THE LIM DOMAIN: FROM THE CYTOSKELETON TO THE NUCLEUS

Julie L. Kadrmas and Mary C. Beckerle

Abstract | First described 15 years ago as a cysteine-rich sequence that was common to a small group of homeodomain transcription factors, the LIM domain is now recognized as a tandem zinc-finger structure that functions as a modular protein-binding interface. LIM domains are present in many proteins that have diverse cellular roles as regulators of gene expression, cytoarchitecture, cell adhesion, cell motility and signal transduction. An emerging theme is that LIM proteins might function as biosensors that mediate communication between the cytosolic and the nuclear compartments.

HOMEODOMAIN TRANSCRIPTION FACTORS
Transcription factors that are crucial for development, and that possess conserved, 60-amino-acid DNA-binding domains.

Huntsman Cancer Institute and the Departments of Biology and Oncological Sciences, University of Utah, 2000 East, Circle of Hope, Salt Lake City, Utah 84112, USA.
Correspondence to M.C.B.
e-mail: mary.beckerle@hci.utah.edu
doi:10.1038/nrm1499

Biological systems mix and match a restricted number of modular protein domains in a cassette-like fashion to generate a proteome with the requisite functional complexity. Protein-interaction domains, including the LIM domain, are now recognized as key components of the regulatory machinery of the cell[1]. By virtue of their ability to recruit specific proteins, protein-interaction domains can localize these targets to discrete subcellular locations, modulate their activities or assemble them into multi-component complexes. Therefore, these protein-interaction domains are essential for integrating diverse cellular circuits. Years after the discovery and initial characterization of the LIM domain, its importance as a facilitator of protein liaisons is beginning to be fully appreciated.

In 1988, Way and Chalfie isolated and characterized cDNAs that encoded *Caenorhabditis elegans* MEC-3, which is required for the specification of mechanosensory neurons. They noted that MEC-3 contained a HOMEODOMAIN as well as another sequence that had no similarity to any sequences that were recorded in the public databases at that time[2]. Subsequent cloning of the gene that encoded the *C. elegans* cell-lineage protein LIN-11 (REF. 3) and the rat insulin gene-enhancer-binding protein Isl1 (REF. 4) led to the identification of a cysteine-rich sequence that was common to all three of these proteins. The new protein motif was termed the LIM domain, derived from the first letter of LIN-11, Isl1 and MEC-3, respectively. The LIM amino-acid sequence, which is present in a wide variety of eukaryotic proteins that have diverse biological functions, contains conserved features that facilitate the formation of a stable structural core, and variable features that impart high-affinity binding to many structurally and functionally diverse protein partners. As we will discuss, it is through specific binding of their targets that LIM proteins fulfil their array of biological functions.

The LIM-domain sequence

Individual LIM domains are comprised of approximately 55 amino acids with 8 highly conserved residues — mostly cysteine and histidine — that are located at defined intervals. Classically, the LIM consensus sequence has been defined as $CX_2CX_{16-23}HX_2CX_2CX_2CX_{16-21}CX_2(C/H/D)$ (where X denotes any amino acid)[5], but analysis of all of the identified human LIM sequences shows a slightly broader consensus sequence (FIG. 1a), and further diversity is evident in LIM sequences from other species. The number and spacing of the most highly conserved cysteine and histidine residues indicated that the LIM domain might be a metal-binding structure, and initial reports speculated that the LIM homeodomain proteins might contain iron–sulphur clusters that are crucial for redox regulation of their transcriptional activities[6]. However, subsequent studies showed that the LIM domain binds to zinc, rather than to iron[7]. The LIM domain coordinates two zinc ions and, as will be discussed in more detail

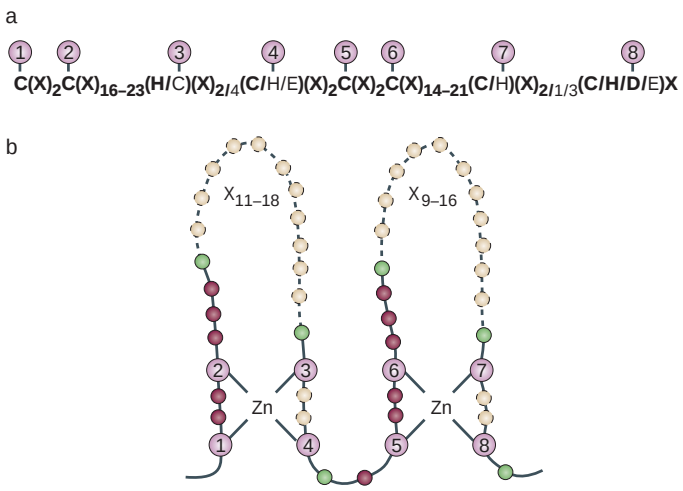


Figure 1 | **Conserved sequence and topology of the LIM domain. a** | The spacing and identity of the eight zinc-binding residues (1–8) based on an analysis of 135 human LIM sequences. Infrequently observed patterns (which are seen in <10% of cases) are denoted by text that is not bold. X denotes any amino acid. **b** | Topology of zinc coordination. Purple circles indicate the zinc-binding residues. Semi-conserved aliphatic/bulky residues are shown as green spheres. Non-conserved residues with invariant spacing are represented as magenta spheres. Dashed yellow circles indicate a variable number of residues (X) that are possible within the sequence.

below, the eight most highly conserved residues are the zinc-binding residues. Residues 1–4 coordinate one zinc ion, and residues 5–8 coordinate the second zinc ion; this establishes the tandem zinc-finger topology[8] that is illustrated in FIG. 1b. A two-residue spacer between these zinc-finger modules is invariant in length and seems to be essential for LIM-domain function[9]. Furthermore, there are several moderately conserved bulky and/or aliphatic residues that are located at defined positions (FIG. 1b). The remainder of the domain sequence is highly variable and confers functional specificity.

Diversity of LIM proteins

The LIM domain is found in proteins from a wide variety of eukaryotic organisms. In the human genome, there are currently 135 identifiable LIM-encoding sequences located within 58 genes (FIG. 2b). Human LIM proteins can contain 1–5 LIM domains. The LIM domain is as abundant as other protein-interaction domains such as Src-homology-2 (SH2) DOMAINS and SH3 DOMAINS (115 and 253 occurrences, respectively)[1]. Invertebrates such as *Drosophila melanogaster* and *C. elegans* express nearly the same complement of LIM-protein families as vertebrates do; but they express LIM proteins in reduced numbers owing to decreased complexity within each family. Although the genomes of organisms like yeasts, slime moulds and plants encode fewer LIM proteins, all eukaryotes for which the genomes have been extensively characterized have been shown to encode LIM-domain proteins. LIM-domain proteins are absent in prokaryotes.

Human LIM proteins and LIM-protein families that have been molecularly characterized are included in the summary shown in FIG. 2a. LIM proteins can be comprised exclusively of LIM domains, or LIM domains can be linked to other domains including homeodomains, catalytic domains, cytoskeletal-binding domains or other protein-binding modules such as SH3, LD or PDZ DOMAINS. The LIM domains can be found internally as well as near the N or C terminus of a protein. These features highlight the modular nature of the LIM domain, as well as the functional diversity of LIM proteins.

LIM proteins have been identified in both the nucleus and the cytoplasm. Several LIM proteins, including the LIM homeodomain proteins, are exclusively nuclear and have clear transcriptional roles during development (for a review, see REF. 10). However, most characterized LIM proteins can interact directly or indirectly with the actin cytoskeleton. Many LIM proteins that were initially identified as cytoskeleton-associated proteins, such as members of the zyxin, four-and-a-half LIM (FHL) and cysteine-rich protein (CRP) families, are now known to shuttle between the cytoplasmic and nuclear compartments of the cell to influence gene expression[11–13]. So, similar to SH2 domains, which are generally considered to function as adaptors in receptor tyrosine kinase signalling[14], the presence of a LIM domain is emerging as a hallmark of proteins that can associate with both the actin cytoskeleton and the transcriptional machinery.

Sequence relationships among LIM domains

Through comparison of their amino-acid sequences, LIM domains can be subjectively classified into four broad categories that reflect their similarity (indicated by shades of red, purple, blue and green in FIG. 2a). Sequence analysis shows some interesting patterns that might provide clues to the evolutionary relationships among LIM proteins. LIM proteins that are exclusively nuclear, such as the nuclear LIM only (LMO) and LIM homeodomain (LHX) proteins, contain amino-acid sequences that are distinct from LIM domains with cytoskeletal roles. The most N-terminal LIM domains of LMO and LHX proteins share a tight cluster of related sequence (red in FIG. 2a). However, the LIM domains that are most closely related to those of LMO and LHX proteins are also present in proteins that have known cytoskeletal roles, such as LIM kinase and actin-binding LIM protein (ABLIM). The C-terminal LIM domains of LMO and LHX proteins also have high sequence similarity (dark purple in FIG. 2a).

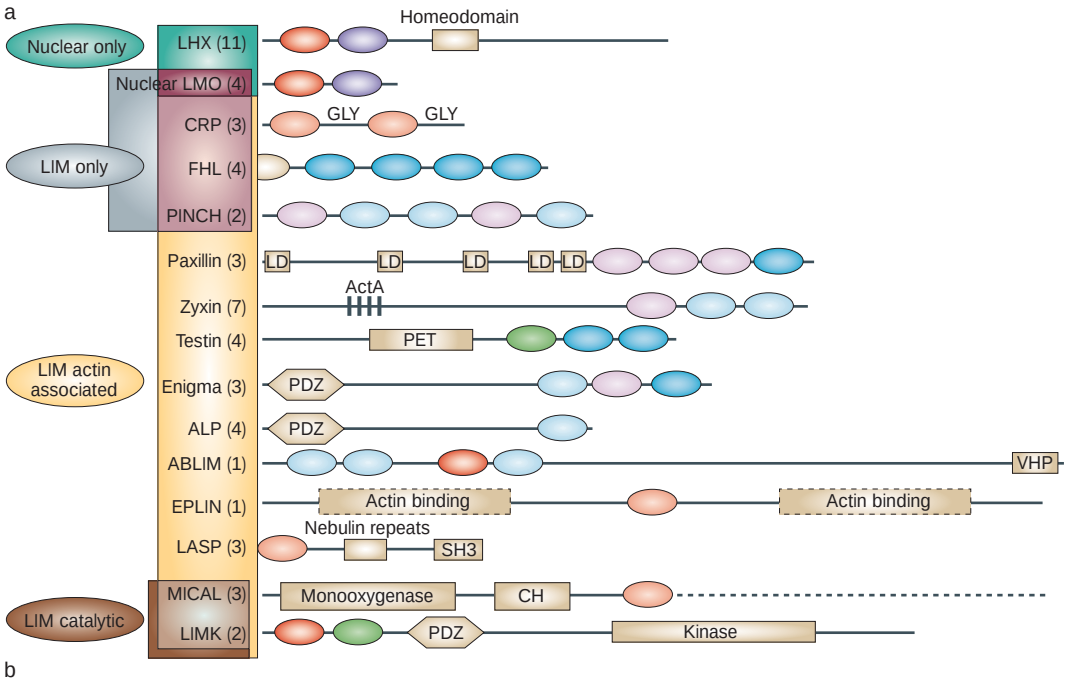
In some LIM-protein families — such as the FHL and CRP families — the multiple LIM domains that are present in each protein family are all closely related, which implies that there are shared ancestral relationships between the individual LIM domains. Therefore, the multiple LIM domains in these proteins might have arisen as a result of relatively recent domain-duplication events. In other cases, such as in the enigma family, the sequences of the three LIM domains are more divergent (FIG. 2a) and might have been assembled from composite sources. Often, LIM-protein-family members share the same arrangement of LIM-domain sequences, which indicates that expansion of these families probably

SH2 DOMAIN
(Src-homology-2 domain). A protein motif that recognizes and binds tyrosine-phosphorylated sequences, and thereby has a key role in relaying cascades of signal transduction.

