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Communication Pathways between the Nucleotide Pocket and Distal Regulatory Sites in Protein Kinases

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ABSTRACT

Protein kinases control many cellular processes via the ATP-dependent phosphorylation of specific amino acids on target proteins. Despite the availability of the three-dimensional structures of a variety of protein kinases, it has been particularly difficult to explain how noncatalytic domains removed from the active site regulate catalytic function. In this review, we describe how solution methodologies complement the available structural data and help explain how protein kinases may utilize medium-to-long-range effects to regulate substrate phosphorylation. For illustration, two protein kinases, cAMP-dependent protein kinase and the C-terminal Src kinase, are presented as paradigms for the serine/threonine- and tyrosine-specific families. While active-site residues provide an optimal environment for fast phosphoryl group transfer in these and other kinases, the overall rate of protein phosphorylation is limited by nucleotide binding and associated structural changes. Hydrogen−deuterium exchange studies reveal that nucleotide binding induces changes that radiate from a central structural assembly composed of the catalytic loop, glycine-rich loop, and helix αC to unique peripheral regions inside and outside the kinase core. This collection of conserved and unique elements delivers information from the active site to distal regions and possibly provides information flow back to the active site. This "push−pull" hypothesis offers a means for understanding how protein kinases can be regulated by protein−protein interactions far from the active site.

Introduction

Protein phosphorylation is recognized as the principal means of controlling cellular signal transduction. Accordingly, the enzymes that catalyze this posttranslational modification (protein kinases) are aggressively pursued as targets for chemotherapeutic agents.[1] Despite their wide-ranging physiological roles, protein kinases catalyze a very simple chemical reaction. As shown in Scheme 1, active-site-bound ATP transfers its γ phosphoryl group to the hydroxyls of serine, threonine, and tyrosine using a direct, in-line mechanism with no apparent chemical intermediates.[2] Therefore, much of what we learned about phosphoryl transfer reactions from simple chemical model systems can be applied to the protein kinases. For example, studies in the 1960s suggested that reactions of phosphate monoesters proceed through metaphosphate-like transition states.[3] Recent experimental and theoretical studies indicate that protein kinases are likely to utilize similar transition states for protein phosphorylation.[4−6] While all eukaryotic protein kinases share a simple kinase domain capable of binding ATP and protein substrates,[7] other noncatalytic protein domains are critical for regulating function. Neighboring polypeptide segments often flank this essential catalytic core profoundly enhancing or repressing catalysis (Figure 1). In other cases, regulation is achieved through protein−protein interactions with heterologous scaffolds, kinases, or docking domains.[8,9]

X-ray structures have revealed a highly conserved protein kinase framework, while solution approaches have provided important links between these structures and catalysis. In this review, we will highlight studies on two important protein kinases, cAMP-dependent protein kinase (PKA) and C-terminal Src kinase (Csk), that illustrate these links. PKA is a heterotetramer composed of a regulatory dimer, R_2, and two catalytic subunits, 2C. A great deal is known about the structure of the C-subunit,[10] the manner in which it is regulated by R_2 and other protein

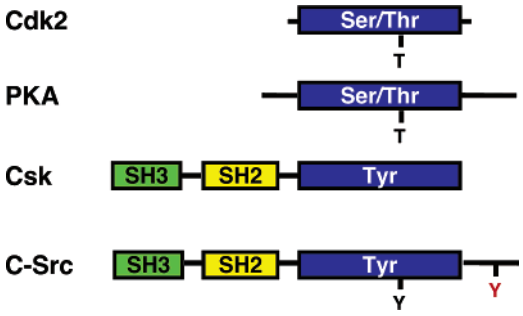
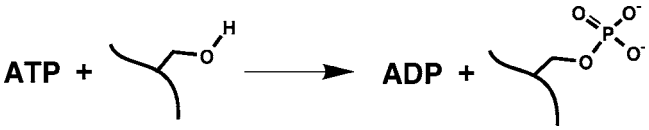


FIGURE 1. Domain structure of several protein kinases. The serine/threonine- and tyrosine-specific kinase domains are shown in dark blue. The phosphorylatable, activating residues in the kinase domains (T or Y) in PKA, Cdk2, and c-Src and the repressing, phosphorylatable residue in the C-terminus of c-Src (Y) are designated.

