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The Conformational Plasticity of Protein Kinases

Review

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Protein kinases operate in a large number of distinct signaling pathways, where the tight regulation of their catalytic activity is crucial to the development and maintenance of eukaryotic organisms. The catalytic domains of different kinases adopt strikingly similar structures when they are active. By contrast, crystal structures of inactive kinases have revealed a remarkable plasticity in the kinase domain that allows the adoption of distinct conformations in response to interactions with specific regulatory domains or proteins.

The importance of protein phosphorylation in eukaryotic signaling is reflected in the fact that protein kinase domains are found in ~2% of eukaryotic genes (Rubin et al., 2000). The spatial and temporal control of phosphorylation of specific serine, threonine, or tyrosine residues is crucial to cellular growth and development, and this control relies on the proper regulation of protein kinases. Kinase activity in the wrong place or at the wrong time can have disastrous consequences, often leading to cell transformation and cancer. Indeed, the first oncogene to be discovered, *v-Src*, encodes an aberrantly regulated tyrosine kinase (Levinson et al., 1978; Eckhart et al., 1979).

The serine/threonine and tyrosine kinases are a fascinating study in molecular evolution. On the basis of sequence and structure, these enzymes form a closely related superfamily that is distinct from the histidine kinases and other phosphotransfer enzymes. While the several hundred eukaryotic protein kinases all contain the same catalytic scaffold, a number of very different regulatory mechanisms have evolved that allow individual members of the family to function downstream of the specialized input signals that turn them on. Protein kinases are usually kept off, and the acquisition of catalytic activity is often buried under multiple layers of control, ranging from the binding of allosteric effectors to alterations in the subcellular localization of the enzyme.

Protein kinases are molecular switches that can adopt at least two extreme conformations: an "on" state that is maximally active, and an "off" state that has minimal activity. All protein kinases catalyze the same reaction, the transfer of the γ-phosphate of ATP to the hydroxyl group of serine, threonine, or tyrosine. Thus, upon activation they all adopt catalytically active on conformations that are structurally very similar (Knighton et al., 1991; Jeffrey et al., 1995; Yamaguchi and Hendrickson, 1996; Canagarajah et al., 1997; Hubbard, 1997). The off states of protein kinases are not subject to the chemical constraints that the active states must satisfy, and so different classes of kinases have evolved distinct off states in which the adoption of the catalytically active conformation is impeded in different ways.

In this review we survey the conformational changes undergone by Ser/Thr and Tyr kinase domains as they turn on and off. After briefly introducing the structure of the kinase domain in the active state, we discuss two key regulatory elements within the domain, the activation loop and the αC helix. We then discuss several kinases that have been analyzed structurally in their inactive states.

The Structure of the Kinase Domain in the On State

The three-dimensional structure of a eukaryotic protein kinase domain was first visualized by X-ray crystallography in the case of cyclic AMP-dependent protein kinase, a Ser/Thr kinase also known as protein kinase A (PKA) (Knighton et al., 1991). Of particular importance for understanding the on state of kinases is the structure of PKA in complex with Mn-ATP and the substrate mimic PKI, which captures the protein kinase in a conformation that is primed and ready for phosphotransfer (Knighton et al., 1991; Bossemeyer et al., 1993; Zheng et al., 1993).

The protein kinase fold, which is extremely well conserved among serine/threonine and tyrosine kinases, is separated into two subdomains, or lobes (Figure 1). The smaller N-terminal lobe, or N lobe, is composed of a five-stranded β sheet and one prominent α helix, called helix αC. The C lobe is larger and is predominantly helical. ATP is bound in a deep cleft between the two lobes and sits beneath a highly conserved loop connecting strands β1 and β2. This phosphate binding loop, or P loop, contains a conserved glycine-rich sequence motif (GXGXϕG) where ϕ is usually tyrosine or phenylalanine. The glycine residues allow the loop to approach the phosphates of ATP very closely and to coordinate them via backbone interactions. The conserved aromatic side chain caps the site of phosphate transfer. The glycine residues make the P loop very flexible in the absence of ATP, a fact that facilitates the binding of small molecule inhibitors. Some of these inhibitors induce large structural distortions in the loop by interacting with the conserved aromatic residue (Mohammadi et al., 1997; Schindler et al., 2000).

