The intricacy and diversity of cell functions require an incredible complexity and specificity of protein interactions (1). LIM domains are one of a growing number of structural motifs implicated in mediating protein-protein interactions required for regulation of transcription and maintenance of the actin cytoskeleton (2). This review will focus on the role of LIM proteins in cell signalling pathways.

LIM is an acronym of three transcription factors lin-11, isl-1 and mec-3, in which the motif was first identified. LIM domains contain 50 - 60 amino acids forming a double zinc-finger motif with the consensus sequence \((C-X_2-C-X_16-H-X_2-C)_2\) (Fig. 1).

The conserved cysteine, histidine and aspartic acid residues form two tetrahedral zinc-binding pockets, which stabilize the secondary and tertiary structure of the protein. Mutation of either the conserved cysteine or histidine, disrupts both zinc binding and the function of the LIM domain.

Fig. 1. Schematic representation of a LIM domain. The highly conserved cysteine (C) and histidine (H) amino acids form two zinc-binding pockets. The two zinc fingers are always separated by two amino acids. The more variable sequences of between 16 and 23 amino acids form the two fingers and contain the protein binding sites.
cardiomyopathy in mice (5).

The CRP family members have a joint localisation in the nucleus and associated with the actin cytoskeleton. Group 3 LIM proteins contain 1-5 predominantly C-terminal Type D and E LIM domains in association with other signalling domains such as PDZ or proline-rich motifs. Group 3 LIM proteins are predominantly cytoplasmic, many interacting with the cytoskeleton. They include the important focal adhesion proteins, paxillin and zyxin.

It has been proposed that LIM proteins, which contain up to 5 LIM domains, form a scaffold upon which the coordinated assembly of signalling proteins occurs (4). LIM-homeodomain proteins bind DNA via their homeodomain, whilst their associated LIM domains bind other protein transcription factors. The LIM domain appears to have an inhibitory effect on the associated homeodomain's ability to bind DNA. Nuclear LIM-homeodomain and LIM-only proteins regulate the interaction of transcription factors, to fine-tune gene expression, particularly of genes involved in tissue differentiation.

LIM proteins have been associated with both congenital and acquired human diseases. Mutations of the gene encoding the LIM-homeodomain protein, Lmx1b, which regulates dorsal limb patterning, cause Nail-patella syndrome (6). Heterozygosity of the LIM-kinase1 gene is responsible for the visual cognition defects occurring in Williams syndrome (7). Lmo2, a nuclear LIM protein containing only two LIM domains, is associated with the most common translocations in T-cell acute lymphoblastic leukaemia (T-ALL). Lmo2 is also essential for normal erythropoiesis; knockout mice die in utero due to severe anaemia (8).

The LIM domains of Lmo2 enable the synergistic interaction of erythroid transcription factors. Although Lmo2 knockout mice fail to develop red cells, overexpression of Lmo2 in a proerythroblast cell line inhibits red cell maturation and haemoglobinisation (9). Lmo2 might enable a population of early erythroid cells to continue proliferating by inhibiting terminal differentiation. This may explain why unregulated Lmo2 expression in T-lymphocytes causes leukaemia.

**LIM proteins in cell signalling**

Non-nuclear LIM proteins bind cytosolic signalling and cytoskeletal proteins. In a manner analogous to transcription factor complexes, cytosolic LIM proteins enable the interaction of proteins important for signal transduction and regulation of the cytoskeleton. In the cytoplasm, LIM domain binding may regulate intracellular localisation or link different signalling pathways.

For example, individual LIM domains of PINCH, comprised of five LIM domains, bind integrin-linked kinase and the adaptor protein Nck-2, comprised of SH2 and SH3 domains (10). Nck-2, binds activated tyrosine kinase growth factor receptors such as the platelet-derived growth factor (PDGF) receptor. PINCH is able to form a complex between ILK and Nck-2, and therefore links integrin and growth factor receptor signalling.

LIM-domains do not appear to bind a single consensus sequence. Instead, a number of binding partners with no overall common sequence or structural motifs have been identified. The highly conserved cysteine and histidine confer the overall structure and stability of the domains but the intervening amino acids are more variable.

These intervening amino acids determine the protein binding specificity of the domain, thus explaining the wide range of binding partners. LIM domains have been shown to homodimerise and heterodimerise, and to bind various tyrosine-containing sequences, PDZ domains, ankyrin type repeats, PKC isoforms and kinase domains. The factors influencing LIM domain binding and the role of LIM-mediated interactions in signal transduction are starting to be elucidated.

**LIM-tyrosine motif binding**

Phosphorylated tyrosine motifs are a widely utilised signalling mechanism for mediating protein-protein interactions, binding both SH2 and PTB (phospho-
tyrosine binding) domains. Other tyrosine-containing motifs, which do not require phosphorylation, have been shown to interact with LIM domains.