Scheme 1



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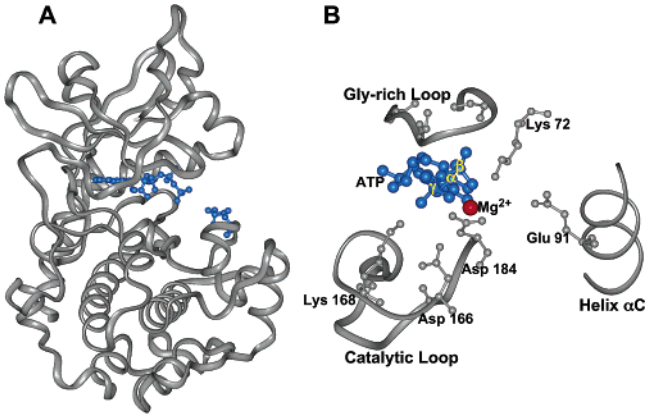

FIGURE 2. X-ray structure of PKA (PDB code 1ATP). Panel A shows a ribbon diagram of the kinase domain of PKA. ATP and the phosphothreonine in the activation loop are shown in blue. Panel B shows the active site of PKA with Mg•ATP bound. Asp-166, Lys-72, Asp-184, and Glu-91 are conserved across species and related homologues. The activating metal (Mg^{2+}) chelates Asp-184 and the β and γ phosphates of ATP.

factors,[11] the kinetic pathway for substrate phosphorylation,[12−14] and the subcellular localization of the inhibited R_2C_2 complex.[15] The binding of cAMP to the R_2-subunit leads to dissociation of the R_2C_2 complex and release of the active C-subunit. In contrast, Csk possesses regulatory SH2 and SH3 domains that are on the same polypeptide strand as the tyrosine kinase domain (Figure 1). The SH2 and SH3 domains, common to many signaling molecules, are important for protein−protein interactions that regulate in vivo function.[16] Csk down-regulates the catalytic activities of all members of the Src family of nonreceptor protein tyrosine kinases (PTKs) by phosphorylating the C-termini of each respective kinase.[17] The SH2 domain binds a phosphotyrosine in the adaptor protein, Cbp (Csk binding protein), localizing Csk to lipid rafts where it can then regulate the Src enzymes.[18] We will discuss how solution methods are used to identify the roles of phosphoryl transfer, product binding, and conformational changes in controlling substrate turnover and providing communication links between the active-site neighborhood and distal regulatory regions.

Structural Frameworks for PKA and Csk

PKA was the first protein kinase structure to be elucidated using X-ray diffraction methods[19] and remains a paradigm for this enzyme family. The kinase domain of the C-subunit of PKA is composed of two distinguishable lobes: a small, ATP binding and a larger substrate binding domain (Figure 2A). The active site lies between these two lobes and contains several catalytic residues (Figure 2B). The essential activating Mg^{2+} interacts with the γ phosphate of ATP and the conserved Asp-184. The hydroxyl of the substrate serine forms a hydrogen bond with the conserved Asp-166 (catalytic loop) suggesting that it may serve as a general-base catalyst. The inability to detect a pH dependence or solvent deuterium isotope effect on the phosphoryl transfer step suggests that, if Asp-166 is a catalytic base, it abstracts the proton late in the reaction

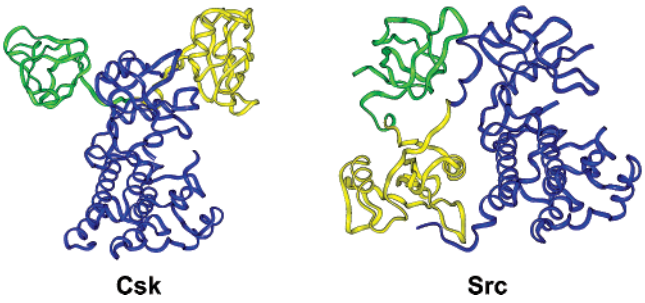

FIGURE 3. Ribbon diagrams of Csk (left panel, PDB code 1K9A) and Src (right panel, PDB code 2PTK). The SH2 (yellow), SH3 (green), and kinase (dark blue) domains are colored as in Figure 1. Notice the dramatic difference in the position of the SH2 domain, while the SH3 domain only shows a slight rotation between the two structures.

