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Editorial overview: How to generate molecular diversity, the most important process in biology

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Gregory Weiss, a professor of chemical biology at UCI, studies and applies molecular diversity to projects ranging from single-molecule enzymology to inventing disease sensors. Recently, his lab has explored using mechanical energy to accelerate protein folding and enzymatic catalysis. He and his wife live in Irvine, CA with their three cats.

Evolution requires just two steps. Diversify to generate a large population (step 1), and then cull through the population to select variants adapted to the conditions (step 2). Maybe "repeat steps 1 and 2" should be included as a third step. But that's it. Just diversify, select, and repeat again and again for the process at the heart of all biology.

So, what do we know about the mechanisms underlying these two steps? Selections, including both their motivations and mechanisms, have been extensively studied by scientists, starting with evolution theory's originator, Charles Darwin. My university, for example, has an entire department dedicated to studying biological selections (a Department of Ecology and Evolutionary Biology), and such research sprawls across an additional six departments. To paraphrase evolutionary biologist Theodosius Dobzhansky, "only through the light of selective pressures and consequent evolution can we understand biology." So, it's not surprising to find the study of selections and their results occupy a central place in biology research for >150 years.

But what about the mechanisms underlying the second step — molecular and its consequent organismal diversification? Here, chemical biology grabs the spotlight. After all, the diversity of the organism ultimately derives from its chemical constituents — DNA, RNA, protein, and other molecules responsible for the phenotypic variation. So, exploring mechanisms to diversify biomolecules is a problem inherent to chemical biology. Furthermore, numerous chemical biology techniques, such as diversity-oriented synthesis, unnatural amino acid incorporation, and molecular display (e.g., phage, mRNA, and yeast display), seek to build collection of diverse molecules with the goal of identifying molecules with new functions. Since this process of molecular diversification is responsible for evolution, it is arguably the most important mechanism in biology.

This special issues focuses on the molecular mechanisms for generating diverse collections of molecules by organisms and, in the laboratory, by protein engineers. Research into such processes is an active and exciting area of chemical biology. So, this special issue offers a snapshot of research at the cutting edge with fascinating examples of unexpected and important discoveries, rather than an exhaustive survey.

Textbooks attribute genome diversification to errors introduced during DNA replication. DNA polymerase is a complex and imperfect machine charged with reproducing perfect copies of organisms' genomes. Thanks to proof-reading, misincorporation of dNTP starting materials happens with

astonishingly low frequencies. Nonetheless, the wrong dNTP starting material can slip by DNA polymerase when it catalyzes DNA polymerization.

In their review, Pugliese and Weiss, describe studies with DNA polymerases missing their proof-reading functions to boost the rate of dNTP misincorporation to a level practical for study. Such enzymes turn out to be surprisingly flexible in their substrate specificity and tolerance to odd, non-wild-type dNTPs. For example, the willingness of DNA polymerases to incorporate heavily modified, fluorescence-labeled dNTPs into the nascent DNA chains forms the basis for DNA sequencing at industrial scales. Single-molecule studies offer a chance to observe the nitty-gritty details of changes to the kinetics and dynamics caused by incorporation of the incorrect dNTP. As the authors report, such studies have uncovered a wealth of new information about the mechanisms used by this class of enzymes, and the steps susceptible to mistakes leading to genomic errors and consequent evolution.

Diversification of DNA is also a process central to protein engineering, and methods to drive such mutagenesis have undergone a mini-revolution in the last decade or so. A large number of new methods have been described and applied to a wide-range of projects. It's often not clear what method to use for each situation. Researchers often resort to attempting one technique after another to find the one that works for their experiments.

Bratulic and Badran offer an incredibly useful survey of state-of-the-art mutagenesis methods, describing the large number of recently reported innovations. Importantly, the authors offer an incisive and critical guide to the different methods. Every researcher pursuing mutagenesis-based protein engineering will want to carefully study this review and especially its excellent Table 1. When I read this paper, I couldn't wait to share it with my laboratory. I suspect many researchers and PIs will have a similar reaction.

