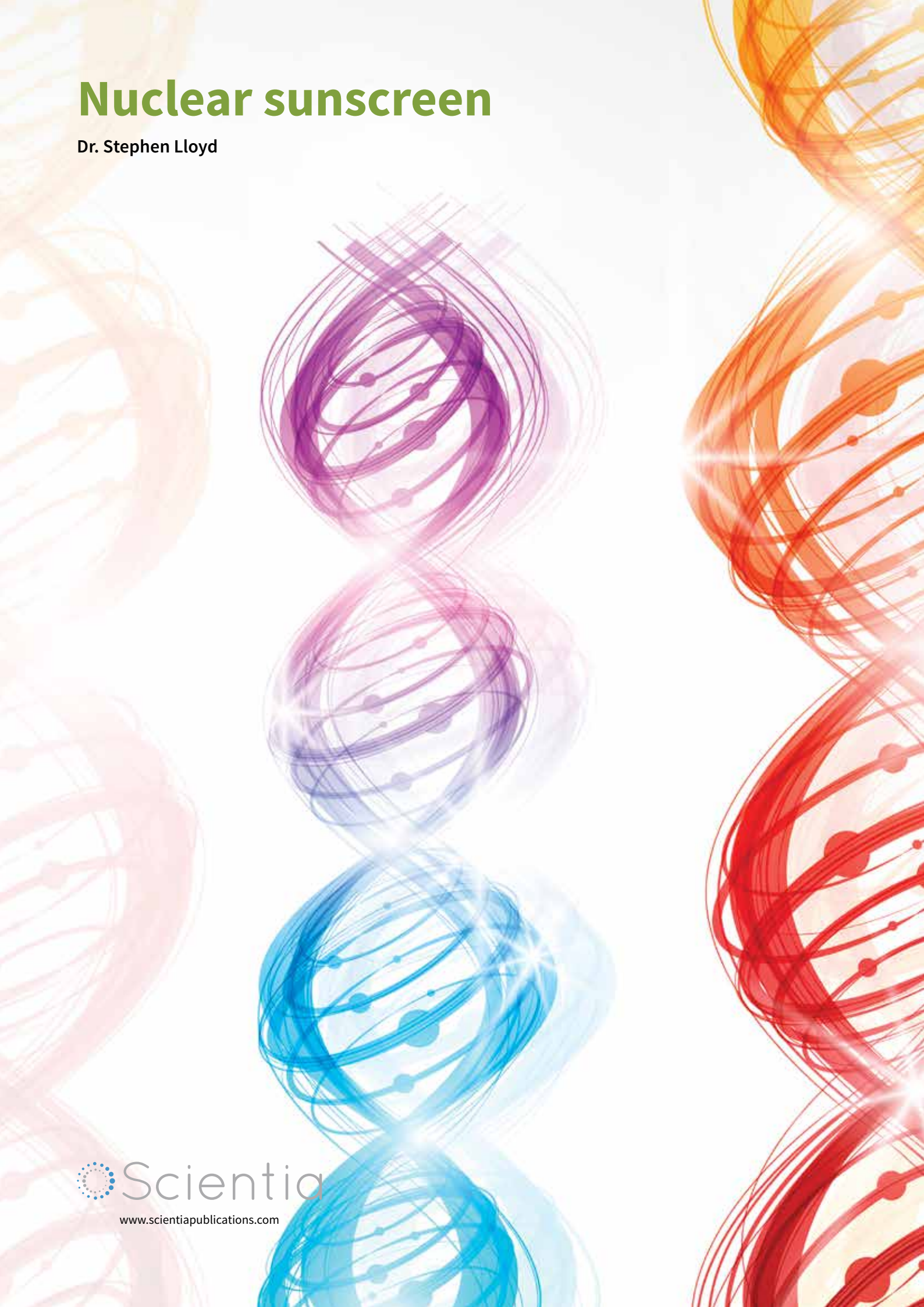
Nuclear sunscreen

Dr. Stephen Lloyd



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Nuclear sunscreen

Dr. Stephen Lloyd has been working on the mechanism of DNA repair after UV damage. Here he talks about the development of a protective enzyme, cv-PDG.