transition state.[5] Lysine-72 positions the α phosphate of ATP and is part of an important electrostatic dyad with the conserved Glu-91 in helix αC. The glycine-rich loop (GRL) covers the triphosphoryl region of ATP and controls the phosphoryl transfer rate and nucleotide affinity/specificity.[20,21] The catalytic activity of PKA is strongly enhanced by threonine phosphorylation in a segment known as the activation loop.[22] The C-subunit contains two segments flanking the catalytic core (Figure 1). The N-terminal extension is myristylated in vivo and makes close interactions with the kinase core but has no effect on catalytic activity in vitro. The unique C-terminus makes contacts with both the N- and C-terminal lobes and appears to modulate peptide substrate binding.[23]

In addition to catalytic function, the kinase domain also serves as a scaffold for the assembly of other regulatory domains and proteins. Csk is a useful paradigm for this higher order complexity. The tyrosine kinase domain of Csk contacts the SH2 and SH3 domains through the N-terminal lobe (Figure 3A). These domains not only provide a template for protein−protein interactions in vivo but also enhance catalysis by as much as 2 orders of magntude.[24,25] Interestingly, c-Src, a nonreceptor PTK with the same domain structure and organization as Csk (Figure 1), uses the SH2 domain for binding its own phosphorylated C-terminus, an interaction important for repressing activity. In this case, the phosphopeptide binding site of the SH2 domain is oriented toward the kinase core and interacts with the large lobe of the kinase domain, not with the phosphopeptide binding site oriented toward solvent and docked on the small lobe as is observed for Csk (Figure 3B). It is compelling to hypothesize that c-Src adopts a domain assembly similar to that for Csk when it is activated via dephosphorylation of the C-terminus. While Csk is considered a constitutively active enzyme because it does not require posttranslational modifications nor regulatory protein binding for activity, its interaction through its SH2 domain with scaffolding proteins such as Cbp enhance catalytic activity.[26] Indeed, a 10-residue phosphotyrosine-containing peptide derived from the Cbp recognition sequence also leads to activation. Thus, the presence of a small peptide in the SH2 domain pocket communicates information to the distal active site. However, the molecular explanation for this

enhancement in activity remains unclear. Later, we will discuss how deuterium exchange experiments have been used to investigate how long-range signaling within the structural framework of Csk may be used to explain these interdomain effects.

Kinetic Tools for Defining Regulatory Elements

While structure offers the visual cue for predicting functional models, kinetics provide the tools for establishing regulatory mechanisms. While steady-state kinetic methods have been the primary analytical tool for understanding mechanism, they cannot reveal the key steps that link specific regulatory events with structure. A classic example underscoring these limitations involves testing the autoinhibitor hypothesis. In this model, the dephosphorylated activation loop physically occupies the active site. Upon phosphorylation, the loop moves out of the pocket enabling the kinase to bind and phosphorylate substrate proteins. This model arose from the X-ray structures for the insulin receptor kinase [InRK] where phosphorylation causes a large conformational shift in the activation loop that improves access to the substrate binding pocket.[27] We originally tested this regulatory model in PKA by analyzing loop-dephosphorylated forms of the enzyme. Although kinetic experiments showed that dephosphorylation leads to a profound increase in K_m for the substrate, it does *not* influence real substrate affinity (i.e., K_d is unaffected by phosphorylation). Thus, the original model forged from the X-ray structures[27,28] was at odds with the solution data.

