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Cytoskeletal repair: Zyxin relieves actin stress from the inside out

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During the dynamic reorganization of the actin cytoskeleton, filaments occasionally break. To maintain the mechanical integrity of the actin structure, the cell must swiftly repair strained filaments *in situ*. New work recapitulates this process using purified proteins to reveal the underlying molecular mechanisms that enable this robust and rapid recovery.

Form and function — at the scale of individual molecules right through to tissues — are inextricably linked[1]. At the cellular scale, cell shape plays a role in everything from polarity determination to mechanics and even gene expression. Regulating cell shape, therefore, is of tantamount importance. In cells, this typically means building and maintaining actin-based structures. While simple in concept, in practice this is quite difficult. The actin cytoskeleton is a highly dynamic structure, constantly changing in response to a cadre of mechanical forces and biochemical signals. Even under the best of circumstances, it occasionally fails, requiring a localized rapid repair response to maintain the mechanical integrity of the structure. While we know some of the key proteins involved in this actin repair process, dissecting the molecular roles of the many components has been challenging due to the complex cellular environment and combination of mechanical and biochemical inputs. Excitingly, as reported in this issue of *Current Biology*, Phua and colleagues[2] have managed to successfully recapitulate this multiprotein mechanosensitive process entirely *in vitro*, shining new light on the molecular interactions that drive actin stress fiber repair.

Cells are continually buffeted by mechanical forces. Externally, these forces can come from interactions with other cells, or from the environment itself. Internally, forces are generated by the activity of proteins like myosin motors. In all cases, these forces largely propagate through the actin cytoskeleton. As a semi-flexible polymer, actin buckles relatively easily under compressive forces but can generally withstand extensile tension[3]. When the pulling force gets too high, however, filaments will ultimately break. In stress fibers, which are crosslinked bundles of multiple actin filaments, these breaks are similar to fraying strands in a braided rope. As an individual strand breaks, the remaining strands take up the load, putting them under increased strain. When all the strands break, the rope will completely fail.

To avoid these sudden catastrophic failures, and the associated shape changes they cause, cells have developed a repair program that can rebuild these fraying strands as they appear. Key to this process is the mechanosensitive LIM domain protein zyxin. Initial studies identified zyxin as one of the many proteins localized to adhesions[4,5]. Notably, however, when cells are exposed to mechanical stress, zyxin relocates from adhesions to stress fibers to reinforce the cytoskeleton[6]: loss of zyxin completely abrogates this mechanoresponsive reinforcement[7] and makes the actin cytoskeleton less elastic[8]. Similarly, when a stress fiber is damaged or fails locally, zyxin immediately binds to the strained region and facilitates repair, helping the cell to maintain its structure[9,10].

Zyxin recognizes strained actin via its three carboxy-terminal LIM domains, whereas it facilitates repair by recruiting the actin polymerization factor Ena/VASP and the actin crosslinker α-actinin via its amino-terminal domains[11,12]. Although previous work established the broad strokes of this process[10,11], the molecular details of repair have remained unclear, including whether other proteins are also necessary. For instance, multiple other LIM domain proteins, including paxillin, Hic-5, FHL2, and testin, have also been shown to recognize strained actin[13–16].

Phua *et al.*[2] turned to reductionist *in vitro* assays to beautifully tease apart these molecular insights. Previous work, including a study from this lab, had demonstrated that purified zyxin could bind actin that was under myosin-mediated tension, but the repair process was not examined[14,15]. By additionally incorporating VASP, α-actinin, and profilin into their previous *in vitro* assay, Phua *et al.*[2] have now successfully reconstituted all the necessary and sufficient components to both initiate and complete stress fiber repair. In doing so, they reveal the molecular interactions that enable stress fiber repair.

These authors start by using a modified version of the traditional gliding filament assay. Instead of a single myosin motor, however, they use a mix of myosin Va and VI molecules, which pull towards opposite ends of actin filaments. By stochastically distributing these motors across the coverslip, actin filaments sporadically encounter both of these motor proteins simultaneously, resulting in the filament getting pulled in opposite directions and becoming strained, and sometimes breaking. By adding additional proteins in a stepwise manner, Phua *et al.*[2] unravel the molecular details, showing that VASP is first recruited to zyxin at strain sites. Somewhat surprisingly, however, instead of solely stimulating polymerization from newly exposed barbed ends, VASP's more prominent function appears to be the nucleation of additional filaments throughout the strain site.

α-actinin, in contrast, does not appear to be recruited directly to zyxin. Instead, zyxin shows a preference for interacting with α-actinin that is already bound to actin. Putting the two findings together, it seems that zyxin recruits VASP to make

