Reading the book of **life**

Molecular biologist, expert biochemist and committed educator **Dr Zachary Burton** shares the details of his background, as well as his current studies towards a better understanding of RNA polymerase, and his efforts to bring forth a new generation of scientists

As an opener, could you give an insight into your background?

I grew up near the beach in Los Angeles; as a child, I was interested in nature, swimming, backpacking and fishing. I was an undergraduate at the University of California, Los Angeles (UCLA), finishing in 1975 with a bachelor's degree in Biochemistry. I stayed at UCLA for graduate studies working with Dr David Eisenberg, and graduated with a PhD in 1980. I spent around three years as a postdoc working for Dr Richard Burgess at the University of Wisconsin. In Dick's lab my studies were focused on bacterial σ factors, and I worked closely with Dr Carol Gross, now a professor at the University of California, San Francisco. Next, I moved to the University of Toronto for a second postdoc with Dr Jack Greenblatt, working on human general transcription factors. After just over three years, I left Jack's lab to take a faculty position at Michigan State University (MSU), where I have been a professor for over 25 years. I am now a Full Professor of Biochemistry and Molecular Biology with tenure. I have worked with the most wonderful and inspiring mentors. Spending a career in academic science has been a constant cycle of inspiration, perspiration and revelation. It is a wonderful career with unimaginable intellectual independence – but not an easy one.

What do you hope the implications of your work will be?

Based on our understanding of RNA polymerases and their accessory transcription factors, in 2014, we are rewriting the book of genesis according to evolutionary principles. A conceptual working model for the genesis of life on Earth is essential for teaching and understanding biology and also for understanding RNA polymerases, which are among the most essential, core functions of life.

Have you encountered any challenges in your work to date?

Everything is a challenge in academic science. Obtaining even subsistence funding for a world-class research project and laboratory is a matter of luck more than expertise, accomplishment or skill. Science fails in America because of societal neglect, ignorance and arrogance. Scientists work on challenging problems of obvious intrinsic interest and importance without adequate societal support. In addition to our contributions to medicine, industry and knowledge, we are a fundamental part of education and training that is not appreciated by society.

Are you currently engaged in any partnerships? What value do they bring to your work?

To facilitate molecular dynamics simulations, the Burton laboratory has partnered with a number of collaborators within MSU: Dr Robert Cukier in the Department of Chemistry, Dr Michael Feig in the Department of Biochemistry and Molecular Biology, and Dr Guowei Wei in the Department of Mathematics. To conduct molecular-genetic and biochemical experiments, the Burton laboratory has longstanding collaborations with Dr Mikhail Kashlev at the National Cancer Institute and Dr Benoit Coulombe at the Institute of Clinical Research (IRCM) in Montreal. Without these valuable collaborations, we could not endure as a functioning laboratory.

Can you discuss your role as a teacher and explain how your teaching and research duties are interlinked?

I have taught a laboratory in recombinant DNA techniques (BMB 472). This is blissful and pure teaching to highly motivated undergraduates, many of whom will go on to jobs in biotech or into graduate school. After students complete the lab, they are ready to conduct serious laboratory research, and I love having these smart and talented individuals work for me. In spring 2015, I will teach a practical course to undergraduates on computer skills in biochemistry (BMB 469) with our department computer genius Dr Kaillathe Padmanabhan. This course is meant to serve as a gateway to high-end bioinformatics, genomics, cancer and molecular dynamics studies, the stuff of modern and evolving biochemistry. It will also be excellent preparation for a student wishing to join my lab, so maybe I will get some skilled recruits.

On a personal level, how do you strive to improve scientific mentorship?

Mentorship in a laboratory is done within a professional network, so a professor must support and protect the network. Based on the network model, the professor is only one of multiple mentors. So, part of being a principal investigator is to simultaneously teach mentorship and protégéship: teaching, listening and asking effective questions.