Peptide substrate binds in an extended conformation across the front end of the nucleotide binding pocket, close to the γ-phosphate of ATP. A centrally located loop known as the "activation loop," typically 20–30 residues in length, provides a platform for the peptide substrate. In PKA, as in most kinases, this loop is phos-

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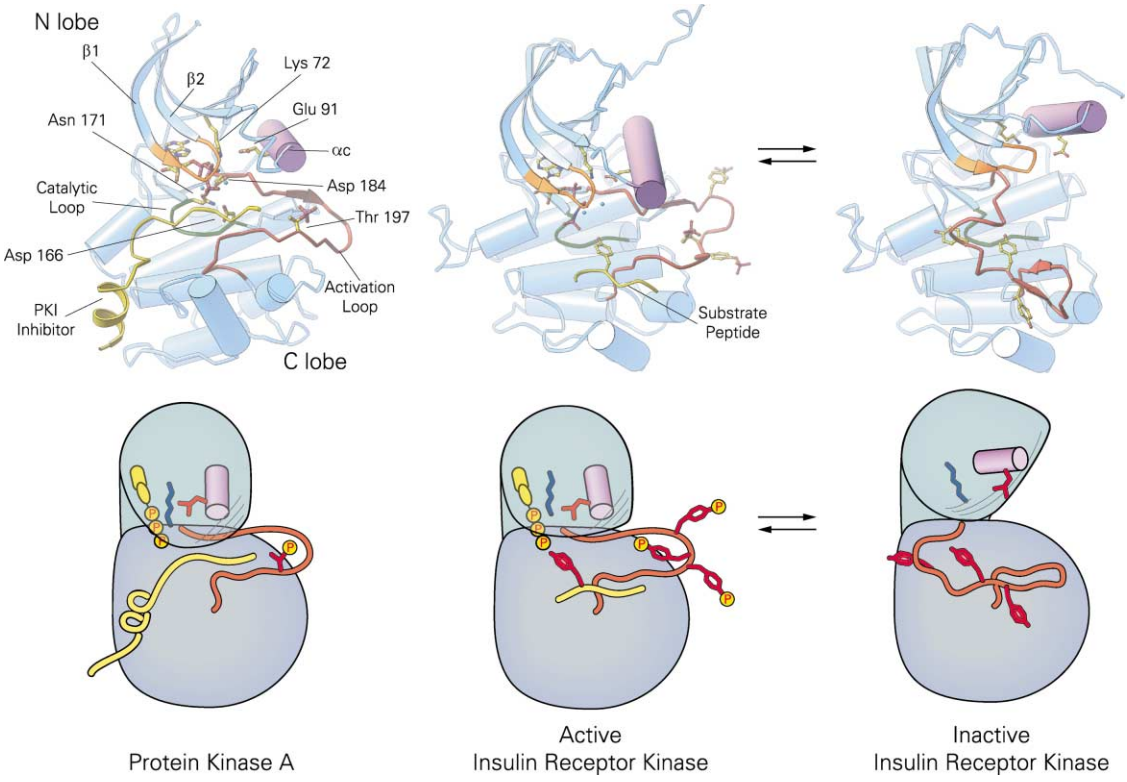

Figure 1. The Catalytically Active Conformation of the Protein Kinase Domain

(Top) The crystal structures of protein kinase A (PKA, a serine/threonine kinase) (Zheng et al., 1993) and insulin receptor kinase (IRK, a tyrosine kinase) (Hubbard, 1997) are shown in ribbon representation. Key structural elements within the kinase domain are colored as follows: activation loop, red; αC helix, purple; P loop, orange; and catalytic loop, green. The peptide substrate for IRK as well as the PKI peptide inhibitor for PKA are colored yellow. Bound nucleotide and several absolutely conserved residues within the active site are indicated. Also shown is the structure of unphosphorylated, inactive IRK (Hubbard et al., 1994). In the unphosphorylated state, the activation segment of IRK adopts a conformation that impedes the binding of nucleotide and peptide substrate.

(Bottom) The three structures have been schematized to clearly depict the conformational transitions involved in the regulation of kinase activity, with particular emphasis on the αC helix and activation loop. The catalytic lysine and glutamate (Lys72 and Glu91 in PKA) are shown in each schematic.

phorylated when the kinase is active. Phosphorylation of the activation loop stabilizes it in an open and extended conformation that is permissive for substrate binding.

Optimal phosphotransfer requires the precise spatial arrangement of several catalytic residues that are absolutely conserved among all known kinases. Asp166 and Asn171 (PKA numbering) emanate from a highly conserved loop structure at the base of the active site, called the catalytic loop. Asp166 interacts with the attacking hydroxyl side chain of the substrate, while Asn171 engages in hydrogen bonding interactions that orient Asp166. Asn171 and another absolutely conserved catalytic residue, Asp184, are also required for the binding of two divalent cations involved in nucleotide recognition. Asp184 forms part of the highly conserved Asp-Phe-Gly motif situated at the base of the activation loop. The structure of this motif, and in particular the conformation of Asp184, is tightly coupled to phosphorylation of the activation loop. In the N lobe, Lys72 makes crucial contacts with the α- and β-phosphate groups, positioning them properly for catalysis. Lys72 is buried deep within the interlobe cleft, where it is stabilized and oriented properly by an ionic interaction with Glu91, a residue in the αC helix. The integrity of the Lys72-Glu91 ion pair is important for activity and will be discussed below.

