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Conditionally and Transiently Disordered Proteins: Awakening Cryptic Disorder To Regulate Protein Function

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1. INTRODUCTION

Proteins, with their seemingly limitless functional diversity, are major players in the maintenance of life. The ability of proteins to fulfill these various biological functions is encoded in their amino acid sequences. Although for a very long time it has been believed that the specific functionality of a given protein is predetermined by its unique three-dimensional (3-D) structure,[1,2] it is recognized now that a large portion of any given proteome is occupied by so-called intrinsically disordered proteins (IDPs).[3–67] These proteins and others with disordered regions (IDPRs) possess a very wide range of biological functions, but are characterized by the lack of a well-defined 3-D structure under physiological conditions.[8–26] Some illustrative biological activities of IDPs include regulation of cell division, transcription and translation, signal transduction, protein phosphorylation and other post-translational modifications, storage of small molecules, chaperone action, and regulation of the self-assembly of large multiprotein complexes such as the ribosome.[8–10,12–30] For IDPs, structural flexibility and plasticity originating from their lack of a definite ordered 3-D structure represent a major functional advantage, enabling them to interact with a broad range of binding partners including other proteins, peptides, membranes, nucleic acids, oligonucleotides, oligosaccharides, and various small molecules.[31–33]

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The binding of some IDPs/IDPRs to specific partners involves a disorder-to-order transition, as a result of which IDPs adopt more structured conformations.[26,34–42] However, many IDPs can remain predominantly disordered in the bound state outside the binding interface. Such mode of interaction is known as “the flanking fuzziness” in contrast to “the random fuzziness” when the IDP remains entirely disordered in the bound state.[43–46] Furthermore, activities of other IDPs do not directly involve coupled binding and folding, but rather are dependent on the flexibility, pliability, and plasticity of the backbone. These are so-called entropic chain activities, as they rely entirely on an extended disordered conformation of a polypeptide that maintains motion and dynamic flexibility while carrying out function.[17]

Functions of many ordered proteins are known to rely on induced fit. In the case of enzymes, the original paradigm was that the active site is continually reshaped by interactions with the substrate. This process continues until the substrate is completely bound, and the amino acid residues that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function.[47] Some ordered proteins, including enzymes, are subject to allosteric regulation, where binding of the effector molecules to the specific allosteric sites, which are different and physically distinct from the protein’s active site, modulates protein function by inducing specific conformational changes.[48,49] On the other hand, the structure of many ordered proteins, for example, nonenzymes that simply mediate interactions, can remain unchanged during their function.

Therefore, biologically active proteins can either have or be devoid of unique 3-D structures, and structures of proteins can either change or remain unchanged during function. The function-related structural changes range from local partial folding to complete folding, and from allosteric transitions to induced fit adjustments in IDPs and ordered proteins, respectively. Generally, the most common outcome of function-related structural changes is the overall increase in the amount of ordered structure. However, functions of some ordered proteins rely on the decrease in the amount of their ordered structure; that is, these functions require local or even global functional unfolding of a unique protein structure. The important features of these functional alterations are their induced nature and transient character. In other words, the function-related changes in these so-called conditionally disordered proteins[50] are induced by transient alterations in their environment or by modification of their structures. They are reversed as soon as the environment is restored or the modification is removed.

On the other hand, one should remember that unwinding (unfolding) of α-helices (i.e., α-helix to irregular structure transitions) does not necessarily correspond to a (transient) order-to-disorder transition. In fact, even if a region adopts an extended conformation (i.e., irregular conformation as opposite to an α-helical or β-structural conformation), disorder is not concerned as far as the polypeptide chain remains visible in the electron density maps obtained in the X-ray crystallographic experiments. Therefore, induced unfolding is not always a synonym of transient disorder. An order-to-disorder takes place when a region being originally well-defined in the electron density map (irrespective of whether it is α-helix, a β-strand, or a coil) becomes undetectable in X-ray structure due to dynamic disorder or when it is shown to become highly dynamic and hence to exist as a conformational ensemble by NMR.

Figure 1 shows that cryptic disorder can be awoken by a wide spectrum of factors, which crudely can be grouped into two major classes, passive and active. Passive factors are environmental factors that are not dependent on any specific interaction between the protein and its partners and correspond to modification of some global parameters of the protein environment, such as changes in pH, temperature, the redox potential, mechanical force, or light exposure. Alternatively, active factors typically involve some specific interaction of a protein with its environment and include interactions with membranes, ligands, other proteins, nucleic acids, or various post-translational modifications or release of autoinhibition (see Figure 1). This Review is dedicated to the description of protein functions relying on the induced unfolding and transient disorder mechanism. Some illustrative examples of such functions are discussed, and a general model of this functional mechanism is proposed.

2. PASSIVE FACTORS PROMOTING TRANSIENT DISORDER: INDUCTION OF FUNCTIONAL PROTEIN UNFOLDING BY GLOBAL ENVIRONMENTAL CHANGES

2.1. Protons in Action: Functional Protein Unfolding Induced by Changes in pH

Protons can affect protein conformation and activity via their interactions with amino acid residues in the polypeptide chain. The vast majority of ordered proteins are known to denature (or unfold at least partially) and lose their biological activity at extremely low pH values (around pH 1 or 2). However, a few proteins are known to be activated by solution acidification. The 9.7 kDa chaperone HdeA, one of the smallest known chaperones, is an example. Unlike most chaperones, HdeA functions as a monomer and does not require any energy factors or cochaperones. This protein is expressed in the bacterial periplasm, where it combats acid-induced protein unfolding and aggregation. At neutral pH, HdeA exists as a very stable ($T_m \approx 75$ °C) but chaperone-inactive dimer. However, this protein specifically senses low pH conditions (pH <3), where it partially unfolds and dissociates into chaperone-active monomers. Thus, HdeA is activated by the same conditions that lead to the inactivation and aggregation of other proteins.[51] This makes HdeA a member of a growing class of conditionally disordered chaperones. This acid-induced partial unfolding and monomerization of HdeA occurs very rapidly ($k > 3.5\ s^{-1}$). Binding studies suggest that HdeA uses its hydrophobic dimer interface for substrate binding.[51] Furthermore, due to its partially disordered conformation, the activated monomeric HdeA is apparently able to adopt different conformations necessary for binding a broad range of substrate proteins.[52,53] These properties result in a chaperone that is rapidly activated by acid stress and is able to efficiently suppress the aggregation of substrate proteins several times its own size.[51]

In the crystal structure of the HdeA dimer, more than 2370 Å^2 of solvent-accessible surface area is buried at the dimer interface.[54] The dimer is stabilized by hydrophobic interactions between the second helix of each monomer, the linkers connecting the second and third helix, and parts of the N-terminus. Another part of HdeA's N-terminus as well as its C-terminus were not resolved in the crystal structure and are likely disordered.[55] On the basis of the computational analysis of the HdeA sequence, this protein was expected to behave as a typical ordered protein at neutral pH but to be at least partially unfolded at acidic pH,[56] a

prediction that was supported also by experimental evidence.[52] In fact, it has been shown that at acidic pH, monomeric HdeA is in a “globally disordered” conformation characterized by the almost complete lack of specific signals in the near-UV CD spectrum and noticeable changes in the intrinsic fluorescence spectrum, suggesting dramatic reduction of ordered tertiary structure.[51] Also, the far-UV CD spectra of this protein at pH values below 2.5 are typical of highly disordered polypeptide chains, being characterized by the large negative ellipticity at 203 nm and the weak ellipticity values at 208 and 222 nm.[51] Finally, in the two-dimensional $^{1}H–^{15}N$ heteronuclear single quantum correlation (HSQC) experiments, dimeric HdeA showed well-dispersed signals in $^{1}H–^{15}N$ HSQC spectra, indicating the presence of well folded structure, whereas the $^{1}H–^{15}N$ HSQC spectrum of HdeA recorded at pH 2.5 was characterized by limited chemical shift dispersion, clearly indicating that HdeA adopts a mostly unfolded conformation at acidic pH.[51]

Because each HdeA monomer contains 28 pH-titratable residues (12 lysine residues and 16 aspartic and glutamic acid residues; HdeA does not possess any histidine or arginine residues), the pH-sensitive conformational switch is determined by the fact that the HdeA dimer is predicted to be stabilized in large part by electrostatic interactions between aspartic and glutamic acid residues on one monomer and positively charged lysine residues on the other monomer.[57] As the pH drops below the pK_a of critical glutamic acid residues, these residues lose their charge, resulting in a decrease in the attractive force between the two monomers. This results in the dissociation of the two monomers, their partial unfolding, and activation of the protein as a chaperone. Mutations in two of these critical glutamic acid residues E20 and E51 have now been shown to convert HdeA into a monomeric, partially unfolded, and constitutively active chaperone.[58] Therefore, the pH-induced conformational changes in HdeA “wake up” the dormant intrinsically disordered nature of this protein crucial for its biological function.[56] This pH-induced functional unfolding of HdeA is triggered by a significant decrease in the periplasmic pH, to pH < 3.0. In other words, this example involves global conformational changes induced by a large and global environmental perturbation.

Other well-known pH-sensors are sodium proton antiporters, which are ubiquitous membrane proteins found in the cytoplasmic and organelle membranes of plant, animal, and bacterial cells. They are involved in cell energetics, play primary roles in the regulation of intracellular pH, cellular Na^+ content, and cell volume, and their structures and functions are characterized by strong pH sensitivity.[59–62] For example, NhaA, the main sodium-proton exchanger in the inner membrane of *Escherichia coli*, regulates the cytosolic concentrations of H^+ and Na^+. It was shown to undergo highly localized structural changes upon its activation by alkaline pH.[63–65] These structural changes are crucial for the biological activity of NhaA, which is inactive at acidic pH, becomes active between pH 6 and pH 7, and reaches maximal activity at pH 8.5 (its ion transport capacity changes by 3 orders of magnitude between pH 7.0 and 8.5).[66,67] The function-related structural changes are localized to the loop VIII–IX region of NhaA and most likely correspond to local unfolding, as indicated by a noticeable increase in the accessibility of this region to proteolytic cleavage.[63–65]

Other examples of pH-sensing proteins are envelope proteins of several viral families (e.g., the *Alphavirus* and *Flavivirus* genera), which fuse with the membranes of endosomal compartments of the host cells in a pH-dependent fashion. It is known that the endosomes, these membrane-bound compartments inside eukaryotic cells, are characterized by acidic pH, which progressively decreases along the endocytic pathway, from pH 6.0–6.5 in early endosomes to pH 4.5–5.5 in late endosomes and lysosomes.[68–70] Upon exposure to mildly acidic conditions (pH ~6.5), these viral proteins undergo extensive conformational and oligomeric state changes, which serve to tether viral and cellular membranes and pull them into close apposition required for lipid mixing.[71,72] For example, fusion of vesicular stomatitis virus (VSV) with the host cell membranes occurs within a very narrow pH range, between 6.2 and 5.8, and is mediated by its envelope glycoprotein, the G protein.[73] This viral G protein has at least three function-related conformational states: the native prefusion state (N), present at the surface of the virus at pH values above 7; the activated hydrophobic state (A), which interacts with the target membrane during the first step of the fusion process; and the postfusion fusion-inactive conformation (I), which is observed after prolonged incubation at low pH and is structurally different from both the native and the activated states.[74–79] The major difference between the VSV G protein and many other fusigenic glycoproteins is in the ability of the VSV G to undergo I→N transition and to recover its native conformation after being reincubated above pH 7.[80]

Many other fusogenic viral glycoproteins experience dramatic conformational changes upon exposure to specific environmental triggers (e.g., a low pH environment and/or cellular receptors), leading to the exposure of hydrophobic motifs (so-called “fusion peptides” or “fusion loops”). These motifs then interact with one or both of the participating membranes, resulting in their destabilization and fusion.[81,82] In a similar manner, the VSV G protein undergoes dramatic structural rearrangements at the fusion pH, likely losing its unique 3-D structure and undergoing a reorganization of secondary structure.[83] To explain the mechanism of low-pH sensing by proteins, the “histidine switch” hypothesis was proposed,[84,85] because protonation of the histidine imidazole ring has profound effects on histidine chemistry under physiological conditions.[86] This hypothesis states that histidine residues, which become protonated and charged upon exposure to moderately acidic conditions (the typical intrinsic pK_a-values of histidine in proteins are in the range of 6.0–7.0),[87] can uniquely serve as pH-sensors and trigger reversible conformational changes in a pH-dependent manner.[84,85] The model proposes that in the inactive (prefusion) conformation, critical histidine(s) are located close to positively charged residue(s), possibly interacting via hydrogen bonds.[84] At low pH (postfusion), the histidine residues become protonated and hence positively charged. This positive charge disrupts any existing hydrogen bonds, causes electrostatic repulsion, and therefore promotes local unfolding. The histidine(s) would form salt bridges with negatively charged residues, thus stabilizing the postfusion, active conformation.[84] To enable this mechanism, the molecular surfaces involved in the corresponding structural rearrangements leading to fusion should be highly conserved.[84] For a number of viral proteins responsible for the pH-sensitive fusion with the host cells, the validity of this hypothesis was established by deleting, modifying, or mutating key histidine residues (reviewed in ref 84).

Members of the inward rectifier K^+ channel family, ROMK channels (Kir1.1 and Kir1.2), illustrate another role that pH-sensing switches play in the regulation of protein function. In fact, the inward rectifier K^+ channels, which appear to control K^+ secretion in renal tubular cells and membrane potential in excitable cells, are known to be strongly inhibited by a decrease in intracellular pH.[88–93] Detailed site-directed mutagenesis analysis revealed that the pH sensing in ROMK channels requires four histidine residues that are distributed within the C-terminus.[94] Earlier studies revealed that pH sensing also required lysine (Lys80) and threonine (Thr70) residues that may not be titratable at physiological pH levels and are located in the N-terminus of the ROMK channels.[90,95] In contrast to the N-terminus, the C-terminal domain of the ROMK channels is characterized by a high density of histidine residues that are accessible for pH-dependent titration.[94] On the basis of these observations, a simple model was proposed, wherein a pH-dependence of the interaction between the N- and C-terminal domains regulates the ROMK channel activity. Here, the C-terminal histidines serve as the pH-sensing sites. Protonation of these sites induces local structural changes in the C-terminal region, which enhances its binding affinity to the N-terminus. Binding of the C-terminal domain to the N-terminal region near residues Lys80 and Thr70 may subsequently inhibit channel activity.[94] Obviously, ROMK channels are not exceptional in their use of histidines as pH-sensors, and there is experimental evidence indicating that histidine residues play a central role in the pH-dependent modulation of a number of other ion channels, including KST1, connexin 43, GABA receptor, Kv2.1, and porin.[96–101]

At neutral pH, the dimeric mycobacterial adenylyl cyclase Rv1264 (an enzyme that is responsible for synthesis of the universal second messenger 3′,5′-cyclic adenosine monophosphate, cAMP) is autoinhibited by its N-terminal domain,[102] because its catalytic and regulatory domains share a large interface involving catalytic residues.[103] Figure 2 shows that acid activation of Rv1264 involves global structural rearrangement of this dimeric protein, where the symmetry-related catalytic domains of neighboring molecules rotate by 55° to form two catalytic sites at their interface. Although activation induces extensive structural rearrangements of the entire Rv1264, the most affected regions are two switch elements, the α1-switch helix (residues 226–231), which is α-helical in the active state and irregular in the inhibited state, and the αN10-switch helix (residues 192–206), which, conversely, is irregular in the activated state and α-helical in the inhibited state.[103] The α1-switch is located within the catalytic domain, whereas the αN10-switch, which undergoes the most dramatic structural change, is located in the linker region. In the active state, this fragment assumes a disordered conformation with a short helical segment (residues 202–206) loosely connecting regulatory and catalytic domains. In the inhibited state, this segment forms an α-helix, extending the αN10-helix of the regulatory domain by 24 Å, or four helical turns, to keep the two catalytic domains apart.[103] Therefore, acid activation of Rv1264 imposes opposite effects on two switch elements, inducing folding of the active site α1-switch and almost complete unfolding of the linker region αN10-switch. On the basis of a high sequence similarity between the catalytic domains of Rv1264 and mammalian adenylyl cyclases, the α1 and the αN10 switch regions were proposed as prototypical regulatory elements for the entire class III adenylyl cyclases.[103]

The pH-specific activation of furin represents another example of how pH-induced local structural changes are involved in the regulation of protein activity. Furins are members of the pro-protein convertase family of calcium-dependent serine endoproteases. They cleave pro-proteins and pro-hormones at pairs or clusters of basic amino acids in a pH- and cell compartment-dependent manner, generating mature active proteins and hormonal processing intermediates that require additional post-translational modifications to gain full bioactivity.[104,105] In fact, the proteolytic maturation of pro-hormones requires the acidic pH of maturing secretory granules, whereas the proteolytic activation of pro-protein substrates by furins is also pH-specific and takes place in the trans-Golgi network/endosomal system. Furin efficiently cleaves its pro-protein substrates at the consensus site Arg-X-Lys/Arg-Arg (where X is any amino acid). Furin-controlled activation of the target pro-protein is mediated by pH-dependent changes in the conformation of its substrates.[105] Intriguingly, furin itself is also synthesized as a pro-protein, making the autoproteolytic cleavages of its cognate pro-domain necessary for activation. Folding and activation of furin itself is compartment- and pH-dependent.[105] A recent study revealed that the conserved His69 of furin serves as the pH sensor that regulates this compartment-specific cleavage of the pro-peptide. Similar to many other pro-proteins, pro-furin folds in the endoplasmic reticulum (ER) under the guidance of its pro-domain and undergoes an autoproteolytic cleavage at the consensus furin site Arg-Thr-Lys-Arg_{107}. This generates an enzymatically masked furin–propeptide complex competent for transport to late secretory compartments. Upon transport to the mildly acidic environment of the *trans*-Golgi network/endosomal system, furin undergoes subsequent maturation steps, where the bound propeptide is cleaved at the internal site $_{69}$HRGVTKR_{75}, unmasking the active furin capable of substrate cleavage.[105] At the neutral pH of the ER, His69 is unprotonated and is involved in stabilization of a solvent-accessible hydrophobic pocket. Exposure to the acidic pH of the late secretory pathway leads to His69 protonation. This disrupts the hydrophobic pocket mentioned above and results in local unfolding, exposure, and subsequent cleavage of the internal cleavage site at Arg75, which activates the enzyme.[106]

In summary, this section illustrates how changes in the pH of a protein's environment can result in either global or local structural changes that are associated with alteration of protein function. There is an obvious correlation between the magnitude of pH changes and the degree of induced structural alterations, with larger changes in the environmental pH typically promoting more global functional unfolding.

