CHAPERONES

Folding against the wind

Many thermodynamically unfavorable processes in biology are powered by ATP, the energy currency of the cell. New evidence suggests that chaperone-mediated protein stabilization may need to be added to that list.

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Energy from ATP is used to power a myriad of essential processes in the cell, including many transport processes, so that cells can fight uphill thermodynamic gradients. In this issue, Goloubinoff, de los Rios and co-workers[1] ask a provocative question: does this also apply to protein folding? It has long been known that major classes of molecular chaperones utilize ATP. However, most studies to date have investigated how chaperones use the energy of ATP binding and hydrolysis to regulate their own conformational changes, not how they may use it to energetically drag other proteins toward their native states. In their study, Goloubinoff et al. postulate that the ATP-dependent chaperonin GroELS can actually harness the energy derived from ATP hydrolysis to maintain substrate proteins in their native states even under conditions in which the native state is energetically disfavored[1].

Many proteins are marginally stable. This puts these proteins on the edge of a precipice that may rapidly lead to many bad outcomes, including misfolding and aggregation. Molecular chaperones, particularly those that utilize ATP during their chaperone cycle, are tasked not only with folding newly synthesized proteins, but also with rescuing proteins from misfolded conformations and allowing them to refold back into their biologically active native states. However, most models of chaperone action agree that chaperones do not alter the stability of their substrate proteins. They are instead thought to guide the folding of proteins, allowing them to avoid misfolded states on their way to the native state, whose free-energy minimum is determined not by the chaperone but by the intrinsic properties of the protein itself[2,3].

GroELS is arguably the most well-studied ATP-dependent chaperone system. Given that the ATP used during GroELS cycling releases such a large amount of energy, Goloubinoff et al.[1] decided to investigate whether GroELS could actually use this energy to pull proteins toward their native states, even under denaturing conditions. To accomplish this, they deliberately selected 37 °C, a temperature that favors the unfolding of a GroELS substrate protein, malate dehydrogenase (MDH). At this temperature, in the absence of GroELS and ATP, MDH loses enzymatic activity over time. However, if GroELS and ATP are included, MDH activity is maintained against the equilibrium that would normally lead to its unfolding. In fact, if GroELS and ATP are added while MDH is being heat treated, MDH activity actually increases, which indicates that nonequilibrium stabilization is occurring. The level of native-state stabilization can be tuned by changing the ratio of ATP/ADP in the system, showing that the amount of energy available from ATP hydrolysis is important. A mathematical model consistent with the data suggests that GroELS uses the energy from ATP hydrolysis to disfavor the non-native substrate, driving the substrate population into its native state. The authors speculate that under conditions wherein the native state is destabilized beyond the maximum energy available in ATP, GroELS might operate as a holding chaperone, acting mainly to prevent aggregation until conditions that are more energetically favorable to the native state return.

Fig. 1 | Proteins tend to unfold when facing adverse conditions. The ring-shaped chaperone GroE is used to rescue these proteins using the power of ATP. Credit: Billy Chrisnada from B'signs

The generality of this finding remains to be demonstrated, and it is still unclear how much of the cellular proteome relies on nonequilibrium stabilization in vivo. Nevertheless, it is interesting to speculate about the implications of chaperone-dependent, nonequilibrium stabilization of proteins. First, this may allow proteins to retain function under conditions in which the native state is unstable, such as during heat or chemical stress. Second, proteins may not need to be intrinsically stable under normal growth conditions in all cases, because the energy provided by ATP can potentially be used to enhance protein stability. Instability might be a prerequisite for optimal function, as proteins often need some degree of flexibility to function properly[4]. Third, these findings also raise the possibility that some proteins may have co-evolved with chaperones, to the point that they may even be considered chaperone addicted. Indeed, the porcine mitochondrial

MDH used in this study is unstable in vitro at 37 °C, a temperature below a pig's normal body temperature, implying that MDH may need chaperone assistance to be stable in its normal environment. It remains to be determined whether proteins, like MDH, that appear to depend on nonequilibrium stabilization lose activity in vivo under ATP-depleted conditions, such as those that occur during oxidative stress[5]. Finally, the findings of Goloubinoff et al.[1] may also help explain the tremendous abundance of ATP-dependent chaperones seen under normal growth conditions, where they may be needed to stabilize proteins by injecting energy from ATP.

The simple observation that chaperones both utilize ATP and facilitate folding led Goloubinoff et al.[1] to reinvestigate whether there is a direct thermodynamic link between ATP hydrolysis and protein stability; their initial results imply that there is. Schrödinger and many others have taught us that life is not at equilibrium[6]. Protein folding in vivo may now need to be added to those many processes that, at least sometimes, need to swim against the flow (Fig. 1). ❐

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