A theme that emerged from preparing this special issue is that DNA polymerase offers just one of many biological machines responsible for diversification of biomolecules during organismal evolution. Organisms have evolved other tricks to evolve more quickly than waiting for appropriate mistakes to appear in their genomes. For example, Hendrickson and co-workers describe a fascinating system of molecular evolution used by many bacteria, which lack asparaginyl- and/or glutaminyl-tRNA synthetase. These bacteria rely on amidotransferases to indirectly synthesize these tRNAs by modifying tRNAs misacylated with aspartic or glutamic acids. These bacteria apply this system for rapid evolution allowing immediate response to stressful conditions, such as the appearance of antibiotics. Studies of this area are in their early days, and this review should encourage future research to examine this process *in vivo* and how diverse organisms take advantage of its cleverness.

A more typical method for turning on and off genes in organisms involves methylation and other epigenetic annotations to the inherited genome. Yung and Elsässer describe the mechanisms for the evolution of epigenetics. Such processes contribute important effects to the stability and randomness of the genome, and provide a type of rheostat for molecular diversification. A fascinating discussion in this review considers the reversibility of epigenetic modifications. Importantly, the time-frame of epigenetic reversibility determines the impact of the modifications on heritability. Thus, this chemical process underlies the processes used for organismal evolution and adaption.

Like DNA, RNA can be modified through methylation. Such post-transcriptional modifications have important consequences for the health of the organism and its responses to environmental conditions. In their review, Stojković and Fujimori, describe the weird world of RNA methylation, a modification catalyzed through both S_N2 displacement and a unique radical mechanism. In prokaryotes, loss of RNA methylation, through mutation to the enzymes catalyzing the modification, is associated with antibiotic resistance. The weird part is that the loss of RNA methylation doesn't seem to affect the fitness of the bacteria. So, here we find examples of lost molecular diversity and mutated enzymes with lost function, but improved fitness for the specific purpose of dodging an antibiotic.

The picture of RNA methylation in eukaryotes is quite complicated with situations where up- or down-regulation of RNA methylases can correlate with a large number of diseases, including forms of neurodegeneration and cancer. Then, there's the expansive and growing list of targeted RNAs–mtRNAs, tRNAs, rRNAs, and, of course, mRNAs. These run not quite the whole gamut of forms of this versatile biopolymer, but getting close. Table 2 in Fujimori's review is particularly eye opening in its rich chemistry and its disease associations. In his review Dominissini expands on the topic of eukaryotic, mRNA modification. The >100 known modifications are described as adding another layer of transcriptome regulation and consequent gene expression. The many questions yet to be answered in this area make life and chemical biology interesting. Thus, these two reviews offer timely and important updates to a very exciting area.

On the topic of RNA and its versatility, Ren, Micura and Patel review the latest structural research in the always surprising area of catalysis by self-cleaving ribozymes. The review highlights the beauty of evolution and molecular diversity to access catalytic function. Yet again, the

picture is not entirely clear despite a number of known structures. For example, a divergence of views exists concerning the mechanisms and even cleavage sites for the twister and twister-sister ribozymes.

Of course, molecular diversity in the cell encompasses more than simply DNA, RNA and proteins. Dennis describes the evolution of glycosylation, the cell's pre-eminent post-translational modification, which augments the diversity of proteins. The author offers a provocative hypothesis that the structure of the genetic code can drive such diversification by favoring sequences targeted for glycosylation. This exciting review directly links the benefits of increased molecular diversity with mechanisms for driving it. The review's conclusion expands to include other post-translational modifications acting upon different time-scales from short (e.g., phosphorylation) to long (e.g., histone modifications). These conclusions, supported by nicely reviewed data, offer a road map for future exploration to understand the links between generating molecular diversity — step 1 — and gaining the improved fitness required for selection — step 2 in evolution.

In summary, this issue reviews the molecular mechanisms underlying the single most important event in biology. The diversity of different systems and methods employed indicates how exploring this area can uncover a rich vein of important and fundamental insights central to biology and chemical biology.