Since the original studies on the activation loop of PKA, functional investigations revealed that other protein kinases also do not obey the autoinhibitor model. This led to a reshaping of the hypothesis and the inclusion of two functional classifications for protein kinases: gated and nongated activation loops.[29] The gated activation loops up-regulate function by controlling both substrate access and the phosphoryl transfer step, whereas the nongated loops have no effect on substrate binding and rely solely on changes in phosphoryl transfer rate for regulation. Given the impact of these types of kinetic experiments, it is important to appreciate how they provide regulatory insights. For the interpretation of the effects of activation loop phosphorylation, we will consider the simple catalytic mechanism in Figure 4. The K_m for the substrate in this pathway is described in eq 1:

$$K_m = \frac{K_d + k_3/k_2}{k_3/k_4 + 1} \quad (1)$$

where $K_d = k_{-2}/k_2$. For the phosphorylated form of PKA, the K_d is approximately 20-fold higher than the K_m due to a large value for k_3/k_4, which effectively diminishes K_m. However, for the dephosphorylated form, the K_m is similar in value to K_d since the phosphoryl transfer step is now rate-limiting ($k_3/k_4 \ll 1$) and the substrate exchanges rapidly with the active site in both enzyme forms ($k_3/k_2 \ll K_d$). Despite the observed effects on K_m and k_3/k_4, phosphorylation of the activation loop does not impact

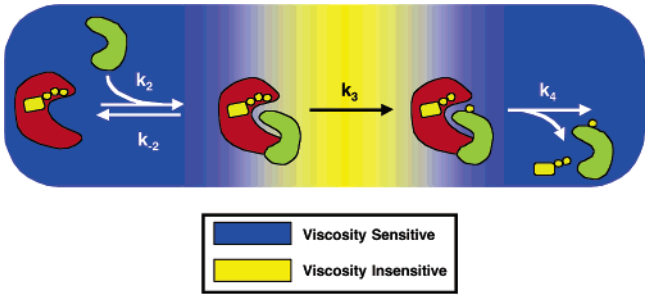

FIGURE 4. Kinetic pathway for a protein kinase at high physiological levels of ATP. The binding of substrate (green) is conducted through association (k_2) and dissociation (k_{-2}) rate constants. Under initial velocity conditions, the net product release step (k_4) is irreversible. The phosphoryl transfer (k_4) is highly favorable, so the reverse (ADP phosphorylation) is ignored.[40] The kinase domain and ATP are shown in red and yellow.

K_d. Thus, attaining information on the individual steps in protein kinase catalysis, a process known as kinetic reductionism,[29] is the best way to directly test regulatory models. This approach requires methodologies that exceed the sophistication of simple activity assays. In the following, we describe two kinetic techniques that have been most successful for the study of protein kinase function.

1. Viscosity Method. In the viscosity method, the individual steps in the kinetic sequence are classified based on molecularity. Bimolecular events (substrate binding and product release) are expected to be influenced by solvent viscosity whereas the unimolecular steps (phosphoryl transfer step) are expected to be unaffected by solvent viscosity (Figure 4). The values of the rate constants can be discerned by the degree to which viscous additives or viscosogens (e.g., glycerol, sucrose) influence the steady-state kinetic parameters.[12,30] A plot of either k_{cat} or k_{cat}/K_m as a ratio in the absence and presence of added viscosogens versus the relative solvent viscosity is a straight line with the following slope values shown in eqs 2 and 3.

$$k_{cat}^{\eta} = \frac{k_3/k_4}{k_3/k_4 + 1} \quad (2)$$

$$(k_{cat}/K_m)^{\eta} = \frac{k_3/k_2}{K_d + k_3/k_2} \quad (3)$$

For PKA, the presence of a large viscosity effect on k_{cat} [$k_{cat}^{\eta} = 1$] implies that the phosphoryl transfer step does not limit substrate turnover (i.e., $-k_3/k_4 > 1$).[12]

2. Pre-Steady-State Kinetic Methods. While implementation of the viscosity method is relatively facile, it is is not without limitations. The assignment of the steps in Figure 4 is normally straightforward, but other physical events (e.g., conformational changes) may or may not be perturbed by added viscosogens. For example, the absence of a viscosity dependence on k_{cat} due to a slow conformational change may be erroneously interpreted as the result of slow phosphoryl transfer. Therefore, while the presence of a viscosity effect on turnover implies fast phosphoryl transfer, caution must be taken in interpreting

the absence of this effect. The solution to this problem lies in the direct measurement of phosphate incorporation in the first enzyme cycle (i.e., pre-steady-state kinetics). Using rapid quench flow instrumentation, we showed that PKA phosphorylates its substrate in two discrete kinetic phases: a fast, exponential "burst" phase followed by a slow linear phase.[13] The latter phase corresponds to k_{cat} in the steady state, whereas the faster phase corresponds directly to the substrate phosphorylation in the active site. This method does not rely on the assignments of molecularity but rather on the chemical formation of a bond between the γ phosphate of ATP and the substrate hydroxyl.

