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Can Hsp70 Proteins Act as Force-Generating Motors?

Minireview

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In recent years there has been intense interest in a group of proteins known as molecular chaperones (Georgopoulos and Welch, 1993). One class of molecular chaperones is the Hsp70 family, which comprises several closely related proteins of about 70 kDa (McKay et al., 1994). Members of the Hsp70 family are found in the cytosol of prokaryotes and eukaryotes and in the lumenal spaces of mitochondria, chloroplasts, and the endoplasmic reticulum. Hsp70 chaperones have been implicated in protein folding, the assembly and disassembly of oligomeric complexes, protein synthesis and degradation, and the translocation of polypeptides across cellular membranes. Despite this wealth of information, the mechanism of Hsp70 action is still poorly understood.

Hsp70 proteins have a two-domain structure: the N-terminal domain binds adenine nucleotides, and the C-terminal domain binds short segments of extended polypeptide (Hightower et al., 1994; McKay et al., 1994). In the presence of ADP, Hsp70 associates tightly with polypeptide substrates. This interaction is weakened by the addition of ATP. Release of polypeptides from Hsp70 is caused not by ATP hydrolysis, as was originally proposed, but rather by ATP binding (Hightower et al., 1994). It is generally believed that this nucleotide-dependent cycle of polypeptide chain binding and release can account for all of the diverse actions of Hsp70 proteins. For example, during protein folding, Hsp70 molecules might bind transiently to unstructured regions of polypeptide chain and thereby prevent the incompletely folded polypeptides from aggregating (Georgopoulos and Welch, 1993). Thus, Hsp70 chaperones would not serve as catalysts to speed up folding, but instead would inhibit unproductive side reactions. However, as described below, recent studies of protein translocation suggest that certain functions of Hsp70 chaperones involve more than mere binding to polypeptide substrates. It seems that Hsp70 proteins can sometimes accelerate reactions that would otherwise be prohibitively slow.

Lumenal Forms of Hsp70 and Protein Translocation

Many proteins are synthesized as precursors in the cytosol and subsequently translocated across membranes. Translocation takes place through proteinaceous channels (Hannavy et al., 1993; Jungnickel et al., 1994). Some precursor proteins remain associated with cytosolic chaperones before engaging the translocation machinery (Deshaies et al., 1988). In addition, protein import into mitochondria and the endoplasmic reticulum depends upon Hsp70 chaperones in the organellar lumen. Both mHsp70 (mitochondrial matrix-localized Hsp70) and Kar2p (the Hsp70 species of the yeast endoplasmic reticulum) are essential components of the translocation systems in their respective organelles (Brodsky and Schekman, 1994; Stuart et al., 1994). Translocation requires ATP inside the organelle, apparently for the operation of lumenal Hsp70 (Voos et al., 1993; Wachter et al., 1994). It is likely that lumenal Hsp70 molecules bind to a precursor chain as it emerges from the translocation channel. Hsp70 proteins are ideally suited for this task, since they recognize a wide range of peptide substrates (Hightower et al., 1994). Indeed, mHsp70 and

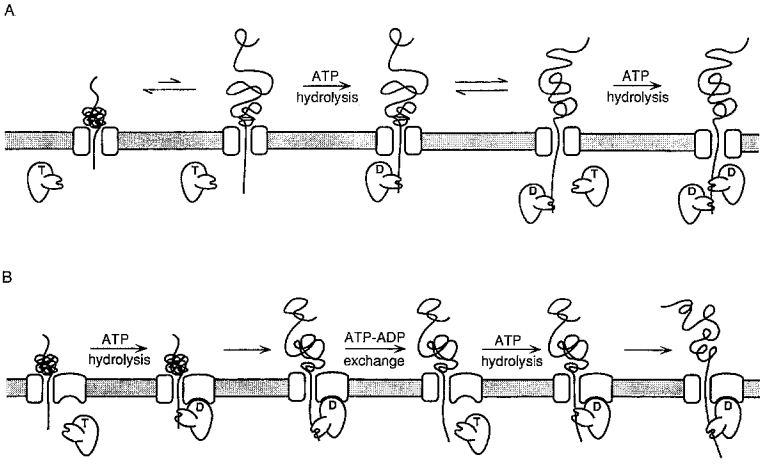

Figure 1. Possible Mechanisms by Which Lumenal Hsp70 Could Drive the Unfolding and Translocation of a Precursor Protein