SH3 DOMAIN
(Src-homology-3 domain). A protein sequence of 50 amino acids that recognizes and binds amino-acid sequences rich in proline.

LD MOTIF
A short sequence found within proteins that has the consensus sequence LDXLLXXL and functions as a protein-binding interface.

PDZ DOMAIN
A protein-interaction domain that often occurs in scaffolding proteins, and is named after the founding members of this protein family (PSD95, Discs large and ZO-1).



b

LHX		LMO	CRP	FHL	PINCH	Paxillin	Zyxin	Testin	Enigma	ALP	LASP	MICAL	LIMK	Other
ISL1	LHX4	LMO1	CRP1	ACT	PINCH	HIC5	Ajuba	Dyxin	Cypher	ALP	LASP	MICAL	LIMK1	ABLIM
ISL2	LHX5	LMO2	CRP2	FHL1	PINCH2	Leupaxin	LIMD1	LMO6	ENH	CLP-36	LIM-nebulette	MICAL-like	LIMK2	EPLIN
LHX1	LHX6	LMO4	CRP3	FHL2		Paxillin	LPP	RILP	Enigma	Mystique	NRAP	MIRAB		LMO7
LHX2	LHX9	LMOX		FHL3			Migfilin	Testin		RIL				Sciellin
LHX3	LMX1a						TRIP6							ZNF185
	LMX1b						WTIP							
							Zyxin							

Figure 2 | **Human LIM proteins. a** | The domain structures of the founding member and/or the best characterized example of the main LIM-protein families are shown. The number of known members of each family is indicated in parentheses. The coloured boxes represent several commonly used categorization schemes. Individual LIM domains are shown as coloured ovals that have been subjectively grouped according to the similarity within the LIM sequence. Heterologous domains such as the LD motif, the monooxygenase domain and actin-binding domains are described in the main text. Domains with boundaries that are not precisely defined are shown as dashed boxes. Dashed lines indicate that scale is not preserved. **b** | A list of the identified members of each LIM family. ABLIM, actin-binding LIM protein; ACT, activator of cyclic AMP response element modulator (CREM) in the testis; ALP, α-actinin-associated LIM protein; CH, calponin homology; CRP, cysteine-rich protein; EPLIN, epithelial protein lost in neoplasm; FHL, four-and-a-half LIM; GLY, glycine-rich region; LASP, LIM and SH3 protein; LHX, LIM-homeodomain protein; LIMK, LIM kinase; LMO, LIM only; MICAL, molecule interacting with CASL protein-1; PDZ, postsynaptic density-95, Discs large, zona occludens-1; PET, prickle, espinas and testin; PINCH, particularly interesting new cysteine and histidine-rich protein; SH3, Src-homology-3; VHP, villin head piece.

occurred after the establishment of the common ancestor. As more detailed information about individual LIM domains becomes available, we might be able to discern the correlations between LIM sequence and molecular function with more accuracy.

Structure of the LIM domain

The structures of several LIM domains have been determined by multidimensional NMR spectroscopy[15–21] and, most recently, by X-ray crystallography[22]. As illustrated by the structure of the C-terminal LIM domain of chicken CRP1, the LIM domain consists of two zinc fingers, each of which comprises two orthogonally packed antiparallel β-hairpins (FIG. 3a; for a detailed description of zinc fingers, see BOX 1). The first zinc finger contains β-hairpins 1 and 2. The second zinc finger contains β-hairpins 3 and 4 and terminates in a short α-helix. RUBREDOXIN-TYPE ZINC KNUCKLES connect the shorter strands of β-hairpins 1 and 3, whereas the longer strands of β-hairpins 2 and 4 are connected by tight turns that often contain a moderately conserved glycine. Conserved tetrahedral zinc coordination establishes the secondary structure and the tertiary fold of the LIM domain, and several hydrophobic core residues (FIG. 3b) assist in the packing of the two zinc fingers[15,23]. Examples of other LIM-domain NMR structures from mouse LMO4 and human PINCH (particularly interesting new cysteine- and histidine-rich protein) are shown in FIG. 3c, and illustrate the conservation of the LIM fold. An overlay of secondary-structure features onto the primary sequence of the LIM domain is shown in FIG. 3d. Differences in non-conserved side chains within the LIM sequence confer unique surface features to these proteins[19,20] (FIG. 3c).

Structural analysis of a LIM domain derived from CRP1 showed a striking similarity between the C-terminal zinc-coordinating module (the second zinc finger) and the DNA-interactive zinc fingers of the GATA and

RUBREDOXIN-TYPE ZINC KNUCKLES
A tight-turn structure within proteins formed by coordination of a metal ion by two closely spaced cysteine residues in the primary sequence. Rubredoxin is a small iron-sulphur protein whose structure was among the first to be solved by X-ray crystallography.

GATA-TYPE TRANSCRIPTION FACTORS
A family of transcription factors that contain a zinc-finger motif that was first identified in the vertebrate GATA1 protein. These transcription factors bind the consensus sequence GATA in the regulatory regions of genes.

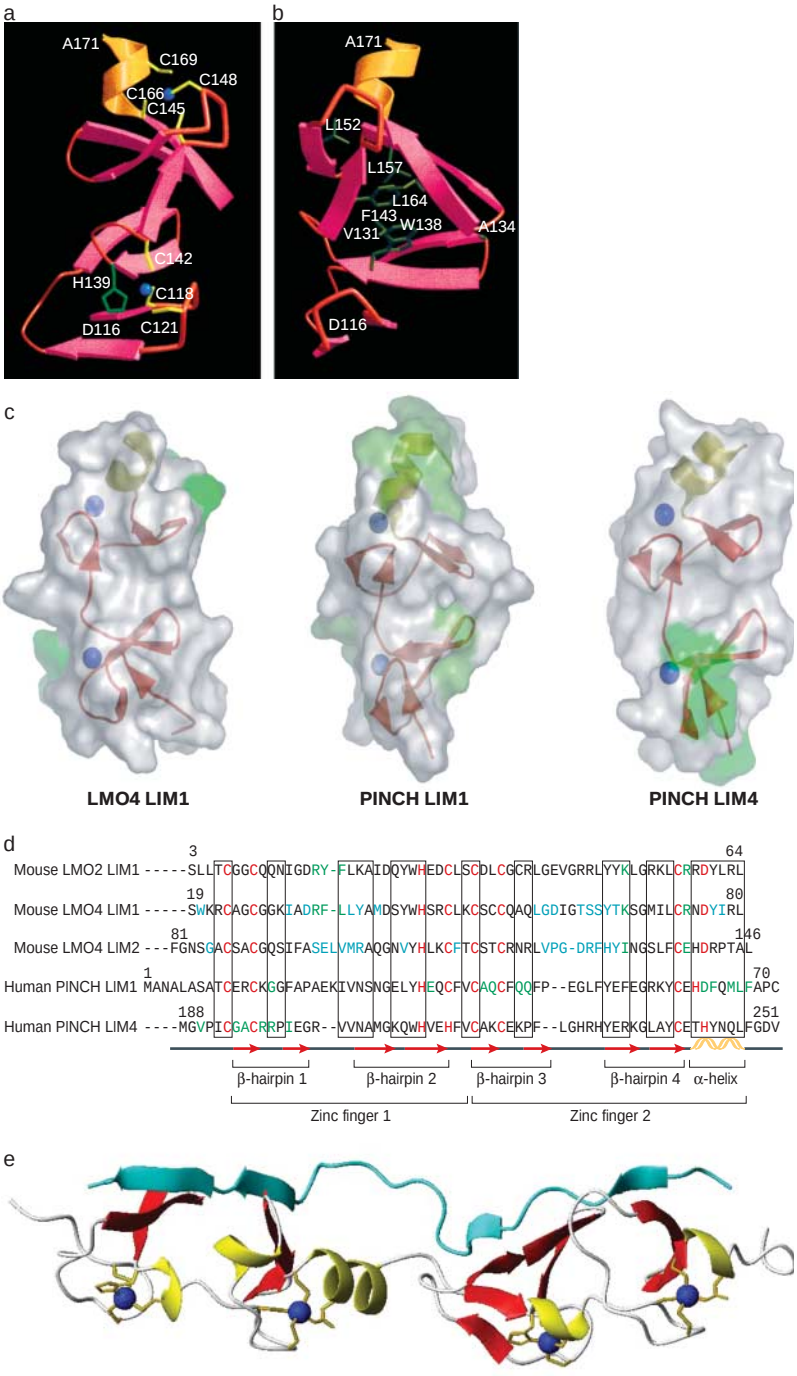


Figure 3 | **Structure of the LIM domain.** Schematic diagrams depict β-strands (red), α-helices (orange in **a**,**b**, yellow in **c**–**e**) and zinc (blue spheres). NMR structure of LIM2 from cysteine-rich protein-1 (CRP1), illustrating the zinc-binding residues and the tertiary structure specified by the LIM sequence (**a**), and core hydrophobic residues (**b**). NMR structures (**c**) for the LIM1 domain of LIM only 4 (LMO4), and the LIM1 and LIM4 domains of PINCH (particularly interesting new cysteine- and histidine-rich protein). Surfaces are overlaid on the ribbon structure, and areas in green represent the surfaces of residues that are involved in binding to the target protein, as determined by NMR. For LMO4, several of these residues are located on the reverse side of the structure. Alignment of the amino-acid sequences of LIM domains (**d**); the structural information is taken from LIM domains that are bound to target proteins. Zinc-binding residues (red), and residues at the binding interface as determined by NMR (green) or X-ray crystallography (blue) are indicated. The secondary structure for these sequences is described below the alignment. The crystal structure of LMO4 LIM domains bound to the LIM-interaction domain (LID) of LIM-domain-binding protein-1 (LDB1; shown in cyan) contains a β-zipper interface (**e**). Parts **a** and **b** are reproduced with permission from *Nature Structural Biology* REF. 15 © (1994) Macmillan Magazines Ltd. Part **e** is modified with permission from REF. 22 © (2004) Macmillan Magazines Ltd.