Problematic **polymerases**

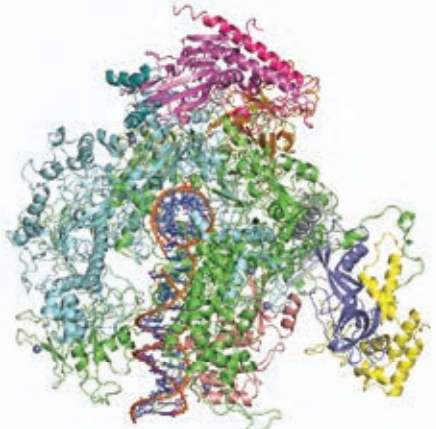
Molecular biologists at **Michigan State University**, USA, have been examining the function and evolution of one particular biological enzyme with the hope of answering diverse questions relating to human health, basic biology and the genesis of life on Earth

DNA MAKES RNA makes protein: it is a simple theory of information flow in biological systems, and one known to most people with a basic grounding in molecular biology. In practice, however, this process is supported by an intricate array of molecular machinery, much of which is still not fully understood. The first step is to transcribe DNA into RNA – specifically, messenger RNA or mRNA, which is the species of RNA responsible for directing protein synthesis. In order to make mRNA, RNA polymerase, a complex organic enzyme and mobile machine, must run on and read DNA. In Bacteria, a kind of protein transcription factor called a σ factor is required, which brings RNA polymerase and the DNA strand together at the promoter region. In Archaea and Eukaryotes, TFB/TFIIB appears to fulfil a similar function to σ factors. In humans, many general transcription factors cooperate with RNA polymerase to initiate a RNA chain.

To commence RNA synthesis, RNA polymerase must split apart or unzip the two strands of the DNA double helix, and 'read' one strand. As it does so, it adds matching RNA nucleotides to the RNA chain, or polymer, before zipping the DNA strand back into contact with its partner. In this way, the end product is an RNA sequence unique to the DNA information that has been read, and this mRNA then goes on to interact with other molecular elements, such as the protein-manufacturing machinery in the ribosome.

MOLECULAR MICHIGAN

Because this basic process is at the heart of the startling diversity of life on Earth, it is easy to see that when it goes wrong, big problems are likely to ensue – and this presents the intriguing question of how, and by what mechanisms, RNA polymerase can operate so accurately and precisely. This enzyme is fundamental to the transfer of biological information, and could potentially reveal many important details, paving the way to understanding and controlling biological systems, and yet many questions about its operation remain unanswered. How does it translocate along the DNA template? How does it correctly select the appropriate nucleoside triphosphates that it uses to assemble new RNA strands? And how have RNA polymerases evolved through the history of life to fit the diverse organisms they are so integral to?



HUMAN RNA POLYMERASE II HOMOLOGY MODEL

One laboratory at Michigan State University (MSU) has set out to tackle all of these questions – and more besides. Dr Zachary Burton, a full professor of Biochemistry and Molecular Biology, leads the Department's Burton laboratory in addition to his role as a teacher and mentor. Using molecular dynamics simulations of RNA polymerase complexes acting on DNA, Burton's team systematically generates hypotheses about the enzyme's structure and function that can subsequently be tested using *in vitro* mutagenesis and the production of mutant proteins. With these studies, the researchers have already been able to shed significant light on what Burton calls 'the largest, most complex, most enigmatic and dynamic enzymes in the human biosphere'.

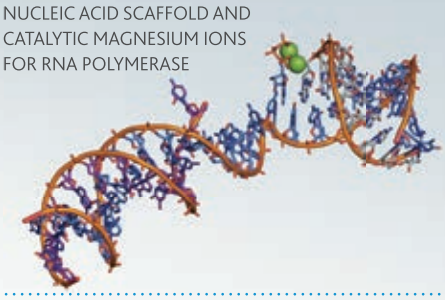
FIRST LIFE

The majority of the history of life on Earth – roughly the last 4 billion out of its total 4.5 billion years – can be traced through the structures of RNA polymerases, because multi-subunit enzymes of this type have been conserved since the last universal common ancestor. All extant organisms therefore share these complex but closely related proteins – and in 2014, Burton published a paper that summates billions of years of evolution in just a few pages by looking at changes in RNA polymerase structures. Eukaryotic cells make use of three distinct versions of the enzyme rather than one. Specialisation of RNA polymerase II with a unique scaffold for evolutionary innovation termed the carboxy terminal domain or CTD, appears to be fundamental to eukaryotic (plant, animal and human) complexity.

