact that it can be switched on and off easily means this method could represent a much more accurate approach to treat cancer, with a low incidence of the type of side-effects common in other therapies. 'It's quite a cool technology. With these tools, we can now not only answer fundamental questions of science that we never could before but also translate them into the clinic for disease intervention,' added Professor Zhou.

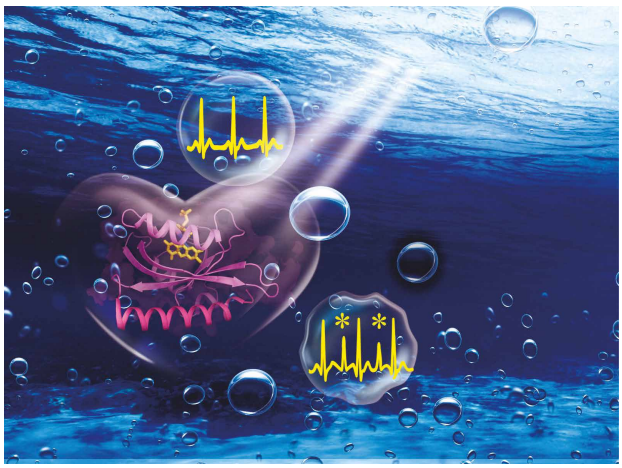
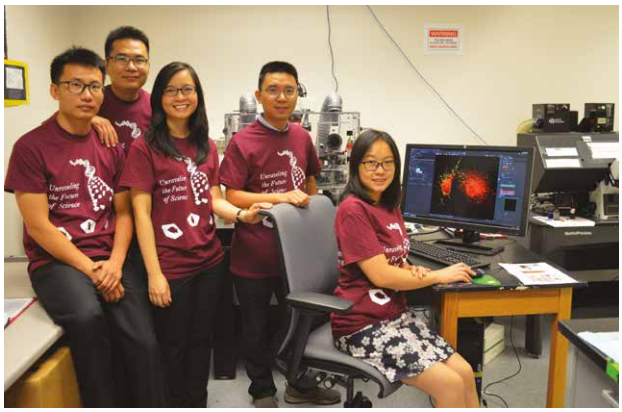


*Figure 5. Non-invasive Control of Voltage Gated Calcium Channel to Intervene with Cardiac Arrhythmia.*



*Professor Yubin Zhou and His Team at the Texas A&M University Institute of Biosciences & Technology.*

**Cellular Communication**

Professor Zhou and his collaborator Dr Yun Huang very quickly realised there was a variety of potential uses for their light-induced method. Their first approach was to couple light-sensitive tags to proteins bound to membrane lipids which are involved in forming the bridges between cellular organelles (Figure 3).

Following blue light illumination, this new system – named OptoPB or LiMETER by the researchers – forces the target to undergo conformational changes to expose the region that binds to membranes. This new method proved to be quite a success and the team even found that they could manipulate the contact region and the distance between membranes, as well as control the movement of proteins in the membrane with a simple blue light.

This work focused on the link between the cell membrane and the endoplasmic reticulum, but the team's ambition is to build bridges between other organelles, such as between the endoplasmic reticulum and mitochondria. For Professor Zhou, this is just a glimpse of what can potentially be achieved in the future in cell biology and other fields.

**Time for a Cider**

The next use of light and optogenetics involved controlling epigenetic changes. To put it simply, these changes modify the activity of certain genes, but only on a superficial level and do not involve modification to the actual genetic code sequence. Researchers know these are involved in multiple conditions, including cancer, Alzheimer's disease, and developmental disorders, but it's still not obvious whether these changes are the cause or a consequence of such conditions.

This question has been impossible to answer because the tools to generate the desired DNA modifications are very limited. But Professor Zhou has developed a system that may provide a workable solution. The new tool allows researchers to control with precision specific epigenetic changes. Crucially, the system can be controlled with a drug and reverses without it, giving Professor Zhou and his team absolute control over epigenetic changes. They named it CiDER, which stands for chemical-inducible epigenome remodelling tool (Figure 4). By replacing the chemical-responsive modules with photosensitive domains, the human epigenome can be conveniently re-sculptured with a simple flash of light.

