LETTER TO THE EDITOR

A LIM Motif Is Present in a Pollen-Specific Protein

We have recently described a sunflower cDNA sequence coding for a pollenspecific protein (SF3) with putative zinc finger domains (Baltz et al., 1992). In a more recent analysis we have found that these domains correspond to the conserved LIM motif identified so far only in a family of metal binding, cysteine-rich proteins from animals. This motif, ~55 amino acids long, is characterized by a unique organization of cysteine and histidine residues into two adjacent putative zinc fingers. LIM motif-containing proteins include developmental regulators such as the rat insulin gene enhancer binding protein ISL-1 (Karlsson et al., 1990), the Caenorhabditis elegans proteins LIN-11 (Freyd et al., 1990) and MEC-3 (Way and Chalfie, 1988), the Drosophila AP-TEROUS protein (Cohen et al., 1992), the Xenopus XLIM-1 protein (Taira et al., 1992), and the mammalian oncoproteins TTG-1 and TTG-2 (also known as RHOM-2) of the rhombotin family (McGuire et al., 1989; Boehm et al., 1990, 1991; Royer-Pokora et al., 1991). The mammalian cysteine-rich proteins CRIP (Birkenmeyer and Gordon, 1986), hCRP (Liebhaber et al., 1990; Wang et al., 1992), and ESP-1 (Nalik et al., 1989), all of which are of yet unknown function, also contain LIM motifs. LIM motifs are found either alone (in CRIP, TTG-1, TTG-2, ESP-1, and hCRP) or in association with a homeodomain (in MEC-3, ISL-1, LIM-11, XLIM-1, and APTEROUS).

Figure 1 shows an alignment of the LIM motifs of the pollen-specific protein SF3 with those of the animal LIM proteins. Conserved residues are shown in bold type. A close examination of a number of semi-

conserved positions (see boxed residues) shows evidence for the existence of two subfamilies of LIM proteins: subfamily A, which includes SF3, hCRP, CRIP and ESP-1, and subfamily B, which comprises the seven other proteins.

The most frequently occurring metalchelating residues in the potential zinc fingers are cysteines and histidines. However, in the majority of the LIM proteins, aspartate (D) is the last residue in the second finger (position 57). This is not necessarily surprising because aspartate has been identified as a metal-chelating residue in zinc-containing enzymes (Vallee and Auld, 1990).

As potential zinc finger domains, the LIM motifs could be directly involved in DNA binding, although a possible role in protein-protein interactions has been

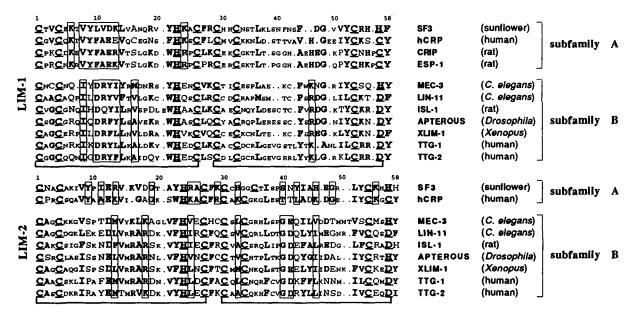


Figure 1. Alignment of the SF3 LIM Motifs with Other LIM Sequences.

Bold type letters indicate conserved and semiconserved residues. Unconserved residues are in standard type. Putative zinc fingers in the LIM domains are delimited by horizontal brackets. Potential metal-chelating residues are underlined. LIM subfamily-specific residues are boxed. CRIP and ESP-1 have only one LIM motif, whereas all of the other proteins have two LIM motifs. CRIP and ESP-1 are highly related, as are TTG-1 and TTG-2 (which is identical to RHOM-2).

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proposed (Boehm et al., 1990). The observation that LIM motifs and DNA binding homeodomains can coexist in the same protein does not preclude the LIM motif from also being a DNA binding domain. Together with the homeodomain, the LIM domain could bind to the regulatory regions of developmentally controlled genes, as has been proposed for the paired box, a conserved sequence motif first identified in the paired (PRD) and gooseberry (GSB) homeodomain proteins from Drosophila (Treisman et al., 1991). The PRD box is also able to bind DNA in the absence of the homeodomain.

