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MINI REVIEW

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LIM domain proteins in cell mechanobiology

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Abstract

The actin cytoskeleton is important for maintaining mechanical homeostasis in adherent cells, largely through its regulation of adhesion and cortical tension. The LIM (Lin-11, Isl1, MEC-3) domain-containing proteins are involved in a myriad of cellular mechanosensitive pathways. Recent work has discovered that LIM domains bind to mechanically stressed actin filaments, suggesting a novel and widely conserved mechanism of mechanosensing. This review summarizes the current state of knowledge of LIM protein mechanosensitivity.

KEYWORDS

actin cytoskeleton, focal adhesions, LIM proteins, adherens junctions

1 | CELLS SENSE AND RESPOND TO MECHANICAL FORCES

Mechanical force plays an essential role in the control of cell shape and motion and serves as a key input in mechanotransduction pathways controlling cell survival, growth, and fate. Cells are subject to a myriad of external forces, including those from neighboring cells, fluid flow, or osmolarity. In addition to these, mechanoenzymes within the cell interior generate forces that are transmitted across cellular scales via the cytoskeleton. These internally generated forces enable cell shape change and are critical to cellular mechanosensing (e.g., environmental stiffness sensing; Trappmann & Chen, 2013). Cells sense and convert mechanical stimuli into chemical signals to initiate downstream signaling pathways (Wang, Tytell, & Ingber, 2009). Examples of force-sensitive chemistries of cytoplasmic proteins include force-dependent changes in binding affinity (e.g., integrins, actin binding proteins) or enzymatic activity (e.g., myosin II; Greenberg, Arpağ, Tüzel, & Ostap, 2016; Jégou & Romet-Lemonne, 2021). These molecular-scale transducers can then give rise to mechanical sensitivities of cytoskeletal arrays and/or regulate signaling and transcriptional pathways. While mechanotransduction pathways are well appreciated in cell physiology, we are just beginning to understand the diversity of force-sensing mechanisms within the cytoskeleton.

2 | MECHANOSENSING IN ADHERENT CELLS

Cells are mechanically coupled to their local environment through adhesions to the extracellular matrix (ECM; e.g., focal adhesions, FAs)

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and surrounding cells (e.g., adherens junctions, AJs; Figure 1a). The actin cytoskeleton connects adhesions and transmits forces across the cell. Force sensitivity of adherent cells underlies adhesion regulation, cellular force generation, and mechanical properties of cells and tissues (Bieling et al., 2016; Courtemanche, Lee, Pollard, & Greene, 2013; Fletcher & Mullins, 2010; Moore, Roca-Cusachs, & Sheetz, 2010; Ohashi, Fujiwara, & Mizuno, 2017; Wang, Butler, & Ingber, 1993; Yusko & Asbury, 2014; Zhong et al., 1998). The mechanical properties of a cell's environment are reflected by the actin cytoskeleton architecture. For example, F-actin networks in cells that are growing on rigid matrices, or within tissues that are being stretched, respond by self-organizing into thick bundles and larger FAs, which is thought to be important for generating and withstanding increased force (Smith et al., 2010; Yoshigi, Hoffman, Jensen, Yost, & Beckerle, 2005).

The actin cytoskeleton includes many different actin filament (F-actin)-based networks that vary in organization and composition. The architecture of FAs and AJs is comprised of stratified layers of distinct proteins that work together to transmit forces sensed by membrane-spanning adhesion receptors to actin filaments (Chen & Singer, 1982; Franz & Müller, 2005; Kanchanawong et al., 2010; Zaidel-Bar, Itzkovitz, Ma'ayan, Iyengar, & Geiger, 2007). Both FAs and AJs exhibit force-dependent changes to their composition and size, which is typically mediated by myosin-II activity within the actin cytoskeleton (Kuo, Han, Hsiao, Yates Iii, & Waterman, 2011) but can also be driven by external force (Riveline et al., 2001).

