Double J-domain piloting of an Hsp70 substrate

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Heat shock 70 kDa protein (Hsp70) chaperones play a crucial role in the biogenesis of tail-anchored proteins (TAs), starting a downstream cascade to the endoplasmic reticulum (ER) *via* the guided-entry-of-tail-anchored protein (GET) pathway. J-domain proteins (JDPs) are generally known to assist Hsp70s, but their specific role in TA targeting remains unclear. Cho *et al.* now identify two separate functions for JDPs in the process, in the initial capture of the TA and the transfer into the GET pathway. These data suggest that several Hsp70 cycles could be involved at distinct steps during protein maturation.

Molecular chaperones assist protein folding in the cell, with heat shock 70 kDa protein (Hsp70) and Hsp90 being the most abundant and conserved chaperone families (1). In general, chaperones bind to folding intermediates to prepare them for further maturation, such as folding into the native state or sorting into the right pathway. Their activity is controlled by an ATP cycle, which is regulated by cochaperones. In the case of the Hsp70 family, this cycle is assisted by two types of cochaperones: JDPs that promote substrate entry into the Hsp70 binding cleft by stimulating ATP hydrolysis and nucleotide exchange factors that promote ADP release and subsequent ATP rebinding, allowing substrate leaving the cleft (1). When the substrate is released from Hsp70, cochaperones can channel it into one of several downstream pathways, such as folding, proteasomal degradation, or targeting to the membrane (Fig. 1). One class of substrates, the TAs—integral membrane proteins containing a C-terminal hydrophobic transmembrane domain (TMD) —are known to be channeled into the GET secretory pathway (2, 3).

TA maturation requires several steps for the proteins to reach their target membrane, the ER *via* the GET pathway (2, 3). TAs initially interact with Hsp70 upon their release from the ribosome and are then handed off to the cochaperone Small glutamine-rich tetratricopeptide repeat-containing protein 2 (Sgt2) followed by Guided entry of tail-anchored proteins factor 3 (2, 3). After subsequent transfer to ER membrane receptors Get1/2, TAs are inserted into the ER membrane. While passaging through the cytosol, Hsp70 keeps the TMD soluble, preventing misfolding and aggregation, and also, it targets the TAs to the appropriate membrane. The initial targeting of Hsp70 to a particular substrate is assisted by JDPs, using variable substrate-binding domains of their own (4, 5).

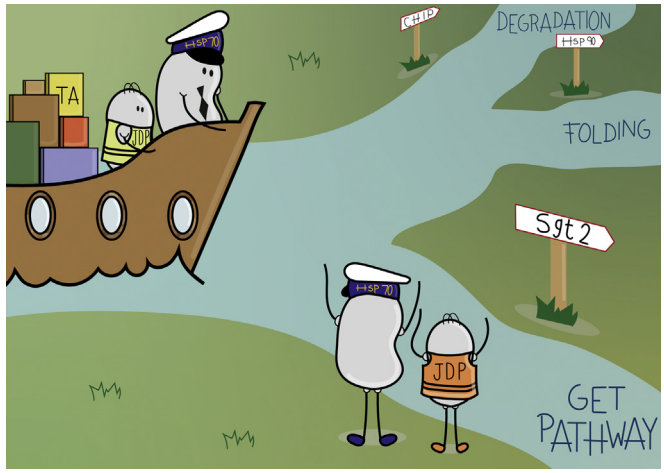

Figure 1. Double JDP targeting of TA proteins into the GET pathway. Hsp70 and a first JDP capture the TA substrate to start the voyage, and the fate of the TA cargo is decided at the river junction. A JDP-triggered second Hsp70 ATPase cycle, possibly including a second Hsp70 molecule, initiates entering the GET stream *via* small glutamine-rich tetratricopeptide repeat-containing protein 2. GET, guided-entry-of-tail-anchored protein; JDP, J-domain protein; TA, tail-anchored protein.

However, whether JDPs perform this role in TA targeting, how the multiple cellular JDPs are partitioned into this *versus* other processes, and whether the JDPs assist with other functions remain unclear. Publishing in JBC, Cho *et al.* (6) propose a model for the Hsp70/JDP-mediated transport of TAs into the GET pathway in which Ydj1 and Sis1, the two most abundant yeast JDPs, contribute to regulating the conformation of Hsp70-bound TAs during membrane protein biogenesis.