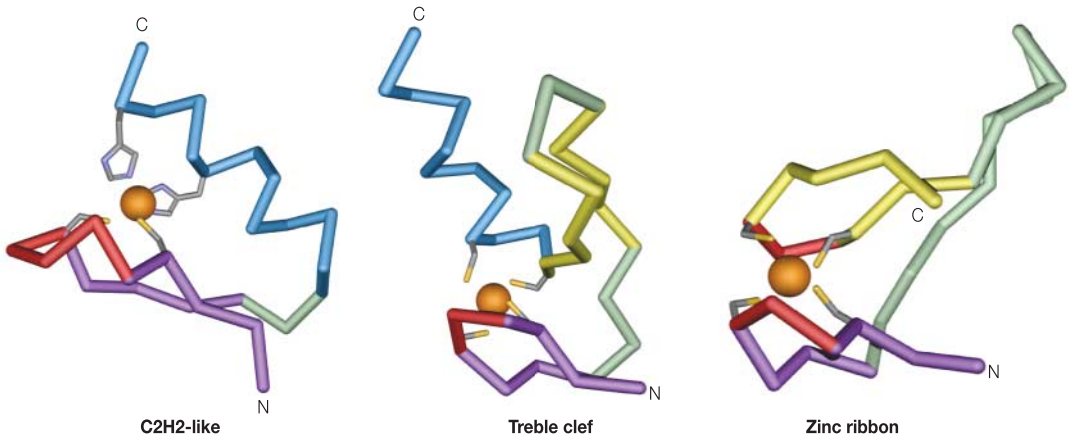
steroid-hormone-receptor classes of transcriptional regulators[15]. One of the zinc fingers of the LIM domain adopts a fold that is theoretically compatible with nucleic-acid binding. So far, there is no compelling evidence for physiologically significant DNA binding by a LIM domain although, intriguingly, many LIM proteins participate in transcriptional control. As discussed further below, in the case of CRP1, the LIM domains coordinate protein partners to assemble a robust transcriptional activator, and the possible contribution of direct LIM-domain contact with the DNA template remains unresolved.

The structures of three multi-LIM proteins — avian CRP1 (REF. 24), CRP2 (REF. 25) and mammalian LMO4 (REF. 22) — have been determined. CRP1 and CRP2 contain two LIM domains, both of which fold independently with no preferred orientation or stable contacts between them,which supports the idea of LIM-domain modularity[5]. The linker region between the LIM domains in CRP1 and CRP2 is a flexible, glycine-rich region of >50 amino acids, but the linkers in multi-LIM proteins typically comprise 8–10 amino acids. LMO4 comprises two LIM domains that are joined by a short linker. In the LMO4 crystal structure, there are no stable contacts between the two LIM domains, but when LMO4 is complexed to its binding partner, the orientation of the two LIM modules is fixed[22]. It is not clear if the orientation becomes fixed when it binds with the target protein, as structural data are not available for full-length LMO4 alone.

LIM domains are protein-binding interfaces

A large body of literature supports the view that the LIM domain functions as a modular protein-binding interface to mediate protein–protein interactions[5,26,27]. For a detailed description of protein-binding interfaces, see BOX 2. Although many classic protein-binding domains, such as SH2, SH3 and WD MOTIFS, have discrete and highly conserved binding preferences[1], efforts to identify the sequence preferences of LIM domains have met with only modest success. An early study using the LIM domains of the enigma protein in conjunction with a random peptide library reported the recognition of a tyrosine-containing motif in binding partners[28]. However, this does not seem to be a general feature of other LIM domains and, so far, no discrete consensus binding sequence or structural element has emerged. At present, the LIM domain seems to provide a stable structural framework on which many kinds of binding specificities can be superimposed. Common features of LIM-domain binding partners might well be resolved when more structural data are available for comparative analysis, but a short linear motif is unlikely to emerge as a general predictor of LIM-domain affinity. Although in many cases, a single LIM domain is necessary and sufficient to support docking of a specific protein partner, this is not a universal feature of LIM-domain recognition. It has been shown that the protein sequence adjacent to the LIM domain can potentiate

Box 1 | Zinc fingers



Zinc fingers comprise any of a wide variety of small protein domains in which zinc (orange sphere) is used within the structural core of the domain to facilitate folding and to increase stability[112]. Zinc fingers can mediate diverse interactions with nucleic acids, proteins or lipids. Nonetheless, the majority of zinc fingers can be categorized into three different protein folds: the classical C2H2-like finger, the treble-clef finger and the zinc ribbon (see figure)[112,113].

Both of the zinc fingers within any LIM domain show the treble-clef fold: two cysteines within a rubredoxin-like turn (shown in red and purple), together with two further ligands within the C-terminal region (often in the N terminus of an α-helix; shown in blue), separated by an intervening β-hairpin (shown in yellow). Treble-clef folds are used to coordinate a molecule of zinc. Although, so far, the LIM domain has only been shown to bind proteins, other treble-clef fingers can bind nucleic acids and lipids. Simply on the basis of amino-acid sequence, straightforward determinants of partner specificity have remained elusive.

The LIM domain contains two treble-clef fingers that are invariantly separated by two amino acids. Other domains, such as RING, PHD and FYVE, also contain obligate tandem treble-clef structures. However, the zinc ligands in the tandem fingers of these domains, in conjunction with their spacing and topology, distinguish them from the LIM domain. For example, the ring domain uses CCCH(C/H)CCC ligands, which overlap with observed LIM-domain ligands. However, the spacing between ligands 3 and 4 is a single amino acid in the RING domain, whereas it is two or — in rare instances — four amino acids in the LIM domain. The RING sequence also produces a different zinc-binding topology, which illustrates that relatively minor differences in sequence can make large contributions to structure and function. The figure was reproduced with permission from REF. 112 © (2003) Oxford University Press.

WD MOTIF
A repeat of ~40 amino acids with a characteristic central tryptophan–aspartic-acid motif that can recognize and bind protein targets containing phosphorylated threonine.

RING DOMAIN
A cysteine-rich tandem zinc-finger domain of 40–60 amino acids often found in E3 ubiquitin ligases.

PHD
The plant homeodomain (PHD) zinc finger is found in many nuclear proteins that are thought to be involved in chromatin-mediated transcriptional regulation.

FYVE
A zinc-finger-containing protein motif that binds the membrane lipid phosphatidylinositol-3-phosphate. The protein that contains FYVE is thereby targeted to the membrane.

NMR CHEMICAL SHIFT ANALYSIS
Changes in the NMR signature of a protein that are induced on addition of an NMR silent binding partner. Amino-acid residues that participate in binding have altered chemical shifts.

binding[26]. Furthermore, there are instances in which a single LIM domain can simultaneously bind several proteins[29,30], or tandem LIM domains can synergistically bind a single partner[22].

Although many protein partners of LIM proteins have been identified, there are only a limited number of known structures of LIM domains bound to their protein targets. These structures have variability in both the sequence and location of the binding-partner interface (FIG. 3c–e). NMR analysis of fusions between the first LIM domain of either LMO2 (also known as rhombotin-2) or LMO4 and the LIM-interaction domain (LID) of LIM-domain binding protein-1 (LDB1) identified residues at the binding interface[21] (FIG. 3c,d). Likewise, NMR CHEMICAL-SHIFT ANALYSIS has revealed the regions in human PINCH LIM1 that might be important for binding to the ankyrin-repeat domain of integrin-linked kinase (ILK)[19], as well as residues in PINCH LIM4 that are important for binding to the proper SH3 domain of the adaptor protein NCK2 (REF. 20; FIG. 3c,d). The LIM motif seems to recognize different protein targets in a variable manner that is dependent on the specific targeted proteins[20].

As with LMO2 and LMO4 (REF. 21), the LIM domains of all LMO and LHX proteins can interact with the nuclear LDB1 (REFS 31–34). However, cytoplasmic LIM proteins — even those that are sometimes present in the nucleus — cannot interact with LDB1 (REF. 32). A recently published crystal structure of tandem LMO4 LIM domains fused to the LDB1 LID (FIG. 3e) provides a high-resolution structure of an extended, high-affinity binding interface[22]. In addition to the contacts that were previously identified by NMR[21], we now have a more complete picture of the residues involved in LIM-domain binding. The LDB1 LID synergistically binds both LIM domains of LMO4 as a β-zipper interface that makes a number of backbone contacts, which extends the second β-hairpin in all four zinc fingers by a further strand. LDB1 also contacts specific residues that are conserved among the nuclear LIM domains[21,22], which explains how LDB1 might discriminate between nuclear and cytoplasmic LIM proteins. LDB1 binds to a single face of the nuclear LIM structure, which is consistent with experimental data that show simultaneous binding of more than one protein per LIM domain — similar to the binding of

Box 2 | Protein-binding interfaces

The ability to form multi-protein complexes is essential for many biological processes. An abundant diversity of protein-binding interfaces is required to form, maintain, remodel and disassemble these complexes in a temporally and spatially regulated manner. The mode of target recognition has been identified for some protein-binding modules. For instance, Src-homology-2 (SH2) domains recognize a short sequence that contains phosphotyrosine, and these domains participate in tyrosine kinase signalling cascades[114]. The PDZ domain can recognize a short C-terminal peptide and often functions in the membrane targeting of its partners[115]. Modular protein-interaction domains can also be designed to recognize a particular target protein. For example, calponin-homology domains[116] and villin headpiece domains[117] function in the recognition of filamentous actin within the actin cytoskeleton. However, all of the above binding interfaces are subject to complexities that sometimes defy the simple definition of target identity.

As illustrated by the LIM domain, not all modes of target recognition are so easily defined, but the LIM domain is certainly not unique in this regard. Domains such as the leucine-rich repeat (LRR)[118] and ankyrin repeats[119] also function as protein-binding interfaces for various protein partners that contain no obvious shared sequence or structural feature that facilitates recognition. The stable framework that zinc coordination imparts to the LIM structure makes LIM domains key elements that have been used repeatedly to build an assortment of LIM proteins. The variability within non-conserved regions of the LIM sequence has permitted specialization, which generates binding affinities for a wide array of protein partners.

the transcriptional regulators CtIP and BRCA1 to LMO4–LDB1 (REF. 30), or the binding of the TAL1–E47 and GATA1 transcription factors to LMO2–LDB1 (REF. 29).