Owing to the ability to identify the chemical step, pre-steady-state kinetic methods are preferred over viscosity methods particularly when the kinase under investigation lacks a viscosity dependence on k_{cat}. In one important example, we showed that Csk displays no appreciable viscosity effect on k_{cat} yet pre-steady-state kinetic experiments demonstrate that the phosphoryl transfer step is fast.[31] In addition to providing direct information on this step, these kinetic studies hint to added complexity in the kinase reaction not depicted in Figure 4. In general, the amplitude for the "burst" phase should be close in value to the total enzyme concentration when the phosphoryl transfer step is fast. This is, indeed, the case for PKA where the "burst" amplitude reflects 100% of the total enzyme.[13] In the case of Csk, however, this amplitude accounts for only 20%, suggesting that high- and low-activity enzyme forms are populated.[31] In this scenario, it is difficult to interpret the magnitude of the viscosity effect since two ternary enzyme complexes may contribute to the turnover step.

Net Flux through the Phosphoryl Transfer Step

Through the application of viscosity and pre-steady-state kinetic techniques, the role of the phosphoryl transfer step in controlling substrate turnover has been addressed in a representative number of protein kinases. Figure 5 summarizes the current data available for 11 protein kinases from the serine (PKA, cdk2, Sky1, ERK2, p38 MAP kinase, phosphorylase kinase) and tyrosine (v-Fps, Csk, HER-2, InRK, Tie2) kinase classes. The data in this bar graph compare kinetic data from only fully activated protein kinases. While some protein kinases are constitutively active (e.g., Csk, Sky1) and require no activation steps after translation, others require regulatory protein binding (e.g., Cdk2) or activation loop phosphorylation (e.g., PKA, v-Fps, Her-2, etc.) or both for high activity. In regard to this comparison, it is worth noting that some protein kinases are assayed under different conditions. For example, the data for Csk was attained using Mn^{2+} rather than Mg^{2+}. It is possible that the lower activity of Csk in the presence of the physiological metal may reflect a change in rate-limiting step.[32] Nonetheless, the summary in Figure 5 suggests that the residues in the kinase active site (Figure 2B) are optimally arranged to facilitate rapid protein phosphorylation. Thus, events outside of the chemical transfer of the γ phosphate of ATP must be considered in developing regulatory models for the protein kinases.

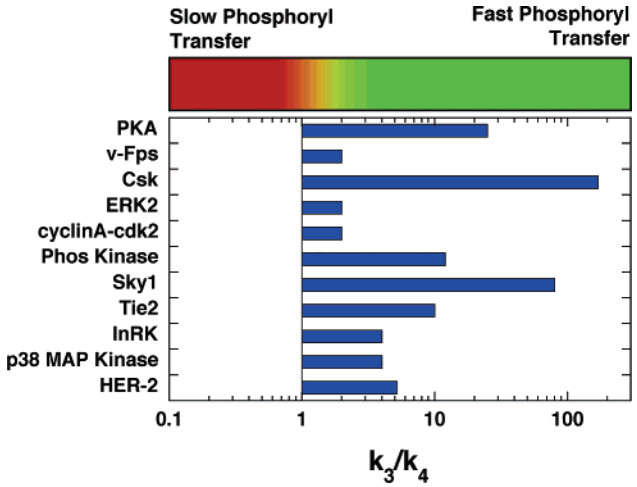

FIGURE 5. Rate of the phosphoryl transfer step in several protein kinases. The value is expressed as a ratio of the phosphoryl transfer rate (k_3) and the net product release step (k_4). Values of k_3/k_4 greater than 1 imply that the phosphoryl transfer rate is much faster than the product release step and does not limit turnover. Values less than 1 imply that k_3 is slow and limits turnover. The data for this bar graph were assembled using previously published literature values.[13,31,38,47,49–55]