The two-lobed structures are Hsp70 molecules; T and D represent the ATP and ADP forms of Hsp70, respectively. The rectangular structures are components of a translocation channel spanning one or more membranes. In the Brownian ratchet model (A), the precursor undergoes a spontaneous, reversible unfolding. The precursor chain then oscillates in the translocation channel. Binding of a lumenal Hsp70 molecule after an inward oscillation traps the precursor in an unfolded state. A further inward oscillation of the precursor chain results in the binding of another Hsp70 molecule, and this process continues until the entire precursor has been imported. ATP hydrolysis is needed for the association of lumenal Hsp70 with the precursor chain; replacement of the bound ADP with ATP recycles the Hsp70 molecule for another round of association (not illustrated). In the translocation motor model (B), lumenal Hsp70 binds to the precursor chain and also to a partner protein in the organellar membrane. This dual binding reaction requires ATP hydrolysis. The Hsp70 molecule then undergoes a conformational change, which exerts force on the precursor chain and causes unfolding and inward translocation of the precursor. (This conformational change in Hsp70 is represented as a relative movement of the N-terminal and C-terminal domains, but other types of motion are also possible.) Finally, replacement of the bound ADP with ATP releases lumenal Hsp70 from the precursor chain and from the membrane partner protein. The cycle begins again when ATP is hydrolyzed by the same or a different Hsp70 molecule.

Kar2p can be cross-linked to incoming precursors (Scherer et al., 1990; Sanders et al., 1992), suggesting that these Hsp70 proteins participate directly in translocation.

The Brownian Ratchet Model

How might lumenal forms of Hsp70 drive protein translocation? One possible mechanism has been termed a "Brownian ratchet" (Simon et al., 1992) (Figure 1A). This model is based on the prediction that a polypeptide chain in a translocation channel will oscillate within the channel because of Brownian motion. There is experimental evidence for such bidirectional movement of translocating polypeptides (Ungermann et al., 1994). Once a precursor chain has undergone a spontaneous inward movement, a lumenal Hsp70 molecule could bind to the chain. If this association is sufficiently stable, retrograde movement of the precursor chain will be prevented, and the chain will eventually penetrate further into the organellar lumen and bind another Hsp70 molecule. A series of such events would lead to translocation of the entire precursor across the organellar membrane (Neupert et al., 1990; Simon et al., 1992). The Brownian ratchet hypothesis fits with the notion that Hsp70 proteins act simply by binding to their polypeptide substrates.

Results published within the past year have forced a rethinking of the Brownian ratchet concept. When this model was originally formulated, there was an underlying assumption that precursor proteins remain unfolded before translocation. Folded domains cannot pass through most protein translocation channels (Hannavy et al., 1993; Jungnickel et al., 1994), so it seemed plausible that one function of cytosolic chaperones might be to inhibit the folding of precursor proteins (Deshaies et al., 1988). However, several artificial mitochondrial precursors fold even when they are synthesized in the presence of cytosolic chaperones (Wachter et al., 1994). This phenomenon has now been documented in detail for cytochrome b_2, an authentic mitochondrial protein. The cytochrome b_2 precursor contains a folded heme-binding domain that must be unfolded during translocation (Glick et al., 1993; Voos et al., 1993; Stuart et al., 1994). An extension of the Brownian ratchet model has been suggested to explain the import of proteins such as cytochrome b_2 (Stuart et al., 1994). In this view, when a folded domain reaches the entrance of a translocation channel, the precursor chain will still be able to oscillate in the channel owing to random thermal fluctuations ("breathing") of the folded polypeptide. A sufficiently large inward oscillation would allow a lumenal Hsp70 molecule to bind to the chain (Figure 1A).

The peptide-binding cleft of Hsp70 holds seven or more amino acid residues (Hightower et al., 1994); thus, at least seven residues would have to be translocated during this initial step in the import of a folded domain. Because protein folding is a highly cooperative process, unraveling even a small number of residues from the end of a folded structure would most likely involve loss of its native conformation (Jaenicke, 1987). Therefore, according to the Brownian ratchet hypothesis, the translocation of a folded domain is preceded by spontaneous unfolding of the entire domain. Subsequent import would be driven by biased Brownian motion, as originally proposed (Figure 1A).