When the LIM domain was first shown to bind zinc, the only known interaction of small zinc-binding protein motifs involved nucleic acids[35], and it was speculated that proteins with LIM domains might function in DNA or RNA recognition. As noted above, the structural similarity of the second zinc finger of LIM domains to GATA-type zinc fingers[15], which can bind both protein and DNA targets, raised the possibility that LIM domains might interact with DNA *in vivo*. However, a failure to detect direct DNA binding by LIM domains — even those present in known transcriptional regulators such as the LHX proteins[36] — cast doubt on this theory. But, there are several *in vitro* studies that show non-sequence-specific binding of LIM domains to DNA[37,38], which indicates that, at present, a function for LIM domains in DNA binding cannot be dismissed. Binding of the LIM domain to DNA might be regulated by association with specific protein partners or by post-translational modification. As more and more LIM proteins are found to enter the nuclear compartment, this possibility becomes more intriguing.

Diverse biological roles of LIM proteins

Through the binding of their partners, LIM proteins participate in an array of biological processes, encompassing aspects of cytoskeletal function and the control of gene expression. Although some LIM proteins have roles that are exclusive to either the nucleus or the cytoplasm, more and more LIM proteins are now known to shuttle between these cellular compartments. Here, we provide examples that show the roles of LIM proteins in the regulation of actin structure and dynamics, neuronal pathfinding, integrin-dependent adhesion and signalling, cell-fate determination and tissue-specific gene expression. The processes that rely on LIM protein function initially seem diverse, but the theme of interplay between the actin cytoskeleton and the nucleus is evident in many cases and might yet be shown for others.

Regulation of actin organization and dynamics. In the cytoplasm, several LIM proteins have been shown to directly regulate actin polymerization and depolymerization reactions. EPLIN (epithelial protein lost in neoplasm) is a cytoskeletal LIM protein that shows downregulated expression in various cancers. It crosslinks actin filaments into bundles using two actin-binding moieties that flank a centrally located LIM domain of undefined function[39,40]. EPLIN inhibits the depolymerization of actin filaments as well as branching nucleation that is mediated by the Arp2/3 COMPLEX[40], and so increases the number and size of stable actin STRESS FIBRES at the expense of more dynamic structures, such as Rac-induced MEMBRANE RUFFLES, which are characteristic of motile cells. So, the loss of EPLIN activity could lead to enhanced motility, which is characteristic of invasive tumour cells.

Members of the ALP (α-actinin-associated LIM protein) and enigma families are closely related proteins that have a single PDZ domain and one or three LIM domains, respectively. ALP was first identified as a partner of the actin-crosslinking protein α-actinin[41,42]. It colocalizes with α-actinin at sites of actin anchorage, such as the intercalated discs of cardiac muscle cells. ALP enhances the ability of α-actinin to bundle actin filaments, and mice that lack ALP develop CARDIOMYOPATHY[43]. Interestingly, ALP has also been observed within cell nuclei under some conditions (C. McKeown, P. Pomiès and M.B., unpublished observations). Cypher (also known as ZASP or oracle), a member of the enigma family, also functions in the maintenance of actin-based structures[44,45]. Cypher is found at the Z-LINES of striated muscle where actin is tethered at the borders of each SARCOMERIC UNIT for the transmission of force. Cypher binds directly to α-actinin as well as to signalling molecules such as protein kinase C (PKC)[46] and calsarcin, a binding partner of the transcriptional regulator calcineurin[47]. So, cypher seems to function as both a structural and signalling component of the Z-line. Mutations in human cypher can produce cardiomyopathy[48,49], and mouse cypher mutants have defects in their striated

Arp2/3 COMPLEX
A complex that consists of two actin-related proteins ARP2 and ARP3, along with five smaller proteins. When activated, the ARP2/3 complex binds to the side of an existing actin filament and nucleates the assembly of a new actin filament. The resulting branch structure is Y-shaped.

STRESS FIBRE
An axial bundle of F-actin and myosin that traverses the cytoplasm. The formation of stress fibres is typically induced by the activity of the GTPase RhoA.

MEMBRANE RUFFLES
Processes that are formed by the movement of lamellipodia in the dynamic process of folding back onto the cell body from which they have extended.

CARDIOMYOPATHY
A disease of the heart muscle.

Z-LINE
A region at the boundaries of muscle sarcomeres in which the actin filaments are anchored. It appears as a dark transverse line in electron micrographs.

SARCOMERE
The basic structural and functional contractile unit of muscle, composed of actin and myosin.

muscle architecture that become progressively more severe with muscle use[50], which is consistent with a role for cypher in the maintenance of muscle actin cytoarchitecture. The nuclear localization of ALP and the binding of cypher to regulators of gene expression raise an intriguing question: do the muscle phenotypes that develop when ALP or cypher function is compromised reflect the loss of a muscle structural element, the failure in the transcriptional response of a muscle to stress, or a combination of both?

Neuronal pathfinding. During neuronal pathfinding, GROWTH CONES at the distal tip of extending axons sense extracellular guidance cues and respond by modulating their actin cytoskeleton to affect the direction of migration. MICAL (molecule interacting with CASL) is a large protein that contains a MONOOXYGENASE DOMAIN, a CALPONIN-HOMOLOGY DOMAIN which binds actin, and a central LIM domain of undefined function. MICAL associates with the adaptor protein CASL[51], which is a member of the p130CAS family of integrin-associated proteins that regulates integrin-dependent signalling and motility[52]. Genetic studies in *D. melanogaster* have shown that MICAL is expressed in axons and functions in repulsive axon guidance[53]. Like MICAL, ABLIM is a LIM protein that contains an actin-binding domain and is essential for high-fidelity axon pathfinding. The physiological role of UNC-115, the *C. elegans* homologue of ABLIM, has been examined genetically. *unc-115* mutants show a developmental defect in which a subset of neurons, although competent to migrate, makes guidance errors[54]. UNC-115 has been shown to function downstream of the RHO-FAMILY GTPase Rac and is postulated to regulate actin dynamics in response to guidance cues[55].

Integrin-dependent adhesion and signalling. Integrins interact with extracellular matrix proteins and relay information bidirectionally across the plasma membrane to regulate cell survival, proliferation, adhesion and motility. Integrins cluster at focal adhesions — specialized regions of the cell surface where actin filaments are anchored. A high percentage of LIM proteins is found at focal adhesions and at analogous structures such as muscle-attachment sites[56]. Examples include migfilin (which is also known as filamin-binding LIM protein) and nebulin-related actin-binding protein (NRAP). Migfilin, a distant zyxin-family member, contains three C-terminal LIM domains through which it binds the focal-adhesion protein MIG2, an integrin effector that is required for cell spreading[57]. The N terminus of migfilin binds the actin-crosslinking protein filamin[57,58], and thereby provides a mechanism to link integrin and cytoskeletal function. NRAP is a muscle-specific protein of the LASP (LIM and SH3 protein) family that is concentrated in the myotendinous junctions in skeletal muscle and in intercalated discs in cardiac muscle. Its single N-terminal LIM domain binds talin — a focal-adhesion protein that regulates integrin activation[59] — whereas the C-terminal NEBULIN SUPER-REPEATS bind actin. NRAP is part of a complex of proteins that anchors the terminal actin filaments of MYOFIBRILS to the SARCOLEMMA to transmit tension[60].

In addition to physically linking the actin cytoskeleton to the cell membrane, focal-adhesion LIM proteins can mediate integrin signalling. Paxillin is a focal-adhesion phosphoprotein that contains five N-terminal LD sequence motifs and four C-terminal LIM domains, through which it binds to various cytoskeletal and signalling proteins. It is proposed to function in coordinating signal transduction at sites of cell attachment through a mechanism that is modulated by its phosphorylation state[61]. Paxillin is an important component of dynamic adhesion structures that are present at the leading edge of migrating cells. Turnover of these adhesions is essential for motility. Tyrosine phosphorylation of paxillin by complexes of focal adhesion kinase (FAK) and Src promotes the disassembly of these structures, perhaps by changing the complement of proteins that bind to the modified paxillin[62]. Paxillin has also been observed in the nucleus and, like its highly related family member HIC5, can interact with the androgen and glucocorticoid nuclear-hormone receptors *in vitro*[63–65]. Given the established role of the paxillin family of proteins at focal adhesions, perhaps the cytoskeletal or nuclear localization of these proteins in tissues that are responsive to steroid hormones is regulated by the adhesion state of the cells. This could provide a mechanism for regulating nuclear hormone receptors using cues from the cytoskeleton.

PINCH, which consists solely of five LIM domains, has roles in cell adhesion, spreading and survival[66]. PINCH interacts with both ILK and NCK2, which implies a potential role in linking integrin and growth-factor-receptor signalling cascades[67,68]. Impaired PINCH function leads to embryonic lethality with defects in actin–membrane linkages and integrin-dependent adhesion[69,70]. PINCH is primarily cytoplasmic, but has also been observed in the nucleus at low frequency[70]. When microinjected into the nucleus of SCHWANN CELLS, PINCH rapidly translocates to the cytoplasm through nuclear export[71], but the biological function of PINCH in the nucleus remains largely unexplored.

Cell-fate determination and tissue-specific gene expression. LIM proteins from the LHX and LMO protein families have established roles in tissue-specific gene expression. LHX proteins are transcription factors that have a prominent role in gene expression in the nervous system where, in a combinatorial fashion, they activate distinct transcriptional programmes to specify the fate of motor neurons, sensory neurons and INTERNEURONS. The homeodomain portion of LHX proteins interacts with DNA in a sequence-specific manner, whereas the LIM portions have an essential regulatory role[72]. Specifically, LHX LIM domains bind directly to the C-terminal LID of the nuclear adaptor LDB1. As LDB1 also homodimerizes through its N-terminal region, LHX proteins have been shown to function as

GROWTH CONE
Motile tip of the axon or dendrite of a growing nerve cell, which spreads out into a large cone-shaped appendage.

MONOOXYGENASE DOMAIN
A protein domain that catalyses oxidoreduction reactions using a flavin cofactor.

CALPONIN-HOMOLOGY DOMAIN
A protein domain, often found tandemly arrayed, that functions in the binding of actin.

RHO FAMILY GTPases
Ras-related small GTPases involved in controlling the polymerization of actin.

NEBULIN SUPER-REPEATS
A 35-residue motif, found in a uniform repeating pattern along the length of the sequence of nebulin. The motif has a role in binding and stabilizing F-actin.

MYOFIBRIL
The structural unit of striated muscle fibres. Several myofibrils make up each fibre.

SARCOLEMMA
The plasma membrane (plasmalemma) of a muscle cell.

SCHWANN CELLS
Cells that produce the myelin sheath around axons in the peripheral nervous system.

INTERNEURONS
Small neurons within the central nervous system that function as connectors between two neurons.

