To Fold or Not to Fold ...

David A. Agard
Molecular chaperones are cellular factors that shepherd newly synthesized proteins along the hazardous journey to the folded state. Their discovery has taken the study of protein folding from the arena of the biophysicist into the cell biological limelight. The results of both in vivo and in vitro studies suggest that these molecular chaperones are crucial for the folding and assembly of many multi-domain and multi-subunit proteins. In fact, it is through a combination of biophysical and cell biological approaches that the mechanism of action of the chaperones is now becoming clear.

Protein folding, especially in the extremely concentrated environment of the cell, must sufficiently accelerate the on-pathway folding rate to kinetically out-compete aggregation. In fact, nature has exploited each of these approaches. The blockade of exposed hydrophobic patches requires a factor that can reversibly bind to them. The molecular chaperone Hsp70 and its relatives [Hsc70, DnaK, BiP, Kar2p, and Ssa1-4p (1)] meet this requirement and are ubiquitous monomeric proteins found in the cytosol, endoplasmic reticulum, and mitochondria. The Hsp70 class of chaperones binds to unfolded and partially folded states of a variety of proteins but shows little interaction with native, folded proteins (2). Binding of Hsp70 stimulates an endogenous ATPase, which causes the release of bound protein. However, sufficient...
state, Hsp70s appear primarily to prevent aggregation or premature folding until the substrate protein can assemble into the appropriate multi-subunit complex (7), be translocated across a membrane (8), or be passed on to a different chaperone, Hsp60 (9).

The completion of folding requires a factor that can sequester partially folded molecules from each other but still allow folding to proceed. This requirement is fulfilled by the Hsp60 class of chaperones, exemplified by the protein GroEL (10, 11). The Hsp60s are organized as large assemblies arranged in two rings, each composed of seven 60-kD subunits. Transiently associated with the Hsp60 proteins is the GroES protein, a single ring of seven 10-kD subunits (12). In vitro, the Hsp60 protein GroEL forms complexes with unfolded proteins and facilitates their folding in an ATP-dependent fashion. One, or at most two, protein molecules can bind to each GroEL (13) in or near the large central cavity of the 14-subunit protein (14). As with Hsp70, ATP hydrolysis promotes the release of bound protein. Some proteins, such as rubisco, only require GroEL and ATP hydrolysis to refold (15), whereas others such as rhodanese, which is extremely prone to aggregation, require both GroEL and GroES (16). The refolding of rhodanese requires the hydrolysis of ~130 molecules of ATP per rhodanese molecule (16), indicating that multiple cycles of binding and release occur during folding.

This information can be integrated into a simple functional model (see figure). The central cavity of GroEL could provide a “box” in which an individual polypeptide chain would be free to fold without risk of aggregation. Partially folded proteins, perhaps delivered to GroEL by the Hsp70 chaperones (9), would bind to the hydrophobic walls of the box by means of exposed hydrophobic patches. Binding to GroEL becomes an irreversible commitment to proper folding and the formation of a stable substrate-GroEL complex. ATP binding to the GroEL-GroES complex causes the lid to close, allowing the protein to be refolded through many cycles of binding and release within the protected environment of the box. Once the protein is folded, the box must open to release the protein. A key aspect of this model is that the chaperone does not directly participate in the folding reaction but merely creates an environment of “infinite dilution” in which the protein is free to fold by itself.

A third way to facilitate folding—increasing the on-pathway folding rate—seems to be used by many bacterial and eukaryotic proteases that are synthesized as preproteins (a hydrophobic signal sequence linked to a proregion linked to a mature protease domain). Although there are now many examples of both amino- and carboxyl-terminal pro regions, the best mechanistic studies have been those of α-lytic protease and subtilisin. These small, bacterial serine proteases have extended amino-terminal pro regions of 166 (18) and 77 (19) amino acids, respectively, that are not a part of the active proteases. However, these pro regions are required for the proper folding of the mature protease domains (20–22). In contrast to the chaperones, they do not suppress aggregation but directly facilitate folding. Indeed, folding intermediates of α-lytic protease (21) and subtilisin (23) have been isolated under non-denaturing conditions by the omission of the pro region from in vitro refolding reactions. The α-lytic protease intermediate is stable for weeks without aggregation or folding to the native state. The addition of the pro region at any time leads to rapid folding of the intermediate. Thus, the pro region is required for folding without aggregation or other off-pathway reactions. The α-lytic protease pro region directly stabilizes the folding transition state (21), thereby accelerating these simple refolding reactions, the mechanisms derived from in vitro studies are likely to be physiologically valid. Not only are the native states produced in vivo and in vitro identical for almost all proteins, but the importance of the chaperones suggests that the starting points for folding may also be similar. There had been concern that, in vivo, folding could start at the amino terminus and proceed as the molecule is synthesized but that, in vitro, only full-length molecules refold. However, the binding of Hsp70 to nascent chains (27) and its slow release of bound proteins (4) indicate that in vivo the temporal order of synthesis is unlikely to correspond to the temporal order of folding.

Despite our limited understanding of protein folding, the exciting synergy that has developed between the cell biologist and the biophysicist will lead to rapid progress.

References