Such interactions have been demonstrated for the LIM protein Enigma, which contains an N-terminal PDZ domain and three C-terminal LIM domains. LIM2 of Enigma binds Ret (11) and LIM3 binds the insulin receptor (12). The respective LIM domains bind tyrosine-containing sequences, which although similar, are not interchangeable.

LIM2 of Enigma interacts with an Asn-Lys-Leu-Tyr sequence at the C-terminus of the tyrosine kinase receptor Ret (11). Germ-line mutations which inactivate Ret kinase cause Hirschsprung’s disease, resulting in defective parasympathetic innervation of the colon and bowel obstruction in infants. Conversely, mutations which activate Ret have been associated with inherited multiple endocrine neoplasms. Activated Ret mutants, which have the Enigma binding tyrosine deleted, or mutated, lose their mitogenic signalling capacity. Ret-mediated mitogenesis is also dependent on the kinase activity of Ret, whereas Enigma binding is independent of tyrosine phosphorylation.

The adaptor protein Shc binds to the same tyrosine residue of Ret as Enigma, but the binding of Shc requires the tyrosine to be phosphorylated. Binding of both Enigma and Shc to Ret, is necessary for Ret mitogenic signalling. Enigma constitutively binds Ret and localizes the activated receptor to the appropriate subcellular compartment. Whereas activation of Ret tyrosine kinase activity is needed for complex formation between Ret and Shc, which then triggers the mitogenic signalling cascade.

LIM3 of Enigma binds a different tyrosine-containing motif, Gly-Pro-Leu-Gly-Pro-Leu-Tyr, which forms a tyrosine tight-turn in the insulin receptor (InsR) (12). Tyrosine tight-turns are essential for the endocytosis of various receptors. Substitution of proline or tyrosine residues within the tyrosine tight-turn, abolishes this interaction. InsR mutants, which fail to be endocytosed, do not bind Enigma, implicating Enigma in InsR internalisation.

LIM3 of Enigma does not bind tyrosine tight-turns in the endocytic sequence of other receptors, indicating that the interaction is specific.

As for the interaction with Ret, kinase activity and phosphorylation of the tyrosine motif are not required for LIM binding. Wu and co-workers noted that the LIM-binding sequence of Ret may also form a tyrosine tight-turn because its sequence is similar to the endocytic sequence of the LDL receptor which itself forms a tight-turn (13).

A consensus tyrosine tight-turn sequence containing two copies of Asn-Asn-Ala-Tyr-Phe arranged in a helix-turn-helix, which mediates receptor endocytosis, interacts with a broader range of LIM domains, possibly indicating a more general interaction between specific LIM proteins and tyrosine tight-turn motifs.

LIM-Protein kinase C interaction

LIM domains have been shown to bind various protein kinase C (PKC) isoforms (14). PKC is a widely expressed serine/threonine kinase, which has 11 isoforms, classified into three groups, according to their sensitivity to cofactors (15). The activity and substrate specificity of PKC is also regulated by anchoring proteins, which localise PKC to distinct subcellular compartments and thereby mediate the substrates to which the enzyme has access (16). LIM protein binding of PKC may perform such an anchoring role.

The LIM domains of ENH, Enigma and LIM kinase 1 (LIMK1) bind certain PKC isoforms but not others. ENH interacts with PKC β1, γ, and ε. Enigma with PKC α, β1 and ε, and LIMK1 complexes tightly with PKC γ and ε, but weakly with α, β1, δ, and ε. These results suggest a general interaction between LIM domains and particular PKC isoforms. PKC phosphorylates ENH, although the functional significance of this phosphorylation is unknown.

Complex formation between ENH and PKC is not affected by PKC activity. However, PKC activity results in the translocation of ENH from the membrane to the cytosol. Translocation of ENH, may also translocate complexed PKC, and thereby provide access to specific substrates. Stimulation of PKC results in translocation of each isoform to and from specific subcellular compartments, thereby allowing access to specific substrates. A family of Receptors for Activated C-kinase, RACKs has been identified, which anchor PKC in different compartments (16). Similar to the LIM protein association, different RACKs bind certain PKC isoforms. However the association of PKC with RACKs is altered by cell stimulation, whereas the association with ENH is not. LIM protein binding of PKC may stabilise the interaction between PKC and its anchoring RACK protein.

LIM-serine/threonine kinases

LIM domains have been shown to associate with other serine/threonine kinases. For example, LIM1 of PINCH binds the ankyrin repeats of integrin-linked kinase (ILK), a serine/threonine kinase, implicated in integrin signalling (10).