Conformational Changes in the Activation Loop

The activation loop has the capacity to undergo large conformational changes when the kinase switches between inactive and active states (Johnson et al., 1996). For example, insulin receptor kinase (IRK) is activated by phosphorylation of three tyrosine residues within its activation loop (Figure 1). In the unphosphorylated state, the activation loop collapses into the active site, blocking the binding of both nucleotide and peptide substrate (Hubbard et al., 1994). Upon phosphorylation, it moves away from the catalytic center and adopts a conformation that allows substrate binding and catalysis (Hubbard, 1997). In a number of kinases, including IRK, these conformational changes involve crankshaft-like motions in the N-terminal portion of the activation loop that result in the proper orientation of the conserved Asp-Phe-Gly sequence within the active site (Hubbard et al., 1998).

The placement and total number of phosphorylation sites within the activation loop varies from kinase to

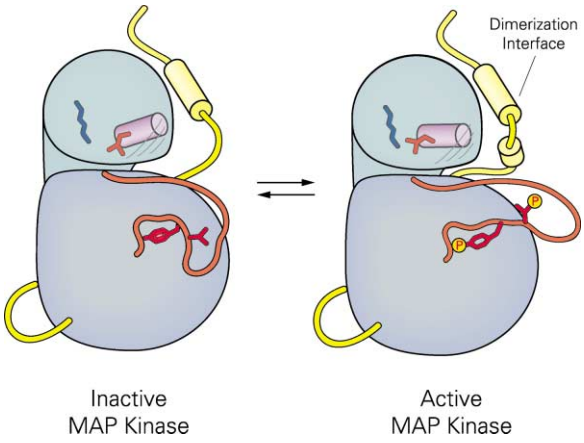

Figure 2. The Regulation of MAP Kinase by Phosphorylation of the Activation Segment

Phosphorylation of the activation segment creates a network of interactions that properly orient the αC helix, promote lobe closure, and organize the C-terminal extension (shown in yellow) into a functionally important homodimerization interface (Zhang et al., 1994; Canagarajah et al., 1997; Khokhlatchev et al., 1998).

kinase. One phosphate usually adopts a central position, where it forms an ion pair with a conserved arginine (Arg165 in PKA) situated in the catalytic loop (Knighton et al., 1991; Russo et al., 1996; Yamaguchi and Hendrickson, 1996; Canagarajah et al., 1997). This ion-pairing interaction is likely to be important in rotating the Asp-Phe-Gly motif into proper orientation for catalysis. Phosphorylation at the central position can also nucleate interactions that propagate well into the N lobe. For example, the mitogen-activated protein (MAP) kinase ERK2 undergoes phosphorylation at a threonine and a tyrosine residue within the activation loop (Figure 2). Of these, pThr183 occupies the central coordinating position, forming contacts that extend to the αC helix, thereby promoting closure of the N and C lobes such that the kinase becomes active (Canagarajah et al., 1997). This network of interactions also plays a key role in modulating the oligomerization state of ERK2. MAP kinases have a C-terminal extension that binds to the surface of the N lobe, near helix αC (Zhang et al., 1994). Phosphorylation at Thr183 in the activation loop induces a conformational change in this C-terminal extension, exposing a hydrophobic surface that facilitates the formation of homodimers (Canagarajah et al., 1997). Dimerization via this interface is required for the nuclear localization of the enzyme (Khokhlatchev et al., 1998).

The ability of the activation loops of different kinases to adopt distinct conformations when the kinase is off has recently been exploited to great medical benefit. The anticancer drug Gleevec is a potent therapeutic in the treatment of chronic myelogenous leukemia, and it acts by inhibiting the kinase activity of the Bcr-Abl oncoprotein directly (Druker et al., 2001). Gleevec binds to an inactive form of Abl kinase selectively, stabilizing the activation loop in a conformation that mimics bound substrate (Schindler et al., 2000). The closely related Src family kinases display an entirely different activation loop conformation in their inactive states (Schindler et al., 1999; Xu et al., 1999) and do not bind Gleevec despite close sequence similarity within the nucleotide recognition pocket (Zimmermann et al., 1997). Thus, the specific binding of Gleevec to Abl and not to Src depends on the capacity of the activation loop to adopt a particular conformation.