To begin, could you tell us more about yourself? What brought you into this field?

As a graduate student at MD Anderson Hospital and Tumor Institute, I worked on the mechanism of action of the anticancer drug, bleomycin, which creates a plethora of DNA damage leading to effective tumour cell killing. After learning about an agent that creates so much DNA damage, I wanted to learn how cells respond to this damage and their strategies to repair it. As a postdoctoral fellow with Philip Hanawalt at Stanford University, I began studies to characterize enzymes that would potentially augment DNA repair of ultraviolet light-mediated DNA damage. These investigations led to a career-long pursuit to ultimately develop these UV repair enzymes into therapeutically relevant molecules.

Thirteen years passed between the characterisation of cv-PDG in 1998 and the cv-PDG-NLS-TAT variant reported in 2011. What made you decide to come back to the protein and continue development?

The progression and timing of research investigations are largely driven by successes in obtaining peer-reviewed funding. For more than two decades, our NIH R01 funding from the National Institute of Environmental Health Sciences had UV-induced carcinogenesis as a central focus. However, these investigations were primarily directed toward understanding the structure-activity relationships for this class of enzymes, and the contexts in which activation of the repair pathway for pyrimidine dimers could be beneficial to eukaryotic cells. In none of those studies were we specifically trying to develop a drug for therapeutic trials.

However, given the clinical successes of AGI Dermatics in using the bacteriophage T4 pyrimidine dimer glycosylase in xeroderma pigmentosum patients, and knowing that the Chlorella virus enzyme was superior in catalytic efficiency, thermal stability, and substrate specificity, we considered that this enzyme might be significantly more effective than their T4 enzyme. Further, data showed that when the T4 enzyme was introduced into cells, very little of it became localized to the nucleus where it needed to function.

To address this problem, we genetically modified the enzyme to contain nuclear localization sequences and, to overcome challenges associated with the transdermal delivery of an intact enzyme, we adopted the TAT delivery peptide as a modification for efficient delivery to cells. However, to move these types of studies from the basic to the applied sciences, it was necessary to form Restoration Genetics, Inc. and avail ourselves of NIH-sponsored small business grants. Through these funding mechanisms, we have been able to guide studies that take these enzymes through large-scale fermentation, purification, and encapsulation, with preclinical efficacy, pharmacology and toxicology trials.

Do you think the Cv-pdg protein will be incorporated into products such as sunscreen for general audiences, or will it be a prescription product for those at higher risk of skin cancer?

As a genetically modified form, containing the nuclear localization sequence or the TAT delivery peptide, these enzymes would be classified by the FDA as drugs. Thus, they would not be sold in an over-the-counter formulation. Even if the enzyme was purified from virally infected green algae, with no genetic modifications, it is unclear that it could be sold in a sunblock formulation. The reason for this is that the function of the enzyme is to change the structure of DNA from a damaged form (the cyclobutane pyrimidine dimer) to its original, undamaged state – and the FDA classifies any molecule that alters the structure of DNA as a drug. We believe that it will be important for the FDA to consider whether natural product-based therapeutics that restore DNA to its previous undamaged state warrants classification as a drug. If this hurdle could be overcome, then commercial sunscreens could contain this purified repair enzyme.

You have started a spin-off company, Restoration Genetics, to commercialise this discovery. Could you tell us more about your thoughts and experiences during this process?

Even though there are many parallels between directing and managing a basic research laboratory and the founding and operations of a small business such as RGI, there are unique challenges to maintaining the success of the company. The first is that there are distinctly different goals for our company versus the specific aims of a basic research grant: milestones versus hypothesis-driven investigations are significantly different and required changes in management that we had not previously experienced in academic research.