Nucleotide Binding and the Catalytic Cycle

Current X-ray data indicate that protein kinases can adopt multiple conformations. For example, several enzymes in this family have been crystallized in "open" and "closed" forms that differ by rotation of the nucleotide and substrate binding lobes.[33] In addition to these rotations, movements in individual loops and helices have also been observed. Cyclin A binding to cdk2 causes large movements in helix αC and the activation loop.[28] In this case, the regulatory protein is thought to push helix αC into the kinase domain, a movement that effectively remodels the activation loop. In contrast to this protein-induced change, inward sliding of helix αC is induced by activation loop phosphorylation in InRK and ERK2.[27,34] In the above cases, these helical movements are believed to be critical for the formation of the conserved electrostatic dyad between glutamic acid and lysine (Figure 2B).

While changes observed in X-ray diffraction studies are certainly compelling, they do not confirm that movements occur in solution and are compulsory elements of the catalytic cycle. To address this issue, we fluorescently labeled a mutant form of PKA and found that ATP binding is accompanied by a slow conformational change step close in value to the mutant's k_{cat}.[35] Can the results from this PKA mutant be applied to the wild-type enzyme? The answer to this question came unexpectedly. Since our original kinetic studies on PKA were performed at high free Mg^{2+} levels, we lowered the free concentration from 10 to 0.5 mM to reflect physiological ion concentrations.[36] Under these conditions, we found that the fast phosphoryl transfer step in PKA is flanked by two slow conformational changes linked to nucleotide binding.[14,37] These structural

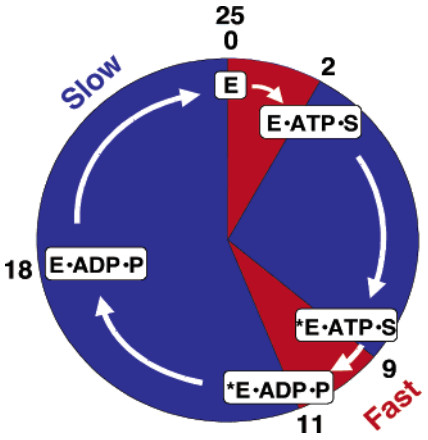

FIGURE 6. Catalytic stop watch for PKA. The mean lifetimes for all enzyme species are shown cumulatively on the perimeter of the watch and are in units of milliseconds. In this cycle, S is a seven-residue substrate peptide, Kemptide, and the two starred complexes represent altered enzyme ensembles.[13,14,37] Regions colored in red represent mean lifetimes of 2 ms or less.

events play a significant role in controlling the time course for substrate phosphorylation. To illustrate this point, we constructed a phosphorylation time clock that relates the mean lifetimes of all enzyme species within the catalytic cycle of PKA (Figure 6). The time needed to generate and release phosphoproduct in the cycle is limited largely by physical events linked to nucleotide binding. It is possible that the time parameters of this catalytic clock are common to other protein kinases. For example, ADP release and associated conformational changes partially limit k_{cat} in the receptor tyrosine kinase Her-2 and Csk.[31,38]

Long-Range Inductive Effects in Protein Kinases

In recent years, enzymologists have grown to appreciate the strong link between conformational dynamics and catalysis.[39] For the protein kinases, conformational changes that alter residue interactions with the nucleotide are well-documented in crystallographic models (see previous section). Given the detailed kinetic data gathered in recent years in our laboratory, it is reasonable to presume that nucleotide binding will be linked to regulation. Thus, information on the structural consequences of nucleotide binding will be useful for characterizing the nature of the rate-limiting step(s) in protein phosphorylation and determining the physical parameters that regulate these steps. Although the three-dimensional structure of the kinase core is well established, the manner in which ATP gains access to the nucleotide pocket is still a mystery. ATP rests deep in the active-site pocket of the kinase domain and in space-filling models appears largely enveloped by the GRL and catalytic loop. While these observations offer the expectation of ordered ligand binding with ATP preceding substrate, most kinetic investigations have curiously demonstrated that ATP has free access to the active site when the substrate is already bound.[40] Clearly, the kinase scaffold must incorporate sufficient flexibility to accommodate physiological ligands but still be able to position the catalytic residues for productive phosphoryl transfer.

