Extreme: An emperor penguin begins a dive in which oxygen levels may reach extraordinarily low levels. (Photo: K. Ponganis.)

Blood sampling catheters or oxygen electrodes were inserted into the aorta or vena cava of penguins under anaethesia. After recovery, the birds were allowed to dive through the isolated dive hole, which ensured that the birds returned to this hole. After one or two days of diving, the equipment was removed, data recorded and the birds allowed to recover.

On analysis of the data from these dives, the researchers found that the penguins were capable of reaching extremely low levels of blood oxygen at the time of their return to the surface of the dive hole. The lowest of the air sac values was significant in relation to the results from other birds. The lowest values in emperor penguins are less than that in air breathed in by birds flying at altitudes of more than 11,500 metres and lower than that found in birds flying at these high altitudes, such as the bar-headed goose.

The researchers found that the remarkably low levels of intervascular and air sac levels of oxygen in emperor penguins is achieved at least in part from the optimum management of oxygen reserves and extreme hypoxemic tolerance. In particular, the birds' haemoglobin with high oxygen affinity enhanced blood oxygen content during hypoxemia and allowed the depletion of respiratory reserves.

The oxygen levels were also extremely low by human comparison, even with patients undergoing treatment for hypoxemia and from climbers at the top of Mount Everest.

As well as revealing a highly specialised mechanism by which emperor penguins can catch their food, the authors believe the novel physiological properties may also help to develop models for improved understanding and treatment of human hypoxemic and ischemic pathologies.

Q & A

Kevin Struhl

Kevin Struhl is the David Wesley Gaiser Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School in Boston, Massachusetts. He did undergraduate work with Boris Magasanik at MIT (SB, SM 1974), graduate work with Ron Davis at Stanford (PhD 1979), and a brief postdoctoral stint at the MRC Laboratory of Molecular Biology in Cambridge, UK with Sydney Brenner. During his graduate work, he was the first to functionally express a eukaryotic protein gene (yeast his3*) in* Escherichia coli *and to use a reverse genetic approach to identify and map eukaryotic promoter elements in cellular genes. This work directly led into a career that has spanned many areas of transcriptional regulation, specifically the intricate interplay of activators, repressors, and co-regulatory complexes with the basic RNA polymerase II machinery, and the role of chromatin structure in gene expression. More recently, he has incorporated functional genomic approaches to complement more classical analyses of individual genes and proteins. Despite a degree in biochemistry and affiliation with a biochemistry department, he is a molecular biologist and geneticist interested in mechanisms that occur in living cells.*

How did you become interested in biology? For most of my childhood, my academic interest was mathematics, and I didn't care much about plants and animals — unlike my brother Gary who was very interested in butterflies, dolphins and other creatures, and is now a developmental biologist at Columbia University. I visited the electron microscope at Rockefeller University and saw images of eukaryotic cells, courtesy of Jim Hirsch, who was a professor of cell biology there and lived across the street. My

interest in biology started around the age of 12, when I read books on heredity, human genetics, DNA and the genetic code (which was rather incomplete at the time). I have no memory of the specific books or how I came to read them. I do remember Vance Packard's book *Animal IQ: The Human Side of Animals*, and giving a 'seminar' on this to my class, complete with home-made visual aids. I was asked to give the same talk to every class in the school, and this started my life-long pleasure in giving scientific talks. The initial sparks from these books didn't immediately translate into a career plan, but they undoubtedly contributed to the excitement I felt upon discovering molecular biology as an undergraduate at MIT. And, learning about the pioneering work of Jacob and Monod initiated my life-long fascination with the field of gene regulation.

Who were important influences in your career? From Boris Magasanik, who combined classical microbiology with genetics and biochemistry, I learned the value of a multidisciplinary approach. Ethan Signer's famed seminar course at MIT on critical evaluation and logical analysis of scientific papers was instrumental in teaching me how scientific progress really proceeds, and it has served as a model for graduate courses in many institutions. From Ron Davis, I developed a life-long appreciation about the experimental details and physical principles that underlie laboratory experiments, and the development of new methods to solve biological problems. Lastly, I was lucky to have pursued my graduate and postdoctoral work in the Stanford Biochemistry department and the MRC Laboratory of Molecular Biology. Both were relatively small with exceptional faculty, postdocs and students who were highly interactive in an atmosphere that was collegial, informal and non-hierarchical, yet scientifically demanding. I particularly liked the fact that my labmates came from different research groups (very rare today, and a missed source of learning) and that funding issues never came up.

How did you start working on eukaryotic gene regulation? Inspired by Jacques Monod's famous comment "anything that is true of *E. coli* must be true of elephants, except more so", I wanted to extend the Jacob and Monod paradigm to eukaryotic organisms. I didn't have a clear idea about how to do this until my day-long interview for becoming a graduate student at Stanford, when I first heard about the pioneering work on recombinant DNA technology. I decided on the spot that this was how I would study eukaryotic gene regulation for my PhD.

When I got to Stanford 6 months later, I wanted to clone a eukaryotic protein-coding gene and decided to do this by functional complementation in *E. coli*. This was viewed as a speculative long-shot given the standard assumption that the functional barrier between eukaryotes and prokaryotes was very high. However, I reasoned that the common genetic code and the ability of *E. coli* to initiate translation at internal AUG codons would permit synthesis of the correct eukaryotic protein (introns had yet to be discovered!), provided there was any reasonable level of transcription throughout the region. This worked, and I cloned the yeast *his3* gene and subsequently showed that *E. coli* was producing the encoded yeast enzyme. I then used a combination of recombinant DNA manipulations and genetic tricks of bacteriophage λ to generate a set of deletion mutants. What was then needed was the ability to transform DNA into yeast, a problem solved by Gerry Fink. I immediately developed the initial yeast vectors, introduced the deletion mutants back into yeast cells, and analyzed the phenotypic consequences, thereby generating the first functional analysis of a promoter region of a eukaryotic cellular gene. Much of my subsequent work for nearly three decades derives from these initial experiments.

