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Review

Regulated unfolding of proteins in signaling

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ABSTRACT

The transduction of biological signals often involves structural rearrangements of proteins in response to input signals, which leads to functional outputs. This review discusses the role of regulated partial and complete protein unfolding as a mechanism of controlling protein function and the prevalence of this regulatory mechanism in signal transduction pathways. The principles of regulated unfolding, the stimuli that trigger unfolding, and the coupling of unfolding with other well characterized regulatory mechanism are discussed.

1. Introduction

Proteins are the work horses of biological systems, performing a plethora of tasks, including chemical catalysis, signal transmission, molecular transportation, cellular movement and forming the structural framework of cells and tissues. Protein function is dictated by the primary amino acid sequence which, in turn, determines the three-dimensional organization and dynamic behavior of proteins. Through evolution, proteins have achieved a fine balance between thermodynamic stability and dynamic fluctuations to optimally perform their biological functions in the environmental setting of their host [1]. It has long been understood that the three dimensional structure of a protein determines its function. Growing evidence, however, establishes the pervasive roles of disorder and dynamics in mechanisms of protein function [2–6]. In fact, nearly a third of all proteins, in all kingdoms of life, contain disordered regions of at least 30 amino acids [7]. Disorder is manifested in different ways, from short, flexible linkers and long "random coil-like" disordered segments to compact but disordered domains and whole proteins termed intrinsically disordered proteins (IDPs) [8]. Structural flexibility and disorder mediates critical biological functions; consequently, these dynamic features are often evolutionarily conserved [9,10]. A noteworthy example is the topologically conserved activation loop in kinases [11]. In the inactive state of Serine/Threonine and Tyrosine kinases (e.g., PKA, IRK) the flexible loop is collapsed on the active site, preventing substrate binding. An evolutionarily conserved kinase activation mechanism involves phosphorylation of this loop, which results in (i) stabilization of an open conformation, and (ii) rearrangement of key catalytic residues, enabling substrate binding and phosphotransfer, respectively [11]. Classic allostery, which mediates signal transduction through the tertiary and quaternary structure of proteins (e.g., hemoglobin, receptor tyrosine kinases), causes structural rearrangements in one functional domain or subunit in response to ligand binding within a distal domain/subunit of the same protein [12]. This regulatory mechanism depends upon the ability of whole proteins or domains to fluctuate between different defined conformations to regulate function. However, accumulating evidence shows that partial or complete protein unfolding is also utilized as a mechanism of regulating function, particularly in signal transduction pathways. Here we introduce the concept of regulated unfolding as a protein regulatory mechanism, provide illustrative examples, and discuss its future implications.

2. Protein unfolding as a type of signaling output

Signaling mechanisms often involve posttranslational modifications and/or protein–ligand (e.g., protein, nucleic acids, lipid, etc.) interactions that couple an upstream input to a conformational

Abbreviations: IDP, intrinsically disordered protein; NLS, nuclear localization signal; NES, nuclear export signal

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change, which alters function and produces a downstream signal. The extent of the conformational change ranges from subtle, local unfolding events to full unfolding of protein domains. For example, the cyclin-dependent kinase (Cdk) inhibitor $p27^{Kip1}$ (p27) regulates progression through the cell division cycle by interacting with and inhibiting several Cdk/cyclin complexes in the nucleus [13]. Cell cycle progression to S phase is characterized by rapid turnover of p27 via the proteasome pathway, a fate which is signaled by phosphorylation of p27 on Thr187 [14]. Counter intuitively, this post-translational modification is performed by the Cdk/cyclin complexes for which p27 is a potent inhibitor [14,15]. Grimmler et al. [14], demonstrated that non-receptor tyrosine kinases phosphorylate Tyr88 of p27, a residue which occupies the active site of Cdk2 [16]. This modification causes an inhibitory 3_{10} helix containing Tyr88 to be ejected from the ATP binding pocket of Cdk2, partially restoring kinase activity. Intrinsic flexibility of the C-terminal domain of p27 allows Thr187 to fluctuate into close proximity to the Cdk active site and become phosphorylated, creating a phosphodegron that leads to selective p27 ubiquitination and degradation, and ultimately full activation of Cdk/cyclin complexes (Fig. 1). Regulated partial unfolding of the inhibitory conformation of p27 through tyrosine phosphorylation triggers this signaling cascade that ultimately drives progression of cells into S phase of the division cycle.

Regulated unfolding mechanisms are also involved in the control of programmed cell death. Cytoplasmic p53 tumor suppressor initiates apoptosis by binding to and activating pro-apoptotic proteins [17]. This lethal function is inhibited by association of p53 with the anti-apoptotic protein BCL-xL [18]. Release and activation of p53 in response to DNA damage is signaled by a BH3-only protein ligand (PUMA) binding to BCL-xL. A π-stacking interaction between His113 in BCL-xL and Trp71 in PUMA, causes unfolding of BCL-xL at an allosteric site comprising two α-helix structural elements and dissociation of p53 from BCL-xL [19]. This example illustrates a signaling mechanism which combines traditional allosteric, ligand binding-induced structural changes with unfolding to release a binding partner.