At the moment, the system still lacks control over where the changes occur, but the researchers want to use the genetic tool CRISPR to improve specificity. Once this issue is overcome, Professor Zhou foresees enormous potential for this tool in any field involving epigenetic changes, and not just for cancer studies.

**After Cider Comes the Carrots**

Keen to pursue the CRISPR avenue, the team's next step was to design a method to use the same light-induced calcium signalling to carry this genome-engineering tool. The method, which the team called CaRROT (for calcium-responsive transcriptional reprogramming tool) – is simple. In the dark, nothing happens. However, when the light is switched on, the passageway controlling calcium signals opens, which in turn triggers expression of specific genes through CRISPR.

This approach allows the researchers to decide exactly how, when, and where genes are programmed to perform (or not) a desired function. The future goal is to control cellular differentiation in stem cells, which can then be used in regenerative medicine. It remains possible that the day will come when doctors can grow a new organ by simply shining a light on a petri dish.

**The Opposite Trick**

Finally, Professor Zhou is also the mastermind behind a new optogenetic tool with the opposite objective: Instead of triggering a calcium signal, when the light shines, calcium influx can be turned off.

**‘With these tools, we can now not only answer fundamental questions of science that we never could before but also translate them into the clinic for disease intervention’**

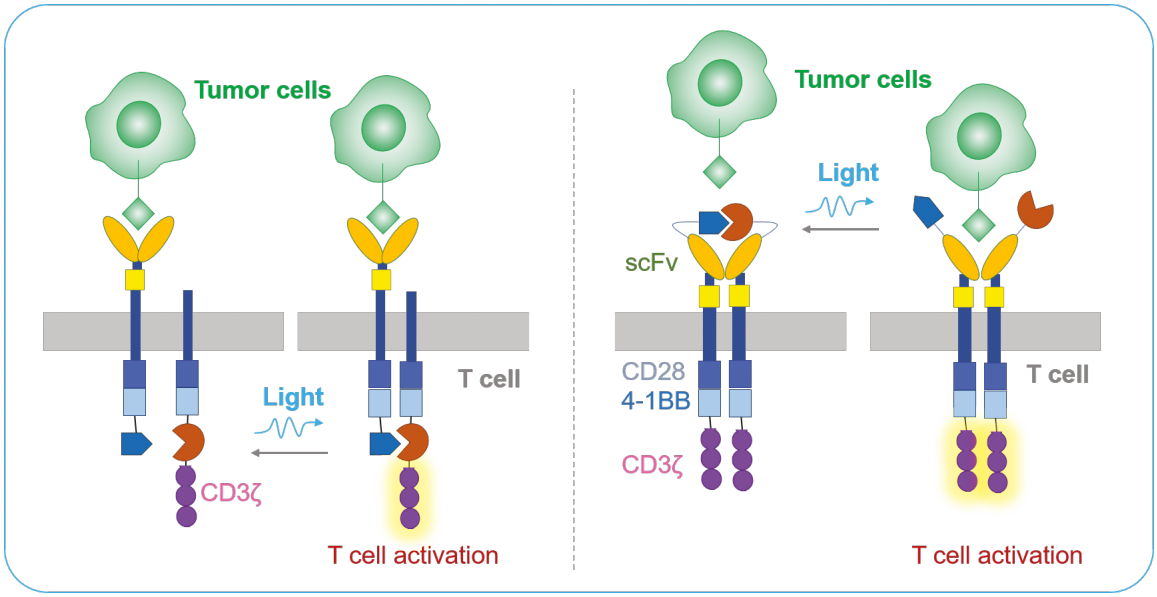



*Figure 6. Design of Photo-switchable CARs for Optogenetic Immunotherapy. Credit Yubin Zhou.*

There are already several calcium-channel blockers commonly used to treat cardiovascular conditions including high blood pressure, arrhythmia, and coronary artery disease. However, with side-effects including headache, dangerously low blood pressure, and palpitations, a new approach to complement traditional medication is eagerly awaited.