Coupling between the αC Helix and the Activation Loop

Helix αC, the only conserved helix in the β sheet-rich N lobe, is an important mediator of conformational changes that take place within the catalytic center. An absolutely conserved glutamate residue (Glu91 in PKA) is located within this helix, and in active kinases this residue forms an ion pair with the lysine side chain (Lys72 in PKA) that coordinates the α and β phosphates of ATP. The αC helix also makes direct contact with the N-terminal region of the activation loop, and its conformation is often linked to that of the conserved Asp-Phe-Gly motif. This interaction, along with the Glu91-Lys72 ion pair, directly couples the conformation of the helix to nucleotide binding. Regulatory mechanisms often modulate kinase activity by altering the conformation of helix αC, thereby affecting the integrity of these interactions.

The cyclin-dependent kinase (CDK) family provides one of the clearest examples of allosteric regulation of catalytic activity via helix αC (Figure 3). CDKs are catalytically inactive on their own but become active upon binding cognate cyclins. In the absence of cyclin, the αC helix (also called the PSTAIRE helix in CDKs for a conserved cyclin recognition motif within it) is rotated outward about its long axis relative to its position in PKA (DeBondt et al., 1993). This swings the conserved glutamate (Glu51 in CDK2) out of the active site and disrupts its interaction with the lysine that coordinates ATP (Lys33 in CDK2). Cyclin binds directly to the αC helix and surrounding elements in the kinase N lobe, inducing the reverse rotation that restores the Lys33-Glu51 ion pair (Jeffrey et al., 1995).

Conformational changes in helix αC are a crucial feature of the activation mechanism of the Src family tyrosine kinases as well (Sicheri et al., 1997; Xu et al., 1997). Src family members bear an SH3 domain and an SH2 domain N-terminal to their kinase core and are regulated by phosphorylation of a tyrosine residue in their C-terminal tail (Tyr527 in Src). Phosphorylation of Tyr527 inhibits Src activity by engaging the SH2 domain in an intramolecular interaction (Figure 3). This positions the SH3 domain so as to interact with the linker connecting the SH2 and kinase domains. The interaction with the SH3 domain induces the linker to adopt the type II polyproline helical conformation, characteristic of SH3 domain ligands. The SH3 domain and linker bind to the surface of the N lobe of the kinase, stabilizing helix αC in an inactive conformation that is very similar to that of the CDK PSTAIRE helix in the absence of cyclin.

Ligands of the SH2 and SH3 domains activate Src family kinases by disrupting the interactions described above and allowing helix αC to assume an active conformation (Moarefi et al., 1997; Gonfloni et al., 2000). While the structural details of the conformational changes in the αC helix are quite similar between Src and CDK, it is interesting that the thermodynamics of the switching