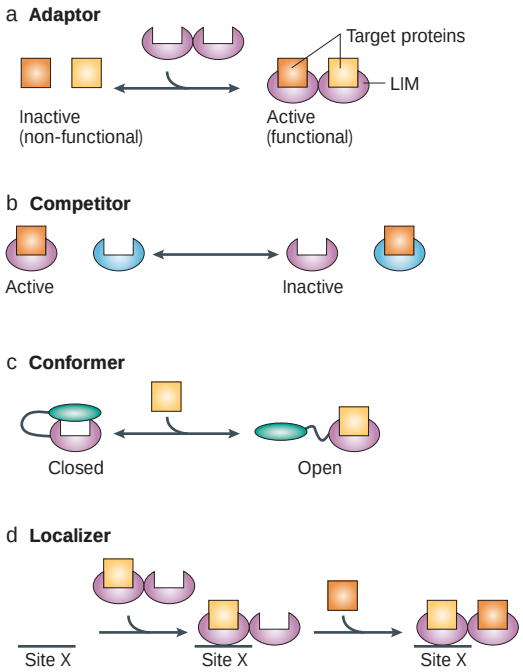


Figure 4 | **LIM contributions to protein function.** LIM domains function by binding to protein partners. **a** | Adaptors can function as scaffolds that assemble binding partners in a way that promotes their activity. **b** | The affinity of a target protein for different LIM domains might result in competition, which could regulate the activity of a LIM complex. **c** | LIM domains can contribute to autoinhibition through intramolecular associations. Such interactions might generate a closed conformer that has an activity that is distinct from an open, or target-protein-occupied, conformer. **d** | LIM proteins can localize themselves and/or their binding partners to a particular subcellular location.

2LHX–2LDB1 tetramers[73]. This presumably facilitates the binding of tandem homeodomains to both promoter and enhancer DNA sequences for maximal transcriptional activation. In cells that express a single type of LHX protein, fate determination is straightforward, as only one active 2LHX–2LDB1 tetramer can be formed. However, in cells that express many LHX proteins, the appropriate cell fate is determined by the combinations of LHX LIM domains that bind to LDB1. For example, in cells that are destined to become motor neurons, LHX3 (which can specify interneuron fate) and ISL1 (which is necessary for motor neuron fate) are both expressed, but under normal conditions the motor neuron fate is selected. LHX3 does not form functional LDB1 interactions because it is displaced to an alternative, high-affinity binding site at the N terminus of ISL1 itself. Evidence from the study of various chimeric proteins indicates that hexameric 2LHX3–2ISL1–2LDB1 complexes are responsible for driving motor neuron fate in these cells[74].

Members of the FHL family of proteins are not transcription factors themselves, but exert their effects on gene expression by interacting with transcription factors such as members of the cyclic-AMP-response-element-binding protein (CREB) family. CREB proteins are the nuclear targets of many converging signalling pathways and are implicated in several cellular responses to extracellular stimuli. One mechanism by which specificity is achieved is through the interaction of CREB proteins with tissue-specific cofactors. Activator of cAMP-response-element modulator (CREM) in the testis (ACT) is a testis-specific FHL protein that binds CREM to powerfully activate CREM transcriptional activity[75]. FHL2 and FHL3, which are expressed at high levels in the heart and skeletal muscle, respectively, can also mediate CREB transcriptional activation[75]. FHL members are also found in association with the actin cytoskeleton. FHL2 has been shown to bind integrins and to localize to focal adhesions in cultured cells[76,77]. After activation of the small GTPase Rho, FHL2 translocates to the nucleus where it can facilitate transcription of target genes[12]. In this way, FHL2 integrates gene expression with Rho-dependent cytoskeletal dynamics.

LIM domains and protein function

The LIM domain has no intrinsic catalytic activity, but LIM proteins mediate many biological processes by binding to target proteins. On the basis of the initial finding that LIM domains are protein-interaction modules, it was postulated that they might localize and modulate the activities of their partners[5] — a prediction that has been borne out in the last decade. By analysing a large number of LIM partnerships, four general themes that describe the consequences of LIM binding have emerged (FIG. 4). LIM domains can function as adaptors, competitors, autoinhibitors or localizers. Often, they carry out combinations of these functions. Here, we provide specific examples to illustrate these four mechanisms by which LIM domains contribute to the biological activity of LIM proteins.

LIM domains as molecular adaptors. In LIM proteins with several protein-interaction domains, the protein-docking capability of the LIM domain enables the protein to function as an adaptor or scaffold to support the assembly of a multimeric protein complex. The association of protein partners with tandemly-arrayed LIM domains provides a mechanism to bring molecules into close proximity, and thereby affect activity (FIG. 4a). In the cases of LMO2 and members of the CRP family, the contributions of the LIM domains to the assembly of functional protein complexes have been established. The principles derived from analysis of these relatively simple, two-LIM-domain proteins probably apply to more complex situations in which several LIM domains or LIM–heterologous-domain pairings occur.

LMO2 is the first well-characterized case in which tandem LIM domains support the assembly of a crucial cell-regulatory complex. LMO2 is required for blood development; homozygous *Lmo2*-mutant mice die owing to failed yolk-sac erythropoiesis[78], and the mechanism by which LMO2 contributes to haematopoiesis has since been elucidated. The LIM domains of LMO2 interact with both the TAL1–E47 and GATA1 transcription factors to form a DNA-binding

complex that is capable of transcriptional activation. TAL1–E47 recognizes E-BOX DNA sequences, whereas GATA1 recognizes the DNA sequence GATA, so the LMO2–TAL1–E47–GATA1 complex can recognize composite E-box–GATA sequences within DNA to activate transcription of specific genes that are required for haematopoiesis. Maximal transcriptional activation is also dependent on both the interaction of the nuclear adaptor LDB1 with LMO2 and homodimerization of LDB1. Such genes could not be activated to the same extent by the various individual subsets of proteins in this complex[79].

Another excellent example of the role of tandem LIM domains as scaffolds is the CRP family. Three CRP-family members have been identified in humans: CRP1 and CRP2 are prominent in smooth muscle; and CRP3, which is also known as the muscle LIM protein (MLP), is expressed in striated muscle. All three proteins interact with α-actinin and are associated with the actin cytoskeleton[80]. CRP-family members also enter the nucleus to promote muscle-specific gene expression[13,26]. How CRPs contribute to the regulation of gene expression has been most clearly established for CRP1 and CRP2, which function through coordinated docking of SERUM-RESPONSE FACTOR (SRF) to the N-terminal LIM domain and GATA factors, specifically GATA4 and GATA6, to the C-terminal LIM domain[13]. This ternary complex of SRF–CRP–GATA strongly stimulates the expression of many smooth muscle-differentiation markers, whereas the pairwise combinations have much less impact on gene expression. Given the dual subcellular distribution of CRP and its clear transcriptional role, it is tempting to speculate that CRPs monitor the integrity of the muscle contractile machinery and contribute to its homeostasis. If this is the case, CRPs that are associated with the actin cytoskeleton might function as sensors to assess the physiological status of the contractile apparatus. When muscle is placed under stress or is damaged, the CRPs might signal to the nucleus to initiate repair through the activation of muscle-specific genes. In support of this hypothesis, CRP3/MLP seems to be a component of a Z-disc-localized stress sensor in cardiomyocytes, which, when it is defective, leads to the development of human dilated cardiomyopathy[81]. The response of cardiomyocytes to mechanical stress involves alterations in gene expression that lead to hypertrophy, and rats in which cardiac hypertrophy has been induced show a nuclear relocation of CRP3/MLP[82]. The CRP family illustrates not only the way in which multi-LIM proteins can function as a framework for the generation of multi-component regulatory machines, but also the theme of LIM proteins as connectors between the cytoskeleton and the nucleus.

Regulation of function by LIM-dependent competition. LIM domains can participate in the control of biological activities by competing with each other for the binding of common partners (FIG. 4b). A striking example can be seen in *D. melanogaster* wing development. The LHX protein apterous confers dorsal-compartment identity on the wing in a temporally regulated manner[83]. Apterous interacts with Chip (Chi; the fly homologue of LDB1) and negatively regulates its own activity late in development by inducing the expression of the LIM-only protein Beadex (Bx), which interacts directly with Chi. As Bx levels rise in a developmentally regulated fashion, Bx displaces apterous from the LIM-interaction domain of Chi, which results in the degradation of the apterous protein by the PROTEASOME[84–86]. A disruption of the balance between Bx, apterous and Chi has deleterious effects on wing development[85–87]. These data, as well as the previously described examples of LHX3 and ISL1 involvement in the specification of neuronal cell fate, and LMO2 function in haematopoeisis, highlight the adaptor functions of nuclear LIM proteins, as well as the importance of competition for LDB1 binding in the biology of nuclear LIM. RLIM (or RNF12), an E3 UBIQUITIN LIGASE for LDB1, promotes proteasome-dependent degradation of free LDB1 to regulate its intracellular concentration and thereby control its activity[88,89].

Autoinhibitory LIM domains. Conformational regulation provides a common mechanism for controlling protein activity. Many transcription factors, cytoskeletal assembly factors, and components of the membrane-fusion machinery undergo autoinhibition[90]. Specific cellular signals that control post-translational modifications, directed proteolysis, or recruitment of further protein partners can relieve autoinhibition and trigger protein activation. In some cases, such as for LIM kinase and testin, a LIM domain mediates the intramolecular interaction that regulates protein activity (FIG. 4c).

LIM kinases are serine/threonine kinases that were first identified by their ability to regulate actin cytoskeletal reorganization by phosphorylation of the actin-disassembly factor cofilin[91,92]. LIM kinases contain targeting sequences that can direct them to either the nucleus or the cytoplasm[93], and they carry out nuclear functions in cell-cycle progression as well as cytoskeletal organizing activities[94]. Autoinhibition is often recognized experimentally when deletion of a single domain results in enhanced activity of another domain. In the case of LIM kinase-1, deletion of the LIM domains or mutation of key zinc-coordinating residues leads to increased kinase activity. Furthermore, LIM domains that are provided in *trans* interfere with kinase activity in a dose-dependent fashion by binding directly to the kinase domain[95]. Identification of the cellular signals that lead to displacement of the LIM region from the kinase domain will provide further insight into the mechanism of LIM-kinase regulation. LIM kinase is activated through phosphorylation by either Rho-associated kinase (ROCK) or p21-activated kinase-1 (PAK1), which are downstream effectors of Rho and Rac, respectively[96,97]. This raises the possibility that phosphorylation might directly affect LIM-dependent autoinhibition.

Human testin (TES) was first identified as a potential tumour-suppressor protein owing to its loss of expression in various tumours and its growth suppressive effects *in vitro*[98]. Subsequently, it was recognized as a focal-adhesion protein that contains three LIM

E-BOX DNA
DNA in the regulatory regions of genes with the sequence CAGATG, specifically recognized by basic helix–loop–helix transcription factors.