Stress fibers (SFs) are contractile bundles of 10–30 actin filaments of mixed polarity and alternating regions of the crosslinker α-actinin and nonmuscle myosin, reminiscent of the sarcomeric organization in striated myofibrils (Cramer, Siebert, & Mitchison, 1997; Hotulainen & Lappalainen, 2006; Tojkander, Gateva, & Lappalainen, 2012). While sarcomere architecture allows for recurring contraction and relaxation cycles, the less organized SF is built for continuous isometric

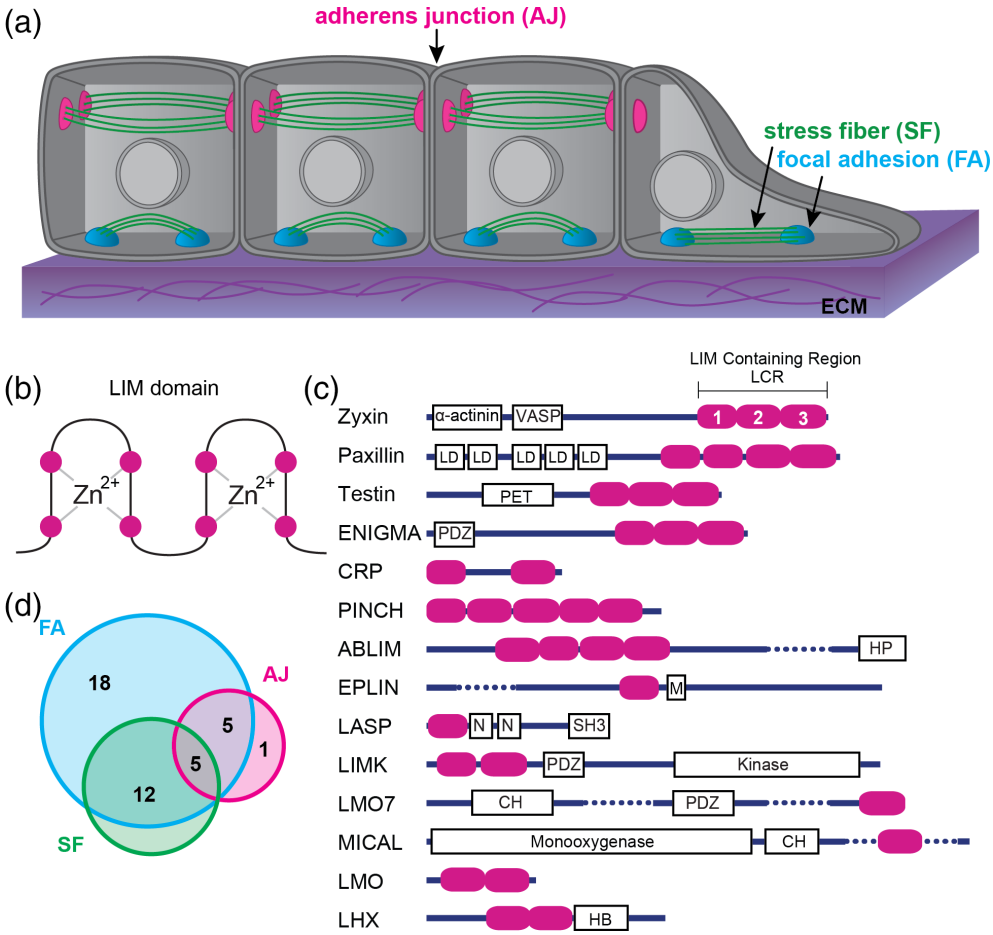

FIGURE 1 Mechanically stressed cells and LIM domain proteins. (a) Schematic of a layer of epithelial cells on top of an extracellular matrix (ECM). (b) Simple schematic of a LIM domain: Two zinc finger motifs. The magenta circles represent the well-conserved residues (typically cysteine or histidine) that chelate the zinc molecules. The remaining amino acid sequence varies between LIM domains. (c) Domain organization of the 14 classes of LIM domain proteins. Magenta ovals represent individual LIM domains. Dotted lines are used to abbreviate a few rather long structures. Other domain abbreviations: LD, Leucine rich aspartate domains; PET, prickle, espinas, testin; PDZ, membrane anchoring domain; HP, headpiece domain for F-actin binding; M, Myo5B interacting domain; N, nebulin; SH3, Src homology 3; CH, calponin homology; HB, homeobox. (d) Venn diagram showing the overlap of LIM domain proteins that associate with the three main networks: FA, Focal adhesions; AJ, adhesion junctions; SF, stress fibers