2.2. Heat Wave: Functional Local Unfolding Promoted by Temperature Changes

Similar to extreme pH, high temperatures are known to denature and inactivate many ordered proteins, causing complete loss of function. However, some proteins, such as the *Saccharomyces cerevisiae* holdase Hsp26 and the wheat holdase Hsp16.9, are activated by high temperatures that cause heat stress-induced unfolding.[107]

The Hsp26 monomer contains four distinct domains, the disordered N-terminal region, middle domain, α-Crystallin domain, and the C-terminal tail.[108] Under nonstress conditions in vitro, Hsp26 exists as a hollow sphere comprised of 12 Hsp26 dimers.[109] Hsp26 is specifically activated by an increase in temperature.[110] Conformational analysis of Hsp26

revealed the existence of two thermal transitions, with midpoints at ~36 and ~74 °C.[111] The first structural transition coincides with Hsp26 activation, whereas the second corresponds to global protein unfolding.[111] Temperature-activated Hsp26 is characterized by a lower content of ordered secondary structure (in comparison with the inactivated state) and by noticeable rearrangement of the environment of its 11 phenylalanine residues, nine of which are located within the disordered N-terminal region of Hsp26.[112]

The Hsp16.9 holdase from wheat forms a dodecameric double disc with the dimensions 95 Å × 55 Å with a central cavity 25 Å in width.[113] This dodecamer is sufficiently flexible to allow rapid exchange of dimeric subunits.[107] At heat shock temperatures (40 °C), the dodecamer-to-dimer equilibrium shifts toward the dimeric species, and the Hsp16.9 complexes disassemble into stable dimers, which are the active species responsible for high affinity binding of client proteins. Upon restoration of the normal environmental temperature, the dimeric subunits reassemble into large oligomeric structures.[113] Importantly, the Hsp16.9 client-binding sites are hidden in the oligomer and become exposed upon disassembly of the oligomer into dimers.[113] Analysis of the Hsp16.9 crystal structure revealed that in both monomers of the dimeric building block of the dodecameric oligomer (which consists of two disks, each comprising six α-Crystallin domains organized in a trimer of dimers), the α-Crystallin domain and C-terminal extension are ordered and resolved. However, the N-terminal arm in only one monomer is fully resolved and composed of helices connected by a random coil segment, whereas the 42 N-terminal residues in the other monomer are disordered.[113] The dodecamer structure is stabilized by a multitude of intersubunit contacts, including contacts between the C-terminal protrusions of the bottom disk monomers and the hydrophobic grooves in the top disk monomers (which are the potential client binding sites), and a series of specific structural locks formed via pairwise intertwining of the six ordered N-terminal arms about the crystallographic 2-fold axes.[113] Here, folding of a pair of N-terminal arms involves an interaction between a monomer from the top disk and a monomer in the bottom disk. Each of these helix–helix interactions results in the formation of a shared helical domain (helical lock) located on the inside of the dodecamer.[113] Obviously, these helical locks are absent when the dodecamer dissociates to dimers. Dissociation also removes C-terminal protrusions from the hydrophobic grooves of neighbors, thereby making them accessible to clients. Therefore, the temperature-induced dissociation of the inactive Hsp16.9 dodecamer into the active dimers is accompanied by the noticeable functional unfolding of this holdase.

2.3. Protein Activation via Redox-Induced Functional Unfolding

Among several classes of molecular chaperones, holdases occupy a unique niche. These ATP-independent holding chaperones serve as high-affinity binding platforms for unfolded proteins. Once activated, holdases bind specifically to aggregation-prone protein folding intermediates, therefore preventing protein aggregation under stress conditions. Holdases are activated via a number of mechanisms and are collectively known as post-translationally regulated, stress-related proteins. The activation mechanisms enable immediate response of chaperone structure to changes in its environment under stress conditions. Among factors leading to the activation of holdases are elevated temperatures (e.g., Hsp26), extreme pH (e.g., HdeaA), and oxidative stress (e.g., Hsp33). Therefore, these stress-specific chaperones

are activated by the very stress conditions that they combat.[107] Typically, they have well-ordered (potentially crystallizable) structures in the inactive form and are activated by stress-promoted partial unfolding.

An illustrative example of a redox-regulated chaperone is Hsp33, which is a well-conserved protein identified in the vast majority of prokaryotic species as well as in some unicellular eukaryotic parasites (e.g., *Trypanosomatidae*).[114] This protein is a highly specialized holdase with a unique activation mechanism that protects bacteria against severe oxidative stress conditions that lead to protein unfolding.[115] Hsp33 activation requires either slow acting reactive oxygen species (ROS) combined with protein-unfolding temperatures (i.e., oxidative heat stress)[116] or protein-unfolding fast acting oxidants, such as hypochlorous acid (HOCl).[117] To be activated by these two stimuli, Hsp33 has two stress-sensing regions located in the C-terminal domain, a redoxsensing zinc-center and a linker region, which responds to unfolding conditions.[118] The redox-sensitive zinc-center contains a conserved motif, Cys_{232}-X-Cys_{234}-X-Cys_{265}-X-X-Cys_{268}. Under nonstress conditions, these four cysteines coordinate one Zn^{2+} ion, whose binding provides significant stability to Hsp33's C-terminus.[119] The zinc binding domain connects to the Hsp33 N-terminal domain via a highly flexible ~52-amino acid linker region. In its inactive form, the linker region is compactly folded and makes extensive contacts with a highly hydrophobic, four-stranded β-sheet platform of the Hsp33 N-terminal domain.[120] This tight binding of the linker region to the N-terminal domain keeps Hsp33 monomeric and functionally inactive.

Once exposed to oxidative stress that leads to protein unfolding, Hsp33's cysteines are oxidized and zinc is released. This leads to the unfolding of Hsp33's zinc binding domain and the adjacent linker region.[121] Therefore, upon activation, the redox-switch domain of Hsp33 adopts a natively unfolded conformation.[116] This large conformational rearrangement and partial unfolding leads to dimerization of Hsp33 and results in the exposure of hydrophobic surfaces in the N-terminal substrate-binding domain, which likely contribute to the binding of partially folded client proteins.[118] Most recent studies also suggested that the unfolded linker region in Hsp33 is involved in client recognition and binding.[122] Structural analysis of complexes formed between active Hsp33 and partially unfolded client proteins revealed significant stabilization of Hsp33's linker region upon client binding, suggestive of client binding-induced refolding of Hsp33. While the enormous flexibility contained in its IDR endows Hsp33 with the ability to bind a wide variety of different client proteins, the high solubility of this region would explain how Hsp33 successfully maintains its client proteins in a soluble state and prevents their irreversible aggregation.[122]

2.4. Protein Photocycles: Light-Mediated Functional Unfolding of Proteins

Light is among the most important environmental factors. Light-dependent organisms developed multiple mechanisms for the detection of the quality and quantity of light in the environment, and for the subsequent control of a wide range of biological responses. Often, photosensitive proteins undergo activation via functional unfolding in response to interaction with light.

To avoid harmful light exposure, both directionality and run length of free-swimming bacteria are modulated by blue light.[123] Photoactive yellow protein (PYP) is a water-soluble

~14 kDa protein that contains a thioester linked *p*-coumaric acid cofactor. It serves as the photosensor in *Ectothiorhodospira halophila*, a motile, alkalophilic, and halophilic bacterium.[124–127] Upon light excitation, trans/cis isomerization of a double bond in the chromophore triggers a cycle of structural events yielding a long-lived, blue-shifted intermediate (known as pB) with a lifetime on the order of 1 s.[128,129] High-resolution NMR spectroscopic analysis revealed that this long-lived pB intermediate exhibits a large degree of disorder and exists as an ensemble of multiple conformers that exchange on a millisecond time scale.[130] Furthermore, the increased exposure of the PYP backbone to the solvent in the presence of light was confirmed by the observation of its increased sensitivity to proteolysis.[130] Figure 3 provides an illustration of this functional unfolding of the photoactive yellow protein induced by light. Although these light-induced structural perturbations affected almost the entire molecule, the ordered structure of PYP was restored once pB converted back to its ground state (pG). This cycle of light-induced unfolding and dark-promoted refolding was proposed to be related to protein function, with the disordered pB state being able to bind partner molecules, allowing the swimming bacterium to operate the directional switch that protects it from harmful light exposure.[130]

A recent review by Zoltowski and Gardner[131] provided an excellent description of the mechanisms of action of several important blue-light photoreceptors from various organisms. In this report, the structural consequences of light activation and the effects of these photoinduced structural changes on protein–protein interactions and other functions of several flavin-binding photoreceptors were reviewed. Among the proteins discussed were self-contained photoreceptors, which harbor both sensory and signaling mechanisms in a single module. These include PYP, light oxygen voltage (LOV), and blue light using flavin adenine dinucleotide, FAD (BLUF), as well as light-activated modules that serve as sensory elements in large multidomain signaling proteins (e.g., cryptochromes, CRYs).[131]

LOV domains are blue-light-sensing modules found in various multidomain proteins that regulate enzymatic activity and signaling cascades in prokaryotic and eukaryotic organisms.[132] Typically, LOV domains have a well-folded sensory domain (containing FAD or flavin mononucleotide, FMN, cofactors) and signaling elements with either α-helix–β-strand–β-hairpin or β-strand–α-helix topologies. Because these signaling elements are commonly located at the N- or C-termini of the LOV domains, they are known as Ncap or Ccap elements. Photoactivation alters the interactions between the core sensory domain and these signaling elements. As a result, the signaling elements are expelled from the structure and partially unfold, preparing the protein for subsequent interactions with various binding partners.[131]

CRYs show an overall structural similarity to DNA photolyases, but do not possess photolyase activity.[133] Most CRYs are composed of two domains: an N-terminal photolyase-related (PHR) region and a C-terminal domain (known as CRY C-terminus, CCT) of varying size, which is largely disordered. This CCT dictates postactivation protein–protein interactions and signaling, and contains regions of residual structure with conserved signaling motifs.[131] It was emphasized that the CCT can act either as a directly repressive element that inhibits interactions at the PHR domain or as an independent recognition element. In both cases, light-induced activation of CRY includes partial protein unfolding

caused by the disruption of intramolecular interactions between the PHR domain and the CCT domain.[131]

The soluble orange carotenoid protein (OCP) acts as a blue-green light-responsive photoreceptor essential for the photoprotection of cyanobacteria.[134–138] Activation of OCP by light induces a shift in the absorbance properties of the protein (the color of the absorbed light changes from orange to red) and induces energy dissipation, resulting in a detectable non-photochemical-quenching of the cellular fluorescence through interaction with the phycobilisome.[134] In the light-activated form, OCP undergoes dramatic structural changes, where compaction of the β-sheet domain is accompanied by the noticeable reduction of the rigid helical structure in a significant portion of OCP.[135] The light-induced structural rearrangements may be a precondition for the OCP interaction with the phycobilisome or other proteins.[135]

Light-regulated gene expression in plants, bacteria, cyanobacteria, and fungi is controlled by phytochrome, a soluble chromoprotein of about 120 kDa.[139–141] Structurally, phytochrome is divided into two modules: the N-terminal conserved photosensory module, which consists of a PAS (PER/Arnt/Sim), a GAF (cGMP phosphodiesterase/adenylyl cyclase/FhlA), and a so-called PHY (phytochrome-specific domain/phytochrome) domain, and the C-terminal module that contains two PAS domains and a kinase-like domain.[142] The C-terminal PAS repeat region (amino acids 594–917) contains a putative cryptic nuclear localization signal (NLS).[143,144] Upon absorption of light, this photoreceptor is converted from the inactive near-redlight-absorbing form to the active far-red-light-absorbing form. Photoactivation induces structural changes in phytochrome, promoting its import into the nucleus by the nuclear localization activity of the C-terminal region of the molecule. Nuclear relocalization is clearly a light-regulated process, because in darkness, the N-terminal chromophoric domain suppresses the nuclear localization activity of the C-terminal domain activity and retains the phytochrome in the cytoplasm.[145] Furthermore, the photosensory GAF–PHY domains interact in a light-dependent manner with the PAS domain.[144] Because nuclear localization signals are commonly located within disordered regions,[146] these findings suggest that interaction with light induces partial unfolding of the phytochrome.

Obviously, denaturing factors are not acting alone and may affect proteins in various combinations. An interesting illustration of this concept is the light-regulated and redox-mediated activation of the chloroplast glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a NAD(P)H-dependent enzyme involved in plant photosynthetic carbon reduction. GADPH of higher plants is a tetramer consisting of two A and two B subunits. The B subunit is 80% identical in sequence to the A subunit. In addition, the B-subunit has a C-terminal extension (CTE) of 30 residues. The CTE is highly conserved among different plant species, and contains two invariant cysteines and nine negatively charged amino acids. The regulatory function of CTE depends on these invariant redox-active cysteines, which undergo dithiol/disulfide exchange reactions with thioredoxin under physiological conditions. In the absence of light, GAPDH is oxidized and exists as a high molecular weight oligomer with low NADPH-dependent activity.[147] In the presence of light, thioredoxin-mediated reduction of the disulfide bond leads to the dissociation of these high molecular weight oligomers into lower molecular weight forms, mostly active

tetramers.[148,149] Analysis of the crystal structure of oxidized A_2B_2–GAPDH complexed with NADP revealed that each disulfide-containing CTE is docked into a deep cleft between a pair of A and B subunits, preventing the functionally important interaction between Arg-77 and NADPH.[150] GADPH activation depends on the reduction of this disulfide bond and on the subsequent local unfolding of the CTE.

2.5. Force Sensors: Mechanically Gated Channels

Because mechanical stimuli are common in nature, mechanosensing represents one of the most ancient sensory transduction processes that evolved in living organisms. A wide range of cellular and organismal sensations, from touching, to hearing and cell swelling, to proprioception, to sensing the osmotic gradients, gravitropism, and control of cellular turgor, are mediated by mechanically sensitive ion channels. These channels detect and transduce external mechanical forces into electrical and/or chemical intracellular signals, and have been implicated in a wide variety of mechanical transduction processes in diverse organisms and species.[151,152] Mechanosensitive ion channels recognize and respond to membrane tension, that is, the mechanical forces applied along the plane of the cell membrane, rather than to the hydrostatic pressure perpendicular to the membrane plane.[153]

The mechanosensitive channel of large conductance (MscL) is one of the best characterized bacterial membrane channels in both structural and functional terms. MscL responds to membrane tensions of about 10–12 mN/m.[154,155] The MscL homologue from *Mycobacterium tuberculosis* was shown to exhibit a homopentameric channel structure in the closed state, with each subunit containing two membrane-spanning α-helices (TM1 and TM2).[156] The N-terminal region forms the S1 helix, and the C-terminal region forms a long α-helix that participates in an intersubunit five-helix bundle at the cytoplasmic side of the membrane. This helical bundle stabilizes the closed structure, which is further stabilized by a hydrophobic lock of the gate.[157,158] Using patch clamp methods, fluorescence resonance energy transfer (FRET) spectroscopy, data from electron paramagnetic resonance experiments, and molecular and Brownian dynamics simulations, a model for the open state of the MscL channel was proposed.[159] In this model, membrane tension was proposed to induce the outward movement of the TM1 and TM2 helices to increase the pore diameter from 2 to 28 Å, accompanied by the noticeable stretching and unwinding of both transmembrane helices (i.e., their partial unfolding).[159] Furthermore, upon full opening, the cytoplasmic helical bundle is thought to disassemble, and individual helices might be involved in interaction with the membrane, whereas the hydrophobic lock is buried in the protein interior by the rotation of TM1.[157,158] It is tempting to speculate that disassembly of the helical bundle leads to the transient unfolding of individual helices, which subsequently fold upon interaction with the membrane surface. The mentioned structural changes associated with the membrane tension-induced opening of the MscL channel are illustrated in Figure 4, which represents the results of the constrained molecular dynamic simulations.[159] Here, the resulting structures retrieved for the closed state and model open states obtained under various membrane tensions are shown. Noticeable unwinding of several helices is evident.