The Wiskott–Aldrich syndrome protein (WASP) provides an example of both posttranslational modification- and ligand binding-induced unfolding involving several protein domains. WASP regulates cytoskeletal actin polymerization through direct interaction of its C-terminal domain with the Arp2/3 and actin complex. However, this domain is auto-inhibited through tertiary interactions with other domains of WASP. Cdc42, a Rho-family GTPase, signals activation of auto-inhibited WASP to initiate actin polymerization. Cdc42 and the C-terminal domain of WASP compete for binding to the WASP GTPase binding domain (GBD). Activation of WASP by Cdc42 involves partial unfolding of the hydrophobic core of the auto-inhibited conformation of WASP and folding of the WASP-Cdc42 complex. Furthermore, the partially unfolded conformation exposes Tyr291, a phosphorylation site for the non-receptor tyrosine kinase Lyn. This modification further relieves inhibition and enables the unfolding required for the structural switch to the Cdc42-bound conformation [20,21]. This activation mechanism (Fig. 2A) is an example of regulated unfolding wherein two input signals, posttranslational modification and ligand binding, synergize to control the three-dimensional organization and function of WASP with switch-like precision. Utilization of two input mechanisms allows WASP to integrate disparate upstream signals [21] and to respond through regulated unfolding.

However, these two mechanisms are not the only inputs that propagate biological signals through regulated unfolding. For example, phototropins, a class of Ser/Thr kinases, play critical roles in signal transduction in plants. Their activation is signaled by exposure to blue light, when a covalent bond forms between a flavin chromophore and the light-oxygen-voltage 2 domain (LOV2), causing unfolding of an inhibitory Jα helix and consequently the activation of the kinase domain [22,23]. A similar mechanism is utilized by a class of bacterial photoactivatable proteins [24]. These examples have illustrated regulated unfolding mechanisms involving relatively subtle alterations of secondary and tertiary structure.

3. Protein shape-shifters

Other examples of regulated unfolding include a class of so-called 'metamorphic proteins' ([25,26], Fig. 2B). The intriguing structural shape-shifting of these proteins mediates multiple cellular functions. For example, the chemokine lymphotactin (Ltn) switches between a monomeric α-helical and dimeric β-sheet sandwich conformation. The monomeric form, which exhibits the classical chemokine fold, binds to the canonical XCRI receptor. In contrast, the dimeric form binds to heparin and localizes to the plasma membrane [27]. The two mutually exclusive functional states exist in equilibrium under physiological conditions and require global unfolding for their inter-conversion [28]. Mad2, a protein involved in regulation of the mitotic spindle assembly, provides another example of metamorphic behavior. This protein undergoes a significant structural reorganization from an inactive to active conformation which requires a partially unfolded intermediate [29]. While the in vitro evidence for the alternative structures of metamorphic proteins supports the observations of functional switching in cells, the exact mechanisms that regulate conformational switching of Ltn and Mad2 in vivo are currently not well understood.

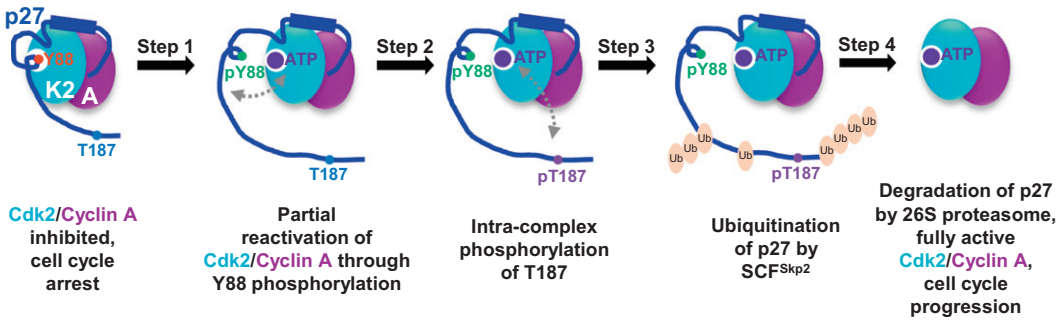

Fig. 1. p27 as a signaling conduit. Tyrosine phosphorylation-dependent partial unfolding of p27 triggers signal propagation through the length of the protein and regulates its degradation. Step 1 involves phosphorylation of Y88 of p27 that is bound to Cdk/cyclin complexes [Cdk2 (K2)/cyclin A (A) here] by non-receptor tyrosine kinases such as BCR-ABL, Src, Lyn, and Jak2, which ejects Y88 from the ATP binding pocket of Cdk2 and restores partial kinase activity. Following Step 1, Step 2 involves phosphorylation of T187 within the flexible C-terminal domain of p27 by partially active Cdk2 through a pseudo uni-molecular mechanism (indicated by gray arrow). Phosphorylation of T187 creates a phosphodegron signal for ubiquitination of Lysine residues within the p27 C-terminus by the E3 ligase, SCF^{Skp2}, during Step 3. Finally, during Step 4, ubiquitinated p27 is selectively degraded by the 26S proteasome, leading to the release of fully active Cdk2/cyclin A, which drives progression into S phase of the cell division cycle.

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