contraction (Burridge, 1981; Pellegrin & Mellor, 2007). SF formation, growth, orientation, and maintenance are sensitive to both externally and internally generated forces (Chrzanowska-Wodnicka & Burr idge, 1996). The constant tension makes SFs susceptible to damage, and localized damaged regions form spontaneously or in response to the application of external forces (Smith et al., 2010). Thus, repair of such SF strain sites (SFSS) is important for maintaining the mechanical homeostasis of the actin cytoskeleton, allowing cells to maintain their integrity and adapt to force fluctuations. It is likely that the rearrangements of actin cytoskeleton networks in response to external force may also be driven by a similar force-induced remodeling. For instance, repeated cycles of uniaxial stretch results in both SF thickening and reorientation perpendicular to the stretch axis (Hayakawa, Sato, & Obinata, 2001; Kaunas, Nguyen, Usami, & Chien, 2005; Kim-Kaneyama et al., 2005; Yoshigi et al., 2005).

Recent progress has elucidated the force-dependent biochemistry of actin binding proteins (e.g., cadherins, vinculin, talin, alpha-catenin; Buckley et al., 2014; Huang, Bax, Buckley, Weis, & Dunn, 2017; Huveneers & de Rooij, 2013; Mei et al., 2020; Vigouroux, Henriot, & Le Clainche, 2020). These studies have primarily considered how forces applied to actin binding proteins (ABPs) alter their binding affinity to F-actin. However, the actin filament itself can twist, stretch, and compress, which may also alter the binding affinity of ABPs (Galkin, Orlova, & Egelman, 2012). In this scenario, the actin filament itself is the force responsive element and could confer mechanical information about the cell and its environment to various signaling and transcriptional pathways (Discher, Mooney, & Zandstra, 2009; Engler, Sen, Sweeney, & Discher, 2006).

3 | LIM DOMAIN PROTEINS IN MECHANOTRANSDUCTION PATHWAYS

Proteomic screens of mechanotransduction pathways have revealed an abundance of proteins that contain one or more LIM (Lin-11, Isl1, MEC-3) domains (Freyd, Kim, & Horvitz, 1990; Karlsson, Thor, Norberg, Ohlsson, & Edlund, 1990; Way & Chalfie, 1988). The LIM domain is a ~ 60 amino acid sequence that forms a double zinc finger protein–protein or protein-DNA binding interface (Michelsen, Schmeichel, Beckerle, & Winge, 1993; Figure 1b). LIM domains occur in diverse multidomain protein organizations and are found in a wide range of eukaryotic proteins (LIM proteins), including ~70 human genes that can be divided into 14 classes (Figure 1c; Koch, Ryan, & Baxevanis, 2012). Early in the evolution of animal multicellularity, there was a large expansion in the number of LIM proteins as well as LIM "promiscuity", that is, LIM has combined within multidomain proteins with many other domains of different structure and function (Basu, Carmel, Rogozin, & Koonin, 2008; Koch et al., 2012). This domain promiscuity has resulted in a functionally diverse LIM protein family whose members play roles in a variety of biological processes but especially those implicated in generating and responding to mechanical forces (Figure 1c; Table 1; Kadrmas & Beckerle, 2004; Smith et al., 2014).

There are 41 LIM proteins found to be enriched at cell adhesions and/or the actomyosin cytoskeleton (Smith et al., 2014; Figure 1d). To date, 26 LIM proteins have been identified in FAs (including zyxin, paxillin, and LIMD1), and the localization of 21 of these is sensitive to myosin II activity (Kuo et al., 2011; Schiller, Friedel, Boulegue, & Fässler, 2011). Similarly, at least 11 LIM proteins display force-sensitive localization to AJs. Numerous LIM proteins co-localize to both FAs and SFs, FAs and AJs, or all three organelles (Figure 1d). Some LIM proteins contain known actin binding domains (e.g., the [CH] domain) that could drive their localization to F-actin networks. However, many that localize to the actin cytoskeleton lack these. Standard biochemical approaches have not detected binding of LIM domains to actin filaments. One notable exception is the CRP class, which canonically binds and bundles actin filaments via their LIM domains (Grubinger & Gimona, 2004; Hoffmann et al., 2014; Thomas et al., 2006). CRP is an ancient class as it is the only mammalian LIM protein class also found in plants, suggesting the possibility that canonical actin binding could be an ancestral function of the LIM domain. For instance, Muscle LIM protein (MLP) is a CRP class protein that has been implicated in mechanoresponse to muscle sarcomere stretching (Vafiadaki, Arvanitis, & Sanoudou, 2015).