Illustrative examples of eukaryotic mechanosensitive ion channels include members of the two-pore-domain (2P-domain) weakly inward-rectifying K^+ channels, TWIK (for "tandem of P domains in a weakly inward-rectifying K^+ channel"), TWIK-1 related K^+ channel (TREK), and TWIK-1 related arachidonic acid-stimulated K^+ channel (TRAAK).[151] These channels are comprised of four transmembrane segments and tandem pore domains, with both N- and C-termini facing the cytosol. The C-terminal domain is essential for mechanosensing, and partial deletion of this region impairs channel activation by membrane tension.[160] The channels are opened by membrane stretching as well as by cell swelling. Although eukaryotic mechanosensitive ion channels have more complex structures in comparison with MscL, they likely utilize a similar gating mechanism where changes in the membrane tension induce local unfolding–folding reactions.

In addition to mechanosensitive channels regulated by lateral membrane tension, some channels are regulated by a linear force applied either through cytoskeletal and through extracellular matrix elements attached to the channel.[161] The transition from the closed to the open state in these channels resembles mechanical force-induced activation of the titin kinase (see below). Two examples of such linear force-modulated channels are found in the hair cells from vestibular organs and in the touch cells from *C. elegans*.[161] Mechanosensitive channels related to hearing perception are located in the inner ear, where the mechanosensory cells (the hair cells) use a bundle of stereocilia to apply force to the channels located at the tips of stereocilia, most likely at both ends of the tip links.[161,162] A positive deflection of shorter stereocilia toward the tall stereocilia causes shearing between adjacent stereocilia that increases tension on the tip links (which serve as gating springs that are stretched and therefore partially unfolded by the stimulus-induced shear), and this tension is apparently directly conveyed to the channels. [161–164]

2.6. Mechanoenzymology: Modulation of Protein Catalytic Activity by Mechanical Unfolding

The ability to process mechanical signals, react to mechanical stress, and convert mechanical force into biochemical signals (i.e., mechanosensing) is a fundamental property of many living cells. In fact, muscle cells, including cardiomyocytes, are constantly exposed to external forces and generate forces themselves. The section below considers some of these strain-sensing proteins, whose mechanically induced conformational changes initiate signaling cascades that mediate changes in cell and tissue properties.

Titins (also known as connectins) are a family of muscle proteins that include some of the largest known vertebrate polypeptides (e.g., human titin is a 4.2-MDa protein consisting of 38 138 amino-acid residues).[165] A single titin molecule spans one-half the sarcomere, with its N- and C-termini being firmly anchored at the Z-disc and M-band of the sarcomere, respectively. Therefore, titins are uniquely positioned relative to the mechanosensory "hotspots", which are located in the Z-disk, I-band, and M-band regions of the sarcomere. In this setting, they are crucial mechanosensing players that sense the mechanical stimuli and transform them into biochemical signals, such as signals triggering cardiac hypertrophy.[166] In addition to mechanosensing, the major biological roles of titins are their functions as "molecular rulers", regulating the assembly of the highly ordered para-crystalline structure

of the sarcomere, and as molecular springs. The latter role is mostly attributed to the I-band region of titin, which is extendable and contains distinct mechanically active elements that respond differently to stretch forces.[166]

Vertebrate titin is a string-like, highly asymmetric, and modular molecule that is about 1 μm long and consists of ~300 immunoglobulin (Ig) domains (i.e., I-set) and fibronectin-like (Fn, type III) domains arranged in tandem. Each of these domains contains ~100 amino acid residues folded in a β-sheet sandwich.[167] At a more global level, a titin molecule has two parts, the A-band, consisting of super-repeats or periodic patterns of the Ig and Fn domains, and the I-band, consisting mainly of Ig-like domains. In addition, the I-band part harbors several unique sequences. The largest of these is the PEVK region, which is rich in proline (P), glutamate (E), valine (V), and lysine (K) residues and is responsible for muscle elasticity. Finally, a specific titin kinase domain is located near the C-terminus of the molecule.[167]

Because titins are firmly embedded in the contractile machinery, their conformation and function is directly affected by mechanical forces.[168,169] This type of mechanosensing is nicely illustrated in the titin kinase domain. This domain is involved in strain-sensing via its unique regulation by dual autoinhibition mechanisms, where a C-terminal regulatory tail blocks the ATP binding site, and a tyrosine residue inhibits the catalytic base.[170] Using atomic force microscopy (AFM)-based single-molecule force spectroscopy, molecular dynamics simulations, and enzymatic studies, the conformational changes induced in the human titin kinase by mechanical strain were studied.[170] These analyses confirmed the existence of strain-mediated activation of this protein and showed that application of mechanical force is able to pull the inhibitory tail from the active site, unfold the tail, and activate ATP binding before unfolding of the structural Ig and Fn domains occurs.[170] Therefore, titin can obviously function as a force sensor. These experimental results are in agreement with the conclusions obtained from earlier force-probe molecular dynamics simulations, which provided evidence for a sequential mechanically induced opening of the catalytic site without complete domain unfolding. The simulations furthermore suggested that the inhibitory C-terminal domain exhibits low force resistance relative to the remainder of the protein.[171] Similar sequential mechanical unfolding was described for the protein kinase domains of the giant *Caenorhabditis elegans* titin-like proteins, twitchin and TTN-1, both of which unfold in a stepwise fashion, where the first step involves the unwinding of the autoinhibitory region, followed by two-step unfolding of the catalytic core.[172]

3. ACTIVE FACTORS PROMOTING TRANSIENT DISORDER: INTERACTION-INDUCED FUNCTIONAL UNFOLDING

3.1. The “Membrane Field” Effect: Functional Unfolding Induced by the Membrane

Many proteins are known to exist in both aqueous and membrane-bound states,[173,174] and some of these proteins can be activated through binding to membranes. Among such dual personality proteins are protein toxins,[175,176] including alamethicin, [177] apolipoprotein H,[178] SecA,[179] cytochrome *c*,[180] and trichosanthin.[181] As early as 1988, it was proposed that the membrane surface can be considered as one of the major protein structure-modifying factors

of the living cell, exhibiting the ability to denature the rigid structure of an ordered protein.[182] In fact, the interaction of some ordered proteins with membranes or even their positioning in the vicinity of a membrane can lead to partial protein unfolding.[183] For example, one of the bacterial protein-toxins, colicin A, was shown to denature during its translocation through membranes in *Escherichia coli* cells.[184–186] It is now clear that in addition to colicine A, a variety of other colicines, including B, D, E1, E3, Ia, M, and N, also undergo binding-stimulated denaturation upon interacting with the membranes of the bacterial cell surface.[187] Denaturation was also detected upon interaction of ricin, a potent cytotoxin from the seeds of *Ricinus communis*, with biological membranes.[188] Ricin is composed of an RNA *N*-glycosidase A-chain (RTA), which is disulfide-bonded to a cell-binding B-chain (RTB). RTA is a member of a large family of plant-derived ribosomeinactivating proteins (RIPs), which cause depurination of a specific adenyl ribonucleotide, thereby inhibiting the binding of elongation factors to the ribosome. Inhibition of protein synthesis eventually causes cell death.[189,190] Similar membraneinduced unfolding is likely to be involved in the transmembrane penetration of other RIPs, such as cholera toxin, Shiga toxin, Shiga-like toxin, and *Pseudomonas* exotoxin.[191–194]

Structural changes accompanying the interaction of filamentous phages with host cells represent another interesting case of global and cooperative membrane-induced unfolding of proteins. The peculiarities of this process were analyzed in detail for the *fd* phage penetrating host cells.[195,196] Here, *fd* phage was shown to undergo large morphological changes, morphing first from the highly extended original form to the contracted I-form, about 1/3 as long as the original phage, then to the micelle-like spherical S-form, and finally to a helical form embedded in membrane bilayers.[196] Detailed biophysical analysis revealed that both I- and S-forms of the *fd* phage represented compact conformations characterized by nonrigid side chain packing and non-native morphologies but native-like secondary structures. In fact, these features correspond to a molten globule state.[196] Therefore, the membrane-induced transition from an ordered protein to a molten globule-like conformation may be necessary not only for translocation of proteins through the membrane, but also for the assembly and disassembly of large protein complexes, such as phage and virus capsids.

Finally, another group of ordered proteins, whose function depends on membrane-mediated denaturation, includes transport proteins, which require a global structural change to release large hydrophobic ligands at the delivery site.[197,198] A recent NMR analysis of the ternary system composed of the bile acid binding protein, bile acids, and a membrane mimetic system revealed that the liver bile acid binding protein undergoes association with the anionic membrane, which induces its partial unfolding and the release of bile salts.[199] These studies are in agreement with earlier model studies, which described the release of retinol from the retinol-binding protein.[200,201]

Mechanistically, the denaturing capacity of a membrane is determined by its effects on the pH and dielectric constant of the solution. In fact, a negative electrostatic potential of the membrane surface can attract protons from the solution, resulting in a noticeable local decrease in pH at the membrane surface and the formation of a pronounced pH gradient extending to the surroundings. Because the local pH decrease does not exceed 2 pH units in salt-free solutions, such mild “acidification” of the media is insufficient for pH-induced

denaturation of the majority of globular proteins, which typically takes place at extremely low pH values (pH 2.0–3.0). Therefore, the local pH decrease cannot be considered as the sole denaturing factor of the membrane surface. However, a local decrease in the effective dielectric constant near the membrane surface can be considered as an additional denaturing factor.[198] Water–organic solvent mixtures at moderately low pH values were suggested as model systems to study the joint action of the local decrease in the solution pH and dielectric constant near the membrane surface (i.e., “membrane field effect”) on the structure of ordered proteins.[197,202,203] In agreement with this hypothesis, an increase in the methanol concentration at moderately low pH values transformed cytochrome *c*,[202] retinol-binding protein, [200,201] and α-fetoprotein into a molten globular state.[204] The existence of the dielectric constant-induced molten globule-like intermediate has also been described for the well-characterized ordered protein β-lactoglobulin.[203]

In conclusion, interaction with biological membranes can induce global conformational changes in many ordered proteins. Typically, the influence of a membrane induces denaturation and transformation of an ordered protein into the molten globule-like conformation. The denaturing activity of membrane is due to its global effects on the solution pH and dielectric constant at the membrane surface. The membrane-induced denaturation is crucial for protein translocation through the membrane, release and binding of the large hydrophobic ligands, and the assembly and disassembly of large protein complexes.

3.2. Functional Unfolding Induced by Ligand (Small Molecule) Binding

Local or global folding is a very general outcome of interaction of many different proteins with many different ligands. The examples of such binding-induced folding events are practically limitless. On the basis of the analysis of available literature data on the effect of ligand binding on protein structure, the following classification was proposed in 1998.[205,206]

Class I: The release of ligands does not result in noticeable changes of the unique protein structure. While the structural characteristics of apo- and holo-forms coincide, the conformational stability of the protein molecule changes.

Class II: The release of the ligand promotes detectable changes in the protein structure. However, the protein molecule still possesses an extensive tertiary structure, and its secondary structure does not change substantially.

Class III: The ligand release transforms the protein into the molten globule state. Whereas the unique tertiary structure is lost upon release of the ligands, the secondary structure is retained, and the protein molecule remains compact.

Class IV: The ligand release initiates noticeable protein unfolding. The ligand-free form of the protein is largely unfolded, has no tertiary structure, the secondary structure content is noticeably less than in the holo-protein, and dimensions of the apoprotein distinctly differ both from statistical coil dimensions and from the dimensions of the holo-protein.

Class V: The ligand release initiates global protein unfolding. The apo-form represents a completely unfolded polypeptide chain.

Class VI: Release of ligands induces large-scale movements of large parts (domains or subunits) of the protein molecule.

For all of these cases (apart from class I), the structural consequences of ligand binding are easy to rationalize, because binding does what it is supposed to do: it induces global or local protein folding. However, there are some proteins, which actually unfold upon ligand binding. Because binding-induced unfolding is a rather atypical phenomenon, examples of such behavior are rare. This conclusion is supported by a recent analysis of the effects of ligand binding on protein structure using the Protein Data Bank structures.[207] Here, 839 nonredundant pairs of crystal structures of proteins with and without bound low-molecular-weight ligand were compared, and protein motions coupled with ligand binding were analyzed and systemized. The authors showed that binding of ligand frequently induces local motions, which in several instances are accompanied by order–disorder or α-helix–coil conformational transitions. Ligand-induced local unfolding typically affects only a few residues, and in the entire set of 839 analyzed pairs, there was only one protein, succinyl-CoA:3-oxoacid coenzyme A transferase (SCOT), where binding of the ligand, 2-(ethylmercuri-thio)-benzoic acid (EMT), produced unfolding of two relatively long regions (loops containing 24 and 19 residues, respectively).[207] There were 15 proteins in which ligand binding induced unfolding of regions longer than 10 residues. Furthermore, in a few cases, ligand binding was accompanied by the simultaneous unfolding of several short regions within the protein.[207] One illustrative example is the human inositol 1,4,5-trisphosphate 3-kinase, where ligand binding induced local unfolding of its N-terminal portion comprising residues 187–196, which was a well-defined α-helix in the apo-form.[208]

3.3. Functional Unfolding via Protein–Protein Interactions

Similar to many other types of protein interactions, binding of two or more proteins is commonly accompanied by their mutual adjustment and folding. In line with the general theme of this Review, examples below show that some protein–protein interactions cause functional protein unfolding.

A recently described example of protein binding-induced local unfolding involves the partial unfolding of BCL-xL upon binding of its interaction partner PUMA.[209] BCL-xL is a helical, globular antiapoptotic protein that binds and sequesters both pro- and antiapoptotic factors in the cytoplasm through protein–protein interactions.[210] Best known to sequester the so-called BH3-only proteins, one of which is the protein PUMA, BCL-xL also interacts with the apoptotic effector proteins, BAX and BAK.[211] In addition, BCL-xL modulates the apoptotic regulatory activity of other cytoplasmic proteins, including the tumor suppressor p53.[212] Under conditions of genotoxic stress, cytoplasmic p53 binds and activates BAX and engages the intrinsic mitochondrial pathway of apoptosis.[213] However, BCL-xL binds and sequesters p53 in an inactive form, preventing engagement of apoptosis. In the absence of PUMA, two α-helices (α2 and α3) within BCL-xL form part of the interface with p53.[209] Strikingly, the binding of PUMA to the BCL-xL/p53 complex causes the binding-induced unfolding of these two helices, leading to p53 release, BAX activation, and subsequent

apoptotic events. These structural changes are illustrated by Figure 5 showing the major events taking place during this process. NMR spectroscopy and X-ray analysis showed that the BH3 domain of PUMA folds into an α-helix upon binding within a hydrophobic groove on the surface of BCL-xL and that a π-stacking interaction between W71 at the N-terminus of PUMA's α-helix and H113 of BCL-xL locally distorts the BCL-xL structure, leading to the destabilization of α-helices 2 and 3. BH3 domains from other BH3-only proteins lack a tryptophan residue at positions equivalent to W71 in PUMA, and, while most can bind to the p53/BCL-xL complex, they fail to cause local unfolding of α2 and α3 (of BCL-xL) and do not release p53. PUMA is an intrinsically disordered protein, and its BH3 domain extensively folds upon binding BCL-xL; it is interesting that this folding-upon-binding event is accompanied by binding-induced partial unfolding of BCL-xL. Whether this entropic compensation scheme is operative in other cases of ligand and protein binding-induced unfolding must be investigated in the future.

