Remodeling protein complexes: Insights from the AAA + unfoldase ClpX and Mu transposase

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Abstract
Multiprotein complexes in the cell are dynamic entities that are constantly undergoing changes in subunit composition and conformation to carry out their functions. The protein–DNA complex that promotes recombination of the bacteriophage Mu is a prime example of a complex that must undergo specific changes to carry out its function. The Clp/Hsp100 family of AAA+ ATPases plays a critical role in mediating such changes. The Clp/Hsp100 unfolding enzymes have been extensively studied for the roles they play in protein degradation. However, degradation is not the only fate for proteins that come in contact with the ATP-dependent unfolding enzymes. The Clp/Hsp100 enzymes induce structural changes in their substrates. These structural changes, which we refer to as “remodeling,” ultimately change the biological activity of the substrate. These biological changes include activation, inactivation (not associated with degradation), and relocation within the cell. Analysis of the interaction between Escherichia coli ClpX unfoldase and the Mu recombination complex, has provided molecular insight into the mechanisms of protein remodeling. We discuss the key mechanistic features of the remodeling reactions promoted by ClpX and possible implications of these findings for other biological reactions.

Keywords: conformational changes; structure/function studies; protein turnover; chaperonins; enzymes; multiprotein complexes; protein remodeling; recombination

Protein folding and unfolding
Remodeling is defined as a protein-assisted change in the structure of a complex that results in a change in the biological activity. The most commonly recognized remodeling enzymes belong to the hsp60 and hsp70 chaperone families. Numerous detailed studies have demonstrated that these folding chaperones prevent off-pathway interactions during protein folding by providing an isolated environment for the folding protein. Some evidence suggests that the hsp70 chaperones may use this same passive mechanism to facilitate the dissociation of multicomponent complexes (for review, see Slepenkov and Witt 2002).

In contrast, the Clp/Hsp100 unfolding enzymes actively direct the structural changes in their substrates. Clp/Hsp100s act on folded and assembled complexes, as well as improperly folded and aggregated proteins. The Clp/Hsp100 proteins belong to the larger AAA+ (ATPases associated with various cellular activities) superfamily of proteins. Most cell types, both prokaryotic and eukaryotic, contain multiple Clp/Hsp100 family members; well-studied members of this protein family include the cytosolic ClpX and ClpA in Escherichia coli, and Hsp104 in Saccharomyces cerevisiae. Crystallographic and electron microscopy studies demonstrate that the active enzymes are homo-hexameric rings in the presence of ATP (Grimaud et al. 1998; Bochtler et al. 2000; Guo et al. 2002; Kim and Kim 2003; Lee et al. 2003) (Fig. 1A).
Thermus thermophilus and ClpB reactivate thermally aggregated proteins, ClpX also participates in some pathway). B pathway) or released into Clp/Hsp100 proteins. (E. coli degradation sequence, an 11-residue peptide that is ap-

ences to the ClpP peptidase (Flynn et al. 2003). The ssrA sequence to ClpX) forms a complex with the HslV pepti-

dase, and the HslU ATPase (Flynn et al. 2001). First, the Clp/Hsp100 component recognizes and selects a target protein. The enzyme binds to a short peptide sequence (e.g., the ssrA degradation tag) usually located near either the C or N terminus of the substrate. Then, in a reaction that requires multiple cycles of ATP-hydrolysis, the enzyme unfolds and directionally translocates the target substrate to the peptidase chamber where it is degraded.

Other studies revealed that these unfolding enzymes also catalyze the recycling of proteins. Thus, not all reactions promoted by Clp/Hsp100 enzymes result in protein degradation (Fig. 2). In vitro, ClpA alone converts the phage P1 origin-binding protein, RepA, from an inactive dimer into active monomers (Wickner et al. 1994). The yeast Hsp104 ATPase, and its closest bacterial homolog, ClpB, have no known partner peptidases, and therefore do not participate directly in protein turnover. Instead, Hsp104 resolubilizes heat-induced luciferase aggregates in vivo, and referees conversion between the prion and nonprion forms of the Sup35 protein (Parsell et al. 1994; Chernoff et al. 1995; Newnam et al. 1999). Likewise, *Thermus thermophilus* and *E. coli* ClpB reactivate thermally aggregated proteins, with the help of other chaperones (Woo et al. 1992; Goloubinoff et al. 1999; Motohashi et al. 1999; Zolkiewski 1999). *E. coli* ClpX also participates in some nonproteolytic reactions, such as the remodeling of the phage Mu transpososome. This well-characterized remodeling event converts the transpososome from a complex that inhibits phage-specific DNA replication into a complex that actively promotes replication initiation (Kruklitis et al. 1996). Thus, the unfoldases, acting independently from peptidase components, can offer misfolded or aggregated proteins a second chance at life, or they can reshape the function of a stable