This model predicts that the translocation of a folded precursor should be no faster than its spontaneous unfolding. However, the spontaneous unfolding of many proteins is extremely slow, with half-times on the order of hours or days (Creighton, 1993). The cytochrome b_2 heme-binding domain is an example of such a stably folded structure, yet cytochrome b_2 is imported into mitochondria within minutes (Glick et al., 1993). It is conceivable that mere contact with the translocation channel causes precursors to unfold, but this possibility seems unlikely because in the absence of ATP, the import of cytochrome b_2 is arrested with the heme-binding domain still folded at the mitochondrial surface (Glick et al., 1993). Import of the heme-binding domain requires ATP in the mitochondrial matrix and the action of mHsp70 (Glick et al., 1993; Voos et al., 1993; Stuart et al., 1994). Thus, lumenal Hsp70 can evidently accelerate an unfolding reaction that takes place at the surface of the organelle.

Organellar Hsp70 Proteins as Translocation Motors

The most straightforward explanation for this active unfolding process is that lumenal Hsp70 exerts a pulling force on the precursor chain. To pull, lumenal Hsp70 would have to be attached both to the precursor and to a component of the organellar membrane. Such an arrangement would allow force to be generated by a conformational change in the Hsp70 protein: a high energy conformation of Hsp70 would first bind to the precursor chain and then relax to a lower energy conformation, causing inward motion of the chain and concomitant unfolding of the precursor (Figure 1B). According to this hypothesis, lumenal Hsp70 is a translocation motor, similar to conventional force-generating proteins such as myosin and kinesin. An Hsp70-based translocation motor would have a direct parallel in bacterial protein secretion, in which ATP-dependent conformational changes in the SecA protein appear to push precursors through the translocation channel (Economou and Wickner, 1994).

The Brownian ratchet and translocation motor models differ in their predictions about the effects of lumenal Hsp70 on precursor unfolding rates. With a Brownian ratchet, Hsp70 binding does not accelerate unfolding, but rather captures the precursor after it has spontaneously unfolded. With a translocation motor, precursor unfolding is coupled to an energetically favorable conformational change in Hsp70; hence, the unfolding reaction is accelerated because its effective activation energy is reduced. In other words, a translocation motor will also be an unfolding enzyme, or "unfoldase" (Rothman and Kornberg, 1986). However, even this mechanism has limitations, since a protein might have an activation barrier for unfolding that is too high to be overcome using the energy gained from a conformational change in Hsp70. Certain artificial precursor proteins contain folded domains that cannot be imported, even though the N-terminal portions of these precursors are bound by lumenal Hsp70 (Scherer et al., 1990;

Sanders et al., 1992). In vivo, some precursors avoid premature folding by following a cotranslational import pathway (Jungnickel et al., 1994). Other precursors were presumably selected during evolution to have unfolding activation energies that can be accomodated by the translocation machinery.

Protein Translocation Is a Multistep Process

A translocation motor can only operate after a precursor chain has penetrated far enough into the organelle to encounter lumenal Hsp70. Therefore, a mechanism must exist to allow the initial insertion of precursors into the translocation channel. Insertion may be driven by the association of a precursor with components of the translocation machinery (Sanders et al., 1992) or, in the case of mitochondria, by an electrochemical potential across the inner membrane (Hannavy et al., 1993). Some precursors will need to unfold before they can insert into the channel. With these precursors, spontaneous unfolding might indeed be an obligatory early step in translocation. For example, a fusion protein consisting of a mitochondrial presequence linked to mouse dihydrofolate reductase (DHFR) can unfold and undergo partial import into mitochondria even in the absence of ATP (Hwang et al., 1991). Because the reversible unfolding of DHFR is unusually rapid, with a half-time of 2–3 min at room temperature (Viitanen et al., 1991), it is likely that the DHFR fusion protein unfolds spontaneously and then inserts into the translocation channel. However, the N-terminal presequence of this protein does not completely cross the mitochondrial inner membrane if the matrix is depleted of ATP; instead, the precursor chain becomes "stuck" in the channel and must be actively dislodged by mHsp70 (Hwang et al., 1991). This observation is probably of general significance, since every precursor chain will interact nonspecifically with the walls of a translocation channel. Some of these interactions are apparently strong enough to block the spontaneous passage of the precursor through the channel. Thus, even in the absence of stably folded domains in a precursor protein, a force-generating motor may be required for translocation to progress beyond the insertion stage.