In their 2004 review, Prakash and Matouschek discussed the roles and mechanisms of in vivo protein unfolding using examples of ATP-dependent proteases and the mitochondrial import machinery.[214] ATP-dependent proteases (proteasomes in eukaryotes and proteasome analogues such as the ClpAP, ClpXP, HslUV, Lon, and FtsH proteases in prokaryotes) are crucial for the timely and controlled degradation of regulatory proteins as well as of misfolded or damaged polypeptides. Furthermore, they are responsible for the production of antigenic peptides. All of these ATP-dependent proteases are large protein assemblies, which are typically barrel-shaped. The proteolytic sites of these proteases are located within the central core and are accessible only through a narrow translocation channel aligned with the long axes of the particles.[215] A crucial functional step of these ATP-dependent proteases is the active unfolding of their protein substrates, as demonstrated for ClpAP,[216] ClpXP,[217] FtsH,[217] Lon,[218] the archaebacterial proteasome-regulatory ATPase complex PAN,[219] and the eukaryotic proteasome.[220] It is likely that these proteases unfold their substrates mechanically by pulling the polypeptide chain into their channel. This conclusion follows from the observation that during degradation of a protein by the ATP-dependent protease, unfolding occurs together with translocation of the polypeptide chain into the degradation channel.[214]

The translocation of cytosolic proteins into mitochondria is controlled by the mitochondrial import machinery complex. This machine includes translocases in the outer and inner mitochondrial membranes termed "TOM proteins" and "TIM proteins", respectively, as well as several other proteins in the matrix. Complexes of TOM and TIM proteins associate to form a contiguous translocation channel that connects the cytosol to the mitochondrial matrix.[221] Mitochondria actively unfold substrates by unraveling them from the targeting signal.[222] During import, most mitochondrial proteins are unfolded by the electrical potential across the mitochondrial inner membrane acting directly on positive charges in the targeting sequences.[223,224]

The commonality of the proteasome- and mitochondria-mediated unfolding of target proteins is that the susceptibility of a protein to unfolding during proteasome-catalyzed degradation or mitochondrial import is determined primarily by the stability of the local

structure adjacent to the targeting signal rather than by the overall stability of a protein to global unfolding.[220,222,225]

An ATP-dependent protease HslVU (heat shock locus VU) found in *E. coli* is a bacterial ATP-dependent protease distantly related to eukaryotic proteasomes. In addition to the ATP-dependent unfoldase activity described above, local unfolding is likely involved in the formation of the active proteasome-like complex. HslVU is one of the two-component ATP-dependent proteases in bacteria, consisting of hexameric HslU ATPase and dodecameric HslV protease packed to align the HslU and HslV central pores.[226–228] HslV exhibits ~20% sequence similarity with respect to the β-subunit of 20 S proteasome, and these two proteins share a conserved fold. HslV forms a barrel-shaped dodecameric complex by head to head stacking of two hexameric rings.[229] Therefore, the HslVU complex contains 12 proteolytic active sites, which are sequestered in an internal protease chamber of HslV. HslV alone shows a very weak peptidase activity, but its activity increases 1–2 orders of magnitude when it binds to HslU in the presence of ATP.[230] Analysis of the HslVU crystal structure revealed that this activation might be caused by localized unfolding of two segments (residues 65–69 and 84–93) within the HslV subunits. In HslU-unbound HslV, the 84–93 segment is an α-helix lining the entrance channel into the HslV protease chamber.[231] Unfolding of this helix induced by binding to HslU might enable the target protein to enter the protease chamber.

The proteasome and mitochondrial import machinery complex discussed above are considered to be unfoldases because they actively unfold their substrates. Several chaperones are also unfoldases and assist the refolding of misfolded proteins that are kinetically trapped in local conformational energy minimum. ATP-dependent molecular chaperones use free energy from ATP binding and/or hydrolysis to assist folding by randomly disrupting non-native interactions (i.e., local unfolding) via repeated cycles of binding and release, allowing the substrate to resume its search in conformational space for the global energy minimum.[31,232–235] By using this mechanism, ATP-dependent chaperones (e.g., Hsp70 proteins) are able to lower the activation energy barrier for the transitions from the misfolded species to the productive intermediates without changing the microscopic rate constant for the folding reaction. Acting as unfoldases, ATP-dependent chaperones reverse the nonproductive reactions leading to the misfolded species, and therefore keep the polypeptide chain in a folding-competent state.[235] Unfoldase activity was studied in detail for several members of the Hsp70 family, such as DnaK,[235] the mitochondrial heat shock protein, mtHsp70,[236] GroEL,[237] and particularly members of the ClpB family.[238–241]

There are three potential mechanisms for the unfoldase activity of chaperones: the entropic pulling model, mechanical unfolding via forcible stretching, and the entropy transfer model.[56] GroEL binds non-native proteins by means of a ring of hydrophobic residues that line the entrance to the central cavity of its heptameric ring.[242,243] When GroEL binds ATP and the GroES cochaperonin, massive structural changes double the GroEL cavity volume and occlude its hydrophobic binding surface.[244,245] In fact, before binding ATP and GroES, GroEL's binding sites are located 25 Å from each other, whereas upon the addition of ATP and GroES, the apical domain of each GroEL subunit twists upward and outward so that the binding sites move apart to a position 33 Å from one another. As a result, neighboring

binding sites move apart by 8 Å and non-neighboring sites by larger increments, up to 20 Å. These large-scale movements provide the means for the mechanical unfolding of the misfolded substrate protein, which, as it is tethered to these sites, will be forcibly stretched and partially unfolded.[246,247]

Another mechanism of the unfoldase activity of chaperones is described by a so-called entropy transfer model:[31] the ability of disordered segments of the chaperone to rapidly and transiently bind the substrate and subsequently create local order may “pay” the thermodynamic cost of local substrate unfolding. In other words, the entropy transfer model implies the ordering of the chaperone with a concomitant unfolding of the substrate. Here, the binding-induced folding of a disordered, nonspecific binding segment of a chaperone may promote the local unfolding of the misfolded segment, and the energy required for the local unfolding of the substrate may be provided by the binding and folding of the chaperone.[31]

Flagella are filamentous organelles that extend from the cell surface and are required for motility. It has been shown that transient unfolding plays a crucial role in the flagellar protein export and in the subsequent assembly of the flagella.[248–250] The bacterial flagellum is a supramolecular structure consisting of about 30 different proteins with copy numbers ranging from a few to tens of thousands of copies. They are assembled into a basal body, a hook, and long helical flagellar filaments with a diameter of ~120–250 Å and a length of up to 15 μm.[251] The assembly of the flagellum is a very complex process that involves multiple proteins and intricate mechanisms.[248–250] Among these mechanisms are the flagellar type III protein export apparatus-driven translocation of the majority of the flagellar components across the cytoplasmic membrane, the diffusion of these components through the narrow channel, and the self-assembly of these components at the flagellar distal end with the help of a cap structure.[248] Flagellar proteins are synthesized in the cytoplasm. They are targeted to the export apparatus by a set of flagellum-specific chaperones and are pushed into the channel by an ATPase.[248] An important step of the flagellar protein translocation is transient unfolding that is required for their directed diffusion through the export channel. The need for transient unfolding of flagellar proteins follows from the mismatch between the folded sizes of these exported proteins and the geometry of the inner channel of the export apparatus. In fact, the diameter of the export path for flagellar proteins, known as the central channel of the flagellum, is only ~20 Å, whereas native hydrodynamic dimensions of proteins to be exported are significantly larger than this opening.[251] A model has been proposed in which successive unfolding and translocation of the long polypeptide chain through the narrow pore of the export gate is driven by the one-dimensional Brownian motion biased by the proton motive force (i.e., the work per unit charge required to move a proton).[249]

4. ACTIVE FACTORS PROMOTING TRANSIENT DISORDER: INDUCTION OF FUNCTIONAL UNFOLDING BY POST-TRANSLATIONAL MODIFICATIONS

Although proteins are generally synthesized from 20 natural amino acid residues, their chemical complexity is dramatically increased by various post-translational modifications, such as acylation (acetylation, isoprenylation, myristoylation), adenosine diphosphate

(ADP)-ribosylation, farnesylation, glycosylation, lipidation, methylation, nitration, *S*-nitrosylation, oxidation, phosphorylation, proteolysis, sulfation, sumoylation, ubiquitination, etc.[252] More than 300 different post-translational modifications are known, which have the potential to change protein size, charge, structure, or conformation, or can introduce new functional groups that can modify interactions with other molecules. Some proteins are known to undergo multiple and different post-translational modifications. An important feature of many post-translational modifications is their reversible nature. Structural and functional consequences of different post-translational modifications are very diverse. In general, post-translational modifications alter protein characteristics such as enzyme activity, binding affinity, or hydrophobicity.[253] They affect and change protein structure and function directly and also modulate protein functionality indirectly, determining compartmentalization, sequestration, degradation, elimination, and protein–protein interactions.[253] Several examples of post-translational modification-modulated functional unfolding of proteins are given below.

4.1. Functional Unfolding Induced by Phosphorylation/Dephosphorylation

Phosphorylation–dephosphorylation cycles are crucial for functional regulation of many proteins in both eukaryotes and prokaryotes, with reversible protein phosphorylation being known to provide a major regulatory mechanism in eukaryotic cells.[254] Phosphorylation sites are frequently found within functionally important protein domains, and about one-third of all eukaryotic proteins are estimated to undergo reversible phosphorylation.[255] At least 575 protein kinases or kinase domains constitute a human kinome. This is the third most populous functional class in the human proteome and covers ~2% of the total genome.[256] Although the crucial role of reversible phosphorylation is most obvious for eukaryotes, in prokaryotes, signaling by phosphorylation is also very important. For example, approximately 1.5% of the entire *E. coli* genome (62 genes) encode proteins related to histidine kinase/response regulator systems.[257] Phosphorylation–dephosphorylation pathways inside the cell can be triggered in response to various extracellular and intracellular signals, such as growth factors, hormonal, or neuronal signals on the outside of the cell. This provides a controllable mechanism for the cell to switch on or switch off many vital processes.[257] Among these phosphorylation-controlled pathways are metabolism, growth, differentiation, transcription, motility, transport, learning, memory, kinase cascade activation, modifications of enzymes, linker molecules, and transcription factors, and many others.[258]

Functionally, phosphorylation can result in protein activation or inhibition. Structurally, it can modify the surface properties of a protein to affect its self-association or propensity to interact with other molecules; it can initiate conformational changes that may be local or remote from the site of phosphorylation; it can act by steric blocking, relief of steric blocking, or by relief of autoinhibition.[258] A systematic computational analysis revealed that phosphorylation sites in proteins are predominantly located within intrinsically disordered regions.[254] The structural consequences of phosphorylation are diverse, and both disorder to order and order to disorder transitions have been observed to follow the phosphorylation event.[257] A few illustrative examples of functional order to disorder transitions induced by phosphorylation are presented below.

In eukaryotes, the cap-dependent translation initiation activity of the eukaryotic translation initiation factor eIF4E is regulated by the family of the 4E-binding proteins (4E-BPs).[259,260] The affinity of the 4E-BPs for eIF4E is regulated by phosphorylation, connecting protein translation initiation to the presence of growth factors, hormones, mitogens, and cytokines.[260] Biophysical analysis revealed that in their unbound forms, the 4E-BP family members (e.g., 4E-BP1) are typical IDPs that contain little or no structure in solution.[261–263] Upon forming complexes with eIF4, their binding regions undergo significant disorder-to-order transitions and become more structured (approximately 50% helical).[262,264] Interaction between 4E-BP1 and eIF4E is controlled by 4E-BP1 phosphorylation, with S65 phosphorylation in 4E-BP1 causing the greatest reduction in eIF4E-binding affinity as compared to all other single phosphorylation events reported for this inhibitor protein.[265] Recently, a systematic biophysical analysis revealed that phosphorylation of this S65, which is located at the C-terminal end of the inducible helical region and required for tight binding to eIF4E, causes a decrease in the propensity of this region to fold. Therefore, phosphorylation acts as an intramolecular structural regulator that modulates the disorder–order transition of 4E-BP1 via the effective destabilization of the nascent α-helix to favor the unfolded form that cannot bind eIF4E.[266]

Recently, phosphorylation-induced unfolding was also shown to enhance chaperone activity of two milk proteins, α_s- and β-caseins.[267] Combining a functional assay with a variety of spectroscopic techniques, the authors established that the alkaline phosphatase-catalyzed dephosphorylation of α_s- and β-caseins resulted in a decrease in the chaperone efficiency of these milk proteins concomitant with an increase in their ordered structure. In other words, naturally phosphorylated αs- and β-caseins are less ordered and more chaperone-active than the dephosphorylated proteins.[267]

In many cases, phosphorylation-induced local unfolding of important inhibitory regions is used as an activating mechanism (i.e., release of autoinhibition). An example for this mechanism is found in the p27/cyclin-dependent kinase (Cdk)/cyclin cell cycle regulatory system.[268] p27 is an intrinsically disordered protein that folds upon binding and inhibits the Cdk/cyclin complexes that are the master regulators of cell division.[269] Here, a conserved, 80 residue long N-terminal kinase inhibitory domain (KID) of p27 binds to the Cdk/cyclin complex through interactions of a short N-terminal motif termed D1 with cyclin A, whereas a longer C-terminal motif termed D2 interacts with Cdk2.[270] These two motifs are connected by a linker domain termed LH. When bound to Cdk2/cyclin A, the D2 subdomain of p27 inserts a tyrosine residue (Y88) into the ATP binding pocket of Cdk2 to inhibit its catalytic activity. Remarkably, despite burial in the Cdk2 active site in crystals, Y88 is accessible for phosphorylation by nonreceptor tyrosine kinases, suggesting that dynamic fluctuations of this residue in and out of the binding pocket are involved.[271] Once phosphorylated, Y88 is unable to resume its inhibitory conformation, restoring partial kinase activity to Cdk2. Restoration of Cdk2 activity triggers a cascade of other post-translational modifications within p27, including phosphorylation of T187. This phosphorylation is followed by ubiquitination through the E3 ligase SCF^{Skp2}, and the degradation of ubiquitinated p27 by the 26S proteasome. The elimination of p27 in turn fully activates Cdk2/cyclin complexes, which then drive progression of the cell division cycle from G_1 to S phase and ultimately through mitosis.[272] Because of the disordered features of isolated p27,

the different subdomains of p27 behave independently within the Cdk/cyclin complexes. Therefore, phosphorylation of Y88 exerts an exquisitely local unfolding effect on the bound conformation of p27 (ejection of Y88 from the Cdk2 active site), while its other subdomains remain bound to Cdk2 and cyclin A. This enables T187, which is located within the 100 residue-long and highly flexible C-terminus of p27, to be phosphorylated by Cdk2 through a pseudo unimolecular mechanism that efficiently propagates the signaling cascade.[271] This example illustrates the regulatory versatility of a disordered protein;[273] folding-upon-binding events accompany the targeting of p27 to inhibit Cdk/cyclin complexes, while phosphorylation-dependent local unfolding of the kinase inhibitory structural element (Y88) relieves this inhibition and triggers a downstream signaling cascade. These phosphorylation-induced local unfolding events and their critical roles in cell cycle regulation are illustrated by Figure 6. Clearly, this process represents an illustrative example of the PTM-induced regulation of the autoinhibition mechanisms described in section 5.1 (see below).

4.2. Local Unfolding of Ordered Proteins and Destabilization of the Unfolded State Induced by Glycosylation

Many biological activities of glycoproteins and glycopeptides are orchestrated by their carbohydrate moieties (or glycans),[274,275] making glycosylation an important regulator of many biological processes, such as protein folding and stability, manipulation of a protein's cellular localization and trafficking, and cell–cell interactions.[276] Glycosylation is an enzymatic process that covalently links oligosaccharide chains to certain amino acids. There are four main categories of glycosylation: (i) N-linked glycosylation (addition of the sugars to the amide group (NH_2) of an asparagine of secreted or membrane-bound proteins), (ii) O-linked glycosylation (addition of the sugar to the hydroxyl group (OH) of a serine or a threonine), (iii) C-mannosylation (addition of a mannose sugar to tryptophan), and (iv) GPI anchors (see lipid anchor modifications).[277] Often, oligosaccharides constitute a significant fraction of the mass of glycoproteins and glycopeptides and therefore can significantly affect peptide backbone behavior.[278–281] Similar to several other PTMs (e.g., phosphorylation), structural consequences of protein glycosylation are difficult to predict, because this modification is known to stabilize some proteins and lead to the destabilization and local unfolding of other proteins. Through their bulky hydrophilic character, glycans often increase solubility and resistance to proteolysis of the protein to which they are attached. Moreover, glycosylation modulates structural and conformational characteristics of proteins by affecting differently the thermodynamics (enthalpy or entropy) of the unfolded and/or folded state, enhancing both the thermodynamic and the kinetic stability of proteins.[282] It was also reported that glycosylation helps in the formation of a secondary structure of a peptide backbone, especially the β-turn,[283–285] and stabilizes the peptide backbone conformation.[286–288] However, carbohydrate moieties were also shown to induce local structural perturbations, affecting the α-helical structure around the glycosylation sites,[289] or even promote complete disruption of α-helical structure.[290] Attachment of glycans inhibits transient interactions in the unfolded state, leading to its destabilization (or the formation of an unfolded state with a higher enthalpy). In other words, glycosylation reduces residual structure typically found in the unfolded proteins, therefore making them more unfolded and resulting in a significant increase in their radius of gyration.[282] The degree of destabilization of the unfolded state increases with the degree of glycosylation. As a result, the unfolded

state ensembles of the glycosylated proteins are more extended in comparison with the conformational ensembles of the nonglycosylated proteins, and their dimensions correlate with the degree of glycosylation.[282]

