Structure
Review

Structural Basis for the Non-catalytic Functions of Protein Kinases

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Protein kinases are known primarily for their ability to phosphorylate protein substrates, which constitutes an essential biological process. Recently, compelling evidence has accumulated that the functions of many protein kinases extend beyond phosphorylation and include an impressive spectrum of non-catalytic roles, such as scaffolding, allosteric regulation, or even protein-DNA interactions. How the conserved kinase fold shared by all metazoan protein kinases can accomplish these diverse tasks in a specific and regulated manner is poorly understood. In this review, we analyze the molecular mechanisms supporting phosphorylation-independent signaling by kinases and attempt to identify common and unique structural characteristics that enable kinases to perform non-catalytic functions. We also discuss how post-translational modifications, protein-protein interactions, and small molecules modulate these non-canonical kinase functions. Finally, we highlight current efforts in the targeted design of small-molecule modulators of non-catalytic kinase functions, a new pharmacological challenge for which structural considerations are more important than ever.

Introduction

By catalyzing phosphorylation, protein kinases play a central role in regulating cellular homeostasis. This enzymatic function is mediated by the conserved kinase domain fold, which is highly preserved among all protein kinases. Despite their structural conservation, protein kinases exhibit remarkable diversity in their ability to recognize unique sets of substrates, as well as other binding partners that might serve as activators or inhibitors. The specificity of these interactions is often encoded in unique binding sites within the kinase domain itself. A kinase domain therefore skillfully couples its role as a catalytic enzyme with a role as a protein scaffold to orchestrate an efficient and specific phosphotransfer.

Studies over the past decades have revealed that scaffolding functions of kinases extend beyond a mere supporting role in phosphorylation. This was initially suggested by observations that inhibition of kinase function can result in fundamentally different biological outputs depending on the experimental approach used to inhibit the enzyme. For example, genetic knockout of the epidermal growth factor receptor (EGFR) results in animal death soon after birth (Miettinen et al., 1995), while loss of EGFR activity through a ''kinase-dead'' V743G mutation produces only mild epithelial defects (Luetteke et al., 1994). Similarly, there are significant phenotypic discrepancies between manifestations of Parkinson's disease observed in mice in which leucine-rich repeat kinase 2 (LRRK2) is knocked out versus inactivated by a D1994S (D166 in protein kinase A [PKA]) mutation (Herzig et al., 2011). Although these discrepancies might be partially attributed to the fact that mutations or inhibitor treatment, as opposed to gene knockdown, might still preserve a low level of kinase activity, they also suggest an alternative, more exciting possibility that the functions of kinases are not limited to catalyzing phosphorylation but extend to other roles that are independent of enzymatic activity. These divergent outcomes of kinase inactivation have been found for an increasing number of kinases, indicating that the roles of kinases in cellular signaling are considerably more complex than previously thought (Figure 1 and Table 1).

The potential of the kinase domain fold to signal through an alternative non-catalytic mechanism is perhaps best exemplified by a subset of protein kinases that have seemingly evolved to lack catalytic activity and have thus been termed ''pseudokinases.'' Pseudokinases are defined by the presence of mutations in critical catalytic residues that prevent catalysis of phosphorylation while preserving the overall structure of the kinase domain (Manning et al., 2002). Although initially thought of as evolutionary remnants that lack biological function, pseudokinases are often highly conserved in evolution and play essential roles in signaling. As such, pseudokinases represent ~10% of all human kinases (Figure 1), underscoring the importance of the non-catalytic utility of the kinase scaffold for cellular signaling. They also provide elegant case studies with which to investigate the molecular basis for non-catalytic kinase functions, since these functions have likely evolved in pseudokinases in isolation from the constraints that catalysis imposes on the structure of the kinase domain.

With our increasing appreciation for the phosphorylation-independent roles of kinases in cellular signaling, several important questions emerge. First, what are the structural components within the kinase domain that mediate these non-catalytic functions, and what are the molecular mechanisms of their regulation? Second, can we modulate the non-catalytic functions of kinases using small molecules in a manner analogous to the ways in which their catalytic activity can be regulated? Lastly, and perhaps most critical from a therapeutic standpoint, how do existing inhibitors of kinase catalytic functions affect kinase non-catalytic functions? In this review, we analyze the molecular mechanisms supporting phosphorylation-independent signaling