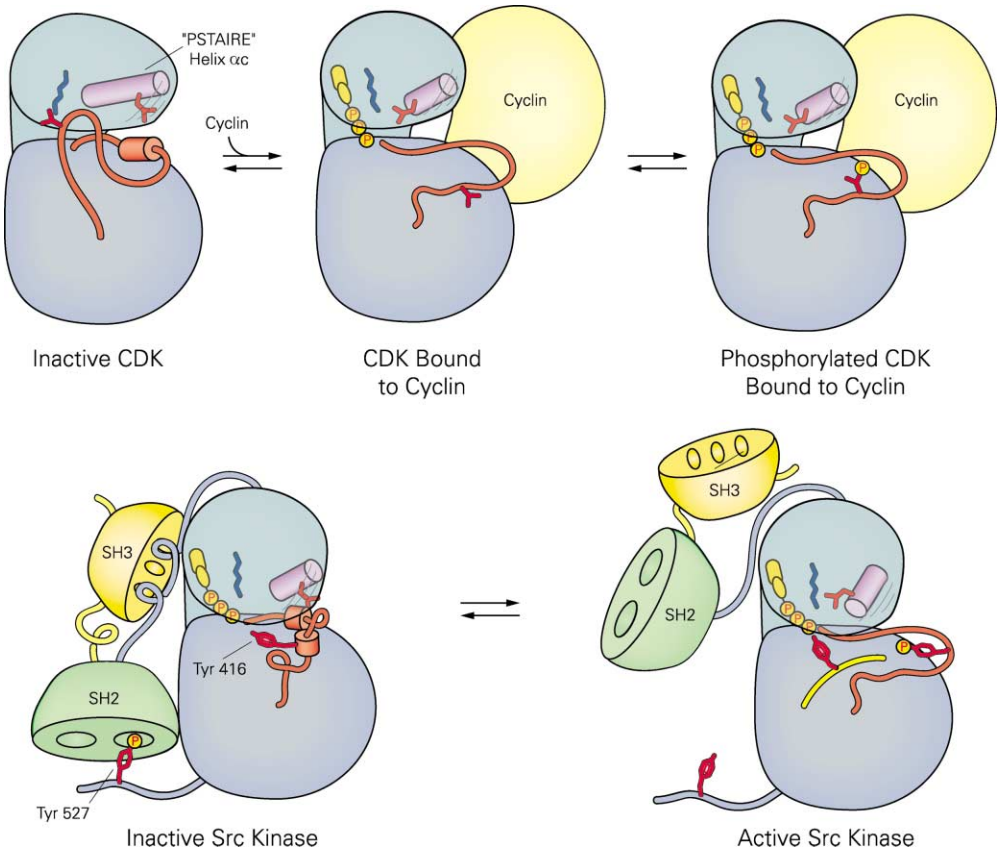

Figure 3. The Regulation of Cyclin-Dependent Kinase (CDK) and Src Tyrosine Kinase

In the absence of cyclin, the αC helix of CDK (also called the PSTAIRE helix) is rotated so as to move a crucial catalytic glutamate out of the active site (DeBondt et al., 1993). This is correlated with an inhibitory conformation of the activation loop. Cyclin binding reorients the PSTAIRE helix so as to place the glutamate within the active site (Jeffrey et al., 1995). The activation loop adopts a near-active conformation upon cyclin binding, and its subsequent phosphorylation further stabilizes the active form (Russo et al., 1996). In Src, intramolecular interactions between the phosphorylated tail and the SH2 domain, and between the SH2-kinase linker and the SH3 domain, stabilize inhibitory conformations of both helix αC and the activation loop (Schindler et al., 1999; Xu et al., 1999). The conformation of αC in the off state is quite similar to that seen in CDK. Disengagement of the SH2 domain by dephosphorylation of the tail (at Tyr527), combined with phosphorylation of the activation loop (at Tyr416), allows the αC helix to move into an active conformation.

process have been tailored differently in these two cases. With CDK, the binding of cyclin activates the enzyme, while with Src, the binding of the SH3 domain and linker stabilizes the inhibited state.

The story is not so simple, however. Both CDKs and Src family kinases also require phosphorylation of the activation loop to achieve full activity. The activation loops of both proteins adopt conformations that block the binding of peptide substrates in the unphosphorylated state (DeBondt et al., 1993; Schindler et al., 1999; Xu et al., 1999). Both activation loops also interact directly with the αC helix, stabilizing its inactive conformation (Figure 3). The activation loop and αC helix appear to be structurally coupled; a switch in one necessitates a switch in the other. The binding of cyclin to CDK, in addition to changing the orientation of helix αC, also induces the unphosphorylated activation loop to adopt a conformation similar to that of the fully active form, which is eventually achieved via phosphorylation by CDK-activating kinase (Jeffrey et al., 1995; Russo et al., 1996). Similarly, activation loop phosphorylation of the Src family kinase Lck bolsters the active conformation of helix αC (Yamaguchi and Hendrickson, 1996).

This structural coupling between αC and the activation loop is also manifested at a functional level. Displacement of the SH3 domain in Hck, for example, stimulates autophosphorylation of the activation loop tyrosine (Tyr416 in Src), presumably via the αC helix (Moarefi et al., 1997). Reciprocally, phosphorylation at Tyr416 weakens the affinity of the SH3 and SH2 domains for their intramolecular targets in the kinase N lobe and tail, respectively (Gonfloni et al., 2000; Porter et al., 2000). The coupling between the activation loop and helix αC allows for allosteric information to pass between the active site and the back of the enzyme, a general phenomenon in kinase regulation. In IRK and ERK2, for example, the conformation of the unphosphorylated activation loop is also correlated with structurally distinct but nonetheless inactive orientations of helix αC (Hubbard et al., 1994; Zhang et al., 1994).

Pseudosubstrate Regulation

One obvious way to inhibit a kinase is to compete directly with one of its substrates (Kobe and Kemp, 1999). This was made clear in the very first structure of PKA,

