Loss of Structure—Gain of Function

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Much of our knowledge about protein function stems from three-dimensional structures of protein ground states. However, there are a growing number of examples where the native ensemble populated by a protein at a given set of conditions is insufficient to explain the mechanisms of biomolecular processes.\(^1,2\) Often, the ground-state conformer represents a nonfunctional state of the protein molecule or just one of multiple functional states. In the course of their action, proteins undergo remarkable transformations, such as those triggered by changing environmental conditions, posttranslational modification, or binding. In some cases, these perturbations elicit drastic conformational changes and/or partial or complete unfolding. Even without external stimuli, multiple nonnative conformers that are transiently sampled by proteins due to thermal fluctuations can play important functional roles.\(^1,2\)

Perhaps the most astonishing example of protein rearrangement leading to a functional conformation is that experienced by influenza hemagglutinin (HA) during virus invasion of the host cell.\(^3,4\) Each subunit of the virus membrane-associated HA homotrimer is trapped in an auto-inhibited native state that transforms into the functional conformation subsequent to the virus binding to the host cell and its internalization in an endosome. The low pH of the endosome triggers large-scale conformational change, which involves a 100 Å movement of protein segments, releasing fusogenic regions buried in the hydrophobic core of the native HA that insert into the host cell membrane. The resulting conformer is an important intermediate where the HA is physically associated with both virus and host membranes, leading to membrane fusion.

Auto-inhibited native proteins that convert to functional conformational states following posttranslational modification or cofactor binding are not unusual in signaling.\(^5\) For example, the DH domain of a guanine nucleotide exchange factor Vav, which mediates signaling through various cellular pathways, exists in equilibrium between an inactive ground state where the substrate-binding site is occluded by an amphipathic helix and a functional low-populated conformer where the active site is open.\(^5\) The latter can be stabilized by phosphorylation of a Tyr residue on the inner face of the helix, resulting in persistent protein activation. A similar mechanism is employed by WASP/Scar proteins that regulate the activity of actin filament-nucleating Apr2/3 complex.\(^6\) The ground state of WASP proteins is auto-inhibited by intramolecular association of their Apr2/3-interacting VCA region with the GTPase-binding domain. The activation is achieved by Cdc42 binding to GTPase-binding domain, which displaces the VCA region and makes it available for interaction with Apr2/3 complex.

An interesting example of an auto-inhibited native protein activated by domain unfolding is provided in the manuscript of Ducett et al. published in this issue,\(^7\) which describes the activation of transcriptional response by the components of ribosome-associated Hsp70 chaperone machinery.\(^8\) Yeast cells can communicate to each other by the means of quorum sensing molecules, whose efflux from the host cell is mediated by Snq2 and Pdr5 plasma membrane transporters encoded by the PDR (pleiotropic drug resistance) genes.\(^9,10\) Upregulation of these transporters increases concentration of the quorum sensing molecules in the surrounding medium, which may lead to cell growth arrest in the nutrient-depleted environment. Intriguingly, such a transcriptional response can be activated by binding of an Hsp70 co-chaperone, Zuo1, to Pdr1 transcription factor,\(^9,10\) implying that cell growth control may be coupled with regulation of protein synthesis.

Zuo1 is a co-chaperone of the Hsp70, Ssb, that facilitates the folding of nascent polypeptide chain newly synthesized at the ribosome. The primary function of Zuo1 as a J-protein is to stimulate ATPase activity of Hsp70. Beyond its role as a co-chaperone, Zuo1 has the ability to directly interact with and activate the transcription factor Pdr1 that regulates transcription of genes encoding the plasma membrane transporters.\(^9,10\) Ducett et al. have shown that
Zuo1–Pdr1 recognition involves 13 mainly hydrophobic amino acid residues at the very C-terminus of the 433-residue yeast Zuo1 that are necessary and sufficient for Pdr1 activation. However, the very same 13-residue region participates in stabilization of the hydrophobic core of an 86-residue Zuo1 CTD (C-terminal domain) that forms a left-handed four-helix bundle. The CTD, along with the preceding positively charged RNA-binding region, is necessary for Zuo1 association with the ribosome. Thus, the truncation of as little as three C-terminal residues of Zuo1 completely destabilizes the CTD, rendering it incapable of ribosome binding. PDR activation assays, however, reveal that only the mutation/truncation variants of Zuo1 with the unfolded or significantly destabilized CTD are capable of productive interaction with Pdr1, suggesting that Pdr1 binding requires the 13 C-terminal amino acids of Zuo1 to be released. The native Zuo1, containing folded CTD capable of association with the ribosome, therefore represents an auto-inhibited form of the protein that cannot initiate PDR response. The second function of Zuo1 as activator of Pdr1, on the other hand, requires its dissociation from the ribosome and unfolding of its CTD.

Even though the key molecular events leading to activation of Pdr1 by Zuo1 have been identified, a number of questions regarding the mechanisms of this process still remain to be answered. The native Zuo1 with an intact CTD is tightly associated with the ribosome, while the mutants with destabilized CTD exhibit reduced ribosome binding. Consequently, the ribosome association would shift the equilibrium toward Zuo1 conformers with CTD folded, and the domain unfolding would be more likely for the ribosome-free form of the protein. What causes the dissociation of Zuo1 from the ribosome? Is it a result of CTD unfolding or a release of an intact domain triggered by another molecular event? Zuo1 was shown to form a stable heterodimer with the atypical human p190A RhoGAP FF1, revealing that domain of both proteins can activate Pdr1. Is there coupling between Pdr1 activation by Zuo1 and Ssz1 or are the two pathways independent?

Positive transcriptional regulation by the means of unfolding of a protein domain is an unusual paradigm, although at least one example of such regulation has been reported previously. The FF domain from p190A RhoGAP, which is one of the two cytoplasmic human proteins containing the FF domains, is tightly associated with the transcription factor TFII-I thus sequestering it in the cytoplasm. The release of TFII-I and its translocation to the nucleus are triggered by phosphorylation of Tyr308 of the FF domain, which is deeply buried in the hydrophobic core of the native protein. The phosphorylation is thought to occur via a thermally accessible unfolded state of the FF domain where the Tyr side chain is exposed. There is a growing body of evidence that nonnative protein states, including disordered ensembles, may play important roles in regulating protein activity. It is likely that more examples of activation by unfolding such as described by Ducett et al. will be reported in the years to come, especially as the technology for characterizing transient or disordered states of proteins matures.

References