Molecular chaperones and the art of recognizing a lost cause

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Molecular chaperones have long been heralded as machines for folding and salvaging proteins. However, not every attempt to fold or refold a protein can be successful. Chaperones are known to participate in the degradation of misfolded polypeptides, but a direct link between folding and degradation pathways has remained elusive. Two recent reports show that the co-chaperone CHIP mediates ubiquitin-dependent degradation of substrates bound to heat-shock protein 70 (Hsp70) and/or Hsp90.

It is well established that molecular chaperones play a key role in assisting cellular protein folding[1]. Several classes of molecular chaperones ensure that newly synthesized proteins, which emerge from ribosomes in an extended conformation, reach the native state rapidly and efficiently. In addition, when proteins unfold as a result of cellular stress, chaperones protect them, prevent their aggregation, and restore them to the native state once the stress has subsided. Indeed, cells greatly increase their concentrations of some chaperones in response to such conditions, hence the term 'heat-shock proteins' (HSPs). However, not all newly synthesized or stress-denatured proteins can successfully attain their native states. Polypeptides that are altered by mutation or modification are targeted for degradation by covalent addition of polyubiquitin, which is subsequently recognized by the 26S proteasome[2]. The process of protein ubiquitination is executed by a complex cellular machinery; the protein to be degraded is specifically recognized by a ubiquitin-ligase enzyme (E3) that cooperates with one or more ubiquitin-conjugating enzymes (or E2s) to transfer multiple moieties of the 76-amino-acid protein ubiquitin to the substrate[2].

Little is known about how non-native polypeptides are delivered to the ubiquitination machinery. The cell must possess components that recognize folding-incompetent proteins and target them for ubiquitination, although a specific ubiquitin ligase for misfolded proteins has yet to be identified. Interestingly, the recently published characterization of Hrd1p as an E3 that functions in endoplasmic-reticulum-associated degradation (ERAD), indicates that its ubiquitin-ligase activity may have a preference for misfolded proteins[3]. In principle, the signals or features in the substrate that would be recognized by such a ubiquitin ligase should be similar to those recognized by molecular chaperones, as both must distinguish between native and non-native conformations. Importantly, it seems that newly synthesized and misfolded proteins are initially recognized by chaperones, the primary function of which is to promote folding. Thus, a critical question concerns the identity of the cellular components and mechanisms that mediate the decision to abort folding attempts and instead to commit a chaperone-bound polypeptide to degradation. Several lines of evidence indicate that chaperones have a role in this triage process, but their precise function is unclear[4–6].

Several models can be proposed to account for the involvement of chaperones in the degradation of misfolded proteins (Fig. 1). The simplest possibility (Fig. 1a) is that molecular chaperones, with their ability to bind to and release protein substrates in cycles driven by ATP-binding and hydrolysis, serve to maintain non-native polypeptides in a soluble state, allowing them to partition to the ubiquitination machinery[7]. In this model, the inability of the polypeptide to fold productively results in multiple rounds of binding and release from the chaperone. Prolonged cycling of the non-native polypeptide with the bulk cytosol

