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HYPOTHESIS

PHOSPHORYLATION OF FILAMIN (ABP-280) REGULATES THE BINDING TO THE LIPID MEMBRANE, INTEGRIN, AND ACTIN

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Actin-binding protein (ABP-280; filamin) is a phosphoprotein present in the periphery of the cytoplasm, where it can cross-link actin filaments, associate with lipid membranes, and bind to membrane surface receptors. Given its function and localization in the cell, the hypothesis that it serves as a substrate for $p56^{lck}$, a lymphocyte-specific member of the *src* family of protein tyrosine kinases associated with cell surface glycoproteins is considered. The results suggest conformationally-induced regulation of filamin (ABP-280).

KEYWORDS: tyrosine phosphorylation; filamin (ABP-280); actin; integrin; lipid membrane.

FILAMIN (ABP-280)—LIPID MEMBRANE ASSOCIATION

Proteins at the interface between the cytoskeleton and the plasma membrane control cell shape and tension and stabilize attachments to other cells and to the substrate. Many of these proteins exist in soluble form in the cytoplasm, which could allow them to associate transiently with the lipid boundary (Luna and Hitt, 1992). In this aggregate form, proteins are likely to interact in a two-step mechanism: an initial electrostatic attraction is followed by lipid insertion after refolding of the protein. This event occurs only when lipid membranes find compatible configurations i.e., α-helices or β-strands on the protein. Surface binding to polar lipid headgroups is usually achieved by exposing amphipathic α-helices; thus insertion into one-half of the hydrophobic bilayer requires a β-barrel or hydrophobic α-helix formation. Normally, when a primary amino acid sequence is known, the method to describe these events affords highly accurate predictions. The hydrophobicity index for each amino acid and the probability, if a protein is membrane-spanning, is derived from hydrophobic plots (Kyte and Doolittle, 1982; Eisenberg *et al.*, 1984). Tempel *et al.* (1995) have used a purpose-written matrix to discriminate between surface-seeking and transmembrane configurations of α-helices. By applying this method, they have been able to predict potential lipid-binding motifs for several proteins with high accuracy including filamin (ABP-280), vinculin, talin, and α-actinin (Tempel *et al.*, 1994a, 1995; Goldmann *et al.*, 1999a). The proposed lipid-binding sites for filamin (ABP-280) have been confirmed by (a) expressing these regions as fusion proteins attached to schistosomal GST (glutathione S-transferase), (b) reconstituting these proteins and intact filamin (ABP-280) into phospholipid layers, and (c) using differential scanning calorimetry, hydrophobic photolabeling, and film balance technique to measure their incorporation (Tempel *et al.*, 1994b; Goldmann *et al.*, 1999b).

FILAMIN (ABP-280) PHOSPHORYLATION

Filamin (ABP-280) is a phosphoprotein present in the periphery of the cytoplasm where it is believed to cross-link actin filaments, bind to membrane

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surface receptors, and associate with lipid membranes (Isenberg, 1991; Isenberg and Niggli, 1998). Based on the reported function and localization of filamin (ABP-280) in lymphocytes (Loo *et al.*, 1998), Sharma *et al.* (1995, 1998) found it localized under the plasma membrane and attached $p56^{lck}$ to the cytoplasmic face through the myristoylated N-terminal glycine. These researchers investigated whether filamin (ABP-280) serves as substrate for $p56^{lck}$, a lymphocyte-specific member of the *src* family of protein tyrosine kinases associated with cell surface glycoproteins (Sharma *et al.*, 1998). They addressed this possibility by utilizing immunopurified filamin (ABP-280) and recombinant $p56^{lck}$ *in vitro* kinase assays and found that tyrosine residue(s) on filamin (ABP-280) are specifically phosphorylated and that phosphorylation is restricted to a single tryptic or V8 peptide. The phosphorylation of filamin (ABP-280) was not due to endogenous kinases associated with filamin (ABP-280), as phosphorylation increased by one order of magnitude when kinasing was performed in the presence of $p56^{lck}$. Using antiserum against $p56^{lck}$, they successfully co-immunoprecipitated filamin (ABP-280) from peripheral blood lymphocytes (PBL) and T-cell line (CEM) lysates; thus co-immunoprecipitation of $p56^{lck}$ was not observed when antibodies against filamin (ABP-280) were used from different cells and detergents. The inability to co-immunoprecipitate $p56^{lck}$ with filamin (ABP-280) is not yet understood, despite the direct interaction of purified $p56^{lck}$ with immunopurified filamin (ABP-280).

Previously, it was demonstrated that protein tyrosine kinase $p56^{lck}$, CD4, and CD8 associate (Zhuang *et al.*, 1984) and that the CD4 -$p56^{lck}$ complex plays an integral role in generating signals that lead to T-cell activation by inducing phosphorylation of a number of transmembrane and intracellular proteins (Kadena *et al.*, 1997). Tyrosine phosphorylation of certain proteins is the earliest event triggered after activation of resting lymphocytes which suggests that protein tyrosine kinases have an important function. The major tyrosine kinases expressed in human T-lymphocytes are $p56^{lck}$, $p59^{fyn}$, and $p62^{yes}$ (Torigoe *et al.*, 1992). Several surface receptors including CD4 have been shown to be associated directly or indirectly with $p56^{lck}$ and recent studies have demonstrated that the *in vitro* activity of $p56^{lck}$ increased six- to eightfold after cross-linking with CD4 (Koyasu *et al.*, 1992; Parolini *et al.*, 1996). One of the substrates phosphorylated by the increased activity of $p56^{lck}$ is the ζ-subunit of T-cell antigen receptors. The tyrosine phosphorylation of the ζ-subunit is based on its affinity after cross-linking with CD4, which in turn increases the catalytic activity of $p56^{lck}$. The phosphorylation of ζ-subunit may also involve other known kinases (Burgess *et al.*, 1992).