Several studies have implicated LIM proteins in cell signaling and gene expression mechanotransduction pathways (Ibar et al., 2018; Martin et al., 2002). For instance, four-and-a-half LIM domains 2 (FHL2) is implicated in mechanical regulation of the cell cycle. On a soft matrix, FHL2 dissociates from F-actin networks and becomes more concentrated in the nucleus where it acts as a transcriptional cofactor to increase p21 gene expression, which regulates cell cycle progression and inhibits growth (Nakazawa et al., 2016). Most force-sensitive LIM proteins display nuclear shuttling raising questions as to whether detection of forces via LIM proteins is connected to localization and function inside the nucleus (Figure 2). Similarly, several LIM proteins in the Ajuba/Zyxin classes exhibit force-dependent binding to AJs to regulate hippo and Yap/Taz signaling pathways (Rauskolb, Pan, Reddy, Oh, & Irvine, 2011; Rauskolb, Sun, Sun, Pan, & Irvine, 2014).

4 | FORCE-SENSITIVE LOCALIZATION OF LIM PROTEINS IN ADHERENT CELLS

The LIM domain-containing region (LCR) has been found to drive the subcellular localization for a large number of LIM proteins (Brown et al., 1996; Hoffman et al., 2012; Smith et al., 2013). This has been dissected most carefully for the LIM protein zyxin, which localizes to SFs, FAs, and AJs in a force-dependent manner. Zyxin is necessary for stretch-mediated SF remodeling, SFSS repair, and FA maturation (Hoffman et al., 2012; Smith et al., 2013, 2014; Yoshigi et al., 2005).

The LCR of zyxin resides at the C-terminus and contains three LIM domains in tandem separated by short unstructured linkers. The LCR is required for zyxin recruitment to SFSS and FAs. For full length zyxin, any one of the individual LIM domains are not sufficient for its localization (Uemura et al., 2011). Recent results demonstrate that at

TABLE 1 A subset of LIM domain proteins and their corresponding mechanotransduction pathways

LIM protein	Localization	LCR-dependent	Binding partners	Mechanotransduction pathway	References
Zyxin	FA, AJ, SF	Yes	α-Actinin, VASP	Ena/VASP	(Drees et al., 2000; Hoffman, Jensen, Chaturvedi, Yoshigi, & Beckerle, 2012; Li & Trueb, 2001; Reinhard et al., 1999; Smith et al., 2013; Uemura, Nguyen, Steele, & Yamada, 2011)
Paxillin	FA, SF	Yes	Vinculin, FAK, Src	Rho GTPases, Microtubules	(Brown, Perrotta, & Turner, 1996; Deakin & Turner, 2008; Efimov et al., 2008; López-Colomé, Lee-Rivera, Benavides-Hidalgo, & López, 2017; Smith et al., 2013; Turner, Glenney Jr, & Burridge, 1990; Watanabe-Nakayama et al., 2013; Weng, Taylor, Turner, Brugge, & Seidel-Dugan, 1993)
LIMD1	AJ, FA	Yes	WTIP, LATS1	HIPPO	(Huggins & Andrulis, 2008; Ibar et al., 2018; G. Sun & Irvine, 2013)
Ajuba	AJ	Yes	α-Catenin, retinoic acid receptor	HIPPO, Rac	(Das Thakur et al., 2010; Hou et al., 2010; Ibar et al., 2018; Marie et al., 2003; Pratt et al., 2005; Razzell, Bustillo, & Zallen, 2018)
FHL2	FA	Yes	Integrin, actin, titin, β-catenin	Wnt, cell cycle, p21	(Johannessen, Møller, Hansen, Moens, & Van Ghelue, 2006; Nakazawa, Sathe, Shivashankar, & Sheetz, 2016)
Testin	FA		Calcium sensing receptor	Rho kinase	(Magno et al., 2011; Smith, Hoffman, & Beckerle, 2014)
Prickle	FA		CLASPs, LL5-β, disheveled, membrane	Microtubules and CLASPs, frizzled/Dischevelled, Wnt	(Han Cheng et al., 2016; Lim et al., 2016; Sweede et al., 2008; Veeman, Slusarski, Kaykas, Louie, & Moon, 2003)
Pdlim5	FA		α-Actinin, protein kinase C, protein kinase D, ID2	TGF-beta	(Cheng et al., 2010; Cheng et al., 2016; Kuo, 2013)
Pdlim7	FA			YAP	(Elbediwy et al., 2016; Kuo, 2013)
TRP6	AJ		Vinculin, LATS1/2		(Dutta et al., 2018)

least two tandem repeats of LIM1 or LIM3 are sufficient for LCR localization to SFSS (Winkelman, Anderson, Suarez, Kovar, & Gardel, 2020), but further work is needed to demonstrate this sufficiency for the full-length protein. Once localized, zyxin's N-terminal functionality mediates SFSS repair by recruiting factors that promote actin filament polymerization (Ena/VASP) and crosslinking (α-actinin; Smith et al., 2014). Therefore, the LCR regulates force-sensitive recruitment, while the functional role is dependent on the additional domains (Smith et al., 2010).