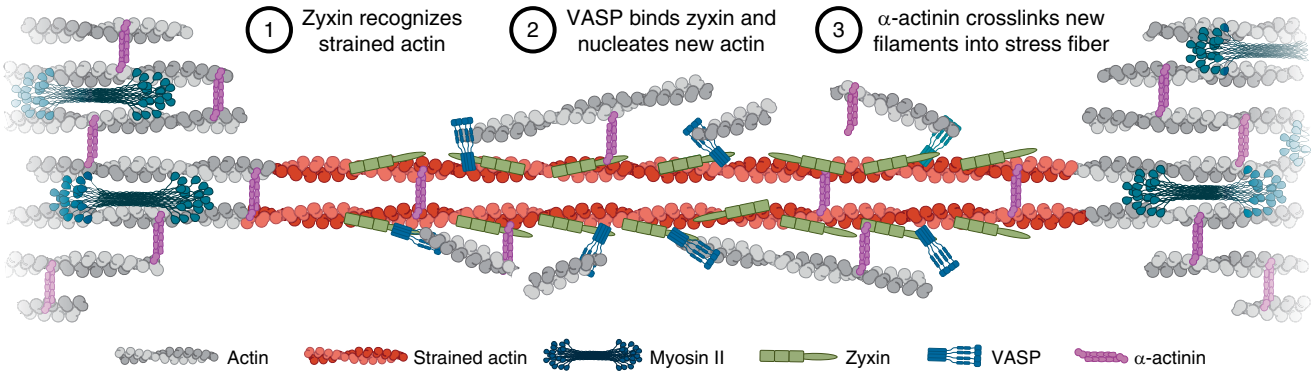

Figure 1. Zyxin-mediated stress fiber repair.
When stress fibers break or are damaged, the remaining actin becomes strained under the forces generated by myosin motors on the rest of the filament. Zyxin recognizes these strained actin filaments via its three LIM domains. Once bound to actin, zyxin recruits VASP to polymerize new filaments along the length of the strain site. Finally, α-actinin binds to these newly polymerized filaments, crosslinking them to the zyxin-coated strained filaments to rebuild the stress fiber.

new filaments, which α-actinin binds and then crosslinks into the structure, allowing repair to proceed simultaneously along the entire length of the strain site (Figure 1). This approach targets the weakest part of the stress fiber and likely allows for much faster and more robust repair.

While these experiments successfully recapitulate stress fiber repair and reveal key molecular interactions, they also raise intriguing new questions. Chief among them is the surprising observation in this study of zyxin 'bridges' that span two actin filaments. Similar one-sided structures called 'tails' are also seen anchoring a single filament to the substrate. The authors hypothesize that these actin-independent accumulations of zyxin represent a novel force-dependent zyxin–zyxin interaction. Such accumulations have yet to be observed in cells, however, and the formation of the 'tails' suggests that at least some non-specific interactions between zyxin and the substrate remain. Teasing apart whether this represents a new class of mechanosensitive biomolecular assembly or is the product of some non-specific binding remains an important and tantalizing question for future experiments.

Phua *et al.*[2] also observe phase separation in mixtures of zyxin with VASP and α-actinin. This builds on previous observations that VASP can phase separate to form concentrated protein droplets that facilitate actin polymerization[17]. The authors hypothesize that these multivalent interactions could potentially assist these proteins in accumulating at strain sites. The caveat to this observation, however, is that the concentrations required (~1 μM) are much higher than those thought to be present in cells. Thus, while this remains an intriguing potential mechanism, its role in cells is yet to be resolved.

How zyxin recognizes strained actin is an open and critical question. Multiple studies have demonstrated that the three LIM domains of zyxin are sufficient to localize to these sites[11,14,15]. A recent study using molecular dynamics simulations suggested that cracks between actin monomers can form in filaments under load[18]. Using docking simulations, those authors showed that LIM domains from testin, a protein closely related to zyxin, preferentially recognize the crack sites and could potentially stabilize the filament. In addition, ultrastructural work from the group behind this current study has revealed that actin under load can take on superhelical structures, which could also facilitate LIM domain binding[19]. Determining the mechanism of this key initiating interaction in stress fibers will undoubtedly reveal additional insights into this process.

Finally, additional unanswered questions include how zyxin's molecular behavior in stress fiber repair relates to its other functions, such as its localization to focal adhesions and within unstrained stress fibers, and whether other LIM domain proteins that recognize these sites help to facilitate repair. It will also be interesting to see whether this same general repair mechanism is happening at smaller scales within other actin structures throughout the cell, including the cortex. Answering these and other questions will likely require a combination of cellular, computational and reductionist approaches. As Phua *et al.*[2] have elegantly shown here, there is still much to learn about this critical mechanotransduction pathway.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Vestibular system: A revolution in understanding a neglected sensory system

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How proprioceptive and vestibular signals are integrated to guide movement and stabilize posture has long been unclear. A new study now demonstrates at cell-level resolution how this is accomplished in the cerebellum of non-human primates.

The vestibular system is the most neglected of major sensory systems that tell us about our relationship to the outside world. The relative neglect may in part be because the vestibular organs — the balance organs of the inner ear, which provide information on the orientation and movements of the head in space — are silent in the sense of giving no localized conscious sensation. The vestibular system is nevertheless crucial for body sense and motor control and influences a perhaps surprisingly broad range of brain functions. For several decades, Kathleen Cullen and colleagues have labored valiantly to give the vestibular system the research limelight it deserves. Ten years ago, she asked a seemingly innocuous question: what are the typical stimulation patterns of the vestibular organs during our everyday lives? The answer[1,2] led to the realization that many laboratory studies that aimed to characterize vestibular function employed stimulus frequencies that fail to capture key aspects of typically encountered frequency domains. This has led to a new appreciation of the response characteristics of the type 1 and type 2 hair cells of the otolith organs and semicircular canals[3], key components of the vestibular system. Now, in a paper in a recent issue of *Current Biology*, the Cullen group[4] reports an important breakthrough for the field. They have discovered how sensory integration of proprioceptive and vestibular information about body motion is accomplished in the nodulus and uvula of the cerebellum, showing that these brain areas are part of a complex network providing multiple reciprocal connections among the vestibular nuclei and fastigial nucleus and other brain regions involved in balance and voluntary control and planning.

The new work of the Cullen group[4] shows how information about dynamic head orientation and body configuration is represented to allow appropriate coordination of vestibulospinal and vestibuloocular reflexes during passive and active movement. In a time of international concern about falling accidents in the elderly, and of burgeoning interest in spaceflight and adaptation to reduced gravity environments, such as the Moon and Mars, this new knowledge is of