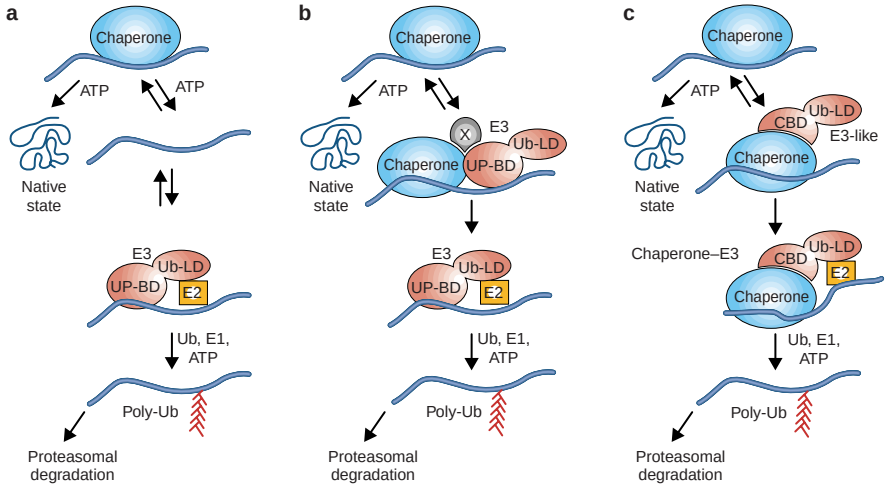

Figure 1 **Models for the involvement of chaperones in misfolded protein degradation.** a, **In this model, an inability to rapidly achieve the native state results in prolonged cycling of the substrate on and off its chaperone. This cycling allows an E3 ubiquitin ligase to recognize and bind to the non-native polypeptide (through its unfolded-polypeptide-binding domain, UP-BD). The E3 contains an E3 ubiquitin-ligase domain (Ub-LD, such as a RING-finger domain), which cooperates with an E2 and with the ubiquitin-activating enzyme E1 to ubiquitinate the aberrant polypeptide and target it for degradation by the proteasome.** b, **Here the chaperone–substrate complex recruits the E3 ligase, either by direct interaction or through the action of an unknown bridging factor (X). Once it has successfully committed its substrate to ubiquitination, the chaperone is released and E3 and E2 enzymes polyubiquitinate the substrate.** c, **Here the chaperone complex itself is transformed into an E3 ligase by interaction with an ancillary protein (E3-like) that contains a chaperone-binding domain (CBD) and an E3 ubiquitin-ligase domain. The ability of the chaperone to bind to non-native polypeptides functions to recognize and present the substrate for ubiquitination. CHIP may be an example of such an ancillary protein, as it contains both a chaperone-binding TPR domain and a RING-finger-like U-box domain.**

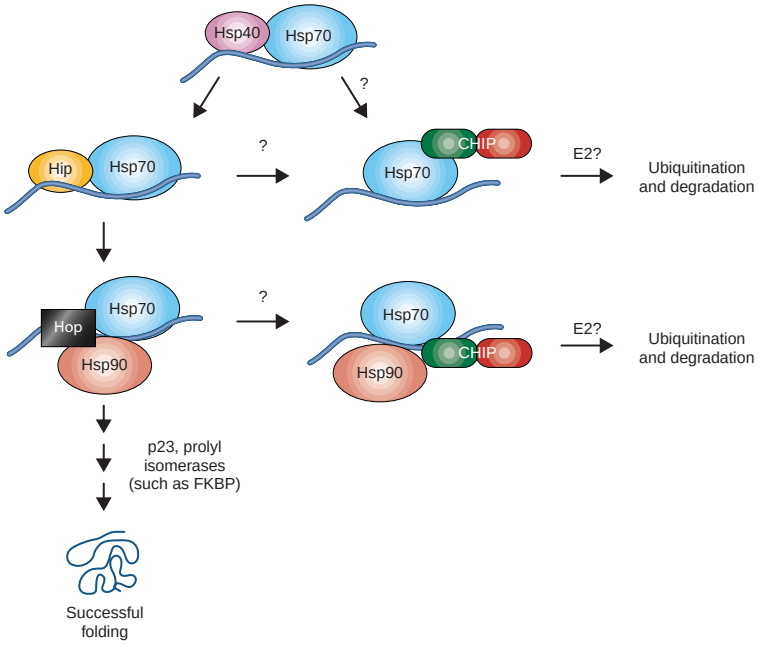

Figure 2 **Model for CHIP-mediated degradation of chaperone substrates. Initial interaction of the non-native polypeptide with Hsp70 and Hsp40 may result in progression along the folding pathway (left branch, reviewed in ref.14). If the substrate is recognized as folding-incompetent, interaction with CHIP (chaperone-interacting TPR domains shown in green, U-box domain shown in red) remodels the chaperone complex and initiates the pathway to degradation (right branch). It is unknown at which stage in the folding pathway, if any, the decision to abort folding can be made. It is also possible that although Hsp70 may be sufficient for CHIP interaction, some substrates may require interaction with both Hsp70 and Hsp90 before interaction with CHIP. The identity of the E2 that participates in this process is not known.**