4.3. Regulatory Unfolding Caused by S-Glutathionylation

Protein S-glutathionylation is a reversible addition of the tripeptide glutathione (GSH) to cysteine residues that have low pK_a values due to their localization in the vicinity of Lys, Arg, or His that create a basic local environment.[291] Reversibility of S-glutathionylation is catalyzed by members of the glutaredoxin family.[292,293] This type of modification is increasingly recognized as an important mechanism underlying redox regulation of signaling pathways with downstream effects on numerous cell functions.[294,295] S-Glutathionylation does not simply block a free thiol, but adds ~305 Da, introduces a net negative charge to the protein (as a consequence of the addition of Glu), and can result in the introduction of steric hindrance due to the addition of this bulky tripeptide. Obviously, these additions are expected to destabilize or even locally unfold protein structure. In agreement with this hypothesis, S-glutathionylation of protein disulfide isomerase was shown to promote partial unfolding and inhibition of this important molecular chaperone.[295] Similarly, glutathionylation also induces partial unfolding of cyclophilin A, causing an overall loss of α-helical structure and a gain in statistical coil in comparison with the nonmodified form.[296] Finally, S-glutathionylation of the *Escherichia coli* Hsp33, which is activated by oxidative conditions, results in partial disruption of ordered tertiary and secondary structure, the formation of solvent-exposed hydrophobic clusters, and functional activation.[297]

4.4. Protein S-Nitrosylation and Transient Unfolding

Protein S-nitrosylation, the covalent attachment of a nitrogen monoxide group to the thiol side chain of cysteine, is another redox-sensitive, thiol-based, and reversible post-translational modification. It plays an important role in a variety of processes, such as mitochondrial metabolic regulation, intracellular Ca^{2+} handling, protein trafficking, regulation of cellular defense against apoptosis and oxidative stress, etc.[298] S-Nitrosylation is produced when cysteine sulfhydryls react with the physiological carrier forms of NO, such as nitrosoglutathione (GSNO), which predominates among the NO carriers intracellularly.[299,300] The interaction of cysteine residues with GSNO is known to play a pivotal role in the regulation of biological activity of various proteins, making S-nitrosylation a precisely regulated process in vivo.[300–303] Among various structural and functional consequences, S-nitrosylation was shown to promote release of zinc ions from zinc fingers,[304] therefore disrupting zinc–sulfur clusters in proteins and causing unfolding of this structural unit.[305,306] Similar oxidation-mediated zinc release and unfolding of structural zinc sites has also been observed in response to reactive chlorine species and peroxide, making zinc centers, whose major biological function is to interact with nucleic acids and other proteins, one of the primary oxidation targets in the cell.[305,306]

4.5. Transient Unfolding Promoted by Methionine Oxidation

All amino acids are susceptible to oxidation to various degrees,[307] with methionine being one of the most readily oxidized amino acid residues apart from cysteine. Methionine is

easily oxidized to methionine sulfoxide, MetO, by different oxidants produced in biological systems, including H_2O_2, hydroxyl radicals, hypochlorite, chloramines, and peroxynitrite.[308] Like most cysteine modifications, methionine sulfoxides can be repaired by methionine sulfoxide reductase, which catalyzes the thioredoxin-dependent reduction of MetO to methionine.[309,310] On the basis of these observations, it was proposed that methionines might serve as internal redox scavengers, as the reversible oxidation of surface-exposed methionines may protect other functionally essential residues from oxidative damage.[311] In human α-synuclein, an abundant presynaptic protein in the brain, misfolding and aggregation of which are implicated as critical factors in several neurodegenerative diseases,[312–315] there are four methionines (Met1, Met5, Met116, and Met127). All of these were shown to be highly susceptible to oxidative modification (methionine sulfoxide formation) in vitro.[316–319] The oxidized form of α-synuclein was shown to be more unfolded than the nonoxidized protein as manifested by the larger contribution of unordered structure to both Fourier-transform infrared (FTIR) and far-UV circular dichroism (CD) spectra,[318] and by a detectable decrease in the α-synuclein-MetO compactness.[319] This was attributed to the decreased hydrophobicity of oxidized methionine leading to a decrease in the overall hydrophobicity of the protein.

5. ACTIVE FACTORS PROMOTING TRANSIENT DISORDER: RELEASE OF AUTOINHIBITION

5.1. General Consideration

Autoinhibition is a very common and potent regulatory mechanism, where functions of proteins are switched on and off in response to various signals utilizing “built-in” inhibitors, autoinhibitory domains, which are the protein regions that negatively regulate the function of other domains via specifically controllable intramolecular interactions and keep the targeted domains in a secure off state. The inhibitory intramolecular interactions between the autoinhibitory domain and the reminder of a protein either directly or allosterically interfere with the function of a “targeted” domain. Generally speaking, autoinhibition can be considered as a reversible barrier that prevents unwanted activation of signaling pathways. Although the autoinhibitory intramolecular interactions are relatively weak and often characterized by relatively small surface areas, their efficiency is determined by the relatively high effective local concentration that is generated by covalent tethering of the regulatory and regulated domains.[320]

Autoinhibition and its release represent a simple and robust mechanism for the control and regulation of protein functions. The efficiency of this mechanism is determined by a simple architecture of the autoinhibited proteins, where a functional domain (FD) and an inhibitory module (IM) are located within one polypeptide chain, by the ability of a system to exist in at least two states: an inhibited (inactive) state, in which the IM is bound to the FD, and an active state, in which FD and IM do not interact, and by the variety of means that can be used to release or enhance the autoinhibition. Figure 7 shows that the FD activity is inhibited by a direct occlusion of its active site (which could be a binding site or a catalytic site) by IM and illustrates that the activation of the autoinhibited protein can be reversible or irreversible. Here, the reversible activation relies in the temporal removal of IM from the FD

proximity either due to the IM binding to activating partner(s), or its post-translational modification (PTM), whereas the irreversible activation is typically caused by the IM proteolysis (see Figure 7).[320] Also, Figure 7 shows that PTMs and interaction with a specific partner or some specific PTMs can reinforce the autoinhibition.[320] Another important observation is that transitions between the active and inhibited states are associated with some structural changes either in IM or in FD. Also, certain flexibility in the polypeptide segments that tether the IMs to the FDs is required for this mechanism to work.[321]

A recent comprehensive bioinformatics study revealed that the inhibitory modules of autoinhibited proteins are enriched in intrinsic disorder.[321] Typically, these intrinsically disordered inhibitory modules contain multiple phosphorylation sites and are common subjects of alternative splicing. These two mechanisms provide means for the fine-tuning of the equilibrium between active and inactive states of autoinhibited proteins. Furthermore, activation of the autoinhibited proteins is commonly accompanied (or is associated with, or promoted by) changes in secondary structure in their inhibitory modules.[321]

Some of the examples where proteins were activated by the local unfolding of self-inhibitory domains (i.e., due to the release of autoinhibition) were already considered throughout this Review. Several of the cases discussed above were related to the activation of proteins by functional unfolding of autoinhibitory domains induced by passive environmental factors, such as changes in pH (e.g., mycobacterial adenylyl cyclase Rv1264 and furin), mechanical forces (e.g., titin and titin-related molecules), and light-induced changes in the redox state (GAPDH). However, many other mechanisms are utilized for the release of autoinhibition. A comprehensive review by Puffal and Graces provides an outstanding compilation of studies on the auto-inhibitory phenomenon and on the role of this process in regulation of protein interaction with various ligands.[320] Although many autoinhibited proteins are activated via spontaneous local unfolding, many proteins can be activated via the local unfolding induced by the active interactions of autoinhibitory domains with various modulators or by the post-translational modification of the autoinhibitory domains. Some of the illustrative examples of the release of autoinhibition via conformational switches, interactions with binding partners, or post-translational modifications are presented below.

The Src family of nonreceptor tyrosine kinases provides additional examples of release of autoinhibition through post-translational modification- and protein binding-dependent unfolding events. In the case of Src, its regulatory SH3 and SH2 domains bind to linker segments between these domains and the kinase domain, as well as C-terminal tail that contains a critical, phosphorylated tyrosine residue (pY527), to impose a compact, autoinhibited conformation.[322] While contacts between many different segments within Src contribute to the stability of the autoinhibited structure, those between pY527 and the SH2 domain anchor this conformation. Dephosphorylation of Y527 or competition for its interaction with the SH2 domain by pY-containing peptides (and proteins) disrupts this interaction, leading to the disassembly of the autoinhibited conformation, and release and activation of the kinase domain. Interestingly, the related Abl kinase lacks a Y residue analogous to Y527 in Src; however, N-terminal myristoylation of Abl enables the formation of an autoinhibited conformation involving its SH3, SH2, and kinase domains that is virtually identical to that of Src.[323] The N-terminal myristoyl group of Abl binds within a

deep hydrophobic cleft on the kinase domain surface, and this interaction functionally substitutes for the “clamping” pY527–SH2 domain interaction observed in Src by imposing conformational changes in the kinase domain that enable inhibitory SH2 domain–kinase domain interactions. As with Src, peptides containing a pY residue bind to the SH2 domain of Abl and disrupt the autoinhibited conformation. Recently, a small molecule was shown to activate Abl by binding to the myristoyl binding cleft and presumably disrupting the autoinhibited conformation.[324] The common features of these tyrosine kinase regulatory mechanisms are that a rigid and compact, auto-inhibited conformation holds the tyrosine kinase domain in an inactive conformation and that this conformation can be released by various means that disrupt or compete for a subset of the stabilizing interdomain interactions. Once these inhibitory interactions are destabilized, various rigid interactions between the SH2, SH3, and kinase domains, as well as the intervening linker segments, are disrupted by unfolding of the linkers, giving rise to a dynamic, multidomain structure with flexible interdomain linkers in which the kinase domain is active. This type of complex regulatory mechanism not only allows control of kinase domain activity but also control of interactions mediated by the SH3 and SH2 domains through sequestration of these domains within the autoinhibited conformation and their exposure upon its disruption.

5.2. Protein Activation via Conformational Switch-Induced Local Unfolding of Autoinhibitory Domains

Autoinhibition is achieved via the interactions between a regulatory element (an autoinhibitory domain) and a functional domain located in a distinct region of the same polypeptide chain. Therefore, the active sites of the autoinhibited proteins are occluded by the autoinhibitory elements that physically block sites of interactions and/or catalytic activity. Because the relief of autoinhibition typically requires activators to bind and/or covalently modify sites in the regulatory element that are buried in the autoinhibited structure, autoinhibited proteins typically require large structural changes to switch between inactive and active states. Obviously, internal dynamics of such proteins provides access to such sites and therefore is likely to be crucial for the regulation and activation of autoinhibited systems.[325] The autoinhibition of the Dbl homology (DH) domain of the protooncoprotein and guanine nucleotide exchange factor Vav1 via interactions between its catalytic site and a helix from an N-terminal acidic region represents an illustrative example of the important role of a spontaneous conformational switch in regulation of autoinhibition. In fact, although the phosphorylation of the inhibitory helix relieves Vav1 autoinhibition, the phosphorylatable residue, Tyr174, is completely buried in the DH–helix interface.[326] NMR analysis revealed that Vav1 exists in equilibrium between a ground state where the inhibitory helix is bound to the DH domain and a weakly populated excited state where the helix is released and unfolded.[325] This analysis clearly shows that the conformational switching and internal dynamics are required for and control both basal activity and the rate of full activation of the autoinhibited DH domain.[325]

A fibroblast growth factor receptor (FGFR) extracellular domain represents another illustration of the role of conformation switching in regulation of autoinhibition. Although the prototypical FGFR extracellular domain consists of three Ig domains (D1–D3), only the two membrane-proximal D2 and D3 domains and the interconnecting D2–D3 linker are

important for ligand binding, whereas D1 and the D1–D2 linker play autoinhibitory roles in the regulation of FGFR. Analysis of the crystal structure of the three-Ig form of FGFR3c (which is a canonical form of the human fibroblast growth factor receptor 3) in complex with the fibroblast growth factor 1, FGF1, revealed that ligand binding results in the complete unfolding of D1 and the D1–D2 linker.[327] Because, despite the fact that the autoinhibitory domain blocks the interaction of the FGFR with its ligand, the FGF1–FGFR3c complex is still formed, one can assume that the FGFR3c exists in equilibrium as a mixture of binding-competent and binding-incompetent (autoinhibited) conformations and that there is a conformational switch that regulates transitions between these two forms.

5.3. Protein Activation via Interaction-Induced Unfolding of the Autoinhibitory Domains: Interaction with Nucleic Acids

Binding affinity and/or specificity of many transcription factors and other DNA-binding proteins are altered by specific inhibitory regions. In one example, DNA binding specificity is regulated via DNA binding-induced structural changes in the inhibitory region. Interaction of the dimeric *Bam*HI endonuclease (from *Bacillus amyloliquefaciens* H) with DNA illustrates an intricate regulatory mechanism, where a C-terminal α7-helix, which is ordered in the free protein, partially unfolds and assumes an extended, partially disordered conformation upon binding to DNA.[328] Typically, α-helices in proteins are only marginally stable by themselves, whereas their structure and configuration within protein folds are stabilized via tertiary interactions. In free *Bam*HI, α7 was the most mobile and solvent accessible helix of the enzyme,[329] suggesting a low-energy threshold between folded and unfolded states. As a result, DNA binding-induced perturbations in the core of *Bam*HI contributed to the destabilization of the C-terminal helix and its unfolding and release from the core.[328]

Members of the ETS family of transcription factors further illustrate functional unfolding induced by DNA-binding. These proteins contain a specific 85-amino acid region termed the ETS (for “E twenty-six”) domain, which adopts a secondary structure similar to the winged helix–turn–helix (wHTH) motif, consisting of three α-helices and four antiparallel β-strands. The “wing” is a loop between adjacent β-strands.[330,331] Adjacent to the ETS domain there are flanking regions that fold into an inhibitory domain, which, in the absence of DNA, is composed of four α-helices and interacts with the ETS domain via a set of intramolecular contacts.[332] Using far-UV CD analysis combined with limited proteolysis, it was shown that DNA binding to Ets-1 was accompanied by transient unfolding of an α-helix and the transient relief of inhibition.[333] It was also hypothesized that unfolding of the inhibitory region could expose a putative protein–protein interaction domain, and the ETS–DNA complex could be stabilized by interactions with a protein partner.[333] In essence, this interaction mode illustrates the “cascade-binding mechanism”, where the first interaction with the first partner alters structure of one or both interactors to prepare the complex for secondary and tertiary interactions with other partners.

Generally, in these and many related cases, DNA acts as an allosteric ligand whose binding alters the affinity of a protein for other ligands, such as coactivators or corepressors.[334]

5.4. Protein Activation via Interaction-Induced Unfolding of the Autoinhibitory Domains: Protein–Protein Interactions

Activity of the mammalian Na^+/H^+ exchange regulatory factor 1 (NHERF1), a multidomain scaffolding protein essential for controlling intracellular trafficking and macromolecular assembly of transmembrane ion channels and receptors, is regulated by an autoinhibitory domain.[335] Among numerous NHERF1 functions are the assembly of signaling complexes and the regulation of endocytic recycling of the cystic fibrosis transmembrane conductance regulator (CFTR), cell surface adhesion and antiadhesion proteins, G-protein coupled receptors, and tyrosine kinase receptors.[335] NHERF1 consists of two modular protein–protein interaction domains, PDZ1 and PDZ2, which bind to the cytoplasmic domains of transmembrane receptors and channels, and a C-terminal domain that binds the membrane-cytoskeleton linker protein ezrin. The interaction of NHERF1 with ezrin and other ezrin-radixin-moesin (ERM) proteins is crucial for establishing a communication bridge between plasma membrane proteins and the actin cytoskeleton.[336,337] Ezrin modulates the PDZ domains of NHERF1 to assemble multiprotein complexes; for example, when ezrin is bound to the ezrin binding domain of NHERF1 (residues 329–358), the binding affinity of PDZ2 for the C-terminal domain of CFTR increases 24-fold.[338] The autoinhibition of NHERF1 is achieved through intramolecular interactions between PDZ2 (residues 150–240) and the CT domain (residues 242–358), and ezrin binding is believed to be crucial for the release of this autoinhibition.[335] Recently, a detailed structural analysis of the PDZ2CT domains of NHERF1 by high-resolution NMR and small-angle X-ray scattering revealed that there are only weak intramolecular interactions between the largely disordered CT domain and the PDZ ligand binding site.[335] The autoinhibited state is held together via a series of transient interactions between the well-folded PDZ2 domain and the different parts of the mostly disordered CT domain. In fact, an autoinhibited conformational ensemble of the CT domain includes two major types of structures. In the one type of structure, the CT domain consists of the PDZ2-bound single N-terminal helix (residues 323–334) with the remaining part of the CT domain, including the ezrin binding region, adopting a random coil conformation. In the other type of structure, the most C-terminal ezrin binding region of the CT domain adopts a helical conformation and is docked into the PDZ2 domain, whereas the remaining part of the CT domain is unfolded.[335] Importantly, neither the N-terminal helix (residues 323–334) nor the ezrin binding domain of NHERF1 (residues 329–358) adopt well formed α-helical conformations in the isolated CT domain, suggesting that the helical structures observed in PDZ2CT are induced and stabilized in the otherwise largely disordered CT domain through weak intramolecular interactions with PDZ2. The autoinhibition is successfully removed by the PDZ2CT interaction with ezrin, which leads to the release of the C-terminal domain from interactions with PDZ2 and the noticeable extension of the largely disordered CT domain. As a result, the PDZ2 and ezrin are separated by a distance of 80 Å and hence not in contact with each other.[335]