Several Clp/Hsp100 family members can also form hetero-oligomeric complexes with peptidases. For example, ClpX and ClpA can each form a complex with the ClpP peptidase, and the HslU ATPase (~50% identical in sequence to ClpX) forms a complex with the HslV peptidase. The peptidases are composed of two stacked hexameric or heptameric rings. Hexameric rings formed by the ATPases stack on the outsides of the peptidase to make up barrel-like structures (Sousa et al. 2000). The proteolytic active sites are sequestered in the inner cham-

bers of these peptidases (Wang et al. 1997). The ATPase actively translocates protein substrates to the peptidase through the centrally located axial pore that runs through the stacked complex (Ishikawa et al. 2001).

The Clp/Hsp100 ATPases are responsible for selecting protein targets. For example, the two different bacterial ATPases ClpX and ClpA impart distinct substrate preferences to the ClpP peptidase (Flynn et al. 2003). The ssrA degradation sequence, an 11-residue peptide that is ap-


**Fig. 1 live 4/c**
complex. It is the purpose of this review to convey the molecular insights gained from analysis of ClpX-mediated remodeling of the phage Mu transpososome, and to explore how application of a similar mechanism to other protein complexes may explain how they are remodeled.

Mu transposase–DNA complexes are destabilized by a protein catalyst

The best-characterized remodeling substrate of *E. coli* ClpX is the stable multimer made up by the transposase phage Mu. Mu is a virus that uses transposition to propagate its genome; in roughly 1 h of lytic development, Mu amplifies its genome over 100-fold by replicative transposition (Chaconas et al. 1981). This robust transposition allowed the development of a highly tractable in vitro transposition system, which minimally requires Mu genome end sequences on a supercoiled DNA, the phage encoded transposase (MuA protein), a host encoded DNA bending protein (HU protein), and divalent metal ions (Craigie et al. 1985). Studies of Mu transposition in vitro have revealed that Mu faithfully completes transposition in a regulated manner. Organized catalysis and regulation depends on recombining the correct DNA sequences, transposing at the right time, and avoiding disruption of its own genome (Baker and Mizuuchi 1992; Aldaz et al. 1996; Savilahti and Mizuuchi 1996; Mizuuchi and Mizuuchi 2001). Mu has solved these problems by utilizing a vectorial process that employs increasingly stable transpososome complexes and a chaperone-regulated transition to direct the stages of recombination. These regulatory steps ensure the phage will ultimately have complete copies of its own genome to package into phage heads.

The principal protein responsible for recombination is the transposase, MuA. MuA is a large (75 kDa) protein that can be divided into three proteolytically distinct domains. Each domain has a specific function; DNA binding, catalysis, and accessory protein interaction (Fig. 3A). The extreme carboxy terminal residues of MuA form a recognition tag, which (like the ssrA tag) is bound by ClpX.

**Mu** directs recombination of phage Mu DNA through a series of reaction steps defined by distinct complexes that are termed transpososomes (Craigie and Mizuuchi 1987; Surette et al. 1987) (Fig. 3B). First, the transposase binds to specific sequences at the left and right ends of the Mu genome (Craigie et al. 1984). Interactions between transposase subunits bring the Mu DNA ends together to form the stable synaptic complex (SSC) consisting of four core subunits. An accessory protein called MuB brings a new target DNA to the transposition complex. To initiate movement into the new host DNA, transposase subunits nick one DNA strand at each end of the Mu genome, thus generating the cleaved donor complex (CDC) (Mizuuchi 1992). The exposed 3′ hydroxyl groups at the Mu DNA...
ends then attack a target DNA molecule in a reaction called DNA strand transfer (and generates the strand transfer complex, STC). As a result of these two chemical reactions, a branched DNA structure is formed at each end of the Mu DNA. Within these branches, one strand of Mu DNA is covalently attached to the old host DNA, whereas the other strand is joined to the new host DNA. This configuration allows for a complete copy of the Mu genome to remain at the donor location, while also being copied into its new host location after DNA replication. After completion of recombination, the recombined DNA strands remain tightly bound by MuA in a complex known as the strand transfer complex or STC.