The study of RNA polymerase evolution also relates to the ancient RNA and RNA-protein worlds. The RNA world hypothesis posits that life as we know it, governed by the central dogma of DNA making RNA making protein, was preceded by a world in which self-replicating RNAs were both genomes and enzymes. The RNA viruses, which occupy the peripheries of life today, function in some similar ways. Burton's RNA polymerase evolution studies most clearly relate to events at the last universal common ancestor (LUCA) and the last eukaryotic common ancestor (LECA).

XSEDE-ING EXPECTATIONS

In order to gain an insight into RNA polymerase in an evolutionary, as well as a health and basic biological context, the Burton lab runs advanced dynamics simulations on the national Extreme Science and Engineering Discovery Environment (XSEDE) computer grid, using the University of Texas Advanced Computer Center's Stampede computer. "These are big, expensive calculations done on a big machine, sometimes by undergraduates," Burton explains – and these methods are paired with a battery of *in vitro* assays designed to analyse the catalytic



NUCLEIC ACID SCAFFOLD AND CATALYTIC MAGNESIUM IONS FOR RNA POLYMERASE

activity, nucleoside triphosphate substrate binding, translocation and fidelity of different polymerases. Currently, the group's attention is turned towards defining the evolution and function of bacterial σ factors.

σ factors are general transcription factors that help bacterial RNA polymerase to recognise promoter regions on the DNA template, and the Burton lab has developed models for the evolution and function of these and related archaeal and eukaryotic factors. The MSU researchers' studies into σ factors will help to further inform their overview of the genesis of life and the evolution of RNA polymerases. Another current goal is to complete and refine the preliminary homology models of human RNA polymerase I, II and III that are used in the absence of actual X-ray crystal structures of these enzymes. With refinement, optimisation and stress-testing, Burton hopes that his homology models can be as useful as good-quality x-ray crystal structures.

FINDING THE FUTURE

RNA polymerases are among the most important biological enzymes, and the Burton laboratory is at the forefront of research in this dynamic and high-impact area. Plans for the future include more of the same – as Burton himself puts it: "I will continue to do what I am doing until they force me to stop".

Varieties of polymerase

There are many different kinds of RNA polymerase, many of which are only found in certain organisms. Bacteria and archaea both have their own single version of RNA polymerase. Bacterial RNA polymerase is relatively simple, but archaeal RNA polymerase resembles eukaryotic RNA polymerase. Eukaryotes make use of a total of five separate types of nuclear RNA polymerase, two of which are specific to plants. In humans, there are just three types:

RNA POLYMERASE I

RNA polymerase I is responsible for transcribing ribosomal RNA, which is used for protein synthesis. It therefore accounts for the majority of the total RNA synthesised by cells. Mutations in the gene that encodes RNA polymerase I cause Treacher-Collins syndrome, a relatively rare disease characterised by facial deformities.

RNA POLYMERASE II

RNA polymerase II is the most commonly studied species, and is responsible for most of the transcription that occurs from DNA to micro RNA, messenger RNA and small nuclear RNA. RNA polymerase II mutations have not been associated with human disease, but this may well be because such mutations would be lethal.

RNA POLYMERASE III

RNA polymerase III is responsible for transcribing many genes commonly used in the everyday life of cells, and produces transfer RNA and other small RNAs. Mutations in the gene encoding this protein cause degeneration of the brain's white matter in a condition known as leukodystrophy.

INTELLIGENCE

ACTIVE SITE DEHYDRATION IN CATALYSIS BY RNA POLYMERASE

OBJECTIVE

To understand the atomistic workings and evolution of multi-subunit RNA polymerases.

KEY COLLABORATORS

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PROFESSOR ZACHARY BURTON received his BS in Biochemistry from UCLA in 1975 and his PhD in Biochemistry from UCLA in 1980, working with David Eisenberg. His first postdoctoral was done with Richard Burgess at the University of Wisconsin, Madison, where Burton also worked closely with Carol Gross, now of the University of San Francisco. Burton's second postdoc was with Jack Greenblatt at the University of Toronto. Since 1987, Burton has been a professor at Michigan State University in the Department of Biochemistry and Molecular Biology.

MICHIGAN STATE UNIVERSITY