SecA, the 204-kDa ATPase motor of the Sec translocase, is autoinhibited via interaction of the C-terminal region with the elongated groove used by SecA to recognize and bind a diverse set of signal sequences. The biological function of translocases is to recognize and secrete extra-cytoplasmic polypeptides, which are usually synthesized as “preproteins” carrying amino terminal, cleavable signal peptides,[339] across membranes. The Sec

translocase is a highly conserved proteinaceous machine responsible for processing the vast majority of bacterial and ER exported proteins.[340] The bacterial Sec translocase contains the SecYEG protein-conducting channel and the peripheral ATPase motor SecA.[341,342] SecA is a large, 901 residue-long, protein that forms homodimers. The helicase-like motor is located at the N-terminus of SecA. This motor is comprised of the discontinuous nucleotide binding domain (NBD) and the intramolecular regulator of ATP hydrolysis-2 domain (IRA2). The nucleotide binds at a cleft formed at the interface of NBD and IRA2.[343,344] Preproteins and signal sequences interact with the preprotein-binding domain (PBD), which "sprouts" out of the body of NBD through an antiparallel β-sheet forming a series of grooves separating PBD and other domains.[345] The C-terminal domain of SecA encompasses four subdomains: the long α-helical scaffold domain (SD), the IRA1 hairpin, the winged domain (WD), and a highly flexible C-tail region.[344] In turn, this extreme C-terminal tail (residues 834–901 in *E. coli*) comprises two regions, with the first region (residues 834–855) interacting with the core of SecA and ending up in a β-strand (residues 849–854) that forms a β-sheet with the two antiparallel β-strands linking NBD and PBD,[344] and the second region containing a zinc-finger motif (residues 879–897) that constitutes the primary binding site for the SecB chaperone.[346] SecA is autoinhibited via the N-terminal region of C-tail (residues 834–848), which occludes a portion of the peptide binding groove.[347,348] This autoinhibition is relieved by the SecB chaperone that binds to the zinc-finger site of the C-tail and displaces it from the binding groove.[347]

Diaphanous-related formin (Drf), a member of the formin family, promotes the nucleation and polymerization of unbranched actin filaments via the formin-homology domains 1 and 2. Interaction of the Drf with the Rho·GTP releases the autoinhibition induced via the interaction of the C-terminal diaphanous-autoregulatory domain (DAD) with the N-terminal regulatory region.[349] The core DAD region is folded as an α-helix, which binds in the highly conserved regulatory N-terminal region via a set of mostly hydrophobic interactions. Rho·GTP displaces DAD by ionic repulsion and steric clashes,[349] likely causing unfolding of the diaphanous-autoregulatory domain.

5.5. Protein Activation via Interaction-Induced Unfolding of the Autoinhibitory Domains: Interaction with Membranes

In addition to numerous factors, such as hormones, growth factors, neurotransmitters, antigens, and other external stimuli, interaction with lipid membranes is assumed to play a role in the activation of phospholipase C (PLC) isozymes.[350] The major function of the PLC isozymes is to hydrolyze phosphatidylinositol 4,5-bisphosphate (PtdIns[4,5]P_2) into the second messengers diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3).[351] Analysis of the crystal structure of PLC-β2 revealed that this protein is autoinhibited due to the occlusion of the active site by a loop (known as an X/Y linker) separating the two halves of the catalytic TIM barrel.[350] Although the X/Y linker of PLC-β2 spans approximately 70 amino acids (466–537), only 22 of them (516–537) are ordered. Residues 516–529 of this region form an α-helix capped at its C-terminus by a tight turn and an extended region (residues 530–537) containing a short 3_{10}-helix (residues 530–534). The C-terminus of the α-helix and the extended region make a set of extensive contacts with the phospholipase active site.[350] The X/Y linkers of different PLC isoforms were noted to vary greatly in both

length and sequence, and even within any given PLC subgroup, no significant conserved regions exist within the sequences that separate the TIM barrels.[350] However, in many PLC isoforms, the X/Y linkers provide a mechanism for occlusion of the active site that is generally dependent on the overall length and negative charge of the linker region. Activation of different PLCs is usually achieved by the physical removal and/or unfolding of the X/Y linkers. The only common feature of otherwise highly diverse X/Y linkers is an unusually high density of the negatively charged residues. On the basis of these observations, it has been hypothesized that the highly negatively charged X/Y linkers can provide an important control of the PLC activities at negatively charged substrate membranes.[350] Here, the negatively charged X/Y linkers are sterically and electrostatically repelled from the negatively charged phospholipid membranes upon recruitment of PLC. This eliminates active site occlusion and activates PLC. In other words, the ordered region of the X/Y linker is needed for regulation of the fine-tuned access to the active site, whereas the negatively charged disordered regions serve as the major autoinhibitory determinant.[350]

5.6. Activation via Phosphorylation-Induced Unfolding of Inhibitory Regions

In several protein classes, phosphorylation-induced local unfolding plays a crucial role in the relief of intramolecular autoinhibition. For example, in most autoinhibited kinases, the relief of intramolecular autoinhibition is essentially a partial unfolding event of the autoinhibited conformation, driven by ligand binding or post-translational modification, including phosphorylation.

On the basis of NMR analysis, the nonphosphorylated form of the N-terminal inactivation domain (ID) of the voltage-gated K^+ channel Kv.4 possessed a compact structure with high affinity for its receptor.[352] This tight interaction blocks the pore on the cytoplasmic side and therefore results in channel inactivation. Phosphorylation of the ID by protein kinase C (PKC) on any of three putative sites (serine residues 8, 15, and 21) is proposed to cause a loss of overall structural stability, local unfolding, and relief of this form of inhibition.[352] These ID phosphorylatable sites are in close proximity to two glutamate residues, Glu27 and Glu28, which, upon phosphorylation, would cause steric and electrostatic repulsion and promote local disorder.[257]

Activation of the autoinhibited interferon regulatory factor 3 (IRF-3), a member of the family of transcription factors that are important for host defense against viral infection, the development of the immune system and apoptosis, also depends on phosphorylation.[353] The C-terminal half of this protein contains a conserved IRF association domain (IAD) responsible for the homo- and hetero-oligomerization, whose transactivation function is suppressed by an autoinhibitory region.[353] This C-terminal autoinhibitory sequence of IRF-3 contains seven phosphorylation sites responsible for its virus-induced activation. [354] Analysis of the IRF-3 crystal structure revealed that the IAD and the flanking autoinhibitory elements collapse to form a hydrophobic core.[355] Here, the folded N- and C-terminal autoinhibitory segments are interspersed within the IRF-3 structure, interact with each other, and together cover a large hydrophobic surface of 1200 $Å^2$ on the H3- and H4-helices of the IAD. Phosphorylation reorganizes the autoinhibitory elements, leading to their unfolding

accompanied by the unmasking of a hydrophobic active site and realignment of the DNA binding domain for transcriptional activation.[355]

Phosphorylation of the conserved autoinhibitory elements proximal to tandem Sec7-pleckstrin homology (PH) domains activates Grp1, a member of a family of phosphoinositide-dependent Arf GTPase exchange factors. Grp1 is autoinhibited through a pseudosubstrate mechanism, where a linker region between domains and a C-terminal amphipathic α-helix physically blocks the docking sites for the switch regions of Arf GTPases. Phosphorylation of the C-terminal α-helix induced local unfolding and release of autoinhibition.[356]

Catalytic activation of the S6K1 kinase, a member of the AGC subfamily of serine-threonine protein kinases, requires dual phosphorylation of critical residues in the conserved T-loop (T229) and hydrophobic motif (HM; T389) peptide regions of its catalytic kinase domain (residues 1–398). In addition to the kinase domain, S6K1 contains a C-terminal autoinhibitory domain (AID; residues 399–502), which wraps around the catalytic domain and prevents T-loop and HM phosphorylation. Autoinhibition is relieved via the AID phosphorylation at multiple serine and threonine sites (S411, S418, T421, and S424) and the subsequent unfolding of this regulatory domain.[357]

Members of the X11/Mint family of multidomain adaptor scaffold proteins are composed of a nonconserved N-terminus, a conserved phosphotyrosine binding (PTB) domain, a pair of C-terminal PDZ (postsynaptic density 95, PSD-85; discs large, Dlg; zonula occludens-1, ZO-1) domains arranged in tandem, and a conserved C-terminal tail. Many proteins are known to interact with the tandem PDZ domains of X11 proteins.[358]NMR analysis revealed that the highly conserved C-terminal tail of X11α (and probably those of other X11 proteins) folds back and inserts into the target-binding groove of the first PDZ domain, resulting in an autoinhibited conformation of X11α. This autoinhibition is likely to be controlled through phosphorylation, because substitution of the absolutely conserved C-terminal tyrosine with glutamate abolished binding of the C-terminal tail to PDZ1.[358]

5.7. Activation via Dephosphorylation-Induced Unfolding of Inhibitory Regions

As was already mentioned above, the binding of Ets-1 to DNA is mediated by the ETS domain (residues 331–414), and is modulated by flanking regions (residues 301–330 and 415–440) that fold into an inhibitory module.[332] DNA binding is accompanied by a structural rearrangement that disrupts the inhibitory module and unfolds the marginally stable N-terminal inhibitory helix HI-1.[333] The autoinhibitory module-induced repression of the ETS is further enhanced by the presence of an adjacent intrinsically disordered serine-rich region (SRR, residues 244–300).[359] This attenuation of DNA binding by Ets-1 is controlled by multisite phosphorylation of the SRR in response to the Ca^{2+} signaling.[360] It has been shown that each added phosphate progressively increases repression.[359] Comprehensive NMR analysis revealed that the SRR is predominantly unstructured both before and after phosphorylation. However, addition of phosphates to the SRR subtly decreased its flexibility and enhanced the association of the SRR with the specific Ets-1 region extending from the inhibitory module to the DNA-binding interface. These transient interactions between the phosphorylated SRR and the functional regions of Ets-1 decreased

the conformational flexibility of the ETS domain and inhibitory module required for high-affinity binding, and possibly occluded the DNA interaction site.[361] The regulatory transient interactions are absent when the SRR region is dephosphorylated. On the basis of these observations, it has been concluded that variable phosphorylation of the SRR motif might serve as a "rheostat" for cell signaling to fine-tune transcription at the level of DNA binding.[359]

A phosphorylation/dephosphorylation cycle is important for the regulation of a 15 kDa protein OdhI, which is an inhibitory subunit of the 450 kDa 2-oxoglutarate dehydrogenase multienzyme complex (ODHC). In this system, the unphosphorylated form of OdhI inhibited the OdhA protein, a key enzyme of the TCA cycle, whereas the phosphorylated form of OdhI was inactive.[362] OdhI contains a forkhead-associated (FHA) domain, which is a recognition domain highly specific to phosphothreonine, and an unfolded 40-residues N-terminal extension where the phosphorylation occurs.[363] Comparison of the NMR structures of the phosphorylated and dephosphorylated forms of OdhI revealed that the unstructured N-terminal segment of OdhI underwent considerable conformational changes upon phosphorylation.[362] In fact, the N-terminal part of the unphosphorylated OdhI protein was fully disordered, whereas phosphorylation at a unique site Thr15 induced folding of the N-terminal disordered region from residue Pro10 to Gly31 and triggered the binding of the region surrounding pThr15 to the FHA domain.[362]

6. UNFOLDONS AS A STRUCTURAL BASIS FOR THE FUNCTIONAL UNFOLDING

It has been proposed that the phenomenon of functional unfolding can be considered as a reflection of the extreme structural heterogeneity of a protein molecule.[364,365] In fact, the comprehensive analysis of the equilibrium and kinetic folding processes of cytochrome *c*,[366] apo-cytochrome b_{562}, dimeric triosephophate isomerase, ribonuclease H, the OspA protein of Borrelia,[367] and staphylococcal nuclease[368] revealed that these small, single-domain proteins possess multiple submolecular foldon units that continually unfold and refold even under native conditions, suggesting that the folding of an ordered protein can be described as the stepwise assembly of the foldon units, with previously formed foldons guiding and stabilizing subsequent foldons to progressively build the native protein.[367,369–371] Furthermore, many ordered proteins were shown to contain nucleation motifs, foldons, the number of which defines the number of accessible folding pathways for a given protein.[372] All of this suggests that ordered proteins should be considered as "modular assemblies of competing foldons".[372] Extension of this foldon concept to IDPs/IDPRs, with their structural and spatiotemporal heterogeneities where some IDP regions are spontaneously folded, others can fold (at least in part) at interaction with binding partners, and still others are always in the semifolded state, suggests that an IDP or IDPR can be described as a modular assembly of foldons, inducible foldons, semifoldons, and nonfoldons.[364,365] From the viewpoint of foldons, inducible foldons, semifoldons, and nonfoldons, the phenomenon of protein dormant disorder, which needs to be transiently awakened to make protein functional, can be considered as an unfoldon.[364,365] In other words, due to its inherent structural and thermodynamic heterogeneity, a protein molecule can be considered as a

modular assembly of foldons and unfoldons, with unfoldons representing less stable parts of a protein structure that have to undergo order-to-disorder transition to make the protein active.

7. CONCLUDING REMARKS

It is known that proteins typically undergo some structural changes during function. The function-related structural changes in IDPs/IDPRs range from the local partial folding to complete folding, and from allosteric transitions to induced fit adjustments in ordered proteins and domains. It is believed that the most common outcome of these function-related structural changes is the overall increase in the amount of ordered structure. The fact that many IDPs/IDPRs have to undergo disorder-to-order transitions either during their functions or to become functional was used as an illustration of the notion that these proteins, when folded after binding to their partners, are not too different from typical ordered proteins and should be considered as proteins waiting for a partner (PWPs) that serve as parts of a multicomponent complex and that do not fold correctly in the absence of other components.[373] However, as described in this Review, phenomenon of functional unfolding (or transient disorder, or cryptic disorder) challenges this viewpoint. In fact, many proteins possess regions of dormant disorder that need to be awakened to make these proteins functional. In other words, some functions of ordered proteins require local or even global unfolding of a unique protein structure. The important features of these functional alterations are their induced nature and transient character. In other words, the function-related changes in a protein are induced by transient alterations in its environment or by transient modification of its structure and are released as soon as the environment is restored or the modification is removed. Another crucial feature of the transient functional unfolding is a wide range of molecular mechanisms by which this dormant disorder can be awoken. We show here that among the functional unfolding-activating factors are light; mechanical force; changes in pH, temperature, or redox potential; interaction with membrane, ligands, nucleic acids, and proteins; various PTMs; release of authoinhibition due to the unfolding of autoinhibitory domains induced by their interaction with nucleic acids, proteins, membranes, PTMs, etc.

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Biographies

Ursula Jakob received her Ph.D. in Physical Biochemistry from the University of Regensburg in 1995 for her work on the identification and characterization of Hsp90 and small heat shock proteins as molecular chaperones under the supervision of Dr. Johannes Buchner. She then moved to Michigan where she conducted a 5-year postdoctoral fellowship in the lab of Dr. James Bardwell. There, she discovered the heat shock protein Hsp33 as the first known redox-regulated chaperone. This work helped establish the field of redox-regulation in biology. In 2000, she received the Burroughs Wellcome Fund Career Award and started her independent research career at the University of Michigan one year later. She is now Professor in the Department of Molecular, Cellular and Developmental Biology and in the Department of Biological Chemistry at the University of Michigan Medical School. Her main interest concerns the role of oxidative stress and redox regulation in aging and disease. She has published over 65 peer-reviewed articles and has received awards from the National Institute of Aging (NIA) and the National Institutes of Health.

Richard Kriwacki received his Ph.D. from the Biophysics Division of the Department of Chemistry at Yale University in New Haven, CT, followed by postdoctoral training with Professor Peter E. Wright at the Scripps Research Institute in La Jolla, CA. In 1996 at Scripps, Drs. Kriwacki and Wright discovered that a small protein named $p21_{Waf1/Cip1}$ that regulates kinases involved in controlling cell division lacked secondary and tertiary structure in isolation but folded upon binding to its kinase targets. This, together with a few following reports of functional, unstructured proteins, drew attention to what are now termed intrinsically disordered proteins in biology. In 1997, Dr. Kriwacki joined the Department of Structural Biology at St. Jude Children's Research Hospital (St. Jude) in Memphis, TN, where he is now a Full Member. At St. Jude, Dr. Kriwacki has continued studies of disordered proteins, with focus on establishing relationships between their disordered features and biological functions, and has published more than 70 papers in the field. Dr. Kriwacki helped establish the Disordered Proteins Subgroup at the Biophysical Society, leading advocates for the disordered proteins field, and covers this topic as an Editorial Board Member of the Journal of Molecular Biology.

