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Structural Basis for Domain–Domain Communication in a Protein Tyrosine Kinase, the C-terminal Src Kinase

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The catalytic activity of protein tyrosine kinases is commonly regulated by domain–domain interactions. The C-terminal Src kinase (Csk) contains a catalytic domain and the regulatory SH3 and SH2 domains. Both the presence of the regulatory domains and binding of specific phosphotyrosine-containing proteins to the SH2 domain activate Csk. The structural basis for both modes of activation is investigated here. First, the SH3-SH2 linker is crucial for Csk activation. Mutagenic and kinetic studies demonstrate that this activation is mediated by a cation–π interaction between Arg68 and Trp188. Second, Ala scanning and kinetic analyses on residues in the SH2–catalytic domain interface identify three functionally distinct types of residues in mediating the communication between the SH2 and the catalytic domains. Type I residues are important in mediating a ligand-triggered activation of Csk because their mutation severely reduces Csk activation by the SH2 domain ligand. Type II residues are involved in suppressing Csk activity, and their mutation activates Csk, but makes Csk less sensitive to activation by the SH2 ligand. Both type I and type II residues are likely involved in mediating SH2 ligand-triggered activation of Csk. Type III residues are those located in the SH2 domain whose mutation severely decreases Csk catalytic activity without affecting the SH2 ligand-triggered activation. These residues likely mediate SH2 activation of Csk regardless of SH2–ligand interaction. These studies lead us to propose a domain–domain communication model that provides functional insights into the topology of Csk family of protein tyrosine kinases.

Keywords: Csk; protein tyrosine kinases; domain–domain communication; SH3 and SH2 domains; the Csk-binding protein

Introduction

Protein tyrosine kinases (PTK) are a large family of enzymes that transfer the γ-phosphate group of ATP to Tyr hydroxyl groups in proteins. All eukaryotic PTKs have evolved elaborate mechanisms for regulating their kinase activities. As a structural foundation for regulation, PTKs contain multiple regulatory domains in addition to a catalytic domain. Interactions between the regulatory and catalytic domains may activate or suppress the kinase activity. Regulatory signals, such as a ligand for a regulatory domain, or phosphorylation of certain residues in the regulatory or catalytic domain, often modulate the kinase activity by affecting domain–domain interaction. Elucidating the communication between the regulatory and catalytic domains could reveal the molecular basis of PTK regulation.

C-terminal Src kinase (Csk) is a cytoplasmic PTK[1] that phosphorylates Src family kinases (SFKs) and down-regulates their kinase activities.[1,2] Csk contains an SH3 and an SH2 domain at the N terminus and a catalytic domain at the C terminus.[3,4] Two modes of communication between the regulatory

Abbreviations used: A_{max}, maximum activation; C-lobe, the C-terminal lobe of a kinase catalytic domain; CBP, Csk-binding protein; Csk, C-terminal Src kinase; ΔSH3, a Csk mutant in which the SH3 domain (Met1 through Pro81) is deleted; GST, glutathione *S*-transferase; kdSrc, kinase-defective Src; N-lobe, the N-terminal lobe of a kinase catalytic domain of; PTK, protein tyrosine kinase; SFKs, Src family kinases; wt, wild-type.

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domains and the catalytic domain have been established for Csk.[4] First, the presence of the SH3 and SH2 domains directly activates the catalytic domain. Deletion of the SH3 and SH2 domains by mutation[5] or proteolytic cleavage[6] results in a severe loss of the kinase activity. Incubation of the catalytic domain with the SH3-SH2 fragment activates the catalytic activity.[6] The linker between the SH3 and SH2 domains plays a crucial role in this activation.[6,7] Second, binding of a phosphotyrosine-containing protein, such as the Csk-binding protein (CBP), to the SH2 domain also activates Csk.[8,9] Apparently binding to a ligand causes a conformational change to the SH2 domain, which can be communicated to the catalytic domain.

Although both the Csk and the Src families of protein tyrosine kinases have similar primary domain arrangements with an SH3, an SH2 and a catalytic domain, the regulatory domains play opposite roles in regulating the kinase activities. In Src family kinases, the catalytic domain alone is highly active, which is suppressed by its interaction with the regulatory domains. On the other hand, the Csk catalytic domain alone has approximately 1% of full Csk activity, which can be stimulated when it interacts with the regulatory domains. The crystal structures of Csk[10] and Src[11–12] family kinases reflect the two regulatory strategies. In the Csk structure (Figure 1(a)), the SH3 and SH2 domains are located on top of the catalytic domain, and form several direct interactions with residues from the N-terminal lobe of the catalytic domain (the N-lobe). In the inactivated Src family kinases,[11,12] the regulatory domains are arranged side-by-side with the catalytic domain.