There may be good news coming soon on this front, as tests in cardiac muscle cells have shown a significant reduction or even stopped calcium release altogether in response to the blue light, and then restarted when the light was switched off.

This approach – which the researchers termed optoRGK – promises unprecedented accuracy and, in combination with all the other light-based tools, is ideal for regulating many processes in many different cellular mechanisms (Figure 5). The team believes their work will one day drive a new generation of optogenetic devices for treating a variety of diseases, including cancer and cardiovascular conditions.

**Translational Research**

Chimeric antigen receptor (CAR) T-cell based immunotherapy has demonstrated curative potential for the treatment of leukemia and lymphoma. Recently, the FDA has approved two CAR T-cell therapies (Yescarta and Kymriah) to treat certain types of blood cancer. Nevertheless, due to a lack of predictable and precise control of the dose, location, and timing of T cell activity, CAR-T cell therapy has been associated with significant safety issues, as notably exemplified by ‘on-target, off-tumor’ toxicity and cytokine storm syndromes. These side-effects have had devastating consequences for certain patients in clinical trials, and pose significant limitations on the use of the current CAR-T therapy. The team aims to tackle this challenge by developing the next generation ‘optogenetic immunotherapy’ in which precise control over anti-tumor immune responses will enable personalised tuning of the amplitude, duration, and location of the treatment (Figure 6). Such intelligent cell-based therapy makes it possible to tell when and where to launch a fierce attack toward tumor cells while sparing healthy ones, thereby mitigating the potential toxicity encountered in current anti-cancer treatment.

**A Rewarding Journey**

For Professor Zhou, the optogenetic engineering of calcium channels turned out to be an extremely rewarding journey. It started with calcium signals and ended with a series of optogenetic and chemical biology tools, including OptoPB, LiMETER, Opto-CRAC, CaRROT, CiDER, and optoRGK, to harness the power of light to control calcium entry and gene expression in cells, and ultimately in living organisms.

‘We have been constantly translating what we have learned from basic studies on ion channels and signalling proteins toward the development of molecular toolkit and drug candidates. The invention of innovative optogenetic tools has enabled life scientists not only observe but also to perturb biological processes at unprecedented precision. We are more like Bob the Builder, very keen to craft new toys for biomedical researchers to play with for the purpose of technical and conceptual innovation,’ concludes Professor Zhou.

# Meet the researcher

**Professor Yubin Zhou**
Center for Translational Cancer Research
Institute of Biosciences and Technology
Texas A&M University
Houston, TX
USA

After completing his medical training in 2003 and a PhD in chemistry at Georgia State University in 2008, followed by a 2-year postdoctoral position at Harvard Medical School, Professor Yubin Zhou joined La Jolla Institute for Immunology in 2010 and eventually moved to Texas A&M University where he currently holds the position of Associate Professor. Research on calcium signalling and optogenetics has rewarded Professor Zhou with almost 100 published manuscripts in high-impact journals and four patents. In addition, he has received multiple awards including the John S Dunn Foundation Collaborative Research Award in 2018 and the High-Impact/High-Risk Research Award, Cancer Prevention & Research Institutes of Texas in 2017. Professor Zhou also teaches at Texas A&M University and has mentored a long list of undergraduate and postgraduate students, many of whom have progressed to receive awards themselves.

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TEXAS A&M UNIVERSITY

INSTITUTE OF BIOSCIENCES & TECHNOLOGY

# WORLDWIDE CANCER RESEARCH

Worldwide Cancer Research is a UK-based charity, founded in 1979. It funds research into all types of cancer across the globe, specifically focusing on early-stage basic laboratory science aiming to provide the seeds of discovery which may ultimately lead to innovation in the prevention, diagnosis, and treatment of cancer. In this exclusive interview, we speak with Worldwide Cancer Research's Chief Executive Officer, **Dr Helen Rippon**, to find out more about the organisation's important work and exciting vision for driving forward the battle against cancer.