The LIM domains of the focal adhesion protein paxillin bind a serine/threonine kinase. In addition to four C-terminal LIM domains, paxillin contains multiple N-terminal protein-binding motifs; SH2 and SH3 domains which bind src-related kinases, phosphotyrosine residues and five novel repeats called LD domains, via which it binds vinculin and focal adhesion kinase. Despite its association with multiple cytoskeletal structural and regulatory proteins via its N-terminal signalling domains, it is the C-terminal LIM domains of paxillin, which mediate association with focal adhesions (17).

LIM2 and LIM3 of paxillin bind, and are phosphorylated by, a serine/threonine kinase. Constitutive serine phosphorylation of the LIM domains of paxillin, increases the localisation of paxillin to focal adhesions and augments cell adhesion to fibronectin (18). LIM3 of paxillin also associates with the protein-tyrosine phosphatase-PEST, which may compete with the serine/threonine-kinase for binding and promote focal adhesion disassembly.

Serine/threonine phosphorylation of LIM domains may be a more general mechanism whereby their binding to target proteins is regulated, particularly given the demonstration that isoforms of the serine/threonine kinase PKC associate with LIM domains from a number of proteins (14).

LIM-kinase 1 (LIMK1) itself contains a C-terminal serine/threonine kinase domain in association with two N-terminal LIM domains. The LIM domains bind, and potentially regulate, the activity of the associated kinase domain, which phosphorylates coflin to inhibit actin motility.
The Role of LIM Proteins in Signal Transduction (contin.)

polymerisation. Such interactions potentially perform a critical role in regulation of the cytoskeleton, particularly in nerve cells (19).

LIM-PDZ domain interaction

PDZ domains comprise 80-120 amino acids and mediate protein interactions with the cytoskeleton, particularly submembrane structures. PDZ is an acronym of the three proteins PSD-95, DlgA, ZO-1 in which the motif was first described. PDZ domains form a hydrophobic pocket, particularly recognising the four or five C-terminal amino acids of the target binding protein.

However, PDZ domains have also been shown to bind other internal protein sequences. RIL, reversion induced LIM protein, originally identified as a gene down-regulated in transformed fibroblasts, comprises an N-terminal PDZ domain in association with a single C-terminal LIM domain. The C-terminal LIM domain of RIL interacts both with its own N-terminal PDZ domain, and the PDZ domain of the protein tyrosine phosphatase, PTB-BL (20).

The LIM domain of RIL contains a consensus tyrosine phosphorylation sequence, which is phosphorylated by an unknown kinase and dephosphorylated by the phosphatase domain of PTB-BL. It is not known how the tyrosine phosphorylation or dephosphorylation of RIL’s LIM domain regulates the association between RIL and the PDZ domain of PTP-BL. The self-association between the LIM domain and PDZ domain of RIL may be tyrosine phosphorylation-dependent and thereby regulate LIM binding to other proteins, such as PTP-BL.

Other LIM domain containing proteins, such as Enigma, LIMK1 and CLP36 also contain an N-terminal PDZ domain. The self-association demonstrated between the N-terminal PDZ domain and C-terminal LIM domain of RIL, therefore could represent a more general interaction and regulate LIM binding to other proteins. The PDZ domain of these proteins often binds to the actin cytoskeleton, whereas the LIM domains mediate binding to various kinases and phosphatases.

This suggests a potential role in regulation of the actin cytoskeleton. For example, the PDZ domain of enigma binds to tropomyosin and, as outlined above, its LIM domains bind to PKC isoforms and the Ret and insulin receptor tyrosine kinases.

Conclusion

LIM proteins contain multiple LIM and non-LIM protein-binding domains. They are therefore archetypal molecular scaffolding proteins, enabling the formation of protein complexes. In the nucleus, LIM proteins enable the interaction of transcription factors to regulate differentiation. Outside the nucleus they enable the formation of signalling complexes, particularly associated with the actin cytoskeleton. LIM proteins link different signalling pathways.

LIM domains bind both tyrosine and serine threonine kinases and tyrosine phosphatases, enzymes critical for the dynamic regulation of the cell and cytoskeleton. In several instances, phosphorylation of the LIM domain has not been shown to directly influence protein binding. LIM domain phosphorylation, however, results in changes in subcellular localisation of LIM proteins. LIM proteins often have overlapping binding interactions and, therefore, possibly stabilise or regulate protein interactions.

References

Fig. 1

Group 1

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LIM-homeodomain proteins
Lmo-1
LIM-kinase 1

Fig. 2

Group 2

[ C² | C²]

CRP1,2,3/MLP

Group 3

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Enigma, ENH
Ril
Paxillin
PINCH