Solution studies play an important role in addressing regulation through conformational changes. Protease footprinting studies revealed changes in cleavage profile in the presence of ADP compared to the apoenzyme suggesting that nucleotide-induced changes occur outside the active site of PKA.[41] Furthermore, fluorescence energy transfer studies demonstrate that the degree of lobe rotation in PKA is a function of the nature of the nucleotide.[42] To address the role of the nucleotide on protein kinase conformation in solution, we employed hydrogen−deuterium [H−D] exchange experiments to identify nucleotide-affected regions in PKA and Csk. Figure 7 shows regions in both PKA and Csk that experience changes in deuterium incorporation rate in the presence of bound nucleotides. Effects in the GRL and catalytic loop of both enzymes result from direct interactions with the nucleotide, whereas effects in helix αC occur in the absence of a direct interaction. Using H−D exchange methods, Ahn and co-workers showed that similar regions are also affected by activation loop phosphorylation in ERK2.[43] These mutually affected segments in Csk, PKA, and ERK2 likely reflect medium-range propagations modulated by interactions between two conserved residues, the lysine that binds to the α phosphate of ATP and the glutamate in the helix (Figure 2B; Glu-91 and Lys-72 in PKA). Overall, the effects in the GRL, catalytic loop, and helix αC constitute a core set of dynamically related elements critical for nucleotide association.

Outside the core elements, H−D exchange results hint to a larger network of interacting components that appear unique to each kinase. For example, the binding of ADP affects the C-terminus in PKA (Figure 7), a region that is outside the canonical kinase domain (Figure 1). This tail sequence traverses both lobes of the kinase domain and may report on rotations in the two lobes as the nucleotide enters the deep pocket. Csk, lacking a prominent C-terminal section, possesses other unique long-range effects. The binding of nucleotides to this enzyme influences the activation loop and helix αG in the large lobe of the kinase domain, a phenomenon that may reflect a concerted transmission of effects. Interestingly, long-range effects in Csk are not limited to the kinase domain. Nucleotide binding also influences two key elements of structure important for interactions with the SH2 domain. The linker region between the kinase and SH2 domains (SH2−kinase linker) and helix αA within the SH2 domain are both impacted by nucleotide binding. In Csk, the nucleotide not only influences the core elements (GRL, catalytic loop, helix αC), as expected, but also imparts changes that ripple through the multidomain protein. We have also shown that the magnitude of these effects is sensitive to nature of the bound nucleotide. For instance, loss of the γ phosphate in the ADP compared to the ATP complex results in increased stabilization of the GRL, destabilization of the activation loop, and equivalent

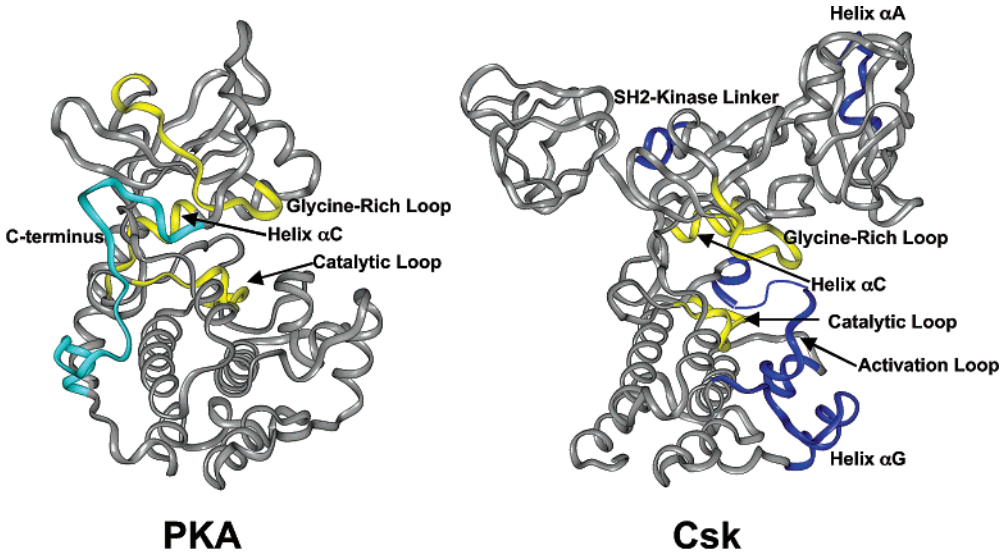

FIGURE 7. Altered solvent deuterium exchange in PKA (left) and Csk (right) upon nucleotide binding. Short-to-medium range effects (core region) in the GRL, catalytic loop, and helix αC are shown in yellow in both structures. Long-range effects induced by nucleotide are shown in light blue (PKA) and dark blue (Csk). A detailed discussion of these effects for both proteins is published elsewhere.[44,56]