**To begin with, please can you tell us about the missions and values of Worldwide Cancer Research?**

Worldwide Cancer Research funds pioneering research projects into any type of cancer, anywhere in the world. The reason? To gain a truly global perspective. Research does not happen in isolation – the answers will not come from one scientist, in one laboratory, in one country.

This worldwide research will enhance our understanding of cancer and help find and develop better, more effective treatments. Since 1979, the charity has invested over £183 million directly into cancer research. We have funded 1,817 projects for 1,120 researchers across 34 countries.

Our mission is to enable researchers to deliver the new discoveries that will save millions of lives and realise our vision of ensuring no life is cut short by cancer.

**What sort of research do you focus on funding?**

Our focus is on discovery research – the basic and fundamental science that will help us explain cancer at a cellular and molecular level. It is well established that clinical advances in cancer only emerge because of the foundations laid by this type of research, and it is at the start of the research journey where we believe we can make the most impact. If we don't support the scientists who want to answer the basic questions about the biology of cancer, we would never get to the position where new treatments or new diagnostic tests are ready to be tested in patients. That journey, from the laboratory to the clinic, can take two or more decades to complete. If we don't take that step out the door, we risk never reaching our destination – and that's the contribution that we hope to make as an organisation.

**Cancer is, of course, a worldwide problem. How do you ensure that your focus is also truly worldwide?**

We believe the best way to beat cancer is to fund the best cancer research in the world. Science is international and it is collaborative. We fund the scientist, not the institute, and have funded UK scientists in France and Australia and Spanish and Greek scientists in the UK. Research is international and this is why we believe we should be. The best scientists move around to gain expertise and knowledge. They collaborate and work with other researchers around the world and move to where they can do the best work they can. For us, it doesn't matter where the researchers are from or where in the world the work will be done, just as long as it is pioneering and will help find the answers to cancer.

We know that the body of knowledge that sits behind diagnostic tests, evidence-based public health interventions and blockbuster cancer drugs involves discoveries made by researchers from many different countries working towards a common goal. Take the new cancer drug olaparib as an example. We are proud to have helped fund initial research in the UK that helped to develop this drug, but we know that the discoveries that underpin how this drug works and its success in the clinic are down to scientists not only here but also in places such as the US and Japan. We can't forget that the evidence that these drugs work in people often comes from clinical trials led by international consortiums involving doctors, researchers, and patients based all over the world.

## ‘We believe the best way to beat cancer is to fund the best cancer research in the world. Science is international and it is collaborative.’

**What are the major obstacles to current research on preventing, diagnosing, and treating cancer? How might Worldwide Cancer Research help overcome these?**

Peering into a crystal ball when it comes to scientific advance is never easy. The exploratory nature of research means you can never really guess at where the next big step is coming from and so it is probably best to resist making any grand predictions! I would guess that the trend towards personalised medicine will continue to gradually figure out which cancer patients will benefit most from which cancer drugs. And that immunotherapy – drugs and cell therapies that harness the power of the body’s own immune system to attack cancer - will become a mainstay of treatment for more and more types of cancer.

But those guesses are based on things we already know – science which has already been done. Worldwide Cancer Research exists to discover the new things, to start new trends, and kick off lines of research that we probably couldn’t even guess at yet. Our role in this is to be a facilitator of scientific evolution and discovery – and thereby to operate in an open-minded way, comfortable with the inevitable risk involved in seed-funding new ideas. If I had a concern about the future it would be this: that the mounting pressure on scientists from funders to make their science immediately translational and articulate from the very start a pathway from ‘bench to bedside’ risks squeezing out the truly novel exploratory work. We know that many of the drugs we use today sprang originally from science that was done simply to understand human biology, not to make a new drug. Indeed, olaparib falls into that category. Solving cancer is still, sadly, not a technical problem of assembling and integrating all the things we know and putting them in the right order. We have fundamental knowledge that is simply missing and generating that key understanding is going to take much, much more curiosity-driven research in the laboratory. Worldwide Cancer Research will always support that, but I hope other, larger funders will too.

worldwide cancer research

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