The reaction pathway proceeds in the forward direction because the transposase–DNA complex increases in thermodynamic stability with each reaction step (Fig. 3B). The SSC progresses to the more stable CDC, which finally becomes a hyperstable STC. In vitro the hyperstable STC resists 6 M urea and temperatures up to 75°C (Surette et al. 1987). Thus, apparently paradoxically, this complex takes on its most stable form precisely at the point at which it has completed its recombinase function, and should be released from the DNA. The next required step in transposition is the replication of the Mu genome. However, the continued presence of transposase at the Mu DNA ends actually inhibits assembly of bacterial DNA replication machinery, and thus lytic growth (Nakai and Kruklitis 1995) (Fig. 3B).

Thermodynamic stabilization of protein–DNA complexes as recombination progresses is likely to be a general feature of reactions promoted by members of
the transposase/integrase superfamily. This protein superfamily includes many DNA transposases and retroviral/retronposase-integrase families with the Tn5 transposase, HIV integrase (Dyda et al. 1994; Rice and Mizuuchi 1995; Davies et al. 1999). The RAG recombinases, which are involved in rearrangement of the immunoglobulin and T-cell receptor genes, also appear to be more distantly related members of this family. Therefore, a thorough understanding of enzyme-catalyzed destabilization of MuA is likely to provide insight into mechanisms important to many recombination pathways.

The ClpX ATPase destabilizes the transpososome

Host factors play an integral part in resolving the recombination–replication transition in the Mu life cycle. Biochemical fractionation studies first isolated a single ATP-dependent activity in cellular extracts that could destabilize the STC (Levchenko et al. 1995). This activity was attributed to the E. coli protein, ClpX.

Early studies of the transpososome–ClpX interaction revealed its potential as a model system for understanding the remodeling of nucleoprotein complexes. Using a simplified in vitro transposition reaction with complexes formed on supercoiled plasmids carrying Mu sequences (mini-Mu), biochemical studies clearly implicated ClpX-mediated destabilization as a major contributor to the recombination–replication transition. Further, the requirement for Mu replication had a striking genetic signature in vivo. Strains deficient in ClpX exhibited a 10^3-fold defect in Mu growth (Mhammedi-Alaoui et al. 1994). In contrast, ClpP-deficient cells support robust phage growth. Thus, ClpX alone appeared to provide the necessary functions for releasing the block between transposition and DNA replication.

Initial characterization of STCs treated with ClpX demonstrated that ClpX destabilizes the hyperstable complex. Whereas the STC remains stable during electrophoresis, complexes treated with ClpX migrated with an altered mobility, similar to that of phenol-extracted DNA (Kruklitis et al. 1996). Stabilization of the complexes by protein crosslinking verified the presence of transposase at the site of recombination after ClpX treatment. Thus, it was concluded that ClpX destabilizes the STC without destroying it, and the ClpX-treated complexes were named “STC2” or “fragile complexes.” Further analysis of the destabilized or “fragile complex” revealed that it was sensitive to both modest ionic strength and heparin, both of which were ineffective at destabilizing the STC (Kruklitis et al. 1996) (Fig. 3B). Therefore, ClpX-treated complexes were not totally destroyed, but clearly exhibited biochemical characteristics that distinguished them from the initial STCs.

Further characterization established that ClpX recognizes the eight extreme carboxy-terminal residues of the transposase, QNRRKKAI (Levchenko et al. 1995). The sequence is similar to the carboxy-terminus of some ClpXP degradation substrates (Flynn et al. 2003). In vitro analysis demonstrated that transpososomes resist degradation by ClpXP but remain susceptible to ClpX unfolding activity, whereas transposase monomers are both efficiently unfolded and degraded (Jones et al. 1998). This apparent prevention of degradation of transpososome-associated subunits was especially interesting since monomeric transposase, free from DNA, is recognized by ClpX through the same C-terminal sequence on the transposase (Levchenko et al. 1995). The mystery became explaining how a destructive enzyme like ClpX could produce a “remodeled” but functional product instead of a destroyed one.