SERUM-RESPONSE FACTOR
(SRF). A MADS-domain-containing transcription factor that binds to the serum-response element in the promoter-enhancer region of many genes.

PROTEASOME
A protein complex that is responsible for degrading intracellular proteins that have been tagged for destruction by the addition of ubiquitin.

E3 UBIQUITIN PROTEIN LIGASE
The third enzyme in a series responsible for ubiquitylation and subsequent degradation of target proteins. E3 enzymes, which are numerous, provide platforms for binding target substrates, thereby conferring specificity to this process.

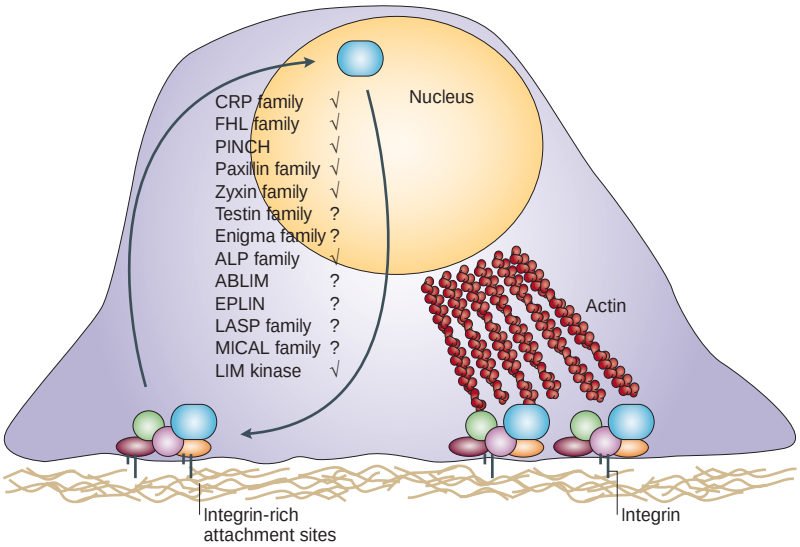


Figure 5 | **Dual localization of LIM proteins.** LIM proteins that have been observed at focal adhesions, muscle-attachment sites or other analogous integrin-rich attachment structures are listed. Many of these proteins can also be found in the nucleus (√), although a nuclear localization for other proteins in this group has not yet been examined (?). Dual localization might function in communication between these cellular compartments. ABLIM, actin-binding LIM protein; ALP, α-actinin-associated LIM protein; CRP, cysteine-rich protein; EPLIN, epithelial protein lost in neoplasm; FHL, four-and-a-half LIM; LASP, LIM and Src-homology-3 protein; MICAL, molecule interacting with CASL; PINCH, particularly interesting new cysteine and histidine-rich protein.

domains and an N-terminal PET (prickle, espinas and testin) sequence — a conserved sequence that was initially identified in TES and *D. melanogaster* PLANAR POLARITY proteins[99]. Full-length TES can interact with actin and its modulators, mammalian orthologue of ENA (MENA) and vasodilator-stimulated phosphoprotein (VASP), but it does not interact with the focal-adhesion components α-actinin, paxillin or zyxin. However, consistent with the idea that TES is conformationally regulated, individual expression of the separate halves of TES promotes binding of α-actinin and paxillin to the PET-containing portion, and zyxin to the LIM portion[100]. The C-terminal LIM region of TES interacts directly with N-terminal TES sequences and this intramolecular association seems to preclude the docking of certain TES partners. The ability of the first LIM domain of TES to interact with zyxin is essential for TES localization at focal adhesions[100], so conformational regulation is also important in the targeting of TES to a specific subcellular location. The biological regulation of conformational changes within TES has not yet been characterized.

LIM domains in protein localization. It is well established that subcellular location is crucial for protein function. Many kinases, phosphatases and transcription factors are subject to regulation by control of their subcellular distribution[101]. LIM domains have been shown to contribute to the targeting of proteins both by directing their own localization and by recruiting partners to specialized subcellular compartments (FIG. 4d). For example, the C-terminal LIM domains of zyxin are necessary and sufficient for the targeting of zyxin to sites of cell–substratum attachment[102]. Likewise, the proper localization of zyxin to focal adhesions is necessary for the accumulation of ENA/VASP proteins to these sites[103,104]. As noted above, zyxin is also required for the localization of TES to focal adhesions[100]. Taken together, these data indicate an important role for zyxin in influencing the composition of focal adhesions on the basis of its ability to dock specific partners at these sites.

Conclusions and perspectives

The LIM domain, which is defined by a conserved cysteine-rich sequence, is a modular protein-binding interface that is found in numerous eukaryotic proteins. The LIM sequence specifies a double zinc-finger structure that is capable of high-affinity binding to a wide variety of protein targets. As more proteins that interact with the LIM domain are identified, and more structures of these partnerships are defined, themes that control the association of partners are likely to emerge. Through binding of target proteins, LIM proteins function in diverse biological processes, the unifying themes of which are nuclear control of gene expression and cytoskeletal function. Moreover, the ability of many LIM proteins to localize to both the nuclear and cytoskeletal compartments is the most striking theme to emerge on global examination of LIM function. Among the LIM proteins that have characterized cytoskeletal functions, most have also been observed, often transiently, in the nucleus (FIG. 5). A number of these proteins contain nuclear export signals[71], which might function to prevent their steady-state nuclear accumulation. Indeed, this is the case with zyxin, which shuttles between the cytoplasm and the nucleus[102,105]. Numerous focal-adhesion LIM proteins have now been shown to be capable of nuclear translocation[12,26,71,94,106–111] (FIG. 5). The extent to which LIM proteins are active in both the nucleus and the cytoplasm remains to be determined. These proteins might have distinct roles in these two compartments. Alternatively, they could be predominantly nuclear factors that are sequestered in the cytoplasm while awaiting activating signals, or they could be cytoplasmic factors that are removed to the nucleus to isolate them from their cytoskeletal targets.

In only a few cases, such as for zyxin (in which mechanical force is important)[11], FHL2 (in which activation of Rho GTPases is paramount)[12] and HIC5 (which involves redox regulation)[111], have the signals that trigger the translocation of an actin-associated LIM protein to the nuclear compartment been defined. The identification of further regulatory mechanisms that trigger the translocation of LIM proteins between the cytoplasm and the nucleus is an important goal for the future. Perhaps the most fruitful area of future research in LIM biology will involve dissecting the precise roles of LIM proteins in both the nuclear and cytoplasmic compartments, and deciphering how the role of a LIM protein that is associated with actin filaments might be integrated with nuclear function and vice versa.

PLANAR POLARITY
The pattern of organization of cells within the plane of an epithelium.

