MINIREVIEW PROLOGUE

Introduction to Thematic Series: Protein Interactions, Structures, and Networks*

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Protein interactions are fundamental to the proper functioning of cells, and aberrant formation or regulation of protein interactions is at the heart of many diseases, including cancer. The advancement of methods to study the identity, function, and regulation of protein complexes makes possible the understanding of how those complexes malfunction in human diseases. New methodologies in mass spectrometry, microscopy, and protein structural analysis are rapidly advancing the amount and quality of the data, as well as the level of detail that can be obtained from experiments. With this progress, the questions that can be addressed and the biological landscape are changing. This series of minireviews highlights methodological advances and how they have been applied in novel ways to explore the function and regulation of pathways and dynamic networks in cells.

The analysis of protein function and regulation is fundamental to the understanding of diseases and how to control them. Inherent in the understanding of protein function and regulation are elucidating protein interactions, determining how complexes modulate signaling, and ascertaining the outcome of responses to stress and disease. Methods for studying protein interactions are rapidly advancing, becoming more sensitive, and providing insights that would have been unthinkable just a few years ago. The minireviews in this Thematic Series address some of those new methods and how they have been applied to investigate protein-protein and protein-nucleic acid interactions. Three minireviews discuss protein interactions that take place in the nucleus, focusing on mismatch repair, chromatin complexes, and nuclear viral DNA sensors. Two minireviews consider the dynamics of protein interactions and the effects of protein misfolding, which are central to several disease states. Lastly, recent findings on signaling complexes downstream of the T cell antigen receptor, which regulate the adaptive immune response, are described.

The first minireview in the series, by Fishel, details how structural biology and real-time single molecule imaging have advanced our understanding of the protein motions involved in mismatch repair (MMR)2 (1). MMR, which is coupled to replication, is an excision-resynthesis reaction that initiates from a strand scission distant from the mismatch and extends to just past the mismatch. Real-time single-molecule analyses have provided critical insights into the time-dependent activities of the MMR proteins, complementing the static structures provided by x-ray crystallography. Importantly, the combined studies have allowed the development of detailed models for mismatch repair by the MutS homologue family of MMR proteins.

The second minireview, by Hoffman, Frey, Smith, and Auble, describes the use of formaldehyde for crosslinking proteins and nucleic acids in cells, with a particular emphasis on the effects mediated by formaldehyde in cells (2). Formaldehyde is widely used in the chromatin field to study protein-DNA complexes and has proven invaluable for stabilizing transient complexes that otherwise could not be isolated. For example, ChIP assays are used to identify the sites of transcription factor binding with high precision. Unbiased approaches to identify proteins that bind to specific DNA sequences, however, remain technically challenging, in part due to the mild reaction conditions used to minimize spuriously crosslink reactions. Several aspects of formaldehyde chemistry are discussed, including specificity and stability in cells and methods used to quench unreacted reagent. These reactions are not trivial, as either too little or too much formaldehyde can lead to low recovery of crosslinked species, due to a paucity of crosslinked material for the case of too little crosslinking agent or possibly the presence of insoluble complexes or masked epitopes when too much is used. A better understanding of the effects of formaldehyde in the cell is imperative as more complex questions are investigated, such as the dynamics and higher-order structures that can form, as well as to ensure that the experimental design does not affect the chromatin structures being studied.

The progression of a viral infection is determined by the dynamic interplay between host defense mechanisms and viral modulatory strategies. Traditionally, it has been thought that detection of viral DNA occurs only in the cytoplasm to prevent detection of “self” DNA; however, that view is shifting, and recent studies have identified sensor proteins that detect viral DNA within the nucleus and activate the expression of antiviral cytokines. In the third minireview, Diner, Lum, and Cristea report on this evolving field, with an emphasis on the interferon-inducible protein IFI16 as the first nuclear sensor (3). Following infection with herpesviruses, IFI16 binds nuclear viral DNA to initiate an immune signaling cascade from within the nucleus. Aside from its role in innate immune signaling, IFI16 has also been shown to use a multiprotein complex, the “inflammasome,” to initiate inflammatory and apoptotic responses to foreign DNA, although this new role is not firmly established. DNA sensing is also emerging as critical for control of RNA viruses, including HIV. In the escalating arms race between

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2 The abbreviation used is: MMR, mismatch repair.
virus and host, distinct viral mechanisms have evolved for inhibiting and/or circumventing the functions of the sensor. Future insights on host-virus interactions could suggest new paths for the development of antiviral therapies.

Many signal transduction cascades are regulated by higher-order assemblies of multiprotein complexes. Among them are the pathways dependent on the binding of the T cell receptor (TCR) to its cognate antigen, which lead to T cell activation, differentiation, and function. The fourth minireview by Balagopalan, Kortum, Coussens, Barr, and Samelson summarizes the current views on the dynamics and function of signaling complexes that form with the membrane-bound adapter protein LAT (liner for activation of T cells) downstream of the T cell receptor (4). A variety of methods, including genetic, biochemical, and biophysical approaches, have been used to study signaling complex formation and their assembly into microclusters that can be visualized by light and super-resolution microscopy. Super-resolution microscopy has further revealed that the microclusters comprise nanoclusters in a distribution of sizes. Insights gained from the studies of LAT-based signaling complexes in T cells not only help inform the development of inhibitors of signaling, but can also be used as a basis to study other signaling systems.

In the fifth minireview, Landreh, Rising, Presto, Jörnvall, and Johansson detail the critical role of specific chaperones in the control of amyloid formation (5). Amyloids can be both beneficial and harmful to the cell; the inherent self-propagation and stability can be beneficial in functional amyloids, such as those that deactivate transcription factors in yeast or function as scaffolds for reaction intermediates, whereas those same features can be quite harmful, as in amyloid diseases. Some protein sequences are more prone to aggregation, requiring mechanisms to protect those regions. One solution is the evolution of specific chaperone pro-protein domains, such as the BRICHOS homologues. For example, the lung surfactant protein SP-C is translated as a pro-protein with a BRICHOS domain that prevents SP-C aggregation by promoting the non-amyloidogenic helical fold and preventing protein-protein interactions that would lead to amyloid formation. New understandings of the mechanisms by which BRICHOS domains prevent amyloid formation provide new strategies for the prevention of the effects of disease-related amyloids and demonstrate the features critical for achieving this function.

In the final minireview, Phillips and Corn present how the conformational dynamics of ubiquitin, the ubiquitin-conjugating machinery, and deubiquitinating enzymes are used in the cell to facilitate signaling and regulation on multiple different levels (6). Ubiquitin is a highly conserved eukaryotic protein that interacts with a diverse set of partners to act as a cellular signaling hub. Although ubiquitin is an extremely stable protein, internal conformational motion and flexibility play important roles in protein-ubiquitin interactions and may be critical to the discrimination of ubiquitin-mediated signals. In addition to the intrinsic dynamics within monomeric and polymeric ubiquitin, the multiprotein complexes that ubiquitinate substrates are also dynamic, such as the conformational rearrangements of some E3 ligases, which are critical for regulation of their activity. Likewise, dynamics within the deubiquitinating enzymes are critical for regulation of their function and preventing promiscuous activity. Protein motion plays a key role in ubiquitin signaling, and the studies of it reveal biological functions for “invisible” conformational dynamics in living cells.

References