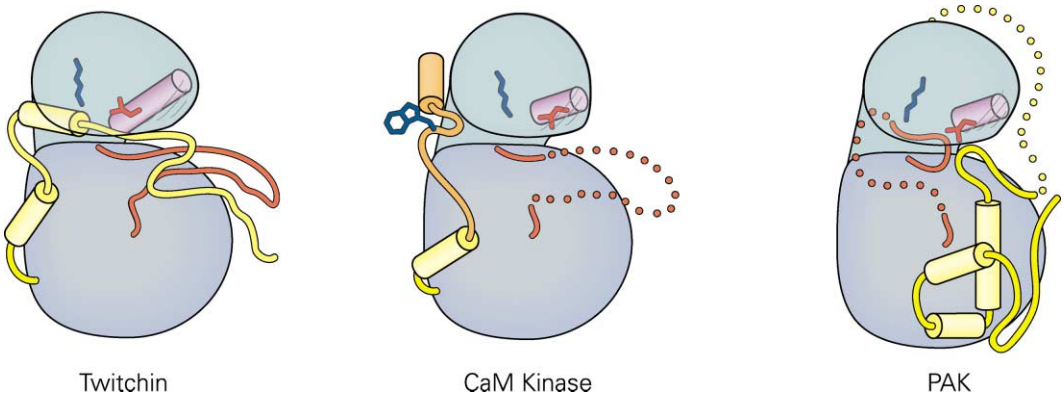

Figure 4. The Regulation of Protein Kinases by Pseudosubstrate Sequences

Twitchin kinase, CaM kinase, and p21-activated kinase (PAK) are depicted schematically with pseudosubstrate sequences colored yellow. Disordered regions are shown in dots. The pseudosubstrate segments in twitchin and CaM kinase adopt distinct structures, but both effectively block the binding of nucleotide and peptide substrate (Hu et al., 1994; Goldberg et al., 1996). The portion of the pseudosubstrate segment in CaM kinase that interacts directly with Ca^{2+}/CaM is shown in orange, and the solvent-exposed tryptophan that anchors the complex is indicated. In PAK, the pseudosubstrate segment extends from the C terminus of a folded inhibitory switch domain that docks onto the C lobe of the kinase (Lei et al., 2000). The binding of the inhibitory switch module to the kinase domain directly blocks interaction with peptide substrate and forces the activation loop to adopt a conformation that prevents the recognition of ATP.

which was solved in complex with the peptide inhibitor PKI (Knighton et al., 1991). PKI mimics a peptide substrate for the kinase by binding directly to the active conformation of PKA, without disrupting the binding of ATP. Indeed, an intramolecular regulatory mechanism involving direct competition with substrate had been proposed prior to the determination of the structure of PKA (Kemp et al., 1987). The myosin light chain kinase subfamily of Ca^{2+}-responsive enzymes contain C-terminal segments that inhibit catalytic activity until they are bound by Ca^{2+}/calmodulin (Ca^{2+}/CaM) or a functionally equivalent Ca^{2+} binding protein. The C-terminal regulatory motifs contained within the kinase were termed "pseudosubstrate" sequences because they appeared to compete directly with substrate for access to the active site. Subsequently, crystal structures of two Ca^{2+}-regulated kinases, twitchin (Hu et al., 1994) and titin (Mayans et al., 1998), were determined, revealing a conserved mode of autoinhibition. The pseudosubstrate segment first forms a helix that fits into the same groove occupied by the helical portion of PKI (Figure 4). The chain then snakes into the ATP binding site and out over the activation loop, blocking the binding of both nucleotide and peptide substrate.

The C-terminal extensions of twitchin and titin also stabilize the kinase domain in a catalytically inactive conformation. The presence of the autoinhibitory sequence within the active site forces helix αC to tip outward, disrupting the crucial Lys-Glu (Lys82-Glu98 in twitchin, Lys53-Glu68 in titin) ion-pairing interaction. In addition, the pseudosubstrate segments of twitchin and titin maintain the N and C lobes in a more open disposition than seen in the structure of active PKA, an arrangement incompatible with catalysis. Thus, by stuffing the mouth of the kinase and prying it open, the C-terminal extensions of twitchin and titin go beyond the simple substrate mimicry of PKI. Interestingly, certain small molecule inhibitors of the MAP kinase p38 stabilize the N and C lobes of the protein in an open conformation that is similar to that observed for twitchin and titin (Wang et al., 1998).

The C-terminal pseudosubstrate segment of CaM kinase I (CaMKI), another Ca^{2+}/CaM-regulated enzyme, induces even more pronounced distortions within the kinase domain (Goldberg et al., 1996). Here, the pseudosubstrate sequence forms the same initial helix that interacts with the groove within the C lobe of the kinase. It then veers sharply and forms a loop and then a second helix that interacts with and deforms strands β1 and β2 in the N lobe, which together form the phosphate binding loop crucial for the recognition of ATP (Figure 4). This effectively blocks nucleotide binding.