The model: Remodeling the transpososome by selective subunit unfolding

To gain insight into how ClpX remodeling of the Mu transpososome generates a fragile DNA-bound complex, rather than completely unfolded or completely degraded MuA subunits, two central questions had to be addressed: First, what mechanism (unfolding, prying, etc.) does ClpX use to promote transpososome remodeling? Second, what is the nature of the products of ClpX-mediated remodeling? A look at the initial steps of protein degradation provided a clear starting point for investigating the mechanism used for remodeling. Following the time course of degradation reactions using GFP-ssrA established that the Clp/Hsp100 proteins globally unfold substrates for translocation to their cognate peptidases for proteolysis (Weber-Ban et al. 1999). Biophysical and molecular probes for protein unfolding revealed that ClpX can unfold both monomeric transposase in solution, and STC-bound transposase during remodeling reactions.

In an effort to understand how both degradation and remodeling could employ the same basic unfolding mechanism, but in one case leave behind an altered and active product, the products of Mu transpososome remodeling were analyzed. ClpX recognition and unfolding of individual subunits in the transpososome did not lead to unfolding of neighboring transposase subunits. This result provided the first suggestion of the mechanistic basis for the distinction between destruction and remodeling. The results of gel shift assays and DNA footprinting indicated that selective removal of one MuA subunit from the complex is the molecular basis of the transition from stable STC to fragile complex (Burton and Baker 2003). In fact, a specific MuA binding site, called L1, along the left end
Selective destabilization: model for ClpX-mediated Mu protein remodeling. MuA binding sites L1–3 and R1–3 are depicted. Work described in this review defines the following important elements for ClpX-mediated remodeling of Mu transpososomes. First, ClpX uses its unfolding activity for remodeling, just as it does for degradation; (2) ClpX recognizes only a subset of the subunits in the complex; and (3) intrinsic asymmetry of the transpososome constrains which subunit(s) is available for interaction with ClpX. The remodeled complex maintains the Mu DNA ends in a synapsed configuration able to recruit replication machinery, as previously demonstrated (Nakai and Kruklitis 1995). Thus, selective interaction with one or a small subset of subunits is sufficient to destabilize but not destroy the complex. We suggest that this is an attractive mechanism for complex remodeling (either coupled or uncoupled to degradation) facilitated by the Clp ATPases and their eukaryotic cousins.

Summarizing the above findings, three salient features of this “selective destabilization” model are: (1) ClpX uses its unfolding activity for remodeling, just as it does for degradation; (2) ClpX recognizes only a subset of the subunits in the complex; and (3) intrinsic asymmetry of the transpososome constrains which subunit(s) is available for ClpX action. Work described in this review defines the following important elements for ClpX-mediated remodeling of Mu transpososomes. First, ClpX uses its unfolding activity for remodeling, just as it does for degradation; (2) ClpX recognizes only a subset of the subunits in the complex; and (3) intrinsic asymmetry of the transpososome constrains which subunit(s) is available for ClpX action. Thus, selective interaction with ClpX or ClpXP, Figure reprinted with permission from Elsevier (Burton and Baker 2003).

Remodeling promotes important biological transitions

Myriad cellular processes involve protein remodeling (Table 1). In fact, a screen for in vivo substrates of ClpX identified some very intriguing remodeling candidates, including Dps and FtsZ (Flynn et al. 2003). The stationary phase protein, Dps, forms very large, torroid structures with chromosomal DNA (Frenkiel-Krispin et al. 2004). These structures are thought to help protect the DNA during starvation, which is often encountered in stationary phase. Such massive, ordered complexes also must be dismantled when the cells return to permissive growth conditions. ClpX, which has been demonstrated to keep Dps levels in check during exponential growth (Stephani et al. 2003), may also play a role in disassembling these large structures at the exit from stationary phase. Similarly, the FtsZ protein assembles into large, ring structures that provide a scaffold for division apparatus at future sites of cell division. This complex too, must have a way to reorganize and disassemble when cell division occurs (Weart et al. 2005). Like the Mu transpososome, chaperone mediated unfolding is
an attractive mechanism to explain how these protein superstructures are remodeled at critical transitions.