The LIM region of the LIN-11 protein is an iron-, sulfur-, and zinc-containing metallodomain (Li et al., 1991). It has been proposed that LIM proteins containing iron-sulfur clusters might act as redox-sensitive transcriptional regulators, modulating their activity in response to a redox signal such as the level of oxygen or another redox-active molecule.

It is possible that the SF3 protein is involved in controlling pollen-specific processes such as male gamete maturation, pollen tube formation, or even fertilization. However, it is also possible that this protein plays another, yet unknown, biological function. Further experiments should clarify this point.

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REFERENCES

- Baltz, R., Domon, C., Pillay, D.T.N., and Steinmetz, A. (1992). Characterization of a pollen-specific cDNA from sunflower encoding a zinc finger protein. Plant J. 2, 713–721.
- Birkenmeyer, E.H., and Gordon, J.I. (1986).

 Developmental regulation of a gene that encodes a cysteine-rich intestinal protein and maps near the murine immunoglobin heavy chain locus. Proc. Natl. Acad. Sci. USA 83, 2516–2520
- Boehm, T., Foroni, L., Kennedy, M., and Rabbitts, T.H. (1990). The rhombotin gene belongs to a class of transcriptional regulators with a potential novel protein dimerization motif. Oncogene 5, 1103–1105.
- Boehm, T., Foroni, L., Kaneko, Y., Perutz, M.F., and Rabbitts, T.H. (1991). The rhombotin family of cysteine-rich LIM-domain oncogenes: Distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. Proc. Natl. Acad. Sci. USA 88, 4367–4371.
- Cohen, B., McGuffin, E., Pfeifle, C., Segal, D. and Cohen, S.M. (1992). Apterous, a gene required for imaginal disk development in Drosophila encodes a member of the LIM family of developmental regulatory proteins. Genes Dev. 6, 715–729.
- Freyd, G., Kim, S.K., and Horvitz, H.R. (1990).
 Novel cysteine-rich motif and homeodomain in the product of the Caenorhabditis elegans cell lineage gene lin-11. Nature 344, 876–879.
- Karlsson, O., Thor, S., Norberg, T., Ohlsson, H., and Edlund, T. (1990). Insulin gene enhancer binding protein IsI-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. Nature 344, 879–882.
- Li, P.M., Reichert, J., Freyd, G., Horvitz, H.R., and Walsh, C.T. (1991). 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.

- Liebhaber, S.A., Emery, J.G., Urbanek, M., Wang, X., and Cooke, N.E. (1990). Characterization of a human cDNA encoding a widely expressed and highly conserved cysteine-rich protein with an unusual zincfinger motif. Nucl. Acids Res. 18, 3871–3879.
- McGuire, E.A., Hockett, R.D., Pollock, K.M., Bartholdi, M.F., O'Brien, S.J., and Korsmeyer, S.J. (1989). The t(11;14)(p15;q11) in a T-cell acute lymphoblastic leukemia cell line activates multiple transcripts, including *Ttg-1*, a gene encoding a potential zinc finger protein. Mol. Cell. Biol. 9, 2124–2132.
- Nalik, P., Panayotova-Heiermann, M., and Pongs, O. (1989). Characterization of an estradiol-stimulated mRNA in the brain of adult male rats. Mol. Cell. Endocrinol. 62, 235–242.
- Royer-Pokora, B., Loos, U., and Ludwig, W.D. (1991). TTG-2, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the t(11;14)(p13;q11). Oncogene 6, 1887–1894.
- Taira, M., Jamrich, M., Good, P.J., and David, I.B. (1992). The LIM domain-containing homeo box gene Xlim-1 is expressed specifically in the organizer region of Xenopus gastrula embryos. Genes Dev. 6, 356–366.
- Treisman, J., Harris, E., and Desplan, C. (1991). The paired box encodes a second DNA binding domain in the paired homeo domain protein. Genes Dev. 5, 594~604.
- Vallee, B.L., and Auld, D.S. (1990). Active-site zinc ligands and activated H₂O of zinc enzymes. Proc. Natl. Acad. Sci. USA 87, 220–224.
- Wang, X., Lee, G., Liebhaber, S.A., and Cooke, N.E. (1992). Human cysteine-rich protein: A member of the LIM/double finger family displaying coordinate serum induction with c-myc. J. Biol. Chem. 267, 9176–9184.
- Way, J.C., and Chalfie, M. (1988). mec-3, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in C. elegans. Cell 54, 5–16.