1. Pawson, T. & Nash, P. Assembly of cell regulatory systems through protein interaction domains. *Science* **300**, 445–452 (2003).
2. Way, J. C. & Chalfie, M. *mec-3*, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in *C. elegans*. *Cell* **54**, 5–16 (1988).
The first report of a LIM sequence, which, along with references 3 and 4, provides the historical context of this sequence.
3. Freyd, G., Kim, S. K. & Horvitz, H. R. Novel cysteine-rich motif and homeodomain in the product of the *Caenorhabditis elegans* cell lineage gene *lin-11 Nature* **344**, 876–879 (1990).
4. Karlsson, O., Thor, S., Norberg, T., Ohlsson, H. & Edlund, T. Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. *Nature* **344**, 879–882 (1990).
5. Schmeichel, K. L. & Beckerle, M. C. The LIM domain is a modular protein-binding interface. *Cell* **79**, 211–219 (1994).
Established LIM function in protein binding.
6. Li, P. M., Reichert, J., Freyd, G., Horvitz, H. R. & Walsh, C. T. The LIM region of a presumptive *Caenorhabditis elegans* transcription factor is an iron-sulfur- and zinc-containing metallodomain. *Proc. Natl Acad. Sci. USA* **88**, 9210–9213 (1991).
7. Michelsen, J. W., Schmeichel, K. L., Beckerle, M. C. & Winge, D. R. The LIM motif defines a specific zinc-binding protein domain. *Proc. Natl Acad. Sci. USA* **90**, 4404–4408 (1993).
8. Michelsen, J. W. *et al.* Mutational analysis of the metal sites in an LIM domain. *J. Biol. Chem.* **269**, 11108–11113 (1994).
9. Schmeichel, K. L. & Beckerle, M. C. Molecular dissection of a LIM domain. *Mol. Biol. Cell* **8**, 219–230 (1997).
10. Hobert, O. & Westphal, H. Functions of LIM-homeobox genes. *Trends Genet.* **16**, 75–83 (2000).
11. Cattaruzza, M., Lattrich, C. & Hecker, M. Focal adhesion protein zyxin is a mechanosensitive modulator of gene expression in vascular smooth muscle cells. *Hypertension* **4**, 726–730 (2004).
12. Muller, J. M. *et al.* The transcriptional coactivator FHL2 transmits Rho signals from the cell membrane into the nucleus. *EMBO J.* **21**, 736–748 (2002).
References 11 and 12 describe triggers for nucleo-cytoplasmic shuttling.
13. Chang, D. F. *et al.* Cysteine-rich LIM-only proteins CRP1 and CRP2 are potent smooth muscle differentiation cofactors. *Dev. Cell* **4**, 107–118 (2003).
Experiments that show the assembly of a functional complex on the CRP adaptors.
14. Pawson, T. Specificity in signal transduction: from phosphotyrosine–SH2 domain interactions to complex cellular systems. *Cell* **116**, 191–203 (2004).
15. Perez-Alvarado, G. C. *et al.* Structure of the carboxy-terminal LIM domain from the cysteine rich protein CRP. *Nature Struct. Biol.* **1**, 388–398 (1994).
The first report of LIM-domain structure.
16. Perez-Alvarado, G. C. *et al.* Structure of the cysteine-rich intestinal protein, CRIP. *J. Mol. Biol.* **257**, 153–174 (1996).
17. Konrat, R., Weiskirchen, R., Krautler, B. & Bister, K. Solution structure of the carboxyl-terminal LIM domain from quail cysteine-rich protein CRP2. *J. Biol. Chem.* **272**, 12001–12007 (1997).
18. Kontaxis, G., Konrat, R., Krautler, B., Weiskirchen, R. & Bister, K. Structure and intramodular dynamics of the amino-terminal LIM domain from quail cysteine- and glycine-rich protein CRP2. *Biochemistry* **37**, 7127–7134 (1998).
19. Velyvis, A., Yang, Y., Wu, C. & Qin, J. Solution structure of the focal adhesion adaptor PINCH LIM1 domain and characterization of its interaction with the integrin-linked kinase ankyrin repeat domain. *J. Biol. Chem.* **276**, 4932–4939 (2001).
The first report of a target-occupied LIM-domain structure describing the identification of a binding interface.
20. Velyvis, A. *et al.* Structural and functional insights into PINCH LIM4 domain-mediated integrin signaling. *Nature Struct. Biol.* **10**, 558–564 (2003).
21. Deane, J. E. *et al.* Structural basis for the recognition of ldb1 by the N-terminal LIM domains of LMO2 and LMO4. *EMBO J.* **22**, 2224–2233 (2003).
22. Deane, J. E. *et al.* Tandem LIM domains provide synergistic binding in the LMO4:Ldb1 complex. *EMBO J.* **23**, 3589–3598 (2004).
Describes the first crystal structure of a LIM protein.
23. Kosa, J. L. *et al.* Common metal ion coordination in LIM domain proteins. *Biochemistry* **33**, 468–477 (1994).
24. Yao, X. *et al.* Solution structure of the chicken cysteine-rich protein, CRP1, a double-LIM protein implicated in muscle differentiation. *Biochemistry* **38**, 5701–5713 (1999).
25. Konrat, R., Krautler, B., Weiskirchen, R. & Bister, K. Structure of cysteine- and glycine-rich protein CRP2. Backbone dynamics reveal motional freedom and independent spatial orientation of the LIM domains. *J. Biol. Chem.* **273**, 23233–23240 (1998).
26. Arber, S. & Caroni, P. Specificity of single LIM motifs in targeting and LIM/LIM interactions *in situ*. *Genes Dev.* **10**, 289–300 (1996).
27. Feuerstein, R., Wang, X., Song, D., Cooke, N. E. & Liebhaber, S. A. The LIM/double zinc-finger motif functions as a protein dimerization domain. *Proc. Natl Acad. Sci. USA* **91**, 10655–10659 (1994).
28. Wu, R. *et al.* Specificity of LIM domain interactions with receptor tyrosine kinases. *J. Biol. Chem.* **271**, 15934–15941 (1996).
29. Wadman, I. A. *et al.* The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. *EMBO J.* **16**, 3145–3157 (1997).
30. Sum, E. Y. *et al.* The LIM domain protein LMO4 interacts with the cofactor CtIP and the tumor suppressor BRCA1 and inhibits BRCA1 activity. *J. Biol. Chem.* **277**, 7849–7856 (2002).
31. Jurata, L. W. & Gill, G. N. Functional analysis of the nuclear LIM domain interactor NLI. *Mol. Cell. Biol.* **17**, 5688–5698 (1997).
32. Jurata, L. W., Kenny, D. A. & Gill, G. N. Nuclear LIM interactor, a rhombotin and LIM homeodomain interacting protein, is expressed early in neuronal development. *Proc. Natl Acad. Sci. USA* **93**, 11693–11698 (1996).
33. Breen, J. J., Agulnick, A. D., Westphal, H. & Dawid, I. B. Interactions between LIM domains and the LIM domain-binding protein Ldb1. *J. Biol. Chem.* **273**, 4712–4717 (1998).
34. Agulnick, A. D. *et al.* Interactions of the LIM-domain-binding factor Ldb1 with LIM homeodomain proteins. *Nature* **384**, 270–272 (1996).
35. Coleman, J. E. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annu. Rev. Biochem.* **61**, 897–946 (1992).
36. Xue, D., Tu, Y. & Chalfie, M. Cooperative interactions between the *Caenorhabditis elegans* homeoproteins UNC-86 and MEC-3. *Science* **261**, 1324–1328 (1993).
37. Baltz, R., Evrard, J., Bourdon, V. & Steinmetz, A. The pollen-specific LIM protein PLIM-1 from sunflower binds nucleic acids *in vitro*. *Sex. Plant Reprod.* **9**, 264–268 (1996).
38. Nishiya, N., Sabe, H., Nose, K. & Shibanuma, M. The LIM domains of hic-5 protein recognize specific DNA fragments in a zinc-dependent manner *in vitro*. *Nucleic Acids Res.* **26**, 4267–4273 (1998).
39. Maul, R. S. & Chang, D. D. EPLIN, epithelial protein lost in neoplasm. *Oncogene* **18**, 7838–7841 (1999).
40. Maul, R. S. *et al.* EPLIN regulates actin dynamics by cross-linking and stabilizing filaments. *J. Cell Biol.* **160**, 399–407 (2003).
41. Xia, H., Winokur, S. T., Kuo, W. L., Altherr, M. R. & Bredt, D. S. Actinin-associated LIM protein: identification of a domain interaction between PDZ and spectrin-like repeat motifs. *J. Cell Biol.* **139**, 507–515 (1997).
42. Pomies, P., Macalma, T. & Beckerle, M. C. Purification and characterization of an α-actinin-binding PDZ–LIM protein that is up-regulated during muscle differentiation. *J. Biol. Chem.* **274**, 29242–29250 (1999).
43. Pashmforoush, M. *et al.* Adult mice deficient in actinin-associated LIM-domain protein reveal a developmental pathway for right ventricular cardiomyopathy. *Nature Med.* **7**, 591–597 (2001).
44. Faulkner, G. *et al.* ZASP: a new Z-band alternatively spliced PDZ-motif protein. *J. Cell Biol.* **146**, 465–475 (1999).
45. Passier, R., Richardson, J. A. & Olson, E. N. Oracle, a novel PDZ–LIM domain protein expressed in heart and skeletal muscle. *Mech. Dev.* **92**, 277–284 (2000).
46. Zhou, Q., Ruiz-Lozano, P., Martone, M. E. & Chen, J. Cypher, a striated muscle-restricted PDZ and LIM domain-containing protein, binds to α-actinin-2 and protein kinase C. *J. Biol. Chem.* **274**, 19807–19813 (1999).
47. Frey, N. & Olson, E. N. Calsarcin-3, a novel skeletal muscle-specific member of the calsarcin family, interacts with multiple Z-disc proteins. *J. Biol. Chem.* **277**, 13998–14004 (2002).
48. Vatta, M. *et al.* Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. *J. Am. Coll. Cardiol.* **42**, 2014–2027 (2003).
49. Arimura, T. *et al.* A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C. *J. Biol. Chem.* **279**, 6746–6752 (2004).
50. Zhou, Q. *et al.* Ablation of Cypher, a PDZ–LIM domain Z-line protein, causes a severe form of congenital myopathy. *J. Cell Biol.* **155**, 605–612 (2001).
51. Suzuki, T. *et al.* MICAL, a novel CasL interacting molecule, associates with vimentin. *J. Biol. Chem.* **277**, 14933–14941 (2002).
52. O'Neill, G. M., Fashena, S. J. & Golemis, E. A. Integrin signalling: a new Cas(t) of characters enters the stage. *Trends Cell Biol.* **10**, 111–119 (2000).
53. Terman, J. R., Mao, T., Pasterkamp, R. J., Yu, H. H. & Kolodkin, A. L. MICALs, a family of conserved flavoprotein oxidoreductases, function in plexin-mediated axonal repulsion. *Cell* **109**, 887–900 (2002).
54. Lundquist, E. A., Herman, R. K., Shaw, J. E. & Bargmann, C. I. UNC-115, a conserved protein with predicted LIM and actin-binding domains, mediates axon guidance in *C. elegans*. *Neuron* **21**, 385–392 (1998).
55. Struckhoff, E. C. & Lundquist, E. A. The actin-binding protein UNC-115 is an effector of Rac signaling during axon pathfinding in *C. elegans*. *Development* **130**, 693–704 (2003).
56. Labouesse, M. & Georges-Labouesse, E. Cell adhesion: parallels between vertebrate and invertebrate focal adhesions. *Curr. Biol.* **13**, R528–R530 (2003).
57. Tu, Y., Wu, S., Shi, X., Chen, K. & Wu, C. Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell* **113**, 37–47 (2003).
58. Takafuta, T., Saeki, M., Fujimoto, T. T., Fujimura, K. & Shapiro, S. S. A new member of the LIM protein family binds to filamin B and localizes at stress fibers. *J. Biol. Chem.* **278**, 12175–12181 (2003).
59. Tadokoro, S. *et al.* Talin binding to integrin β tails: a final common step in integrin activation. *Science* **302**, 103–106 (2003).
60. Luo, G., Herrera, A. H. & Horowits, R. Molecular interactions of N-RAP, a nebulin-related protein of striated muscle myotendon junctions and intercalated disks. *Biochemistry* **38**, 6135–6143 (1999).
61. Schaller, M. D. Paxillin: a focal adhesion-associated adaptor protein. *Oncogene* **20**, 6459–6472 (2001).
62. Webb, D. J. *et al.* FAK–Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nature Cell Biol.* **6**, 154–161 (2004).
63. Kasai, M. *et al.* The Group 3 LIM domain protein paxillin potentiates androgen receptor transactivation in prostate cancer cell lines. *Cancer Res.* **63**, 4927–4935 (2003).
64. Fujimoto, N. *et al.* Cloning and characterization of androgen receptor coactivator, ARA55, in human prostate. *J. Biol. Chem.* **274**, 8316–8321 (1999).
65. Yang, L., Guerrero, J., Hong, H., DeFranco, D. B. & Stallcup, M. R. Interaction of the tau2 transcriptional activation domain of glucocorticoid receptor with a novel steroid receptor coactivator, Hic-5, which localizes to both focal adhesions and the nuclear matrix. *Mol. Biol. Cell* **11**, 2007–2018 (2000).
66. Wu, C. Integrin-linked kinase and PINCH: partners in regulation of cell–extracellular matrix interaction and signal transduction. *J. Cell Sci.* **112**, 4485–4489 (1999).
67. Tu, Y., Li, F., Goicoechea, S. & Wu, C. The LIM-only protein PINCH directly interacts with integrin-linked kinase and is recruited to integrin-rich sites in spreading cells. *Mol. Cell. Biol.* **19**, 2425–2434 (1999).
68. Tu, Y., Li, F. & Wu, C. Nck-2, a novel Src homology 2/3-containing adaptor protein that interacts with the LIM-only protein PINCH and components of growth factor receptor kinase-signaling pathways. *Mol. Biol. Cell* **9**, 3367–3382 (1998).
69. Clark, K. A., McGrail, M. & Beckerle, M. C. Analysis of PINCH function in *Drosophila* demonstrates its requirement in integrin-dependent cellular processes. *Development* **130**, 2611–2621 (2003).
70. Hobert, O., Moerman, D. G., Clark, K. A., Beckerle, M. C. & Ruvkun, G. A conserved LIM protein that affects muscular adherens junction integrity and mechanosensory function in *Caenorhabditis elegans*. *J. Cell Biol.* **144**, 45–57 (1999).
71. Campana, W. M., Myers, R. R. & Rearden, A. Identification of PINCH in Schwann cells and DRG neurons: shuttling and signaling after nerve injury. *Glia* **41**, 213–223 (2003).
72. Shirasaki, R. & Pfaff, S. L. Transcriptional codes and the control of neuronal identity. *Annu. Rev. Neurosci.* **25**, 251–281 (2002).
73. Jurata, L. W., Pfaff, S. L. & Gill, G. N. The nuclear LIM domain interactor NLI mediates homo- and heterodimerization of LIM domain transcription factors. *J. Biol. Chem.* **273**, 3152–3157 (1998).
74. Thaler, J. P., Lee, S. K., Jurata, L. W., Gill, G. N. & Pfaff, S. L. LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein–protein interactions. *Cell* **110**, 237–249 (2002).
Elegant use of chimeric proteins to further define the LIM combinatorial code.