Vladimir Uversky obtained his Ph.D. in biophysics from the Moscow Institute of Physics and Technology (1991) and D.Sc. in biophysics from the Institute of Experimental and Theoretical Biophysics, Russian Academy of Sciences (1998). He spent his early career working on protein folding at the Institute of Protein Research and the Institute for Biological Instrumentation (Russian Academy of Sciences). Here, while working on the

experimental characterization of protein folding, Dr. Uversky has found that some mostly unstructured proteins can be biologically active. These findings, together with the similar observations of other researchers, eventually forced him to reconsider the generality of the protein structure-function paradigm and to suggest that natively unfolded (or intrinsically disordered) proteins represent a new important realm of the protein kingdom. In 1998, he moved to the University of California Santa Cruz to work on protein folding, misfolding, and protein intrinsic disorder. In 2004, he moved to the Center for Computational Biology and Bioinformatics at the Indiana University–Purdue University Indianapolis to work on the intrinsically disordered proteins. Since 2010, he has been with the Department of Molecular Biology at the University of South Florida, where he is now an Associate Professor. At the University of South Florida, Dr. Uversky has continued his work on various aspects of protein intrinsic disorder phenomenon and on analysis of protein folding and misfolding. He has published over 500 peer-reviewed articles and book chapters in these fields. Dr. Uversky participated in the establishment of the Intrinsically Disordered Proteins Subgroup at the Biophysical Society and the Intrinsically Disordered Proteins Gordon Research Conference. He is an Executive Editor of the Intrinsically Disordered Proteins journal published by the Landes Bioscience.

ABBREVIATIONS

ADP	adenosine diphosphate
AFM	atomic force microscopy
AID	autoinhibitory domain
BLUF	blue light using FAD
cAMP	3′,5′-cyclic adenosine monophosphate
CCT	CRY C-terminus
CD	circular dichroism
Cdk	cyclin-dependent kinase
CFTR	cystic fibrosis transmembrane conductance regulator
CRY	cryptochrome
CT	C-terminal
CTE	C-terminal extension
DAD	diaphanous-autoregulatory domain
DAG	diacylglycerol
DH	Dbl homology
Dlg	discs large
Drf	diaphanous-related formin
4E-BP	4E-binding protein

eIF4E	eukaryotic translation initiation factor 4E
EMT	2-(ethylmercuri-thio)-benzoic acid
ER	endoplasmic reticulum
ERM	ezrin-radixin-moesin
ETS	E twenty-six
FAD	flavin adenine dinucleotide
FD	functional domain
FGFR	fibroblast growth factor receptor
FHA	forkhead-associated
FMN	flavin mononucleotide
FRET	fluorescence resonance energy transfer
FTIR	Fourier-transform infrared
GABA	gamma-aminobutyric acid
GAF	cGMP phosphodiesterase/adenylyl cyclase/FhlA
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSH	glutathione
GSNO	nitrosoglutathione
HM	hydrophobic motif
HSQC	heteronuclear single quantum correlation
Hsp	heat shock protein
IAD	IRF association domain
ID	inactivation domain
IDP	intrinsically disordered protein
IDPR	intrinsically disordered protein region
IM	inhibitory module
IP_3	inositol 1,4,5-trisphosphate
IRA2	intramolecular regulator of ATP hydrolysis-2 domain
IRF-3	interferon regulatory factor 3
KID	kinase inhibitory domain
LOV	light oxygen voltage
MscL	mechanosensitive channel of large conductance
NBD	nucleotide binding domain

NHERF1	Na^+/H^+ exchange regulatory factor 1
NLS	nuclear localization signal
OCP	orange carotenoid protein
ODHC	2-oxoglutarate dehydrogenase multienzyme complex
PAS	PER/Arnt/Sim
PBD	preprotein-binding domain
PEVK	titin region enriched in proline (P), glutamate (E), valine (V), and lysine (K) residues
PH	pleckstrin homology
PHY	phytochrome-specific domain/phytochrome
PKC	protein kinase C
PLC	phospholipase C
PSD-85	postsynaptic density 95
PTB	phosphotyrosine binding
PtdIns[4,5]P_2	phosphatidylinositol 4,5-bisphosphate
PWP	protein waiting for a partner
PTM	post-translational modification
PYP	photoactive yellow protein
RIP	ribosome-inactivating protein
ROMK	renal outer medullary potassium channel
RTA	RNA *N*-glycosidase A-chain
RTB	RNA *N*-glycosidase B-chain
SCOT	succinyl-CoA:3-oxoacid coenzyme A transferase
SD	scaffold domain
SRR	serine-rich region
TM	transmembrane
TRAAK	TWIK-1 related arachidonic acid-stimulated K^+ channel
TREK	TWIK-1 related K^+ channel
TWIK	tandem of P domains in a weakly inwardrectifying K^+ channel
VSV	vesicular stomatitis virus
WD	winged domain
wHTH	winged helix–turn–helix

ZO-1 zonula occludens-1

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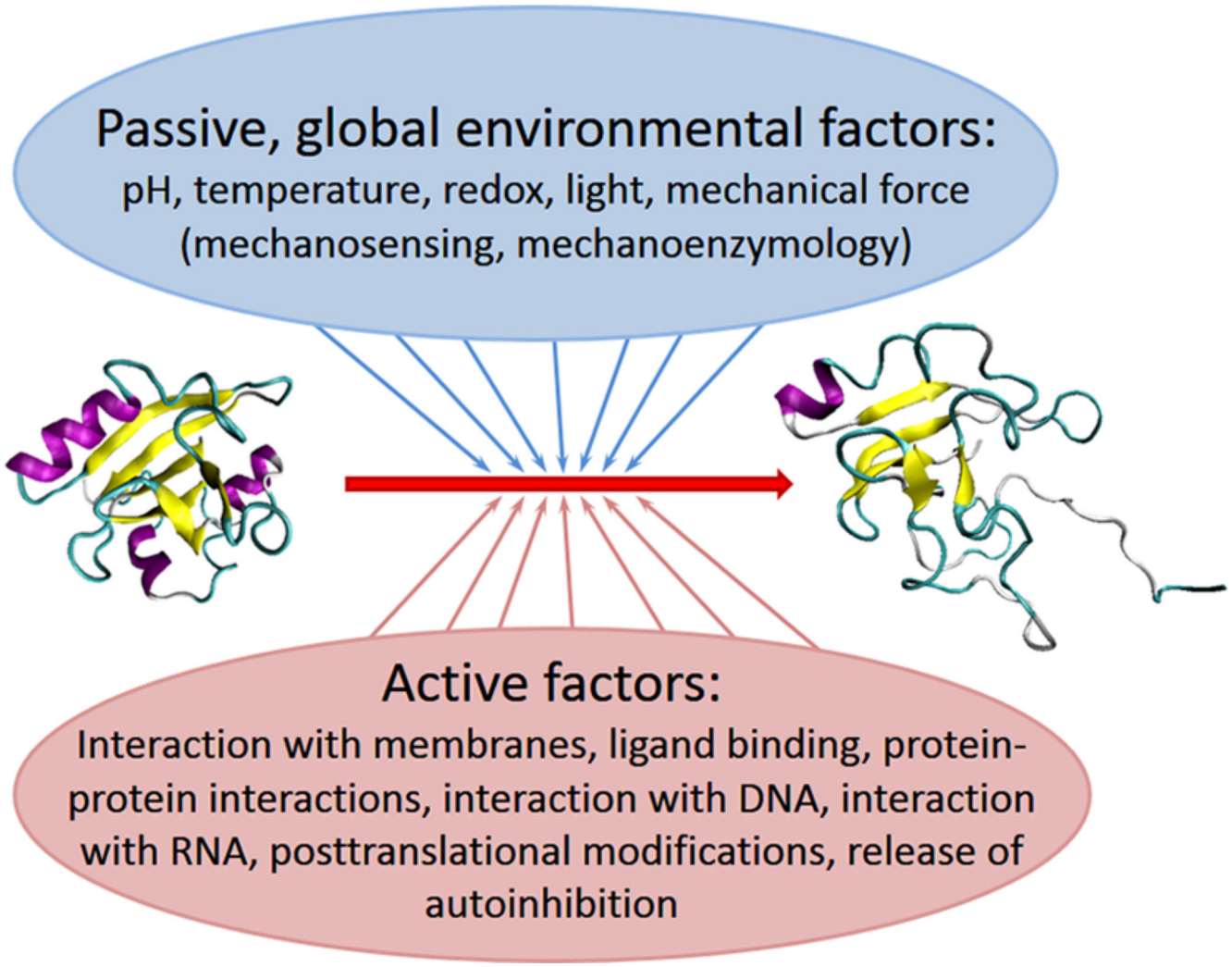

Figure 1.

Molecular mechanisms underlying functional unfolding of proteins. An ordered (inactive) protein can become active via local or global functional unfolding. This transition can be promoted by passive, global environmental factors (such as changes in pH, temperature increase, changes in the redox potential, application of mechanical force, exposure to light). Alternatively, many active factors (such as interactions with various partners, post-translational modifications, and release of autoinhibition) can also result in functional unfolding.

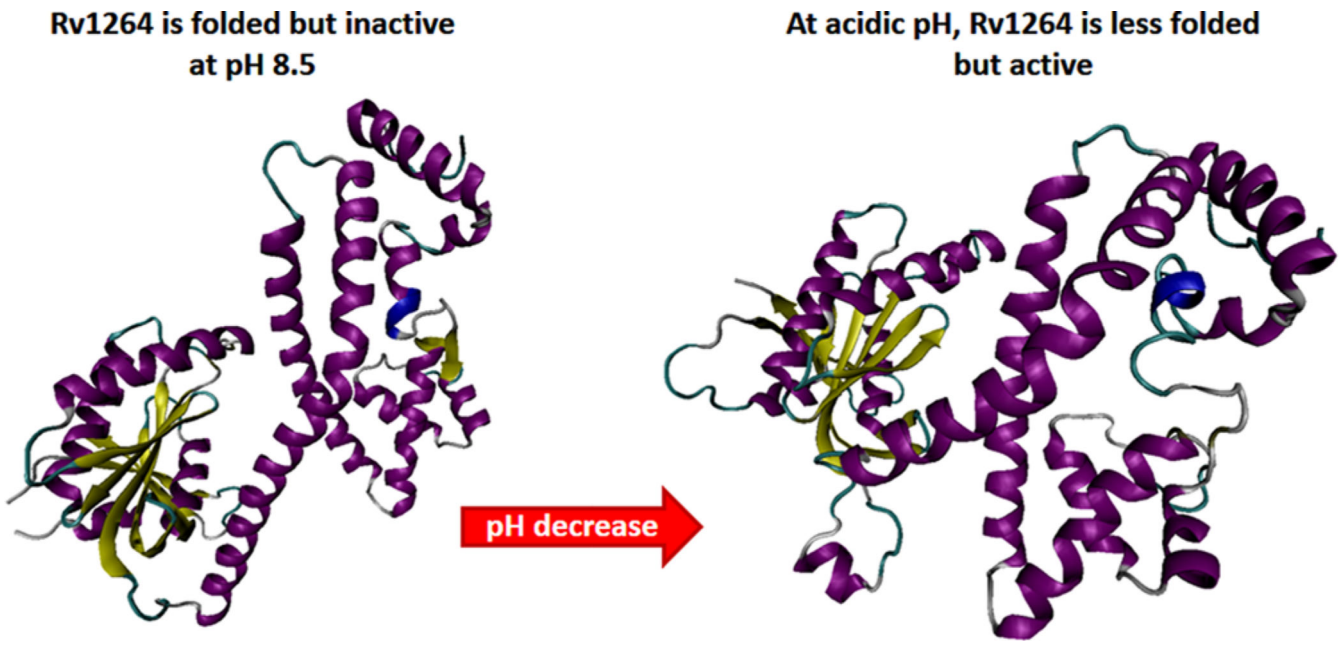

Figure 2.

Acid-induced local unfolding leading to activation. Overall structure of Rv1264 in the inhibited (left; PDB ID: 1Y10) and active states (right; PDB ID: 1Y11). Although Rv1264 exists as a dimer, structures of inhibited and active monomers are shown for simplicity. The regulatory domains remain essentially unchanged upon enzyme activation, but the interface with the catalytic domains differs substantially. In fact, in the structure of the inhibited state, catalytic and regulatory domains share a large interface involving catalytic residues. In the structure of the active dimer, the catalytic domains rotate by 55° to form two catalytic sites at their interface.[103] Although in the acidic environment the entire molecule undergoes noticeable structural alterations, the most dramatic structural changes occur at the linker region containing the αN10-switch. In the inhibited state, this segment forms a long α-helix, whereas in the active state, this fragment assumes a disordered conformation with a short helical segment loosely connecting regulatory and catalytic domains.[103]

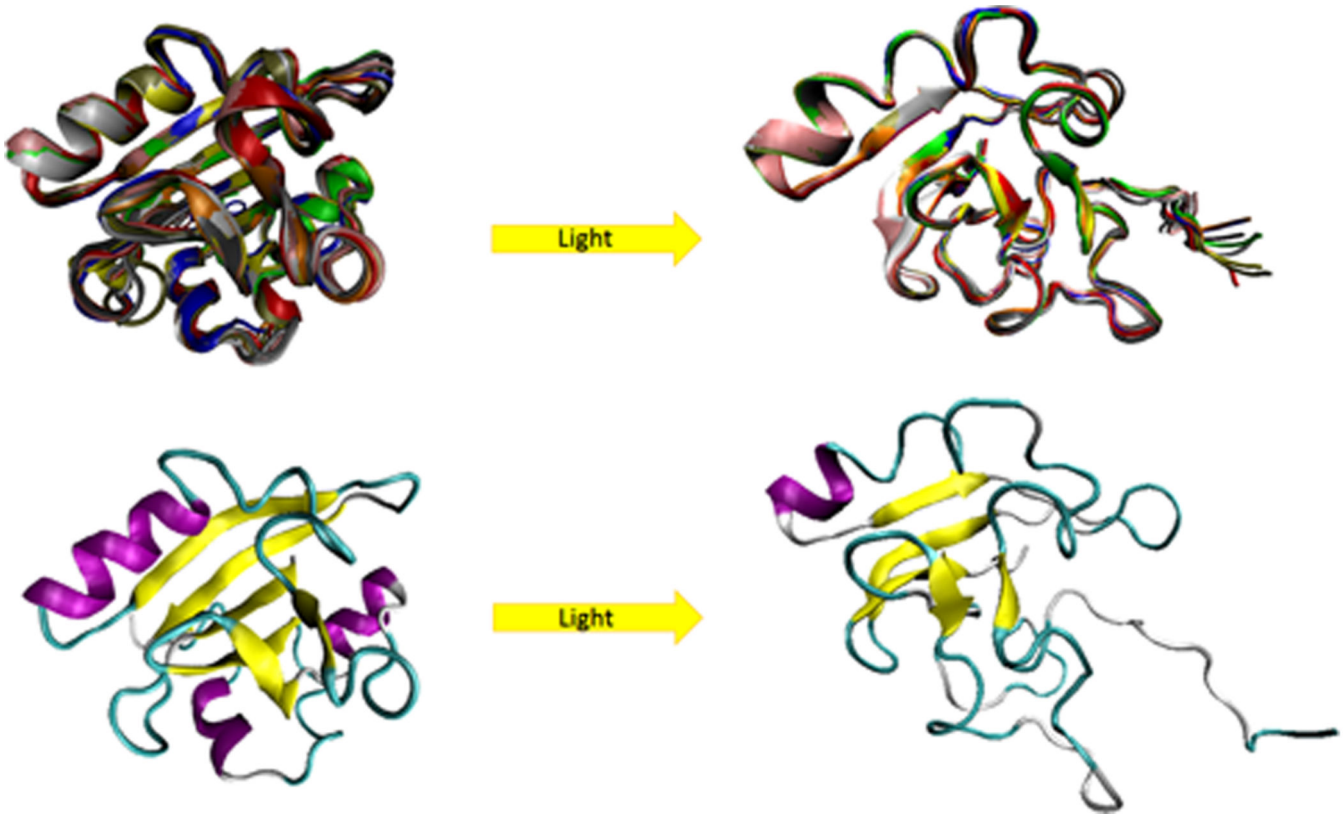

Figure 3.