5 | LIM DOMAINS FROM DIVERSE PROTEINS BIND STRESSED ACTIN FILAMENTS

Recent research has made progress in understanding the mechanism of LIM protein force-sensitive localization to the actin cytoskeleton. Two studies used complementary experimental approaches to screen LIM proteins for force-sensitivity in cells. One employed cell stretching experiments to systematically quantify the enrichment of full length and LCR constructs of LIM proteins on stretched SFs (Sun et al., 2020), while the other quantified LCR recruitment to SFSS (Winkelman et al., 2020). Together, these studies identified force-sensitive LCRs in 18 LIM proteins from Zyxin, Paxillin, Tes, and Enigma classes from both animals and yeasts (Sun et al., 2020; Winkelman et al., 2020). These complementary experimental approaches revealed that cytoskeletal strain sensing via the LIM domains is widespread in cells and existed in the last common ancestor of yeasts and animals.

To isolate the force-sensitive substrate of LIM, both groups used in vitro approaches to reconstitute force-sensitive recruitment with a minimal set of purified components (Sun et al., 2020; Winkelman et al., 2020). Two types of in vitro reconstitution assays were utilized to test the stress sensitivity of a subset of LIM proteins, and both

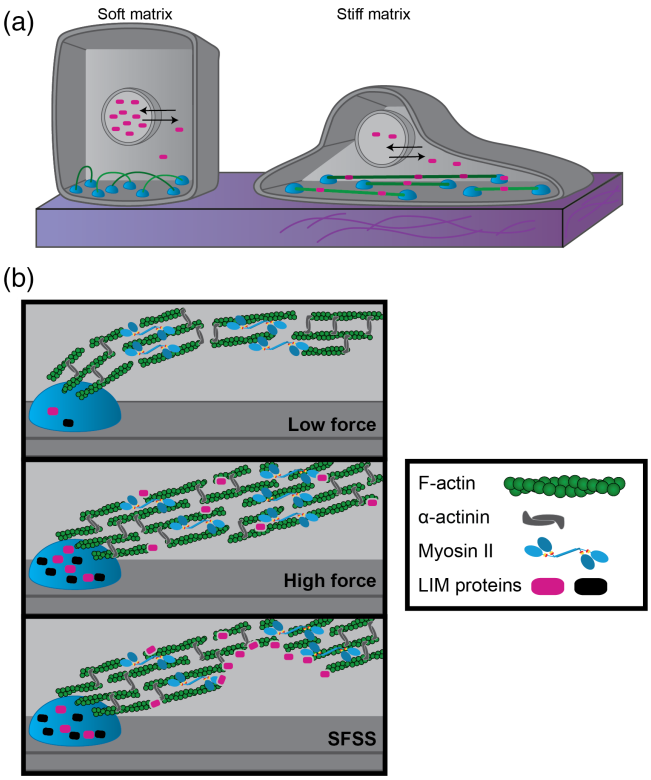

FIGURE 2 Schematic of LIM domain protein localization in cells. (a) Nuclear shuttling of LIM domain proteins (magenta ovals) occurs when cells spread out on stiff matrices. (b) LIM domain proteins (black and magenta ovals) localize to FAs and SFs under high tension. A subset of LIM domain proteins localizes to stress fiber strain sites (SFSS)

