STRUCTURAL BIOLOGY

Allosteric Protein Kinase Regulation by Pseudokinases: Insights from STRAD

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Protein kinases regulate a plethora of diverse cellular functions. Their highly controlled activation is subject to an equally diverse repertoire of regulatory mechanisms. Pseudokinases, a class of proteins that possess a structurally related protein kinase domain that lacks phospho-transfer function, are emerging as critical yet mysterious regulators of other protein kinases. A new structural and functional analysis of the pseudokinase STRAD provides insight into the mechanism by which it allosterically regulates the catalytic function of the protein kinase LKB1 and hints at an evolution from a classical kinase-substrate relationship.

Protein kinases regulate virtually all aspects of eukaryotic cellular biology, typically by catalyzing the covalent attachment of a phosphate from adenosine 5′-triphosphate (ATP) onto specific seryl, threonyl, or tyrosyl residues in substrate proteins. The addition of a phosphate moiety directly impinges on substrate protein function by altering its conformation or promoting interactions with myriad downstream factors that couple activation of the kinase to a specific cellular response (or both). About 2% of all eukaryotic genes encode proteins containing the conserved catalytic core necessary for protein kinase function, referred to as the protein kinase domain (*1*).

Nearly 10% of kinase domain–containing proteins are classified as "pseudokinases" owing to the presence of one or more point mutations of conserved catalytic residues lining the active site, which are predicted to ablate nucleotide binding, phosphoryl-transfer, or both (*1*). Pseudokinases were initially thought to function primarily as organizing centres or scaffolds (or adaptors) in the cell. However, recent structural characterization of kinase domains from pseudokinases has revealed some unexpected findings (*2*–*6*), such as the ability to tightly bind nucleotides in a regulated manner despite the loss of multiple conserved residues that normally mediate kinase-nucleotide interactions (*4*). If pseudokinases simply act as inert docking modules as previously thought, it seems paradoxical that they have retained certain kinase-specific attributes through evolution. An emerging notion that might provide a new framework for reevaluating the role of pseudokinases is the intriguing finding that some pseudokinases possess the ability to allosterically regulate their kinase-competent counterparts (*7*). Zeqiraj *et al.* provide the first structural characterization of the mechanism of action of the pseudokinase STRAD in activating the kinase domain of LKB1, shedding new light on the evolutionary history of pseudokinases (*8*).

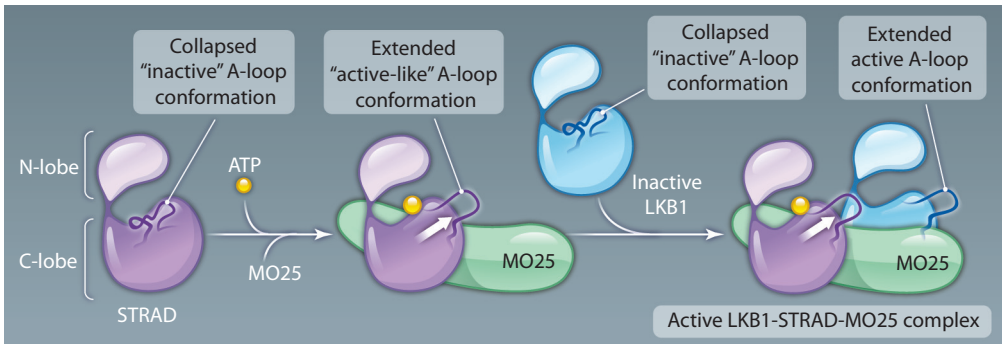

Fig. 1. STRAD allosterically activates the kinase domain of LKB1. The kinase domain of STRAD is presumed to be in an inactive conformation in its isolated state. The binding of ATP and MO25 to STRAD transitions its kinase domain to an active-like kinase conformation that is characterized by the extended conformation of its A-loop and allows STRAD to bind LKB1 as a pseudosubstrate. STRAD binding allosterically induces the kinase domain of LKB1 to adopt an active kinase conformation, which is further stabilized by the binding of MO25 to the A-loop of LKB1 that positions the loop in an extended conformation.

The pervasiveness of protein kinases in regulating cellular functions stems from the ability of the kinase domain to cycle between catalytically active and inactive states. The interconversion between these two states is tightly regulated and exhibits a diversification in the precise repertoire of catalytic switching mechanisms across the kinase superfamily (*9*). In general, catalytic switching mechanisms can involve (i) direct modulation of structural elements within the kinase domain, (ii) intramolecular interaction of the kinase domain with other regions of the protein, or (iii) intermolecular interactions with regulatory or substrate proteins. Often, several regulatory mechanisms are co-deployed in the same kinase to ensure strict control over its activity.

The kinase domain adopts a bilobal structure with the cleft between the two lobes composing the active site for the phosphoryl-transfer reaction (Fig. 1). The N-terminal lobe is characterized by a predominantly β-sheet architecture and a single helix referred to as helix αC, which serves as a critical transducer of conformational change to the catalytic cleft. A large, dynamic element of the kinase domain termed the activation loop (A-loop) is found in the C-terminal lobe. The A-loop is subject to phospho-regulation in most protein kinases whereby specific site(s) in the loop are phosphorylated in the active state of the kinase, leading to stabilization of the loop in an open and extended conformation that is permissive for substrate binding. Perturbation of the conformations of helix αC or the A-loop (or both) represent two common mechanisms of kinase regulation.

