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Lim kinase regulates the development of olfactory and neuromuscular synapses

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Abstract

Lim Kinase (Limk) belongs to a phylogenetically conserved family of serine/threonine kinases, which have been shown to be potent regulators of the actin cytoskeleton. Despite accumulating evidence of its biochemical actions, its in vivo function has remained poorly understood. The association of the *Limk1* gene with Williams Syndrome indicates that proteins of this family play a role in the nervous system. To unravel the cellular and molecular functions of Limk, we have either knocked out or activated the *Limk* gene in *Drosophila*. At the neuromuscular junction, loss of *Limk* leads to enlarged terminals, while increasing the activity of *Limk* leads to stunted terminals with fewer synaptic boutons. In the antennal lobe, loss of *Limk* abolishes the ability of *p21-activated kinase* (*Pak*) to alter glomerular development. In contrast, increase in *Limk* function leads to ectopic glomeruli, a phenotype suppressible by the coexpression of a hyperactive *Cofilin* gene. These results establish Limk as a critical regulator of Cofilin function and synapse development, and a downstream effector of Pak in vivo.

Keywords: Olfactory; Antennal Lobe; Glomeruli; Synapse; Pak; Limk; Cofilin

Introduction

Neuronal pathfinding and synaptogenesis are critical events in the formation of the intricate pattern of connectivity in the brain. At the heart of these events is the precise regulation of the axonal actin cytoskeleton by signals from the environment and from inside the cell. In recent years, the Lim-kinase (Limk) protein has emerged as an important link between regulatory cues and the actin cytoskeleton. Limk belongs to a novel, evolutionarily conserved family of serine/threonine kinases, characterized by two N-terminal LIM motifs, a central PDZ motif, and a C-terminal Kinase domain (Fig. 1A). Numerous experiments, in which members of this family were exogenously expressed in cultured cells, showed that Limks are potent inducers of the actin cytoskeleton (Nagata et al., 1999; Ohashi et al., 2000; Sumi et al., 1999). Associated biochemical studies have also begun to unravel the molecular mechanisms by which Limk directs actin cytoskeletal assembly. In vitro kinase assays using the mammalian Limk1 protein show that it directly phosphorylates and inactivates Cofilin, a major actin-depolymerizing factor (Arber et al., 1998; Lappalainen and Drubin, 1997; Rosenblatt et al., 1997; Yang et al., 1998). Consistent with the notion that *Cofilin* inactivation is a principal mechanism by which *Limk1* promotes actin polymerization, coexpression of *Cofilin* strongly curtailed *Limk1*'s ability to induce actin polymerization (Arber et al., 1998; Yang et al., 1998). A recent biochemical study also shows that human Limk1 is directly phosphorylated and activated by the human p21-activated kinase (Pak) (Edwards et al., 1999), an enzyme that is stimulated by Rac and Cdc42 (Daniels and Bokoch, 1999; Manser et al., 1994). Taken together, the results have led