Several published studies on Csk domain–domain interaction provide important insights into the mechanism of this regulation. First, the kinase activity of the Csk catalytic domain is not activated by the presence of exogenous Csk SH2 domain, but the added SH3 domain and SH3-SH2 fragment activate the kinase activity of Csk catalytic domain by four-to fivefold,[6] indicating that intermolecular interactions between the Csk SH3 domain and the catalytic domain activate Csk. Studies with peptide binding, site-specific mutagenesis[6] and NMR mapping[7] demonstrate that the SH3 surface that interacts with the catalytic domain is distinct from the surface that binds type II polyproline helix peptides. Second, site-specific mutagenesis studies reveal that the SH3-SH2 linker is crucial for Csk activation by the regulatory domains,[5,7] as individual point mutations of a number of residues in this region result in loss of Csk activity similar to those caused by the removal of the regulatory domains.[5–7] Third, the SH2-catalytic linker is also crucial for the domain–domain communication, with mutation of Phe183[13] and Trp188[4] resulting in severe loss of Csk activity, apparently by decoupling the communication between the regulatory and the catalytic domains. Because the SH3-SH2 linker and the SH2-catalytic linker directly interact with each

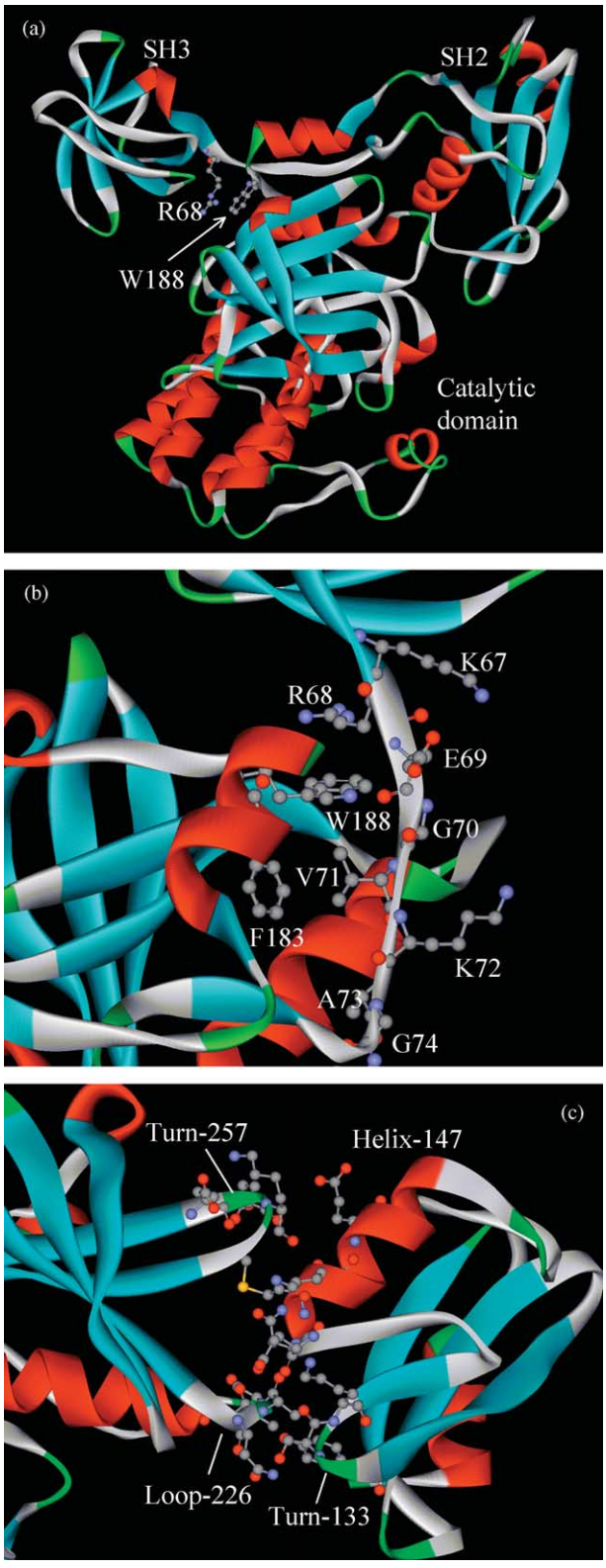

Figure 1. Csk structures depicting the interactions between the regulatory domains and the catalytic domain. (a) Overall Csk tertiary structure based on coordinates of chain A from 1K9A from the Protein Data Bank.[10] (b) Residues in the SH3-SH2 linker region and their interactions. (c) Residues in the interface between the N-lobe and the SH2 domain. The four substructures involved in the domain–domain interaction are indicated. The individual residues from each substructure are given in the text.