FILAMIN (ABP-280) BINDING MECHANISM

One possible function of *in vivo* phosphorylation of filamin (ABP-280) by $p56^{lck}$ is that it regulates its association with surface receptors, i.e. β2-integrins (Sharma *et al.*, 1995), lipid membrane binding (Tempel *et al.*, 1994b) and cross-linking of actin filaments into networks (Marti *et al.*, 1997). The other is the protection of filamin (ABP-280) from proteolysis (Yada *et al.*, 1990). That the phosphorylation of filamin (ABP-280) by $p56^{lck}$ does not alter the susceptibility toward calpain suggests that phosphorylation of filamin (ABP-280) in T-lymphocytes has probably a different function (Sharma *et al.*, 1998). It is therefore tempting to speculate that the increased avidity of T-lymphocytes could be due to the activation of $p56^{lck}$ after cross-linking with CD4, which then phosphorylates filamin (ABP-280) in the periphery of cells. Events of phosphorylation and dephosphorylation of filamin (ABP-280) have been postulated to play a dynamic role in the organization of the cytoskeleton (Kovacsovics and Hartwig, 1996). This was supported by the finding that phosphorylation of filamin (ABP-280) by $p56^{lck}$ is highly effective when filamin (ABP-280) isolated from cells is treated with phorbol myristate acetate (PMA) (Sharma *et al.*, 1998). These results suggest that phosphorylation of filamin (ABP-280) by purified $p56^{lck}$ and induced by PMA probably target different residues on filamin (ABP-280). It is intriguing that PMA may activate or inactivate other kinases or influence the confirmation of filamin (ABP-280) that affects the activity of $p56^{lck}$ (Wang *et al.*, 1997).

These results allow some hypothetical considerations of the interaction of filamin (ABP-280) and $p56^{lck}$. As shown by Sharma *et al.* (1995, 1998) the $p56^{lck}$ molecule is associated through its N-terminus peptide with filamin (ABP-280). This region contains cysteine residues with T-cell surface glycoprotein CD4, which also contains cysteine residues in the cytoplasmic domains to form a bimolecular complex. When bound, the catalytic domain of $p56^{lck}$ (present in the C-terminal half of the molecule) is free to perform phosphorylation of its substrate, thus making this molecule less mobile. This interaction between CD4 and $p56^{lck}$ is

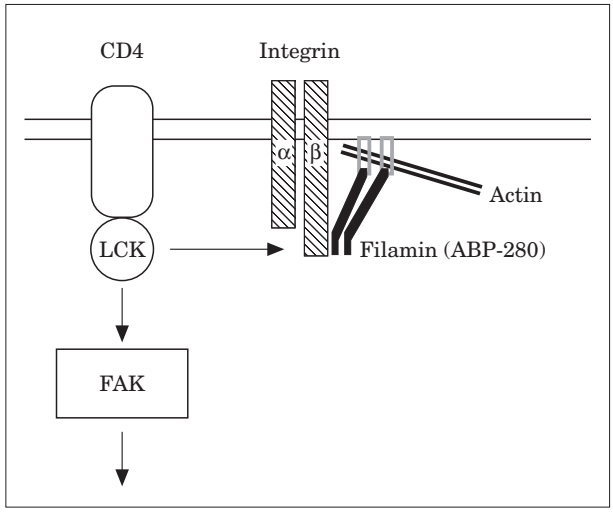

Fig. 1. A working model of a potential stimulatory pathway of $p56^{lck}$ that could regulate tyrosine phosphorylation of filamin (ABP-280) enabling it to associate with the membrane, integrin, and cytoskeleton. Note: for simplicity, this scheme does not include possible serine/threonine phosphorylation pathways by kinases like casein kinase 1, 2, or CaM kinase II.

probably responsible for the inability of $p56^{lck}$ to co-immunoprecipitate with filamin (ABP-280), despite the fact that $p56^{lck}$ phosphorylates filamin (ABP-280). The coordination complex between CD4 and $p56^{lck}$ serves probably a dual purpose: first, it helps the CD4 molecule to remain on the surface, as disruption of the complex leads to rapid internalization of CD4 (Marti *et al.*, 1997); and second, it renders the $p56^{lck}$ molecule less mobile so that it can perform specific functions.

At this point it is not known whether other cytoskeletal proteins are also substrate for $p56^{lck}$, and what effect serine or threonine phosphorylation has on filamin (ABP-280); thus, tyrosine phosphorylation suggests its conformationally induced regulation and binding to the membrane, integrin, and cytoskeleton (Fig. 1).

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