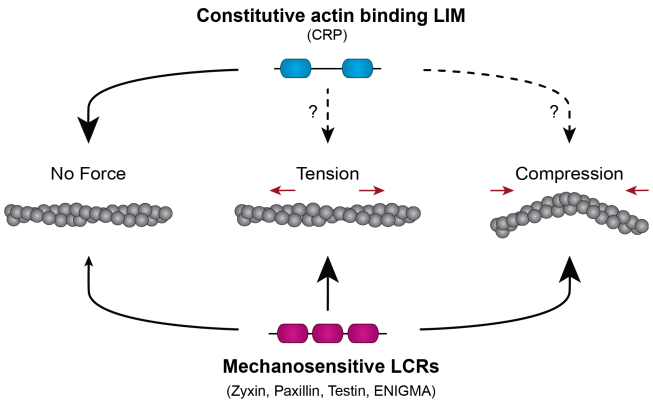

FIGURE 3 Schematic of mechanosensitive LCR localization to stressed actin filaments. The constitutive actin binding CRP class LIM proteins bind actin filaments in the absence or presence of force. The dashed lines indicate that CRP localization is suspected to occur for stressed actin filaments but has not been fully investigated. Mechanosensitive LIM domain protein LCR constructs bind with high affinity to actin filaments under tension or compression but with low affinity to relaxed filaments (adapted from Winkelman et al., 2020)

showed localized recruitment of LIM domains directly to mechanically stressed regions of F-actin. Sun et al. applied tensile stresses to actin filaments with a modified gliding filament assay. Single filaments were pulled in opposite directions via surface-attached myosins with barbed (myosin V) and pointed (myosin VI) end directionality. LIM proteins localize to actin filaments only after initiation of myosin activity facilitates tensed filaments. Actin filament breakage, coinciding with stress relief, results in LIM protein dissociation. Similarly, Winkelman et al. reconstituted contractile actin networks comprised of F-actin, α-actinin, and myosin II. After addition of myosin II to initiate contraction, LCRs localize to stressed regions of the network due to contractile forces, particularly to bundle sites just prior to their rupture, after which the LCR dissociates from the actin filaments.

To understand the mechanism by which LIM domains bind F-actin, these studies identified particular amino acids and LIM domain architectures that are necessary for binding. With the exception of eight well-conserved residues (cysteine and histidine) responsible for Zn^{2+} chelation, the sequence of LIM domains is highly variable. However, a phenylalanine resides at a similar position in all strain sensing LIM domains and was found to be necessary for force sensitivity (Sun et al., 2020). Additionally, force-sensitive LCR all have three or more LIM domains in tandem, each separated by a short linker. Alterations to this organization in the LIM protein zyxin revealed that multiple LIM domains, when organized in tandem and connected by short linkers (serial), but not when oligomerized (parallel), contribute additively to stressed F-actin binding (Sun et al., 2020; Winkelman et al., 2020). Together, these data lead to a hypothesis that multiple LIM domains that are appropriately positioned interact via a hydrophobic interaction with a strained actin filament (Figure 3).

6 | EVOLUTIONARILY CONSERVED MECHANISM OF LIM DOMAIN-BASED FORCE SENSING

Interestingly, despite the lack of sequence conservation, binding to stressed actin filaments appears to be an ancient and conserved function of the LIM domain. Strain sensing LIM domains may have a conserved tertiary structure despite primary sequence variability, similar to other well studied protein folds (Dominguez, 2010). For instance, the LCR of the fission yeast paxillin 1 (Pxl1) binds to both SFSS in mammalian cells and purified mammalian stressed F-actin (Winkelman et al., 2020). Fission yeast do not have stress fibers (or SFSS), but there is a phenomenon analogous to SFSS that occurs within the yeast cell. Pxl1 localizes to the cytokinetic contractile ring (CR), and its deletion results in fragmentation of the ring during contraction (Ge & Balasubramanian, 2008). The rupture of the contractile ring in Pxl1 mutants is reminiscent of increase rupturing of stress fibers observed in zyxin null cells (Smith et al., 2010). Indeed, there are many interesting parallels between CRs and SFs. Both are composed of similar molecular components and are arranged in an architecturally similar way: antiparallel bundled actin filaments crosslinked by α-actinin and pulled on by myosin II. Both may also display a rough

sarcomeric pattern where α-actinin and myosin form complementary domains (Tojkander et al., 2012). The contractility of these networks must be regulated so that they remain tense but do not rip themselves apart. While SFs remain roughly the same length, the CR must shorten during constriction to pinch the mother cell into two daughters. The organization of the CR, SF, and muscle sarcomere may be a coincidence or belie a common origin. Since we first see clear versions of myosin II, α-actinin, and strain-sensing LIM proteins in the unikont branch of eukaryotes, the ancestral version of these contractile networks may have emerged near this branch.