stabilization of the catalytic loop and helix αC. These region-specific changes in dynamics can be interpreted as alterations within the structural ensemble (conformational states defined by fluctuations around the lowest energy state) during catalysis. The coupling energies among different regions are a result of the redistribution of the conformational ensemble due to binding or phosphate transfer.[45] Thus, these nucleotide-specific changes in stabilization and dynamics of conserved regions within Csk upon phosphate transfer suggests that there are discrete communication pathways within the kinase scaffold.

Control from Afar

Noncatalytic regions of Csk (as well as other kinases) that are well removed from the active site (Figures 1 and 3) influence phosphoryl transfer. In addition, the binding of the adaptor protein Cbp to the SH2 domain of Csk upregulates catalytic activity, although the regulator is also well removed from the active site.[26] Other scaffolding proteins such as caveolin-1 bind to the SH2 domain and may also have similar effects on catalysis.[46,47] In a similar vein, chemical modification via phosphorylation of Ser-363 in the large lobe of the kinase domain by PKA in T cells causes catalytic enhancements although the site of phosphorylation is again far removed from the active-site pocket.[48] Thus, the interplay between the nucleotide-linked core and the peripheral regions define communication roadways within the protein kinase during catalysis. Interestingly, our characterization of the effects of nucleotide analogues on the thermodynamic structural ensemble with H−D exchange methods also reveals a nucleotide-linked core that serves as a nerve center for the productive communication of signals across the kinase domain and into neighboring noncatalytic domains. While the H−D exchange experiments do not define the molecular mechanism underlying this interplay, they serve as a useful indicator of distal structural elements that may potentially serve as docking sites for other regulatory elements. Taken together, the thermodynamic and kinetic studies reveal potential routes for the bidirectional flow of information between the active and distal sites (Figure 8). Current experimental and theoretical efforts in our laboratories are directed toward investigating this long-range communication property in Csk and related enzymes.

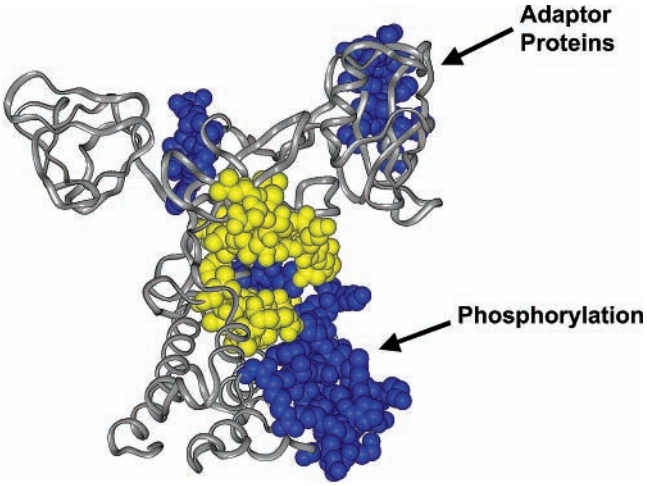

FIGURE 8. Communication Roadway in Csk. The core region (GRL, catalytic loop, and helix αC) is shown in yellow, and the distal regions are shown in dark blue. Csk is displayed in the same orientation as in Figure 3 (left panel). The enzyme is activated by phosphorylation in the large lobe of the kinase domain and by adaptor protein binding to the SH2 domain.[26,48,57]

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Abbreviations

Cbp: Csk binding protein
Csk: C-terminal Src kinase
GRL: glycine-rich loop
PKA: cAMP-dependent protein kinase
PTK: protein tyrosine kinase
SH2: Src homology-2
SH3: Src homology-3.

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