Cho *et al.* started their investigation by asking whether Ydj1 and Sis1 enhance the ability of Ssa1 to capture TAs and keep them in the soluble form. They used a hybrid protein containing Bos1, a *Saccharomyces cerevisiae* membrane protein strongly dependent on the GET pathway, as their TA model. Cho *et al.* specifically mutated Ydj1 in its J-domain to abolish Hsp70 ATPase activation and created a Ydj1 fragment with a truncated substrate-binding domain (JD-GF). Subsequently, they monitored the ability of Ssa1 to maintain Bos1 solubility using turbidity and sedimentation assays. These experiments revealed that ATPase activation mediated by a functional J-domain is required for TA binding by Ssa1. Assays with Sis1 showed it does not prevent Bos1 aggregation, meaning that this cochaperone is not able to target Bos1.

Would its inability to capture Bos1 now prevent Sis1 from playing a role in targeting this TA protein to the membrane?

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The authors next tested both Ydj1 and Sis1 for their ability to stimulate TA transfer from Ssa1 to Sgt2. Monitoring Bos1 transfer from Ssa1 to Sgt2 *via* a photocrosslinker integrated into the Bos1 TMD revealed, unexpectedly, that both Ydj1 and Sis1 enhance substrate transfer. Consistently, also genetic experiments revealed that both JDPs can substitute each other to promote TA membrane insertion. Thus, despite the inability of Sis1 to synergize with Ssa1 during TA capture, it can enhance Ssa1-to-Sgt2 TA transfer. To identify the functional elements in this step, the authors mutated both JDPs in the ATP-stimulating motif in the J-domain and partially deleted the substrate-binding domains. Monitoring TA transfer by cross-linking revealed that the functional J-domain is crucial, and differences between the Ydj1 and Sis1 substrate-binding domains did not have an impact. These data suggest that the ability of the JDPs to enhance TA transfer depends on stimulation of Hsp70 ATPase activity by functional J-domains.

To pilot TA proteins into the GET stream, Hsp70s must capture and then transfer substrates to Sgt2. The data from Cho *et al.* demonstrate that both of these steps are stimulated by JDPs. However, as Sis1 can stimulate only the second but not the first event, this means that there are two independent steps involving ATP hydrolysis by Hsp70 and thus at least two Hsp70 ATP cycles required before the Hsp70 reaches the GET pathway (Fig. 1). If the first ATP cycle is dedicated to capturing the aggregation-prone and highly hydrophobic TMD, the second cycle may serve a different purpose. The authors conclude that the second ATP hydrolysis induces Ssa1 to bind the substrate in a conformation more conducive to transfer. We suggest that these findings may even be taken one step further. Hsp70 ATP hydrolysis is coupled to substrate-binding events, and analysis of the N-terminal domain preceding the Bos1 TMD reveals six potential stretches that could serve as Hsp70-binding sites (7). Thus, a second Hsp70 may bind the substrate, either adding another flag recruiting Sgt2 or potentially further unfolding this domain to possibly increase affinity for Sgt2. Such an unfolding-related mechanism is consistent with earlier findings of bacterial Hsp70, DnaK, rebinding to potential multiple binding sites in luciferase (8) and in rhodanese (9).

This Hsp70 double act beautifully illustrates the necessary versatility of the JDP proteins. The Hsp70 captain knows how to power the boat, but the JDP pilots ensure the vessel navigates to the right target, and this may involve more than one JDP, as in metazoan disaggregation (10). The beauty of the GET entry process is that it shows that Hsp70 binding has different functions, even for the same substrate protein. It will now be exciting to investigate the differences in what happens at molecular level to the Hsp70 substrate in the two different cycles. The double JDP piloting explored by Cho *et al.* may offer an experimental route to this important question.

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Abbreviations—The abbreviations used are: ER, endoplasmic reticulum; GET, guided-entry-of-tail-anchored protein; Hsp70, heat shock 70 kDa protein; JDPs, J-domain proteins; Sgt2, small glutamine-rich tetratricopeptide repeat-containing protein 2; TAs, tail-anchored proteins; TMD, transmembrane domain.

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