Functional unfolding associated with the light-induced activation of the photoactive yellow protein. Comparison of the ground state (left structure, PDB ID: 3PHY) and the transient light activated signaling state of the photoactive yellow protein (right structure, PDB ID: 2KX6). The top row represents ensembles of these two functional states of the photoactive yellow protein, whereas the bottom row shows representative members of the corresponding ensembles. Ground-state structure was determined by multidimensional NMR spectroscopy,[374] and is in agreement with an earlier published 1.4 Å crystal structure.[126] The light activated state structure was obtained by combining double electron electron resonance spectroscopy (DEER), NMR, and time-resolved pump–probe X-ray solution scattering (TR-SAXS/WAXS) data.[375] It consists of an open, twisted, 6-stranded, antiparallel β-sheet, which is flanked by four α-helices on both sides.[126,374,375] On the contrary, the light-activated form is highly disordered. This structure satisfies DEER, SAXS/WAXS, and NMR data simultaneously.[375]

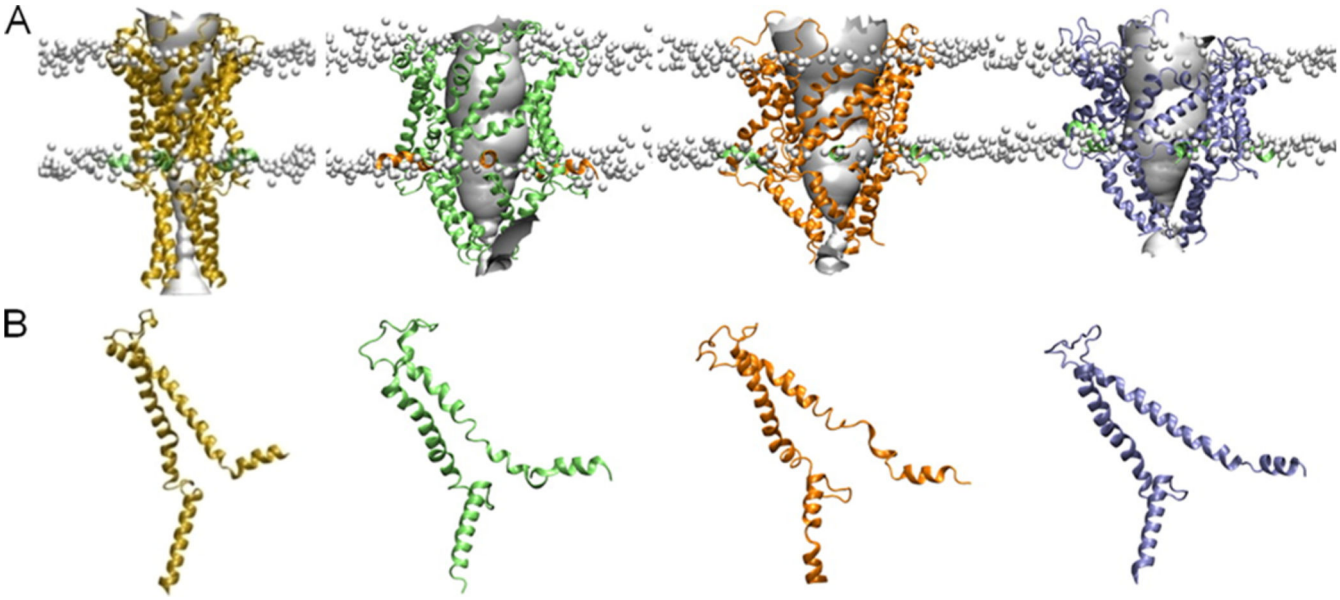

Figure 4.

Mechanical activation of the MS channel. Simulation studies incorporating EPR and FRET data. (A) The closed state (yellow) and model open states of the protein obtained under membrane tension of 0 (green), 5 (orange), and 60 mN/m (blue), respectively, viewed in the plane of the membrane. The location of the lipid phosphate head groups is indicated by the gray balls to give an indication of the position of the membrane. (B) The structure of a single subunit of each structure is indicated. Reprinted with permission from ref 159. Copyright 2010 Rockefeller University Press.

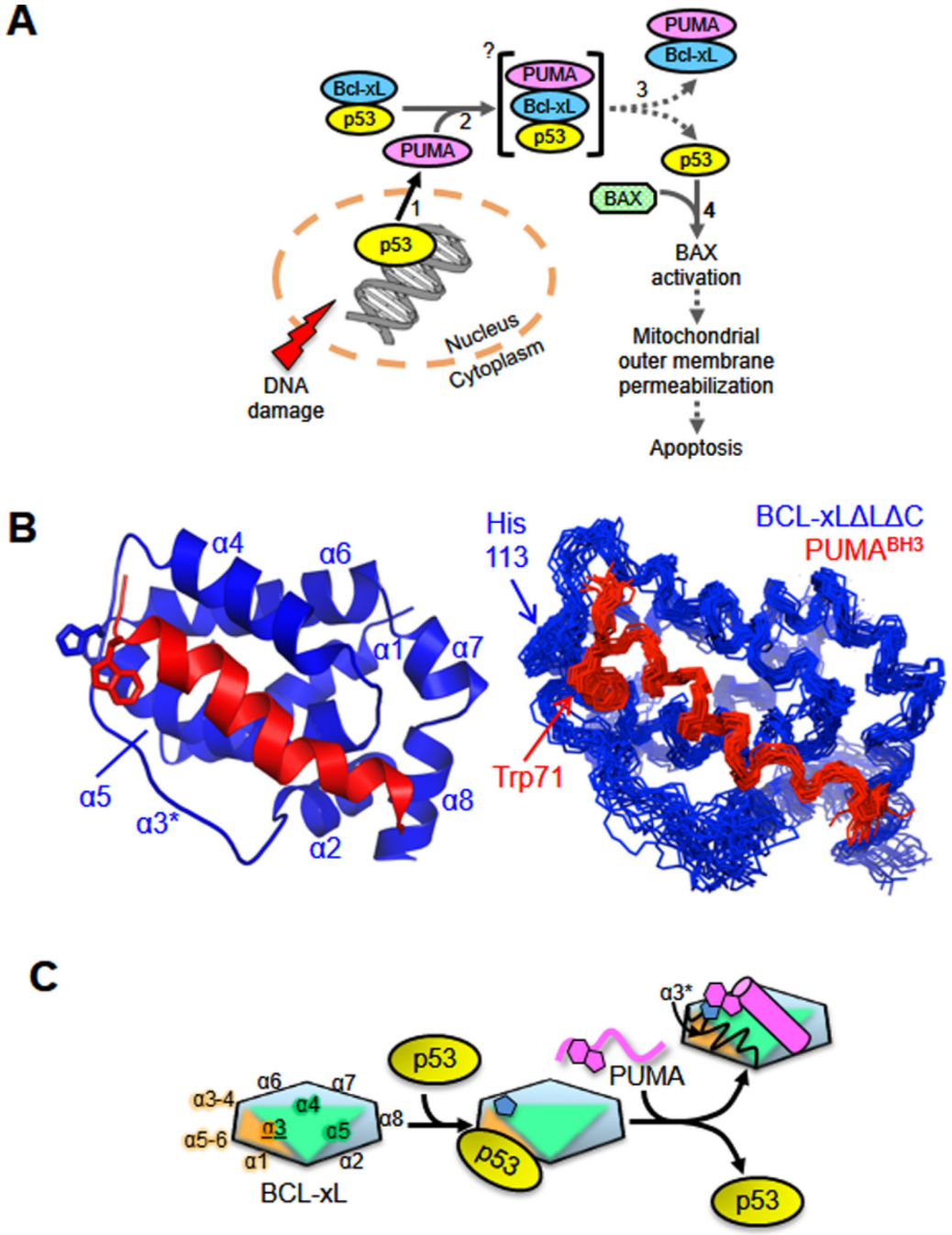

Figure 5.

Local unfolding induced by ligand binding. Ligand binding-associated unfolding of two helices within BCL-xL is associated with p53 release and the induction of apoptosis. (A) Scheme illustrating the mechanism by which p53 regulates apoptosis through interactions with DNAin the nucleus and BCL-2 family proteins in the cytosol. Increasing numerals denote the sequence of events involved in this process. (B) Solution structure of the BCL-xLΔLΔC–PUMA^{BH3} complex; ribbon representation of the lowest-energy structure (left) and alignment of the 20 lowest-energy structures (right). The disrupted α3 region is marked

with an asterisk. (C) Schematic illustration of the mechanism by which PUMA induces unfolding within α2 and α3 of BCL-xL, which is associated with p53 release. The formation of a π-stacking interaction between His113 of BCL-xL (blue pentagon shapes) and Trp71 of PUMA (magenta geometric shapes) is associated with unfolding of α2 and α3 (α3* in the upper right). BCL-xL is represented as a multicolor hexagon, with the edges representing its α-helices, as marked; PUMA in its unbound form is shown in magenta as a wavy line and as a cylinder when bound to BCL-xL. Adapted with permission from ref 209. Copyright 2013 Nature Publishing Group.

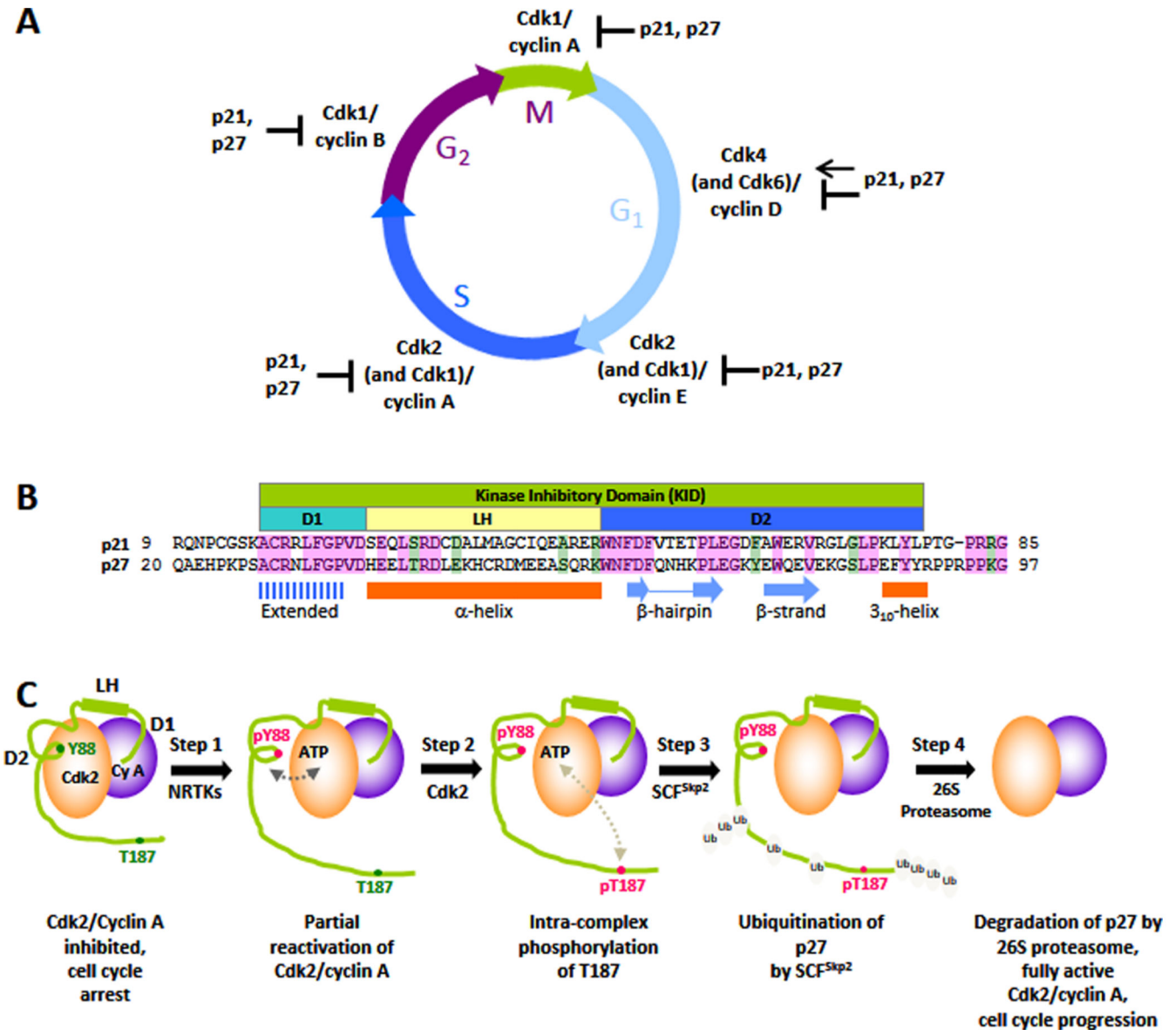

Figure 6.

Local functional unfolding promoted by phosphorylation. The disordered protein, p27, is a regulator of eukaryotic cell division (A) and folds upon binding to Cdk/cyclin complexes. The kinase inhibitory domain (B) mediates binding to Cdk/cyclin complexes, while the C-terminal domain (containing T187) remains disordered (C) and plays a critical role in phosphorylation signaling that controls the transition from G1 to S phase of the division cycle. Phosphorylation of Y88 causes unfolding of a structural element that otherwise blocks the binding of ATP to the Cdk2 active site. This so-called “regulated unfolding” reactivates Cdk2, enabling intracomplex phosphorylation of T187 and subsequent ubiquitination and degradation of p27, which fully activates Cdk2 and drives cell cycle progression. Adapted with permission from ref 376. Copyright 2012 Biochemical Society.

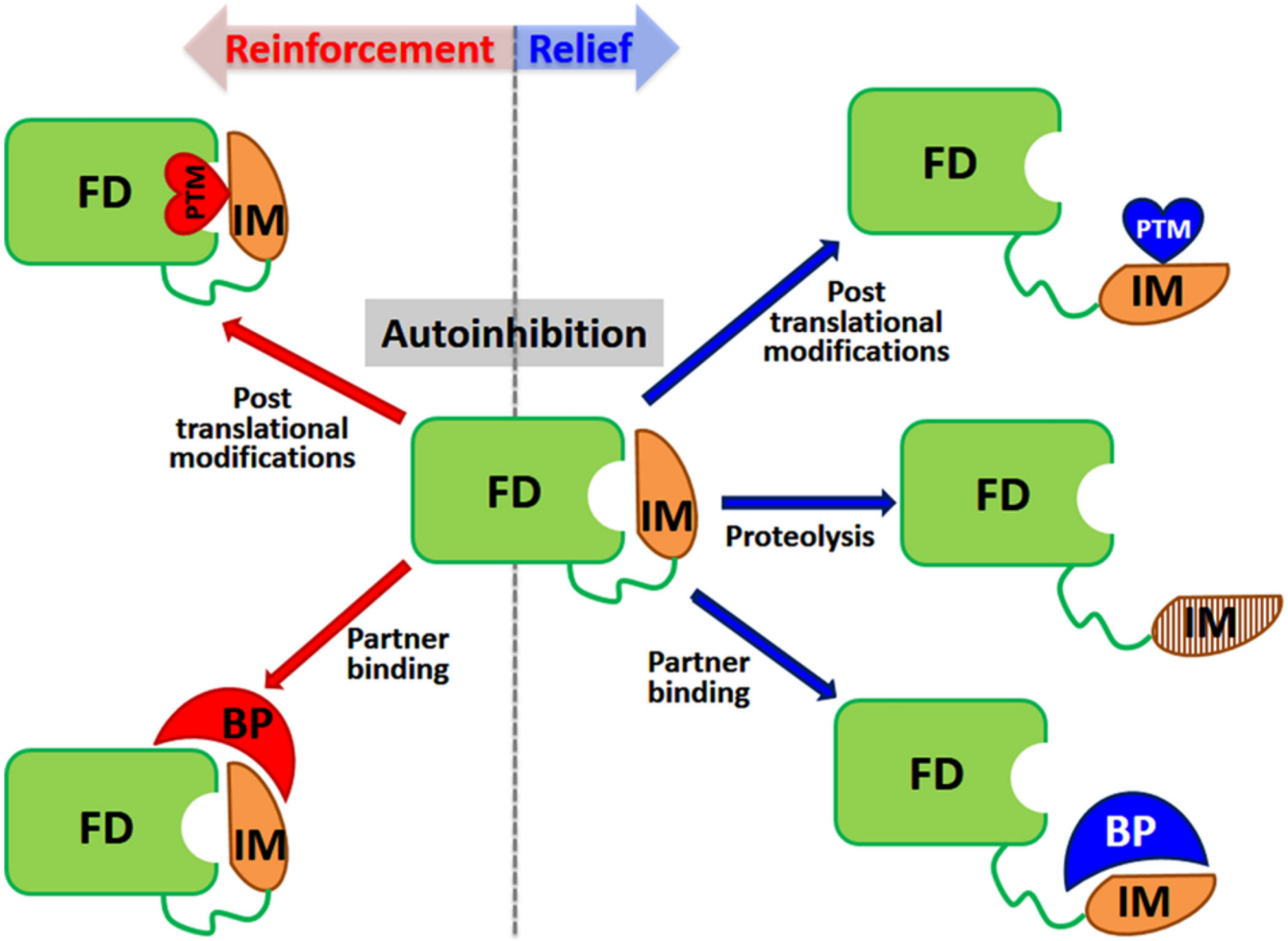

Figure 7.

Regulation of autoinhibition. In autoinhibited proteins, an inhibitory module (IM, yellow) modulates/inhibits the activity of a functional domain (FD, green). Equilibrium between active and inhibited states is regulated; that is, autoinhibition can be actively relieved (right side) or reinforced (left side). Reversal of autoinhibition in most systems relies on post-translational modifications (PTMs, hearts, red for reinforcement or blue for relieve), such as phosphorylation, binding to an activating partner (BP, crescent, red for reinforcement and blue for relieve), or proteolytic cleavage of the IM (right side). Reinforcement of autoinhibition has been shown to be caused by binding of partners or PTMs (left side). Autoinhibition can be caused by direct, steric interactions of the IM with the FD but can also be induced allosterically.