In p21-activated kinase (PAK), the mode of regulation by a pseudosubstrate is quite different from that seen in the Ca^{2+}-regulated enzymes (Lei et al., 2000). Rather than extending directly from the C terminus of the kinase domain, the pseudosubstrate sequence emerges from the C terminus of a folded regulatory module called the inhibitory switch domain, which is N-terminal to the kinase core (Figure 4). The inhibitory switch domain docks onto the C lobe of the kinase, positioning the pseudosubstrate sequence within the active site cleft. As with CaMKI, this binding induces distortions within the kinase domain itself that further inhibit its catalytic activity. The activation loop is trapped within the active site by the pseudosubstrate segment, where it blocks ATP binding. In addition, the presence of the activation loop within the active site disrupts the Lys-Glu (Lys299-Glu315 in PAK) ion pair and forces the αC helix away from the catalytic center.

PAK is activated by the GTP bound forms of certain Rho family G proteins. The inhibitory switch domain contains within it a CRIB motif that binds to Rac and Cdc42. Structural studies of the WASP protein have revealed the conformation of the CRIB motif bound to an activated GTPase, and that conformation is incompatible with the recognition of PAK by the CRIB motif (Abdul-Manan et al., 1999). Thus, the binding of G protein

would dissociate both the inhibitory switch domain and the pseudosubstrate motif from the kinase, liberating the enzyme to undergo activation loop phosphorylation.

The pseudosubstrate sequences function as regulators by having two binding partners, the kinase domain and the activating protein. The binding footprints for kinase and activator usually overlap only partially, a feature that may facilitate fast regulatory switching. If the recognition sites overlap completely, initial access of the activator to the regulatory sequence would be dependent upon transient dissociation of the inhibitory segment from the kinase. With partial overlap, the activator is given access to at least a portion of its binding site as an anchor from which it can nucleate the remainder of the interaction and force off the inhibitor.

The Ca^{2+}/CaM recognition site in the regulatory motif of CaMKI, for example, includes the second of the two helices in the inhibitory segment, as well as portions of the loop connecting the two helices. A solvent-exposed tryptophan residue that anchors the binding of Ca^{2+}/CaM to the inhibitory segment is situated in the loop and extends away from the kinase, where it is available for initial interaction with Ca^{2+}/CaM (Figure 4; Crivici and Ikura, 1995; Goldberg et al., 1996). Similarly, the CRIB motif within the inhibitory switch domain of PAK only partially overlaps those parts of the structure that are required for autoinhibition. The N-terminal portion of the CRIB motif is not involved in the formation of the inhibitory switch core and is available to nucleate interaction with the activated GTPase. As the interaction with the G protein grows, it presumably destabilizes the folded structure of the inhibitory switch domain, inducing its dissociation and the adoption of an entirely new conformation (Kim et al., 2000; Lei et al., 2000).

Autoinhibition and Substrate Recruitment by N-Terminal Segments in EphB2 and TβR-I

Some groups of kinases are regulated by N-terminal segments that do not impinge upon the active site directly, but rather inhibit catalytic activity via conformational change alone. The EphB2 receptor tyrosine kinase is activated by phosphorylation of the activation loop and also by phosphorylation of two conserved tyrosines in a juxtamembrane region just N-terminal to the kinase domain. Mutagenesis indicates that the unphosphorylated juxtamembrane segment acts as a negative regulator of kinase activity (Dodelet and Pasquale, 2000). The crystal structure of unphosphorylated EphB2 shows that the juxtamembrane region adopts a helical conformation that impinges upon the N lobe, stabilizing it in a catalytically inactive conformation (Figure 5; Wybenga-Groot et al., 2001). The αC helix is kinked and moved closer to the β sheet, where it contacts the glycine-rich phosphate binding loop and impedes the proper binding of nucleotide. The juxtamembrane region of EphB2 also sterically blocks the activation loop from adopting an active conformation.

The type I TGFβ receptor (TβR-I), a receptor serine/threonine kinase, is also activated by phosphorylation of an N-terminal regulatory segment. Called the GS region, this segment is named for a highly conserved TTSGSGSG sequence within it, and 3–4 of the serines and threonines within this short stretch of amino acids

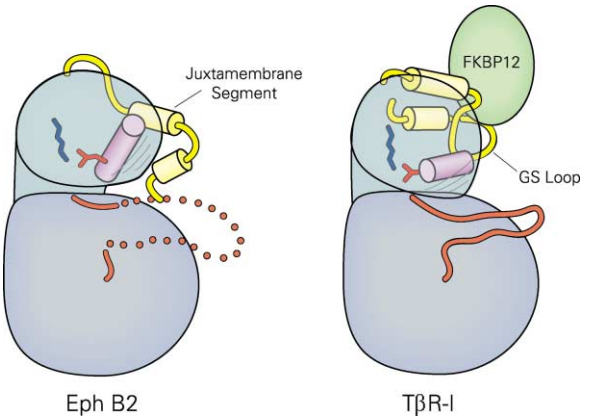

Figure 5. The Regulation of Receptor Kinases by N-Terminal Juxtamembrane Segments