We propose that the selective destabilization model presented here may be instructive when considering remodeling mechanisms for many of these other crucial complexes in the cell. Three examples discussed below all share common features with Mu transpososome remodeling. In each case, interaction of a chaperone with a multicomponent complex changes the biological function of the complex (Fig. 5). Each of the critical features of the model is then discussed in relation to these examples to highlight the recurring themes among these divergent cellular remodeling events.

### Generation of monomeric RepA

Activation of the phage P1 replication initiator protein RepA requires help from the *E. coli* DnaK and DnaJ proteins, members of the hsp70 and hsp40 chaperone families. RepA exists as an inactive dimer in solution. Monomers of RepA are the active form of the protein that binds to the origin DNA, oriP1, with high affinity (Wickner et al. 1991b). The chaperones, DnaK and DnaJ act together to activate the sequence-specific DNA binding of RepA (Wickner et al. 1991a). In fact, when monomeric RepA is added to an in vitro replication reaction, DnaK and DnaJ are not required (Wickner et al. 1992). Thus, it was proposed that in an ATP-dependent manner, the chaperones convert the inactive dimers into high-affinity DNA binding proteins. Later, it was demonstrated that ClpA, a Clp/Hsp100 unfolding enzyme, could substitute for DnaK and DnaJ in vitro (Wickner et al. 1994). Thus, members of two distinct chaperone families remodel RepA.

### Recycling membrane fusion proteins

Another AAA+ family member, NSF (N-ethylmaleimide-sensitive fusion protein), remolds proteins critical for intracellular membrane fusion (for review, see Haas 1998). Structural and biochemical evidence implicates NSF in the dissociation of transmembrane SNARE complexes. Membrane anchored SNAREs interact selectively with one another in a four-helix bundle to form an extremely stable and structurally conserved complex (Fasshauer et al. 1997; Poirier et al. 1998). NSF and soluble NSF attachment proteins (SNAPs) pull apart SNARE complexes in an ATP-dependent manner, thereby allowing SNARE subunits to catalyze multiple rounds of membrane fusion (Hanson et al. 1997). The kinetics of NSF/SNAP/SNARE complex turnover suggest that NSF ATPase activity is important in generation of fusion-competent vesicles (Swanton et al. 2000). Thus, it is thought that NSF-mediated remodeling of SNARE complexes is coupled with SNARE reactivation.

### Activation of transcription complexes

Recent data also support the idea that ATPase subassemblies of proteases function as remodeling chaperones. The eukaryotic 26S proteasome, although much more complex in subunit composition than the Clp/Hsp100-associated proteases, shares a similar structural design and many mechanistic features. The outer regulatory subunits of the proteasome are members of the AAA+ family, and are referred to as the 19S or “cap” complex. The inner proteolytic rings make up the “core” or 20S complex. Genetic studies originally implicated Sug1, a 19S AAA+ subunit, in transcriptional activation (Swaffield et al. 1992). More recent biochemical studies suggest that the 19S complex may play a nonproteolytic role in transcription elongation (Ferdous et al. 2002). Chromatin immunoprecipitation assays demonstrate that a subassembly of that 19S cap is recruited to a Gal4-regulated promoter upon induction with galactose (Gonzalez et al. 2002). Notably, neither certain 19S subunits needed for proteasome function nor 20S sub-

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Table 1. Example of protein complex subject to enzymatic remodeling

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units were found associated with promoter DNA. The APIS (AAA proteins independent of 20S) was thus named to denote this distinct subcomplex of the 19S that acts independently from the other proteasome subunits. The precise mechanism by which the APIS functions in transcription is not known. However, these data suggest a nonproteolytic activity for the eukaryotic proteasome that might parallel the remodeling activity exhibited by the Clp/Hsp100 ATPases.

**Recurring themes in protein remodeling**

The examples presented here demonstrate that several key features may be common to many protein remodeling reactions. First, we examine the case of RepA activation. ClpA is an unfolding enzyme, and like ClpX it is known to actively unfold its degradation substrates. Presumably, it uses that same unfolding mechanism for RepA remodeling as well. The result that the ClpA unfoldase can remodel RepA dimers strongly suggests that the relevant activity in vivo could be DnaK-facilitated protein unfolding. DnaK may not actively unfold the RepA protein like ClpA does. Instead, since DnaK is known to bind exposed regions of unfolded protein, it may more passively bind transiently exposed peptide segments generated by “breathing” in the dimer.