What was it like during the early days of recombinant DNA technology? Like the beginning of any revolution, it was very exciting and constantly changing. Many people recognized that a new era of biology was on the horizon, but even so, I think we underestimated the future impact. Ground-breaking techniques — molecular cloning, enzymatic manipulations of DNA and reverse genetic analysis, Southern, northern, and western blotting, DNA sequencing, monoclonal antibodies — were developed when I was a graduate student, and I was lucky to be part of the small recombinant DNA community that used them with home-made materials and anecdotal knowledge. Shortly thereafter, biological supply companies quickly sprouted, and they disseminated the necessary materials and expertise to the broad community of biologists. It is hard now to imagine biological research without these techniques.

What fundamental principles of transcriptional regulation are specific to eukaryotes? Many fundamental principles are universal, and were established from classical studies in prokaryotes that unfortunately are often now forgotten. But, eukaryotes clearly use new principles and unexpected mechanisms, among which are the following: 1) Combinatorial regulation that makes possible the overwhelming complexity of gene expression patterns. This occurs primarily via synergistic activation by multiple proteins bound at enhancers, but also through interactions within and between multiprotein families of transcription factors. 2) Structurally and functionally autonomous modules in transcription factors for regulatory diversity and evolutionary flexibility. 3) Regulation of transcription factors by shuttling between the nucleus and cytoplasm. 4) Tight

coupling of transcription with post-transcription processes such as mRNA capping, splicing, polyadenylation and nuclear export. 5) Co-regulatory complexes that transmit information from proteins bound at specific genome locations to the core transcription machinery. 6) The chromatin template which acts as a regulated scaffold for transcription factors.

How important is chromatin structure for transcription? The role of chromatin in transcription has had a schizophrenic history. Before 1975, the transcription field largely ignored chromatin, even though a correlation between histone acetylation and transcriptional activity had been noted in the early 1960s. With the discovery of nucleosomes and the use of nuclease sensitivity assays, chromatin became trendy and was frequently invoked to explain unknown aspects of gene regulation. However, a long dark period then followed, in large part because it was unclear how chromatin could explain the specificity of gene regulation. This specificity problem was solved by the discovery that DNA-bound activators and repressors recruited nucleosome-remodeling complexes and histone acetylases to specific genomic locations, thereby creating localized perturbations in chromatin structure. This discovery, together with antibodies against specific histone modifications and advances in purifying protein complexes, has again rocketed chromatin to the forefront of the transcription field.

Nevertheless, while chromatin is clearly important for transcription, gene regulation is highly specific, and the DNA-binding proteins are the only entities with the requisite specificity to decode the genome. In fact, multiple DNA-binding proteins are required to achieve the specificity needed in large mammalian genomes. Furthermore, the pattern of histone modifications is generated by direct interactions of the relevant enzyme complexes with activators, repressors, and elongating RNA polymerase II. In my view, the stability of developmental states and gene regulatory patterns is determined primarily by the constellation of transcription factors in the particular cell type. The primary role of chromatin structure is to reinforce the regulatory decisions that are ultimately governed by transcription factors and the transcription process. Reinforcement occurs in large part by positive feedback loops, in which the true regulators function with 'activating' or 'repressing' co-factors that both modify histones in specific ways and preferentially bind to those modified histones.

What do you think of the manuscript reviewing process? I think it is flawed at several levels. Anonymous reviews assume that reviewers are unbiased, objective and without personal or scientific conflicts of interest; this is not always true, especially in competitive situations, and there is no mechanism to detect such problems. Aside from the potential for abuse, anonymous reviews create an inequality between authors and reviewers that is unfair and scientifically unjustified. At many journals, particularly those run by commercial companies as opposed to scientific societies, disagreements between authors and reviewers are often adjudicated by editors with modest scientific accomplishments and experience. I favor a process in which editorial decisions are made by practising scientific experts, reviewers are identified by name, and the signed reviews and author responses published online along with the paper. Lastly, it is unfortunate that the biology community has permitted commercial companies to control most of the journals. Competition among journals and business-related decisions about scientific publishing has seriously distorted the literature, and it has created an artificial rating system that is used to judge decisions about funding and career advancement.

How has the field of biology changed during your career? When I started, biology was a small and self-contained academic enterprise. Laboratories were small, individual students and fellows typically carried out all aspects of their own research project, and it was much less competitive to obtain faculty positions and research support. Now, biological research has an undeniable industrial flavor, even in academic institutions. Many projects are collaborative, often involving multiple laboratories with very different expertise. Individuals often perform highly specialized tasks, and there are management issues at all levels. Academic scientists now interact with a real biotechnology industry, complete with patents, lawyers, for-profit companies, and the potential to become personally wealthy. There is a trend away from small-scale, investigator-initiated, hypothesis-driven experiments to answer specific questions toward large-scale, high-throughput experiments designed to produce vast amounts of data. These large datasets are not only analyzed for their own sake, but they also serve as a general infrastructure for individual investigators. Personally, I prefer elucidating mechanisms rather than generating data, but both styles of research have great value, and we use them in my laboratory.

What is the future for gene regulation? I think that many, perhaps most, of the basic principles of gene regulation are already understood, although certainly there are interesting unanswered questions. Much less is known about the evolution of gene regulatory systems, both specific and general. In addition, elucidating the details of specific regulatory systems will become increasingly important in medicine, because for diagnosis and treatment of disease, the devil is in the details.

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