The import of an entire precursor almost certainly involves multiple rounds of Hsp70 action. The simplest possibility is that one molecule of ATP is consumed during each round. Based on the available information, it is possible to envision a mechanism by which ATP might be used to drive a translocation motor (Figure 1B). According to this model, ATP hydrolysis allows lumenal Hsp70 to bind strongly both to the precursor chain (Sanders et al., 1992; Stuart et al., 1994) and to a membrane partner protein (see below). Hsp70 would then undergo a conformational change, thereby pulling a segment of the precursor chain through the translocation channel. Finally, replacement of the bound ADP with ATP would release Hsp70 from the precursor and from the membrane partner protein, and the cycle could begin again. Translocation would be unidirectional because lumenal Hsp70 is anchored to the membrane in a defined orientation and because interactions of the precursor chain with the translocation channel would inhibit "backsliding" of the precursor between rounds of Hsp70 binding. This mechanism for protein import is reminiscent of the ATP-driven translocation of actin filaments by myosin (Rayment et al., 1993). Thus, even though the N-terminal domain of Hsp70 is structurally related to actin (Holmes et al., 1993), Hsp70 proteins may be functionally analogous to myosin.

Experimental Tests of the Translocation Motor Hypothesis

To be an effective translocation motor, a lumenal Hsp70 protein would have to perform two operations in addition to binding incoming precursors: it would have to anchor itself to the membrane (for example, by associating with a component of the translocation machinery), and it would have to undergo conformational changes during the ATP hydrolysis cycle. There is experimental evidence for both of these activities.

The best-documented case of an interaction between an organellar Hsp70 protein and a translocation machinery is in the yeast endoplasmic reticulum, where a fraction of the Kar2p molecules are associated with the transmembrane protein Sec63p (Brodsky and Schekman, 1994). The lumenal portion of Sec63p contains a so-called J domain, a conserved sequence found in the Escherichia coli DnaJ protein and in a number of other proteins that form functional complexes with Hsp70 chaperones. Genetic and biochemical data indicate that the specific interaction of Kar2p with Sec63p is critical for translocation (Brodsky and Schekman, 1994). A similar association of lumenal Hsp70 with the translocation machinery occurs in mitochondria, where mHsp70 forms a complex with an inner membrane protein called ISP45 or MIM44 (Kronidou et al., 1994; Schneider et al., 1994; Rassow et al., 1994). Genetic experiments indicate that the mHsp70–ISP45 interaction is essential for translocation (Rassow et al., 1994). In support of the model shown in Figure 1B, the Kar2p–Sec63p and mHsp70–ISP45 complexes are stable in the presence of ADP, but are disrupted by the addition of a nonhydrolyzable ATP analog (Brodsky and Schekman, 1994; Kronidou et al., 1994).

There is evidence that Hsp70 proteins undergo nucleotide-dependent conformational changes (Hightower et al., 1994). Peptide binding to the C-terminal domain of Hsp70 is allosterically regulated by nucleotide binding to the N-terminal domain (McKay et al., 1994). It is possible that the two domains can move relative to one another. Moreover, the N-terminal domain itself may undergo significant rearrangements during the Hsp70 reaction cycle (Holmes et al., 1993). Any of these movements could result in force generation. Structural studies are needed to elucidate the nature and magnitude of the conformational changes in Hsp70 proteins.

Additional tests can be employed to determine whether lumenal Hsp70 proteins truly function as translocation motors. It will be useful to compare quantitatively the import rates of folded precursors with their spontaneous unfolding rates in solution. Eventually, it may be possible to apply techniques from the motor protein field (Walker and

Sheetz, 1993): Hsp70 molecules could be attached to a solid surface and tested for their ability to translocate polypeptide chains in vitro.

Hsp70 Proteins as Generic Force Generators

As mentioned above, some of the processes that are mediated by Hsp70 chaperones probably require only binding of the chaperones to their polypeptide substrates. However, there are reactions in addition to protein import that may be driven by nucleotide-dependent conformational changes in Hsp70. One example is the movement of nascent polypeptide chains through the ribosomal tunnel (Nelson et al., 1992). Hsp70 proteins could associate with a component of the ribosome and then pull on a nascent chain to help overcome its interactions with the walls of the tunnel. This process would be analogous to the action of organellar Hsp70 chaperones during protein translocation. More generally, Hsp70 proteins often act as "molecular crowbars" to promote the dissociation of protein oligomers (Georgopoulos and Welch, 1993). A conformational change in Hsp70 might serve to pry apart the subunits of an oligomeric complex. This idea is similar to the original proposal by Pelham (1986) that conformational changes in Hsp70 proteins can be transmitted to their polypeptide substrates. It now seems appropriate to suggest once again that Hsp70 chaperones are force-generating proteins and that this capacity has been harnessed by the cell to catalyze a variety of important reactions.

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