Once contractile machinery arose in evolution, the cell must have evolved regulatory mechanisms for their maintenance and repair. The strain sensing LIM domain may represent one way in which cells learned to detect stressed F-actin. Other domains may be added to this LIM containing protein to tailor responses to LIM-detected stress, for example, some LIM proteins contain domains that bind actin assembly factors that enable these proteins to recruit actin assembly factors to sites of mechanical stress that has been detected by LIM (Hoffman et al., 2012; Smith et al., 2010). One hypothesis for the development of strain sensitive LIM domains is that general actin binding by LIM was tinkered with by evolution to tune it to bind strained actin filaments. The most ancient and widespread LIM proteins are in the CRP family and have been shown in multiple studies to bind unstressed actin filaments (Grubinger & Gimona, 2004; Weiskirchen & Günther, 2003), suggesting the possibility that generic actin binding may be an ancestral function of LIM domains that was tuned to bind strained F-actin (Figure 4).

7 | THE ACTIN FILAMENT IS A SUBSTRATE FOR FORCE-SENSITIVE BINDING

The load dependent mechanical response of F-actin networks is likely to arise from force-sensitive biochemistry of ABPs. A recent review summarizes evidence for force-sensitivity for several ABPs (e.g., Arp2/3 complex, cofilin, alpha-catenin; Jégou & Romet-Lemonne, 2021). Filament curvature promotes the binding of Arp2/3 complex binding to F-actin, while tension decreases the stability of an Arp2/3 complex-mediated daughter branch (Pandit et al., 2020; Risca et al., 2012). There are conflicting reports of how tension may impact the binding of F-actin depolymerizing factor cofilin (Hayakawa, Tatsumi, & Sokabe, 2011; Wioland, Jegou, & Romet-Lemonne, 2019), while additional research suggests torsion may impact cofilin's F-actin severing rate (Mizuno, Tanaka, Yamashiro, Narita, & Watanabe, 2018; Wioland et al., 2019). Low tension applied directly to an actin filament increases the binding of alpha-catenin to adjacent actin subunits, and the force detection is attributed to a 35 amino acid region at the C-terminus (Mei et al., 2020). We hypothesize that similar sensing may occur in LIM protein, but will require further investigations.

As a common component in these mechanosensitive networks, it is likely that the actin filament itself is a force sensor whereby the force-induced conformation of actin filaments affects the binding interactions of the ABPs. There are many studies and hypotheses about how mechanical forces may alter filament conformation, but there is no explicit structural data comparing stressed and unstressed actin filaments (Galkin et al., 2012). Modeling has shown that due to the twist of an actin filament, strain is not distributed homogenously throughout the filament, and localized regions of strain may result (Schramm, Hocky, Voth, Martiel, & De La Cruz, 2019). Therefore, the filament level force can impact the conformation of and interactions between adjacent subunits. These subunit level alterations could possibly reveal additional binding sites for ABPs. We hypothesize that LCRs recognize a binding site along an actin filament that is revealed under tensile or compressive stress (Winkelman et al., 2020). Additional research will be required to fully understand the binding interface of LCRs and mechanically stressed actin filaments. LIM domain proteins, and even isolated strain sensing LCRs, display overlapping but non-identical localization to stressed actin networks, raising the question of how specificity for particular networks arise. Additionally, stressed actin binding is distributed across several protein families involved in diverse cellular processes. Lastly, an important remaining question that will require extensive investigation is how binding by LIM to stressed actin filaments might regulate these diverse cellular processes.

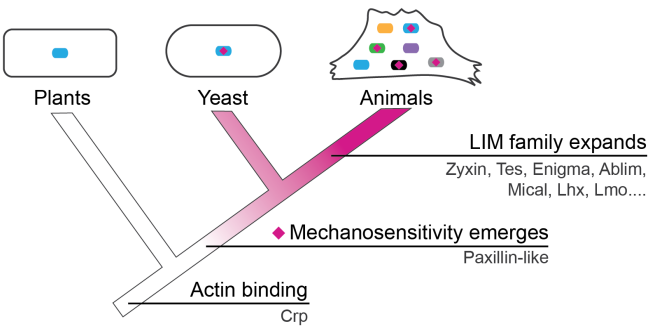

FIGURE 4 Evolution of LIM domain proteins. LIM domains have evolved over time to become mechanosensitive. The family then expanded to include a diverse population of proteins in mammals (adapted from Winkelman et al., 2020)

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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