LKB1 is a tumor suppressor protein kinase, which regulates cellular energy status, proliferation, and cell polarity (*10*). Unlike most kinases, however, LKB1 is not activated by phosphorylation of its A-loop but is instead activated upon binding to the pseudokinase STRAD through a direct interaction of their kinase domains, suggesting a possible allosteric mechanism for LKB1 activation. Zeqiraj *et al.* previously reported that the

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pseudokinase STRAD adopts an "active-like" kinase conformation that underlies to its ability to activate LKB1 (*4*). Specifically, the A-loop of STRAD adopts an extended conformation that is reminiscent of the A-loop of most active protein kinases. Moreover, STRAD retains the ability to bind to nucleotides, albeit through noncanonical kinase-nucleotide interactions, the most striking of which is the absence of a highly conserved Asp residue in the Asp-Phe-Gly motif of the kinase domain that is normally key in coordinating ATP phosphate groups. The ability of STRAD to adopt an active-like kinase conformation is thought to be regulated through its interactions with both nucleotides and the scaffold protein MO25 (Fig. 1). This data suggested that the adoption of an active-like kinase conformation per se is critical for the ability of STRAD to activate LKB1 (Fig. 1).

The new structure of the ternary complex of LKB1-STRAD-MO25 reveals that STRAD engages LKB1 in a manner reminiscent of kinase-substrate interactions in which LKB1 binds as a "substrate" of STRAD (*8*). This mode of binding provides an explanation as to why STRAD must adopt an active-like kinase conformation, most notably one in which the A-loop of STRAD adopts an extended conformation competent for "substrate" recognition as often visualized in co-crystal structures of kinase-substrate interactions. STRAD uniquely recognizes elements on the LKB1 kinase domain that often undergo conformational change between active and inactive kinase states, suggesting a basis by which STRAD allosterically modulates LKB1 activity. Furthermore, MO25 also engages LKB1 and appears to position the A-loop in a productive conformation, thus explaining why LKB1, unlike most kinases, does not depend on phosphorylation of its A-loop for activity (Fig. 1).

The conformational plasticity of the kinase fold has been exploited through evolution for purposes beyond simply carrying out the transfer of phosphate. Indeed, the case of pseudokinases underscores the notion of conformational transitions within the kinase fold acting as switches for cellular events. The structural characterization of STRAD reveals how the adoption of an active-like kinase conformation as a result of ATP and MO25 binding in turn drives the allosteric activation of LKB1, whose kinase domain is itself conformationally receptive to STRAD binding (*8*).

In addition to the mechanism of action of STRAD, recent studies have revealed that, for certain kinases like epidermal growth factor receptor (EGFR) and Raf that are activated by the formation of specific allosteric dimers, closely related pseudokinase variants can also serve as direct activators by retaining the ability to form allosteric heterodimers, despite having dispensed with phosphoryl-transfer function (*11*, *12*). Reflecting the importance of self-interaction in the function of both EGFR and Raf protein kinase families, residues composing the self-interaction surfaces of the kinase domain have been selectively conserved through evolution between kinase-active and pseudokinase members. In effect, the kinase-active and pseudokinase member within each family evolved from a single ancestral progenitor, one that possessed both protein kinase catalytic activity and a noncatalytic allosteric function. Following a gene duplication event, one gene dispensed with the phosphoryl-transfer function to take on the dedicated task of functioning as an allosteric activator of its kinase-active partner.

Pseudokinases appear to have evolved opportunistically. Take, for example, the cases of inositol-requiring enzyme 1 (Ire1) and ribonuclease L (RNaseL), which compose a subfamily of proteins possessing fused protein kinase and ribonuclease domains (*13*, *14*). The ribonuclease activity of both enzymes is regulated by the adoption of a specific dimer configuration through their kinase domains, and dimerization in turn is potentiated by nucleotide binding to the kinase domain (*15*). Thus, the kinase domains in Ire1 and RNaseL merely function as regulated dimerization modules that activate the latent ribonuclease function. In this regard, although Ire1 still retains phosphoryl-transfer function, RNaseL has completely dispensed with phorphoryl-transfer function altogether.

The case of STRAD in the LKB1 activation process represents a distinct evolutionary pathway for a pseudokinase, one in which the trajectory of evolution is more cryptically hidden. LKB1 and STRAD localize to different regions of the kinome tree (*1*), and, hence, STRAD is not simply a kinase-dead version of LKB1. Instead, the observation that LKB1 binds to STRAD in a manner reminiscent of how protein kinases recognize their substrates suggests an origin in which STRAD once phosphorylated LKB1 to regulate its function. This enzyme-substrate relationship could have evolved such that the STRAD-LKB1 interaction per se functioned as a switch for LKB1 activation, which in turn might have led STRAD to lose its phosphoryl-transfer function altogether.

Although the origin of the STRAD-LKB1 regulatory relationship is distinct from others characterized to date, the ability of STRAD to allosterically regulate LKB1 is a common feature of this pseudokinase and is a departure from the conventional dogma that pseudokinases function as passive scaffolds. This provides renewed impetus for revisiting other pseudokinases that may have been overlooked as less-than-interesting topics of study in the past.

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