Also, the timeframe and cost to bring a fundamental basic science discovery to the first clinical trial is intimidating. In this regard, although there are multiple funding mechanisms to launch ideas, the processes to further refine lead compounds, define their markets, and the competition for the next major infusion of funding have been a continuous challenge. In this entrepreneurial environment, tenacity to contribute to the betterment of human health and wellbeing is essential.

Restoration Genetics has been successful in obtaining government development grants. Where to from here?

The governmental grants that we have obtained have been absolutely instrumental in achieving the successes that have accrued to date. The outcomes of the preclinical toxicology and efficacy studies will largely inform and guide the remaining preclinical investigations required by the FDA. We are actively seeking partnering opportunities to complete the preclinical studies and obtain financing for the phase I clinical trials and beyond.

Beyond sunscreen – solar repair

Founded in 1887, the Oregon Health & Science University is a world-class teaching hospital and research institution with the causes and cures of cancer amongst their top research priorities.

Most of us will have had this experience at some stage in our lives, usually right after spending a glorious day at the beach, in the park, or hiking in the mountains. That evening we look in the mirror, realising, as we look at our incandescent red glow, that sunscreen would probably have been a good idea after all. The days following, in which we progress from tomato-red to zombie-skin-flaking, act as a strong reminder of the importance of UV protection. Unfortunately, the effects of sunburn are far worse than the merely aesthetic. Too much exposure can damage skin, witness the leathery look of aged sun-bathers, and in a number of cases, can lead to skin cancer. Indeed, skin cancer is one of the most common types, making up over 40% of all cancer diagnoses, with 1.5 to 3.5 million new cases diagnosed each year in the USA alone with a cost of over 8 billion dollars.

An algal virus may hold the key to repairing UV-related DNA damage.

The majority of damage comes from ultraviolet (UV) radiation, which is energetic enough to cause a number of unwanted chemical reactions directly within our cells. The most dangerous of these, those which act as the precursor to cancer formation, involve reactions between atoms inside DNA strands. One of the most common UV-related consequences is known as pyrimidine dimers, in which two of the molecular letters of our DNA code (C & T, out of the usual bases: GATC), cross-link to each other, forming a distortion in the usual DNA structure. This damaged section in turn interferes with the usual DNA reading and replication processes that can ultimately lead to mutations in the genetic code. Over time, mutations in the genome can give rise to the uncontrolled cell growth which is characteristic of cancer.

Given the danger of UV radiation, and the relative ubiquity of sunlight, it is no wonder that living organisms have a number of methods available to repair this kind of DNA damage. The version that mammals such as humans use

is known as Nucleotide Excision Repair (NER). In this process, the misshapen section of DNA is recognised by enzymes and then a relatively short section of one strand is cut out. As DNA is double stranded, the other undamaged strand can then be used as a template to accurately fill in the remaining gap, leaving a complete and accurate stretch of genome.

SUNBURNT THUMBS

While this process is effective, it involves a number of steps and can thus be somewhat slow. In contrast, a subset of bacteria and viruses, having no protective 'skin' and thus being far more sensitive to UV radiation, use a faster process known as Base Excision Repair (BER) in addition to their own NER system. Here, the unnatural base pair is detected and then 'flipped', being rotated by enzymes such that it points outside the DNA strand rather than, as usual, in towards the core. Poking out like a sore (possibly sunburnt) thumb, these flipped bases are readily detected and cut out by damage control enzymes, after which the remaining gap is refilled with the correct bases. BER is fast and effective, but the first step, detecting and removing UV-damaged DNA, requires specific enzymes to proceed. Enzymes which, curiously enough, are completely lacking in humans.

