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EDITORIAL

Cell and molecular dynamics: visualizing, measuring, and manipulating the chemistry of life

Denise J. Montell

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Whereas the twentieth century was the era of genes and genetics, the twenty first promises to be the epoch of cellular and molecular dynamics. The last century witnessed the discovery of the structure of DNA, solved long-standing biological problems via large-scale genetic screens, revealed the genetic causes of numerous diseases, and culminated in the complete human genome sequence. As powerful as genetic approaches have been and continue to be, they provide a static view of biology that is at best incomplete because life is dynamic.

Innumerable chemical reactions occur in each living cell every second and yet rarely do we get a glimpse of these dynamics. What we know about the biochemical lives of cells comes predominantly from studies of purified or partially purified components in test tubes. While clearly valuable, these studies divorce the components from their native environments, so critical information is lost, such as where in the cell the reaction occurs. A more complete and accurate understanding of the chemistry of living systems requires the ability to observe and manipulate biochemical events as they actually occur in living cells, tissues, and organisms. This field is in a rapid growth phase, and the goal of this special issue is to provide a snapshot of the state of the art, identify current challenges, and anticipate what the future might hold.

Conceptual advances frequently follow technical advances and nowhere is that more evident than in the field of cellular and molecular dynamics. Improvements in microscopy, together with an ever-expanding palette of fluorescent proteins and small molecules, are allowing investigators to develop optical reporters of, and tools for manipulating, specific biochemical reactions in living cells. The article by Terai and Nagano reviews the history of fluorescence as a tool in biology, the development of the first Ca^{2+} indicators, which marked the beginning of a revolution, and recent progress in expanding the small molecule probe set to enable detection of changes in pH, metal ions, and reactive oxygen species in living cells.

The second phase of the cell dynamics revolution came with the discovery of green fluorescent protein and development of its spectrally diverse derivatives. This advance opened the door first to observing dynamic protein localization and later to visualizing the spatial distribution and temporal dynamics of protein activities in living cells. Förster resonance energy transfer sensors offer a general approach to detecting a great variety of biochemical events in living cells. Mutoh and Knopfel describe the "tool box" of genetically encoded indicators and focus on their use in monitoring neuronal activities not only in single cells but also in circuits. The development of a palette of spectrally diverse fluorescent proteins raised the theoretical possibility of observing multiple biochemical reactions simultaneously in the same cells. This is important in order to determine the spatial and temporal relationships between different signaling pathways. Depry et al. describe the possibilities and challenges involved in such "multiplexing." The field of cellular and molecular dynamics is all about pushing limits, and Hensel and Xiao review recent progress in detecting single molecules in living cells with a particular focus on efforts to use this approach to study gene regulation.

In addition to observing and measuring biochemical reactions, it is also important to manipulate them within living cells with the highest possible spatial and temporal resolution. Four reviews in this issue summarize diverse approaches to

D. J. Montell (✉)
Department of Biological Chemistry, Center for Cell Dynamics, Johns Hopkins School of Medicine, 855 North Wolfe St., Baltimore, MD 21205, USA
e-mail: denise.montell@lifesci.ucsb.edu

Present Address:
D. J. Montell
Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106-9625, USA

manipulating protein activities. Yin and Wu review the nascent field of optogenetics, which takes advantage of naturally occurring light receptor proteins from plants and microorganisms, to manipulate ion concentrations or protein activities in animal cells. The simplest approach is to express light-sensitive ion channels, such as channelrhodopsins, in animal cells or in whole organisms and then use illumination to open the channels and thus depolarize or hyperpolarize cells. More sophisticated approaches involve fusing critical proteins, such as Rho family GTPases, to a light-sensing domain, thus rendering the protein activity sensitive to light. While challenging to implement, this approach provides exquisite spatial control of protein activity.

The word dynamic—by definition—implies change over time, so gaining control over the timing of biochemical events is as important as spatial resolution. DeRose et al. describe powerful tools that enable rapid manipulation of biochemistry in living cells. So-called chemically induced dimerization approaches enable protein translocation from one subcellular compartment to another on the timescale of seconds. Such translocation, when carefully designed, can result in rapid activation of an enzyme by bringing it into proximity to its substrate for example. DeRose et al. enumerate the various ways that chemically induced dimerization has been used to modulate not only protein localization and activity but lipid second messengers, which has enabled investigators to address previously intractable questions. They also discuss strategies for multiplexing and improving spatial resolution of chemically induced dimerization, which represent the frontiers in this class of approaches.

As mentioned above, one limitation of genetic approaches is the necessarily static view they provide of biology. An additional limitation is that loss-of-function phenotypes resulting from mutation of a particular gene can be confounding due to a multitude of indirect consequences of the initial loss. Kanemaki describes a novel set of approaches that have been developed to circumvent these limitations and provide more acute loss of function. The basic idea is to replace a gene encoding an endogenous protein of interest with one encoding a fusion protein bearing an inducible protein degradation domain, or degron. The inducer causes rapid degradation of the protein of interest and allows examination of a time course of events that ensues. While this approach has been put to most effective and extensive use in yeast, Kanemaki describes the promise and challenges associated with moving it to animal cells.

The complete sequence of the human genome teaches us that there are still many things that we do not know. For example, the genome encodes numerous proteins that can be predicted to be enzymes but which are completely uncharacterized. Medina-Cleghorn and Nomura describe an exciting new approach that is enabling functional annotation of uncharacterized enzymes. The approach is called activity-based protein profiling. The principle is to use chemical probes that recognize and chemically modify a specific type of catalytic activity. In this way, proteins with hydrolase, kinase, or other enzymatic activities can be specifically labeled, identified, and enriched. This technology complements transcriptional profiling and proteomic approaches because it specifically targets active enzymes and can be used to compare the activity profiles of normal cells or tissues to disease states. Combined with profiling of metabolites, this technique can place new enzymes within known metabolic pathways or identify new ones.

Of course the greatest challenge for biomedical science is to turn understanding of biological processes into diagnostic tools and treatments for diseases. As Medina-Cleghorn and Nomura describe, activity-based protein profiling can facilitate screening of chemical libraries to identify specific enzyme inhibitors. Such inhibitors can serve as lead compounds in drug development if they target an enzyme that is important in the etiology of a particular disease. Alternatively, the inhibitor can be used again in the lab in combination with metabolite profiling to determine the effects of a particular inhibitor on the concentrations of hundreds to thousands of metabolites. Medina-Cleghorn and Nomura finally describe new metabolic flux analysis and metabolomic imaging approaches.

Together, these eight reviews show how we are proceeding in the post-genomic era to move from identifying all the genes and their protein products to studying their activities in living cells, tissues, and organisms. Many of the approaches are as yet very low throughput and require extensive protein engineering to follow one protein activity at a time, whereas other approaches are amenable to medium or high throughput. The challenges are different for each approach, but advances are coming with breathtaking speed. With this set of reviews, we stop to take a breath, assess the state of the art, and evaluate where we need to go next.