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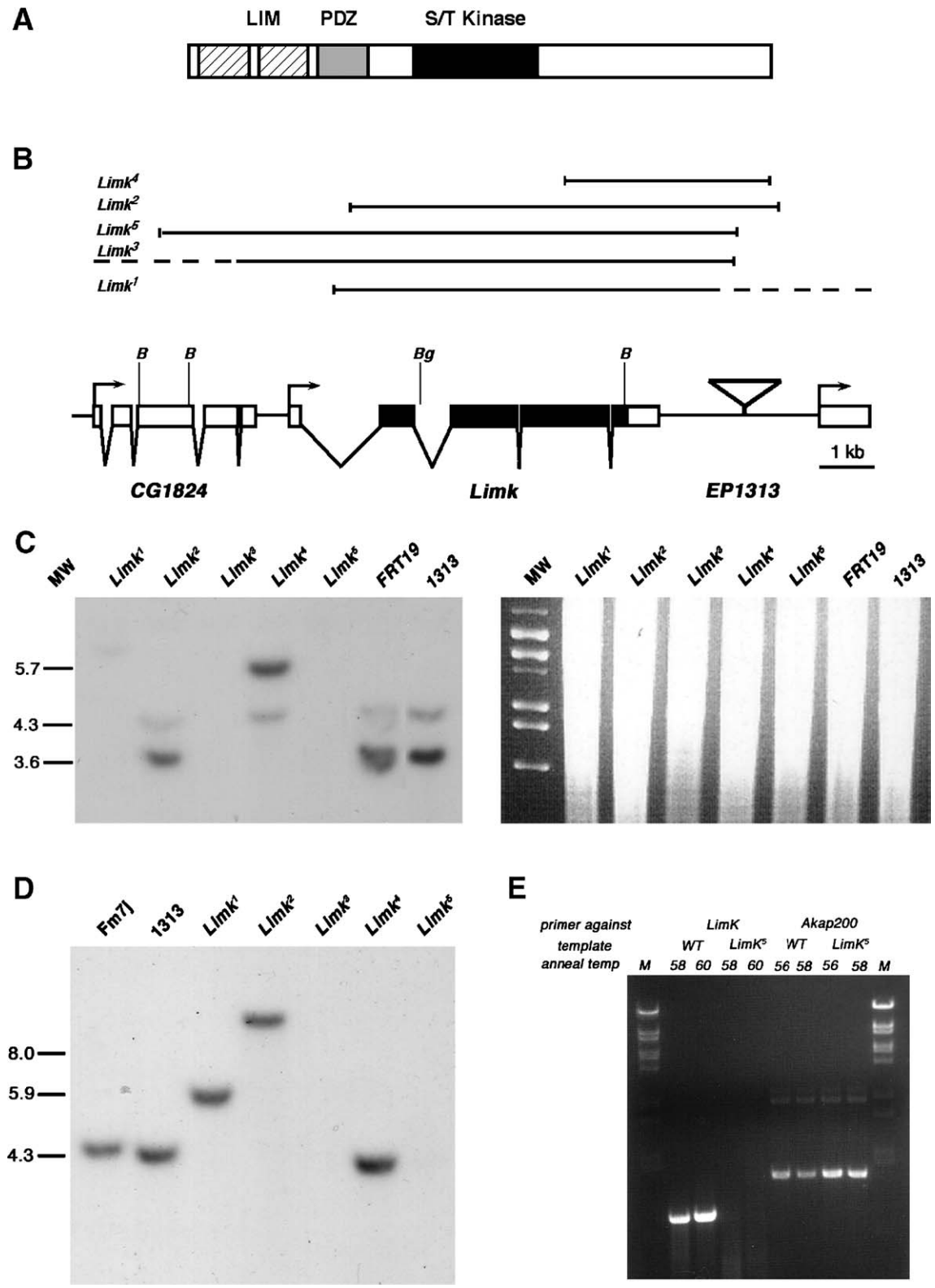

Fig. 1. The fly *Limk* gene is mutated by imprecise transposon excision. (A) The Limk protein consists of an N-terminal regulatory domain with two LIM and a PDZ motif, a kinase domain and a long C-terminal tail. (B) The fly *Limk* gene consists of 5 exons that span 6 kb. By mobilizing a P element, *EP(X)1313* located 2.8 kb downstream of *Limk*, 5 deletion alleles of the *Limk* gene were generated. The extents of the deletions in each of the alleles are indicated above the *Limk* gene. (C) Southern blot of the agarose gel (right). Genomic DNA of *Limk* mutants and control animals were digested with *Bam*H1 and Bgl2 and probed with the *Limk* cDNA. The 3.6 kb band is more intense than the 4.3 kb band because it contains more of the *Limk* cDNA. The gene is deleted in the $Limk^1$, $Limk^3$, and $Limk^5$ alleles. (D) When probed with promoter sequences, alleles $Limk^3$ and $Limk^5$ also show the loss of 5′ regulatory sequences. (E) RT-PCR of mRNA from $Limk^5$ and wild type (using primers against *Limk* or a control gene, *Akap200*) showed that the mutant does not express any *Limk* transcripts.