A new direction for titin pulling

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Many proteins are known to serve roles as structural scaffolds in cells and tissues. These proteins maintain the shape of the cell, act as local force sensors, and are essential to form tissues that are exposed to large external forces. Although seemingly passive elements, these structural proteins have unique physical features that are worthy of a closer look. In particular, it is critically important to understand how scaffolding proteins and protein complexes respond to the high external forces commonly encountered in biology. In this issue of PNAS, Bertz et al. (1) report a remarkable new feature of these load-bearing complexes. Apparently, evolution has molded these proteins to be remarkably resistant to unfolding when pulled in the same direction as they would experience in the cell. However, in other pulling directions the complex is relatively fragile.

Bertz et al. (1) found these “directional bonds” in a complex of titin and its binding partner, telethonin (1). Titin is an enormous protein found in muscle sarcomeres, where it contributes to the organization and passive elasticity of muscle (2, 3). Two titin molecules are anchored together at the sarcomere Z-disk through an antiparallel arrangement of their N-terminal ends. Telethonin is found at this N-terminal overlap region, where it binds to two Ig domains, Z1 and Z2, from each titin molecule (4) (see Fig. 1). This whole N-terminal complex must be particularly robust to withstand the large forces found in muscle.

Bertz et al. (1) focused in on this 2:1 titin Z1Z2:telethonin complex, using an atomic force microscope to pull on and mechanically unfold the complex. Using handles attached to the C-terminal ends of the two Z2 domains, they pulled on the complex in a direction that would mimic the stretching direction within a sarcomere. What they found was surprising: not only was this the most robust protein or protein complex ever measured, but it also could withstand nearly half the force required to break a covalent bond. Clearly, there are some unusual features of this complex that lead to its remarkable stability.

Even more surprising, Bertz et al. (1) found that this stability depended

Fig. 1. A highly simplified cartoon of the 2:1 titin-telethonin complex illustrating the directional bond. Two titin fragments (green and blue) consisting of the Z1Z2 domains are shown with a bridging telethonin molecule (red). When pulled from the C-terminal ends of the Z2 domains, as experienced in the cell, the complex can support high forces. However, when pulled from the N-terminal ends of the Z1 domains, the complex dissociates at significantly lower forces. Note that these ends of the Z1 domains do not directly interact with telethonin.

on the pulling direction, rather than being a global property of the complex. When they switched the handles to the N-terminal ends of the Z1 domains (a pulling direction that would never be experienced in muscle), they found that the complex disassembled at a 1/3 the force, and that telethonin contributed little to the stability of the complex.

Proteins of all shapes and sizes tend to have a very narrow range of thermodynamic stabilities, with a ΔG_{fold} of -5 to -15 kcal/mol (5). How then is it possible to produce proteins or complexes that are robust to large pulling forces? These directional bonds may be the answer. Apparently, Nature can buttress the vulnerable areas along the pulling direction, even while working within the usual constraints of overall protein stability. However, there is no guarantee that these efforts will help against all possible pulling directions.

What is the structural origin of this directionality? In principle, one way would be to construct proteins that mate in a pawl and ratchet assembly. This does not seem to be the case for telethonin, which is an elongated protein that sits in the groove between the two sets of titin Ig domains. However, the crystal structure of the titin-telethonin complex provides some other tantalizing clues.

As Bertz et al. (1) point out, telethonin forms a β -sheet with the β -strand in Z2 that receives the pulling force in the cell. Thus, telethonin is directly at the site of action, where weak

points in the structure need support. Telethonin also forms an extended β -sheet with the Z1 domain, so at first glance it would seem that telethonin would also help with pulling from the N-terminal end of Z1 (the nonnative direction). However, there are no direct interactions between telethonin and the N terminus of Z1, where the force is applied. In this case, the N terminus of Z1 is still dangerously exposed.

Why are the locations of these supporting interactions so critically important? One useful framework for understanding these directional bonds comes from the protein-folding field. Mutational ϕ/ψ analysis can be used to determine whether a particular interaction is formed before or after reaching the transition state of the folding or unfolding pathway (6, 7). Along similar lines of thought here, the interaction of telethonin with Z2 must likely be broken (with a large energetic penalty) before reaching the unfolding transition state. This is the case because the force is applied near the site of telethonin-Z2 interaction. Conversely, when Z1 is being pulled, the telethonin-Z1 interaction is still intact in the transition state because the pulling site is far from the interface. Thus, the presence of telethonin hardly affects the unfolding process of Z1 when pulled from the N terminus. A simple demonstration of this effect can be illustrated by untying your shoes while holding on to one of the two loops. If you pull on the free end connected to the held loop, you will need to pull harder to break your hold on the loop. However, pulling weakly on the other free end will also untie the knot, without needing to break your hold on the loop.

With these key structural features identified, can we now engineer these stabilizing interactions where none existed? In principle, we now have sufficient information for a reasonable attempt. One approach would be to engineer in biHis mutations as metal-binding sites, as used in ψ analysis (7).

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BiHis mutations that stabilize the first β -strand of Z2 (and perhaps further downstream) should dramatically in-

crease the pulling force needed to break the titin dimer, even in the absence of telethonin. Such an approach

could be applied to stabilize other designed proteins that are vulnerable to applied loads.

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