thus increases the chance of substrate recognition by a ubiquitin ligase. In a variation of this model (Fig. 1b), the ubiquitin ligase may be physically recruited by the polypeptide–chaperone complex, possibly with the aid of a 'bridging factor'. Here, the ubiquitin ligase still recognizes and directly interacts with the unfolded polypeptide, but the transfer from chaperone to ligase occurs without release into the bulk solution. A third possibility is that the chaperone complex itself is transformed into a ubiquitin ligase by interaction with components of the ubiquitination machinery (Fig. 1c). A distinguishing feature of this model is that substrate recognition is provided solely by the chaperone. Given our lack of understanding of this problem, it is currently impossible to discern whether one or all of these models are at work in the cell. Two papers recently published in *Nature Cell Biology* provide some surprising insights into the factors that affect the balance between protein folding and degradation[8,9]. Both papers investigate the role of a protein called CHIP (for carboxy terminus of Hsp70-interacting protein)[10] in the degradation of substrates that are normally folded by the Hsp70–Hsp90 chaperone systems, and indicate that CHIP can functionally and physically link these chaperones to the ubiquitination and degradation machineries.

The chaperones Hsp70 and Hsp90 cooperate in the folding and maturation of a subset of proteins that includes protein kinases, steroid hormone receptors (such as the glucocorticoid receptor), and the cystic fibrosis transmembrane regulator (CFTR), a membrane protein that contains large cytoplasmic domains[11–13]. The process by which proteins achieve their native, active states through the action of Hsp70 and Hsp90 is mediated, in part, by chaperone co-factors such as Hip (Hsp70-interacting protein) and Hop (Hsp70–Hsp90-organizing protein)[14]. These accessory proteins have a modular domain structure that is characterized by the presence of tetratricopeptide repeat (TPR) domains, which are highly degenerate, 34-amino-acid motifs that are known to be important for protein–protein interactions in an ever-increasing number of cellular pathways[15]. TPR motifs may occur singly or, as is more often the case, in multiple tandem arrays throughout the length of a protein. Their loose sequence conservation allows for extensive variety in protein interaction and even allows different TPR repeats in the same protein to selectively interact with different proteins. For example, Hop harbors different TPR domains that are specific for Hsp70 and Hsp90. The interaction of TPR domains with chaperones requires sequences in the C terminus of Hsp70 and Hsp90, including the conserved EEVD motif[16].

CHIP was originally identified as a new TPR-containing protein that interacts with the C-terminal region of Hsp70 and negatively regulates its ATPase and chaperone activities[10]. The primary amino-acid sequence of CHIP revealed three amino-terminal TPR domains and, notably, a region that shares ~50% similarity with the proteasome-associated proteins UFD2 and NOSA. This common region, designated the 'U-box', also resembles the RING-finger domain that is characteristic of ubiquitin ligases[17].

The domain structure of CHIP indicates an intriguing connection between Hsp70 and the ubiquitination machinery. The two recent studies[8,9] found that increased cellular levels of CHIP cause a marked shift in the balance between folding and degradation for the glucocorticoid receptor and CFTR, both of which are folded by the Hsp70/Hsp90 pathway. CFTR folds inefficiently, and mutations that lead to cystic fibrosis, such as CFTR(ΔF508), increase its propensity for misfolding. The chaperone pair Hsp70 and Hdj-2 participate in CFTR folding, together with Hsp90 (refs 11, 18). The exacerbated folding inefficiency of CFTR(ΔF508) results in its prolonged association with Hsp70 and Hdj-2, its inability to exit from the endoplasmic reticulum (ER), and its ultimate degradation by the ubiquitin–proteasome pathway[11,19]. CHIP, which has been shown to localize to the cytosol[10], co-localizes with CFTR and Hsp70 at the ER membrane, indicating that it may be involved in CFTR biogenesis[9]. Interestingly, although CHIP overexpression negatively affects the ability of wild-type CFTR to mature to the cell surface, it does not result in accumulation of immature CFTR in the ER membrane. Instead, it seems that CHIP is capable of diverting CFTR to the ubiquitin–proteasome pathway for degradation.