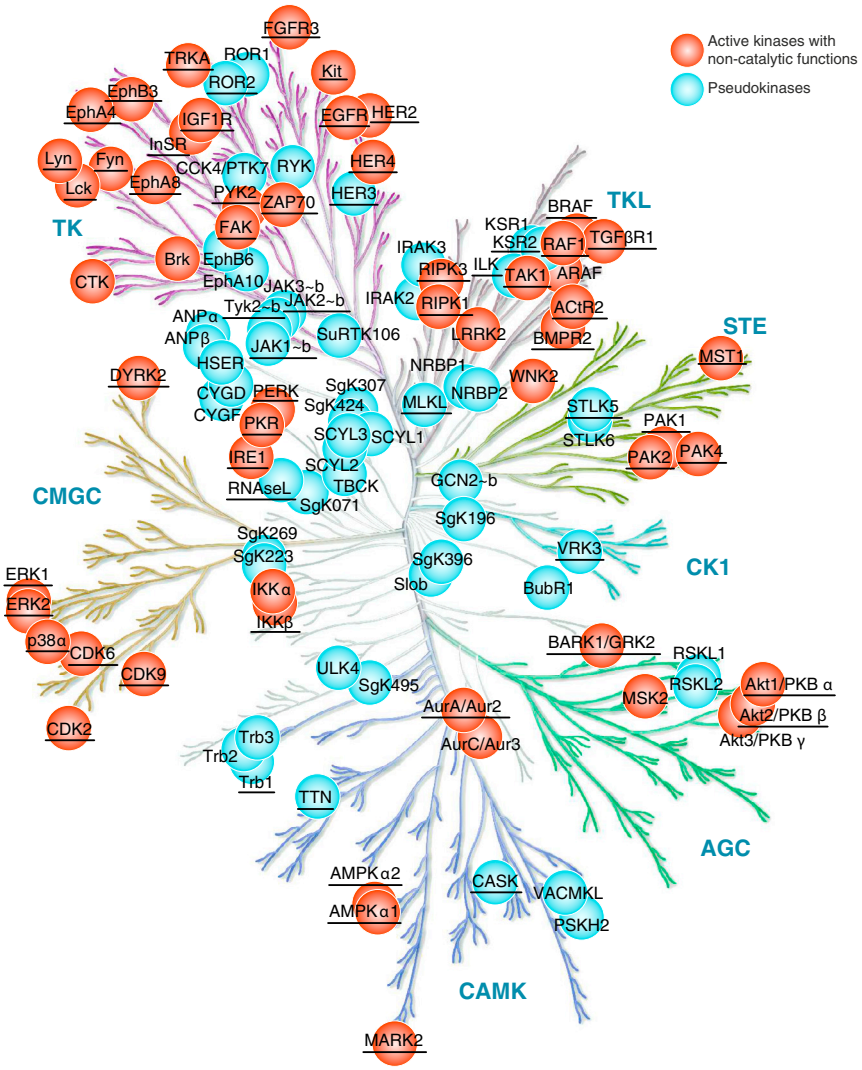

Figure 1. Kinases Reported to Have Non-catalytic Functions

Active kinases (red circles) and pseudokinases (blue circles) that have been reported to perform non-catalytic functions are marked on the human kinome tree. The names of kinases whose crystal structures are available are underlined. Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com).

in kinases and attempt to identify common structural characteristics that enable kinases to perform these non-catalytic functions. We then discuss how these non-canonical functions could be modulated using small molecules, and examine current progress toward the development of compounds that modulate non-enzymatic activities of kinases.

Known Non-catalytic Functions of Kinases

Kinases interact with a surprisingly wide range of binding partners given the high degree of conservation of the kinase domain fold. This versatility enables kinases to perform a diverse array of non-catalytic functions, including allosterically regulating other kinases and/or unrelated enzymes, acting as molecular scaffolds to coordinate interactions between different components of signaling pathways, and regulating transcription through interactions with transcription factors or by binding directly to DNA. Structural and functional studies in recent years have shown that each of these novel kinase functions utilizes a unique molecular mechanism, highlighting an impressive degree of adaptation.

Allosteric Regulation of Other Kinases

Several kinases and pseudokinases allosterically regulate the catalytic activity of other kinases through dimerization (Figure 2A). The human epidermal growth factor receptor (HER/EGFR) family of receptor tyrosine kinases is one example of such regulation. The HER family consists of three catalytically active kinases, EGFR, HER2, and HER4, and one catalytically impaired pseudokinase, HER3. Although HER3 retains very weak kinase activity in vitro (Shi et al., 2010), this activity does not seem to be necessary for HER3 signaling (Mendrola et al., 2013). The canonical model of receptor tyrosine kinase activation postulates that transphosphorylation of kinases in response to ligand-induced receptor dimerization is necessary for kinase activation and, therefore, is contingent on both receptors being catalytically active. However, the catalytically active HER receptors (EGFR, HER2, and HER4) readily form signaling-competent heterodimeric complexes with the catalytically impaired HER3, suggesting that only one kinase in HER receptor dimers needs to be catalytically active. Comparisons of crystal structures of the EGFR kinase domain in inactive and active states revealed that, instead of transphosphorylation, EGFR activation relies on asymmetric dimerization between kinase domains where one kinase allosterically activates the other (Brewer et al., 2009; Jura et al., 2009a; Zhang et al., 2006). This allosteric activator function is independent of kinase catalytic activity but reliant upon the structure of the kinase domain (Zhang et al., 2006). Consequently, it can be carried out by both the catalytically active and catalytically impaired HER kinases, enabling HER3 to form functional signaling complexes with other HER receptors (Jura et al., 2009b; Littlefield et al., 2014; Monsey et al., 2010).

The RAF family of kinases (A-RAF, B-RAF, and C-RAF) also relies on catalysis-independent allosteric activation between two kinase domains, yet through a very different mechanism. Analysis of RAF mutations found in human cancers has identified several substitutions in B-RAF (G466E, G466V, G596R) that impair its catalytic activity but still stimulate cell proliferation by activating C-RAF through heterodimerization (Garnett et al., 2005; Wan et al., 2004). In addition, under subsaturating