EphB2, a receptor tyrosine kinase, and TβR-I, a receptor serine/threonine kinase, are depicted schematically with their juxtamembrane segments colored yellow. In the unphosphorylated state, the juxtamembrane region of EphB2 adopts a helical conformation that prevents the αC helix and the activation loop from adopting active conformations (Wybenga-Groot et al., 2001). Phosphorylation of the juxtamembrane segment disrupts the inhibitory interactions and creates binding sites for downstream proteins that contain phosphotyrosine recognition modules (Dodelet and Pasquale, 2000). The unphosphorylated juxtamembrane segment of TβR-I, which is called the GS region, maintains the N lobe of the kinase in a distorted, inactive conformation, but only when bound by the inhibitory protein FKBP12 (Huse et al., 1999). Phosphorylation of the GS region disrupts the interaction with FKBP12 and creates binding sites for substrate proteins of the Smad family (Huse et al., 2001).

must be phosphorylated in order to fully activate the enzyme (Wieser et al., 1995). When unphosphorylated, the GS region inhibits the kinase activity of TβR-I, but only when bound by the inhibitory protein FKBP12 (Huse et al., 1999, 2001). The unphosphorylated GS region forms a helix-loop-helix structure in which the phosphorylation sites are located in the loop between the two helices (the GS loop). When the GS region is bound by FKBP12, the GS loop adopts an ordered conformation that is positioned to maintain the N lobe of the kinase domain in a distorted, inactive conformation (Figure 5). The αC helix is moved away from the N lobe β sheet at its C terminus and pressed closer to the sheet at its N terminus. As with EphB2, these conformational changes disrupt productive ATP binding.

In the absence of FKBP12, the GS loop becomes flexible and no longer adopts an inhibitory conformation (Huse et al., 2001). Thus, it is the binding of FKBP12 that molds the N-terminal sequence into an inhibitory motif. While this mode of regulation distinguishes TβR-I from EphB2, it is reminiscent of the autoinhibitory mechanism seen in Src family kinases, where the binding of the SH3 domain molds the sequence just N-terminal to the kinase into a type II polyproline helix, positioning it to form inhibitory interactions with the N lobe (Sicheri et al., 1997; Xu et al., 1997).

The N-terminal segments of EphB2 and TβR-I, which both adopt inhibitory conformations when unphosphorylated, are transformed into binding sites for their appropriate physiological substrates in the phosphorylated state. EphB2 binds a number of SH2 domains via its

juxtamembrane tyrosines (Dodelet and Pasquale, 2000). The structure of autoinhibited EphB2 reveals that these binding sites are hidden in the inactive form of the kinase. The juxtamembrane regions of receptor tyrosine kinases such as c-Kit and PDGFR may play a similar dual role (Wybenga-Groot et al., 2001).

The localization of proteins via recognition of phosphoserine/phosphothreonine is also important in eukaryotic signaling (Yaffe and Elia, 2001). Biochemical experiments using TβR-I protein phosphorylated homogeneously on four sites within the GS region have demonstrated that phosphorylation activates TβR-I by creating a binding site for substrate Smad proteins while simultaneously disrupting the binding of FKBP12 (Huse et al., 2001). Smad proteins are transcription factors that are related structurally to the phosphothreonine binding FHA domain (Durocher et al., 2000), and mutagenesis suggests that a positively charged portion of the Smad surface close to the phosphothreonine binding site of the FHA domain is involved in the binding of phosphorylated TβR-I (Huse et al., 2001).

As seen for the overlapping binding sites used in pseudosubstrate sequences, phosphorylation of the regulatory motifs in EphB2 and TβR-I allows the binding of kinase and substrate/effector to be mutually exclusive.

Concluding Remarks

The structural analysis of protein kinases in their various on and off states has underscored the fact that the mechanisms of kinase regulation are as varied and complex as the signaling networks in which these proteins operate. Conformational plasticity, which allows reversible switching between distinct states of the kinase, is a central feature of these regulatory mechanisms. While our current understanding of kinase regulation owes much to X-ray crystallography, static pictures will take us only so far and must be combined with information from other approaches that more effectively capture the dynamics of the switching process. Molecular dynamics simulations of Src, for example, have suggested that the dynamics of an interdomain linker is crucial to effective regulation (Young et al., 2001). Experiments using time-resolved fluorescence anisotropy decay have begun to provide an experimental window into the catalytic dynamics of PKA (Li et al., 2002). Improvements in NMR technology have enabled the striking visualization of an important conformational equilibrium within the bacterial histidine kinase NtrC (Volkman et al., 2001). We look forward to the further application of powerful spectroscopic and computational methods to the analysis of protein kinases.

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