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Cracking under stress: How actin might turn failure into action

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Actin is one of the most abundant, most important, and also most investigated proteins in our body, comprising several percent of the protein mass in both muscle and nonmuscle cells (1,2). Monomeric or globular actin (G-actin) is a 42 kDa protein that can quickly polymerize into its filamentous form (F-actin), with a thickness around 7 nm and a persistence length around 10 μm. Because this is the typical size of an animal cell, F-actin is very well suited to build up cell-scale structures that are both dynamic and mechanically stable at the same time. This includes crosslinked filament bundles in filopodia, branched networks of the lamellipodium, and contractile networks and bundles that form the actomyosin cortex and stress fibers, respectively (3). These structures are at the heart of many essential cellular processes, including adhesion, migration, division, and mechanosensing. Historically, its name goes back to its "action" in muscle contraction (4). Due to its versatile and adaptive nature, actin is also often used to reconstitute cellular function in the test tube (5).

One of the most prominent cellular functions of F-actin is mechanosensitivity, which is usually attributed to actin-binding proteins (ABPs) like zyxin, a protein from the LIM domain family that is known to be recruited by actin under mechanical stress (6–8). However, until now, no binding interface has been identified on the surface of F-actin that would recruit ABPs like zyxin under conditions of mechanical stress. In this issue of *Biophysical Journal*, Vilmos Zsolnay and colleagues report that by using all-atom molecular dynamics computer simulations, they have found an explanation for this riddle: while stress does not change the F-actin surface much, it leads to microcracks, opening up new binding interfaces at the longitudinal contacts between monomers that are adjacent along the filament length (9). Using docking simulations, they demonstrate that 43 members of the LIM domain family can bind to this newly discovered binding interface. The computer simulations in fact suggest that the tandem structures of the LIM domain proteins have evolved to simultaneously bind several different binding epitopes within the cracked interface. Their discovery finally suggests a likely mechanism for the mechanosensitive nature of F-actin. Although direct experimental evidence is lacking, this suggestion fits well with what we know about actin and ABPs.

Stacking of the G-actin monomers in F-actin is chiral and leads to a helical arrangement, which can be interpreted as two protofilaments winding around each other such that the double helix repeats itself every 14 monomers (corresponding to 72 nm) (1,8). In the work discussed here, the authors simulated a short actin filament composed of 13 monomers, corresponding to 9 longitudinal (intra-strand) contacts (compare Fig. 1 *A*). The unstrained distance between two neighboring monomers in one strand was measured to be around 5.53 nm and to not increase much under pulling forces up to 400 pN. However, the authors observed that in this regime of low tension, the so-called D-loop (a flexible part of actin involved in monomer-monomer interactions) in one monomer sometimes flipped away from residue Y169 in a neighboring monomer, making the interface less stable. At tensions between 400 and 600 pN, this flipping was strongly increased, and metastable cracks appeared (compare Fig. 1 *B*), now with monomer-monomer distances around 6.94 nm and with 11 times as much accessible surface area as the uncracked interfaces. Importantly, cracking now allowed for LIM domain proteins to bind, while F-actin as a whole still remained stable due to the presence of the lateral (inter-strand) contacts.

The picture that emerges here is very appealing because it immediately suggests a rationale for the architecture of F-actin. If one untwists F-actin, it appears as two parallel strands of monomers that are staggered by 2.78 nm (half a monomer distance, which is also the effective length increase if the filament is growing by one monomer). Strikingly, this is exactly the architecture one would need

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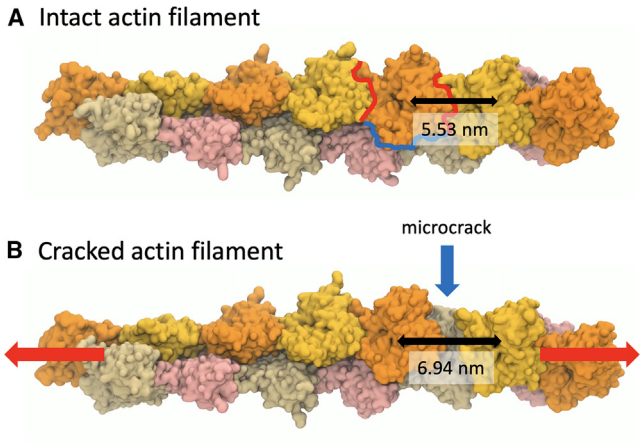

FIGURE 1 Two snapshots from all-atom molecular dynamics computer simulations of actin filaments cracking under force. (*A*) The intact filament consists of two strands that wind around each other and thus form a double helix. Each G-actin monomer is hold by longitudinal (*red*) and lateral (*blue*) contacts with neighboring monomers. The unstrained distance between two neighboring monomers is 5.53 nm. (*B*) Filaments stretched by force can form microcracks, in which one of the longitudinal contacts breaks up. The monomer-monomer distance across this contact increases to 6.94 nm and allows binding of LIM domain proteins. However, the filament as a whole does not lose stability because the cracked monomers are still held by the lateral contacts due to the double-helical architecture. Figure adapted from (9).

if the filament should remain stable under the removal of one monomer. However, before the finding of this paper, this viewpoint was not entirely convincing because actin monomer removal has not been observed. With the prediction of cracks at the longitudinal contacts, the actin architecture suddenly makes much more sense. The double-stranded and staggered architecture of F-actin might be as simple a design principle as the double helical nature of DNA, for which Watson and Crick once wrote that its design "immediately suggests a possible copying mechanism for the genetic material" (10). In a similar vein, we now might conclude that the design of actin immediately suggests a possible mechanism for its mechanosensitive function because, in a staggered double strand, cracks can open without destabilizing the whole filament.

This new view on actin fracture also nicely agrees with similar findings for other central molecules in mechanosensing, including the focal adhesion protein talin, which opens up new binding sites under force (11), and microtubules, which exchange tubulin under external forces (12). Thus, it seems to be a very general principle of mechanosensing to turn plastic changes under force into action by the generation of new binding sites. It is very appealing to assume that the same general mechanism is at work in F-actin. However, as we know from DNA, even if a simple design principle exists, how it plays out in practice might be very complicated, and thus direct experimental proof is needed to confirm the existence and details of the suggested microcracks in F-actin. Because they are predicted to be rare and transient, it will be very difficult to observe them with structural methods such as cryoelectron microscopy, which recently has led to a revolution in our understanding of ATP hydrolysis in F-actin (13,14). Therefore, it is more likely that fluorescence-based methods (including super-resolution microscopy) will provide direct proof for the suggested mechanism (15). Apart from these experimental challenges, it also remains a computational challenge to further investigate the details of the suggested fracture process, including the roles of loading rate, ATP hydrolysis, and coupling between stretch, twist, and bend deformations of F-actin. In view of these open questions and its long and winding history (4), it is clear that F-actin will not yet stop to surprise us and that there is lots of room for more discoveries to come.

DECLARATION OF INTERESTS

The author declares no competing interests.

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