This is where recent work from Stephen Lloyd and Amanda McCullough of Oregon Health & Science University comes into play. It has long been known that humans have an effective and complete BER system for many forms of DNA base damage but lacks only an enzyme

to begin the process of UV damage repair. However these enzymes, known as pyrimidine dimer glycosylases (PDGs), are often found in other forms of life, such as bacteria and viruses. The question Drs. McCullough and Lloyd are answering is: can these enzymes act to fill the gap in the human DNA repair system?

The answer began in 1998, when Drs. Lloyd and McCullough, in collaboration with Dr. James van Etten published the discovery and characterisation of a PDG protein from a Chlorella virus (viruses which infect the single-celled aquatic algae known as Chlorella). This protein, cv-PDG, was unique compared to other PDGs in that it had a wider range of possible substrates (damaged DNA sections which it could recognise). This flexibility, combined with the general stability of the protein, made for significant potential.

A BETTER PAIR OF SCISSORS

Flash-forward to 2011, when a new publication comes out, this time describing a new and improved version of the cv-PDG protein. Firstly, the protein has an extra piece attached, a "TAT" peptide sequence derived from a harmless HIV protein. This peptide allows the protein to pass through the cell membrane, effectively jumping over the fence which separates inside and outside. This is where the second attached peptide comes into play. Unlike bacteria, humans corral their DNA strands into a specific compartment in the cell, known as the nucleus. Proteins can be delivered to the nucleus only if they have the correct 'address' written on them, the nuclear localisation sequence (NLS). By attaching this sequence to the cv-PDG protein, it is automatically sent to the correct location to start repairing DNA.

The important question, then, is: does this protein actually work? Yes, yes it does. Initial tests were performed with cultured keratinocytes, essentially free-living skin cells in a flask, while later work involved tests on lab-grown skin. When these cells are hit with ultraviolet light, there is immediate formation of damaged DNA such as pyrimidine dimers, damage that can be seen over 24-72 hours later. But when the modified cv-PDG protein was added to the mix, it rapidly soaked into the skin cells and was then promptly transported to the nucleus. Upon repeating the ultraviolet blast the damage was repaired extremely rapidly, rapidly enough that very few pyrimidine dimers could be detected even after the time needed to harvest the cells or tissue. Cv-PDG was thus able to enter skin cells and rapidly initiate repair of the UV-induced DNA damage, all without further effort from the researchers.

Given the success of this protein in repairing UV-related DNA damage, Dr. Lloyd began to suspect that it may have a use as a therapeutic for those with higher risk of skin cancer. To follow up on this idea, he and his long-time research and life partner, Dr. Amanda McCullough, set up a spin-off company to develop the technology. Restoration Genetics Inc., as it is known, has successfully raised over 1.5 million dollars in funding from the US government. Their current goal is to extend their knowledge of the protein in various model systems, with an eye to beginning the long process of gaining approval to turn their discovery into a therapeutic.

A long process, to be sure, but a rewarding one. As Dr. Lloyd comments, "melanoma and non-melanoma skin cancers are escalating at alarming rates worldwide, with exposure to harmful UV irradiation being at the root of this epidemic. Decreasing the cellular burden of UV-induced DNA damage is an important component for disease prevention." Through their work with the cv-PDG, Restoration Genetics hopes to assist in this last, challenging step. Perhaps, one day, summer will no longer be a time of incandescent sunburn and worry over skin cancer.

Researcher Profile

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Having completed his BS (Biology) with a major in marine pollution biology, Dr. Lloyd found his interest turning towards chemotherapy and soon gained a PhD in Molecular Biology from the University of Texas. With a career containing stints at Stanford and Vanderbilt Universities, as well as industry employment and two directorships via the University of Texas, Dr. Lloyd has had significant success along the way. He is currently joint-head of a laboratory with his wife, Dr. McCullough, their efforts focusing on the mechanisms of DNA damage and repair.

KEY COLLABORATORS:
Dr. Amanda McCullough, Oregon Health & Science University
Dr. James Van Etten, University of Nebraska

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