75. Fimia, G. M., de Cesare, D. & Sassone-Corsi, P. A family of LIM-only transcriptional coactivators: tissue-specific expression and selective activation of CREB and CREM. *Mol. Cell Biol.* **20**, 8613–8622 (2000).
76. Wixler, V. *et al.* The LIM-only protein DRAL/FHL2 binds to the cytoplasmic domain of several α and β integrin chains and is recruited to adhesion complexes. *J. Biol. Chem.* **275**, 33669–33678 (2000).
77. Li, H. Y. *et al.* Translocation of a human focal adhesion LIM-only protein, FHL2, during myofibrillogenesis and identification of LIM2 as the principal determinants of FHL2 focal adhesion localization. *Cell Motil. Cytoskeleton* **48**, 11–23 (2001).
78. Warren, A. J. *et al.* The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. *Cell* **78**, 45–57 (1994).
79. Xu, Z., Huang, S., Chang, L. S., Agulnick, A. D. & Brandt, S. J. Identification of a TAL1 target gene reveals a positive role for the LIM domain-binding protein Ldb1 in erythroid gene expression and differentiation. *Mol. Cell. Biol.* **23**, 7585–7599 (2003).
Identifies a target gene for intact LDB1–LMO2 regulatory complexes with tandem DNA recognition sites.
80. Louis, H. A., Pino, J. D., Schmeichel, K. L., Pomies, P. & Beckerle, M. C. Comparison of three members of the cysteine-rich protein family reveals functional conservation and divergent patterns of gene expression. *J. Biol. Chem.* **272**, 27484–27491 (1997).
81. Knoll, R. *et al.* The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell* **111**, 943–955 (2002).
Proposes a model for the role of MLP in nucleo-cytoplasmic shuttling.
82. Ecarnot-Laubriet, A. *et al.* Downregulation and nuclear relocation of MLP during the progression of right ventricular hypertrophy induced by chronic pressure overload. *J. Mol. Cell. Cardiol.* **32**, 2385–2395 (2000).
83. Milan, M. & Cohen, S. M. Temporal regulation of apterous activity during development of the *Drosophila* wing. *Development* **127**, 3069–3078 (2000).
84. Weihe, U., Milan, M. & Cohen, S. M. Regulation of Apterous activity in *Drosophila* wing development. *Development* **128**, 4615–4622 (2001).
85. Milan, M. & Cohen, S. M. Regulation of LIM homeodomain activity *in vivo*: a tetramer of dLDB and apterous confers activity and capacity for regulation by dLMO. *Mol. Cell* **4**, 267–273 (1999).
86. Milan, M., Diaz-Benjumea, F. J. & Cohen, S. M. Beadex encodes an LMO protein that regulates Apterous LIM-homeodomain activity in *Drosophila* wing development: a model for LMO oncogene function. *Genes Dev.* **12**, 2912–2920 (1998).
87. van Meyel, D. J. *et al.* Chip and apterous physically interact to form a functional complex during *Drosophila* development. *Mol. Cell* **4**, 259–265 (1999).
88. Ostendorff, H. P. *et al.* Ubiquitination-dependent cofactor exchange on LIM homeodomain transcription factors. *Nature* **416**, 99–103 (2002).
89. Hiratani, I., Yamamoto, N., Mochizuki, T., Ohmori, S. Y. & Taira, M. Selective degradation of excess Ldb1 by Rnf12/RLIM confers proper Ldb1 expression levels and Xlim-1/Ldb1 stoichiometry in *Xenopus* organizer functions. *Development* **130**, 4161–4175 (2003).
90. Pufall, M. A. & Graves, B. J. Autoinhibitory domains: modular effectors of cellular regulation. *Annu. Rev. Cell Dev. Biol.* **18**, 421–462 (2002).
91. Arber, S. *et al.* Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. *Nature* **393**, 805–809 (1998).
92. Yang, N. *et al.* Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* **393**, 809–812 (1998).
References 91 and 92 first established LIM function in actin dynamics.
93. Yang, N. & Mizuno, K. Nuclear export of LIM-kinase 1, mediated by two leucine-rich nuclear-export signals within the PDZ domain. *Biochem. J.* **338**, 793–798 (1999).
94. Roovers, K., Klein, E. A., Castagnino, P. & Assoian, R. K. Nuclear translocation of LIM kinase mediates Rho–Rho kinase regulation of cyclin D1 expression. *Dev. Cell* **5**, 273–284 (2003).
95. Nagata, K., Ohashi, K., Yang, N. & Mizuno, K. The N-terminal LIM domain negatively regulates the kinase activity of LIM-kinase 1. *Biochem. J.* **343**, 99–105 (1999).
96. Ohashi, K. *et al.* Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J. Biol. Chem.* **275**, 3577–3582 (2000).
97. Edwards, D. C., Sanders, L. C., Bokoch, G. M. & Gill, G. N. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nature Cell Biol.* **1**, 253–259 (1999).
98. Tobias, E. S., Hurlstone, A. F., MacKenzie, E., McFarlane, R. & Black, D. M. The *TES* gene at 7q31.1 is methylated in tumours and encodes a novel growth-suppressing LIM domain protein. *Oncogene* **20**, 2844–2853 (2001).
99. Gubb, D. *et al.* The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in *Drosophila* imaginal discs. *Genes Dev.* **13**, 2315–2327 (1999).
100. Garvalov, B. K. *et al.* The conformational state of Tes regulates its zyxin-dependent recruitment to focal adhesions. *J. Cell Biol.* **161**, 33–39 (2003).
101. Cyert, M. S. Regulation of nuclear localization during signaling. *J. Biol. Chem.* **276**, 20805–20808 (2001).
102. Nix, D. A. *et al.* Targeting of zyxin to sites of actin membrane interaction and to the nucleus. *J. Biol. Chem.* **276**, 34759–34767 (2001).
The first careful examination of LIM nucleo-cytoplasmic shuttling.
103. Drees, B. *et al.* Characterization of the interaction between zyxin and members of the Ena/vasodilator-stimulated phosphoprotein family of proteins. *J. Biol. Chem.* **275**, 22503–22511 (2000).
104. Drees, B. E., Andrews, K. M. & Beckerle, M. C. Molecular dissection of zyxin function reveals its involvement in cell motility. *J. Cell Biol.* **147**, 1549–1560 (1999).
105. Nix, D. A. & Beckerle, M. C. Nuclear–cytoplasmic shuttling of the focal contact protein, zyxin: a potential mechanism for communication between sites of cell adhesion and the nucleus. *J. Cell Biol.* **138**, 1139–1147 (1997).
106. Petit, M. M., Meulemans, S. M. & Van de Ven, W. J. The focal adhesion and nuclear targeting capacity of the LIM-containing lipoma-preferred partner (LPP) protein. *J. Biol. Chem.* **278**, 2157–2168 (2003).
107. Wang, Y. & Gilmore, T. D. LIM domain protein Trip6 has a conserved nuclear export signal, nuclear targeting sequences, and multiple transactivation domains. *Biochim. Biophys. Acta* **1538**, 260–272 (2001).
108. Kanungo, J., Pratt, S. J., Marie, H. & Longmore, G. D. Ajuba, a cytosolic LIM protein, shuttles into the nucleus and affects embryonal cell proliferation and fate decisions. *Mol. Biol. Cell* **11**, 3299–3313 (2000).
109. Srichai, M. B. *et al.* A WT1 co-regulator controls podocyte phenotype by shuttling between adhesion structures and nucleus. *J. Biol. Chem.* **279**, 14398–14408 (2004).
110. Akazawa, H. *et al.* A novel LIM protein Cal promotes cardiac differentiation by association with CSX/NKX2-5. *J. Cell Biol.* **164**, 395–405 (2004).
111. Shibanuma, M. *et al.* Hic-5 communicates between focal adhesions and the nucleus through oxidant-sensitive nuclear export signal. *Mol. Biol. Cell* **14**, 1158–1171 (2003).
112. Krishna, S. S., Majumdar, I. & Grishin, N. V. Structural classification of zinc fingers: survey and summary. *Nucleic Acids Res.* **31**, 532–550 (2003).
113. Grishin, N. V. Treble clef finger — a functionally diverse zinc-binding structural motif. *Nucleic Acids Res.* **29**, 1703–1714 (2001).
114. Schlessinger, J. & Lemmon, M. A. SH2 and PTB domains in tyrosine kinase signaling. *Sci. STKE* **191**, RE12 (2003).
115. Nourry, C., Grant, S. G. & Borg, J. P. PDZ domain proteins: plug and play! *Sci. STKE*, RE7 (2003).
116. Gimona, M., Djinovic-Carugo, K., Kranewitter, W. J. & Winder, S. J. Functional plasticity of CH domains. *FEBS Lett.* **513**, 98–106 (2002).
117. Vardar, D. *et al.* Villin-type headpiece domains show a wide range of F-actin-binding affinities. *Cell Motil. Cytoskeleton* **52**, 9–21 (2002).
118. Kobe, B. & Kajava, A. V. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* **11**, 725–732 (2001).
119. Mosavi, L. K., Minor, D. L. Jr & Peng, Z. Y. Consensus-derived structural determinants of the ankyrin repeat motif. *Proc. Natl Acad. Sci. USA* **99**, 16029–16034 (2002).

Acknowledgements

The authors would like to thank M. Ranall and P. Renfranz for critical reading of the manuscript, and H. Schubert for assistance with the illustration of the three-dimensional protein structures. We are also grateful to J. Matthews for sharing structural data in advance of publication. This work was supported by the Huntsman Cancer Foundation and National Institutes of Health grants to M.C.B.

Competing interests statement

The authors declare no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez: http://www.ncbi.nlm.nih.gov/Entrez/
unc-115
Flybase: http://flybase.bio.indiana.edu/
apterous | Beadex
Interpro: http://www.ebi.ac.uk/interpro/
LIM domain
Swiss-Prot: http://www.ca.expasy.ch
ABLIM | CRP1 | CRP2 | EPLIN | FHL2 | FHL3 | | LDB1 | LHX3 | LMO2 | LMO4 | MICAL | paxillin | PINCH | testin | zyxin

Access to this links box is available online.