Since NSF and the 19S proteasomal subunits are also members of the AAA+ family, it is logical to consider that they may use the same unfolding mechanism as the Clp/Hsp100 proteins to promote remodeling. However, in the case of NSF disassembly of SNARE complexes, the prospect of NSF releasing unfolded membrane proteins into solution is not so enticing. It is more likely that NSF disassembles only the soluble domain of SNARE complexes. Such limited unfolding would presumably allow for refolding of the soluble domain, thus generating the recycled subunit that can go on to catalyze a new round of fusion. In the case of MuA, transposase subunits unfolded by ClpX have the capacity to refold and promote another round of recombination (Burton et al. 2001). Similarly, the soluble domains of the SNARE subunits could refold, thus resetting the SNARE proteins to allow a new round of membrane fusion. Moreover, it remains to be seen whether some of the unfolding chaperones may have a folding chaperone activity as well. As for the 19S ATPases, not enough is known about the complexes with which they interact to hypothesize about mechanism. However, since the 19S subunits regulate degradation for the proteasome, much like ClpX does for ClpP, again it is intriguing to consider that they are prone to use the same unfolding mechanism for both remodeling and degradation.

The second hallmark of the selective destabilization model is the recognition of only a subset of the subunits within the substrate complex. For the RepA dimer, selective recognition is a very sensible paradigm, as action on one subunit would leave the other subunit free to bind DNA. In fact, for the ClpA-mediated remodeling pathway, direct interaction with only one subunit has been shown to be sufficient to generate active RepA (Pak et al. 1999). However, recent data suggests that ClpA recognizes and processes both subunits of a RepA dimer (Sharma et al. 2005). Whereas numerous experiments with ClpX provide substantial evidence that the
directly recognized subunit is unfolded and processed, it appears that more work is needed to determine whether action on a single subunit is a reasonable model for the ClpA and DnaK pathways as well.

The structures of the SNAREs indicate that a portion of each complex is accessible to NSF (Hanson et al. 1997; Hohl et al. 1998). SNARE disassembly could involve selective destabilization of individual SNARE components, followed by release of intact components that are capable of reassembly for further rounds of fusion. Such a mechanism has already been proposed for this unfolding/refolding reaction; however, this assertion is based almost entirely upon crystal and EM structures. It remains to be seen from biochemical analyses whether NSF remodeling of SNARE complexes actually follows the selective destabilization model.

The final attribute of the model is that an intrinsic property of the structure of the target complex is responsible for directing the outcome of the remodeling reaction. For example, in the case of the Mu transpososome, inherent asymmetry in the STC has been proposed to control which transposase subunits are accessible to ClpX. In contrast, the RepA dimer structure is likely symmetric, in which case we predict that random engagement of subunits by ClpA would be responsible for remodeling. Similarly, after a dimer of DnA binds to the RepA dimer, recognition by DnA and GrpE could be stochastic, as the outcome would presumably be identical regardless of which subunit is contacted. Thus, we propose that the physical properties of the RepA dimer do not impose constraints on the outcome of the remodeling reaction. Similarly, the structures of the NSF–SNARE complexes do not thus far suggest any constraints for the remodeling reactions. However, minor structural or sequence differences between t-SNAREs and v-SNAREs may provide the asymmetric handle necessary for NSF to preferentially interact with a specific component. Finally, it is highly likely that the multicomponent transcription complexes targeted by the 19S ATPases exhibit inherent asymmetry allowing for specific recognition and targeted unfolding of only certain protein constituents.

Conclusion

The information we have learned from studying ClpX-mediated remodeling of the Mu transpososome has provided a detailed look at a mechanism used by unfolding enzymes to remodel rather than destroy complexes. The emerging picture for how AAA+ proteins work in proteolysis is that they recognize a peptide sequence, and from that site, unfold and translocate the protein through the cylindrical protease complex. Accumulating mechanistic evidence suggests that a similar unfolding and polypeptide threading mechanism can also result in the remodeling of protein complexes, rather than mere destruction. Here, we have considered just a few of the myriad of transitions in biological pathways that require assistance from unfolding proteins and that are likely to work by selective destabilization. Although the specifics of each system will vary, a few common themes for the selective destabilization mechanism are emerging. As the details of other systems are examined, selective destabilization may come forward as a common means to achieve changes in the biological activities of protein complexes.

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