Connell *et al.*[8] recently showed that increased levels of CHIP also reduce the ability of the receptor for the steroid hormone glucocorticoid to attain its active state. In this instance, CHIP not only promotes the ubiquitination of the receptor, but also remodels the co-factor composition of the Hsp70–Hsp90 complex. The action of CHIP to promote degradation of proteins that would otherwise become folded requires the presence of the TPR domain, which can interact with Hsp70 and/or Hsp90. Importantly, the function of

CHIP also requires its U-box domain, and CHIP that lacks the U-box acts in a dominant negative manner to block CFTR degradation[9] As the related RING-finger motif is thought to possess the ubiquitin-ligase activity that leads to poly-ubiquitination, the U-box may serve a similar function in CHIP. Taking these results together, it seems that CHIP links chaperone-mediated protein folding and degradation by virtue of its ability to interact with chaperones and its function in promoting ubiquitination.

These findings offer new insights into the role of chaperones in protein degradation, beyond their previously proposed functions in keeping a misfolded substrate soluble. CHIP may act as an intermediate factor that physically links a chaperone, with its bound substrate, to a novel E3 ubiquitin-ligase complex that promotes its destruction (Fig. 1b). A more tempting speculation is that binding of CHIP to Hsp70 and Hsp90 may transform these complexes to act in the same capacity as E3 ubiquitin ligases (Fig. 1c). In this situation, Hsp70 would carry out the task of substrate recognition, in much the same way as F-box proteins[20] recognize substrates for the SCF-like (Skp1-cullin-F-box) ubiquitin ligases. CHIP, with its RING-finger-like U-box, would provide the catalytic domain for ubiquitination and mediate the transfer of activated ubiquitin from E2 ubiquitin-conjugating enzymes to the chaperone-bound substrate (Fig. 2). In this regard, it is intriguing that CHIP seems to associate both with polyubiquitinated proteins and with the proteasome[8]. This association has been detected for some E3 ligases[21], leading to the idea that E3 ligases both promote ubiquitination and ferry the substrates to the 26S proteasome.

Although this model provides a possible answer to the question of how chaperones facilitate substrate degradation, many new questions must now be posed to further our understanding of this process. For example, substrate degradation in these studies was the result of CHIP overexpression, and it remains unclear how and when CHIP induces the switch from folding to degradation in the context of normal cell function. Under physiological conditions, there must be a trigger that allows CHIP to bind to Hsp70, thwart further folding attempts, and initiate the ubiquitination and degradation of the substrate. This probably requires an interaction with one or more E2s, the identities of which are unknown at present. Also unknown are whether the ubiquitin-ligase activity requires only CHIP or an Hsp70–Hsp90–CHIP complex, and whether further components are involved. Notably, the CHIP–Hsp70 connection probably represents only one of several avenues for the degradation of misfolded proteins in the cell, as degradation of Hsp70-bound ApoB is not affected by CHIP expression[9]. Thus, it is likely that several cellular pathways have evolved to ensure the degradation of damaged or mutated proteins. The characterization of CHIP as a factor that can switch chaperone function from assisting folding to degradation may provide an important clue as to how proteins with a modular domain structure can connect with the folding and degradation machineries. Future studies should ultimately determine whether it is chaperones, their co-factors, or both that recognize when folding is a lost cause and that, for the greater good of the cell, the potentially damaging substrate must be eliminated. □

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