Protein–protein interactions in the allosteric regulation of protein kinases  
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Protein–protein interactions involving the catalytic domain of protein kinases are likely to be generally important in the regulation of signal transduction pathways, but are rather sparsely represented in crystal structures. Recently determined structures of the kinase domains of the mitogen-activated protein kinase Fus3, the RNA-dependent kinase PKR, the epidermal growth factor receptor and Ca\(^{2+}\) or calmodulin-dependent protein kinase II have revealed unexpected and distinct mechanisms by which interactions with the catalytic domain can modulate kinase activity.

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**Introduction**

The clustering of receptor molecules is emerging as a key feature of intracellular signal transduction. Such clustering brings signaling molecules into close proximity at high local concentrations and promotes a diverse array of protein–protein interactions, often through the utilization of specialized domains [1]. Within these clusters, protein kinases, which use ATP to phosphorylate proteins on serine/threonine or tyrosine residues, become activated by being phosphorylated themselves or by interacting with other proteins or ligands.

In the 15 years since the first structure of a protein kinase was determined, that of cAMP-dependent protein kinase (PKA) [2], much has been learnt at the structural level about how conformational control is exerted on the kinase domain. The structure of the cyclin-dependent protein kinase Cdk2 was determined shortly after that of PKA and revealed an inactive conformation of the kinase domain, rather than the active form seen for PKA [3]. The structure of a Cdk2–cyclin A complex showed us how protein–protein interactions can bring about conformational changes in the kinase domain, in this case resulting in activation [4]. Since then, much effort has been focused on filling in structural information for the kinome [5], understanding how intramolecular interactions control the kinase domain (e.g. in the Src [6,7] and Abl [8,9] tyrosine kinases), how the conformation of the activation loop controls kinase activity [10,11], how peptide substrates are recognized [12,13] and how the modulation of kinase structure is reflected in the specificity of kinase inhibitors [14].

Surprisingly little has been revealed so far at the detailed structural level about how protein–protein interactions involving the kinase domain result in changes in kinase activity, with one prominent exception being the early analysis of the Cdk–cyclin interaction [4]. This is now changing with several recent studies providing new information on protein–protein interactions involving the catalytic domain of protein kinases. We summarize the findings of some of these studies here.

**A kinase domain acting alone: activation loop phosphorylation in cis**

We begin by digressing somewhat to describe a remarkable observation concerning the DYR (dual-specificity tyrosine-phosphorylation-regulated) kinases. The most obvious protein–protein interaction involving kinase domains is the formation of a dimer that promotes transphosphorylation of the centrally located activation loop and subsequent activation (Figure 1). The kinase active site appears to be so constructed that phosphorylation cannot occur in cis. This restriction arises because peptide substrates bind to the catalytic domain in an orientation opposite to that of the activation loop backbone [12,15] (Figure 1). Crystal structures of inactive kinases, such as those of insulin receptor kinase (IRK) [16] and c-Abl [8], show that the active site is disrupted when the activation loop folds back so as to present itself as a substrate mimic.

DYR kinases (DYRKs) phosphorylate their substrates on serine/threonine residues, but are themselves autophosphorylated on a critical tyrosine residue in their activation loop [17–21]. Intriguingly, autophosphorylation of DYRKs is a result of intramolecular rather than intermolecular phosphate transfer, and this phosphorylation occurs before the kinase leaves the ribosome [22]. It is proposed that this in cis reaction is catalyzed by a translational intermediate of the kinase domain that differs from the mature and fully folded enzyme in that the intermediate can phosphorylate the activation loop...
2 Catalysis and regulation

Figure 1

Anatomy of a protein kinase catalytic domain. Ribbon diagram representing the main features of a prototypical kinase catalytic domain based on the structure of IRK (PDB code 1R8) [12]. The N-lobe is shown in green, the C-lobe is shown in purple and the activation loop is colored yellow. The substrate peptide, docked onto the activation loop, and an ATP analog are shown in black. Helix-C, which contains residues critical for catalysis, and helix-G, which is involved in docking protein substrates, are labeled.

Figure 2

Comparison of Fus3 in complex with a peptide derived from the scaffolding protein Ste5 and PKA. (a) The Ste5 peptide, shown in dark blue, docks onto Fus3 (PDB code 2F49) [23**], shown in white. (b) The Ste5 peptide follows a path similar to that of the C-terminal tail extension (dark blue) of PKA (PDB code 2CPK) [2].

Tyrosine whereas the mature form cannot. Autophosphorylation by a folding intermediate has so far only been described for this family of kinases, but it raises the possibility that such translational intermediates might provide alternative targets for inhibitor development [22**].

Scaffolds as allosteric regulators of kinases

By bringing signaling components into close proximity, the yeast scaffold protein Ste5 contributes to the speed and specificity of the mating pheromone signaling pathway. In addition to assembling components of the mitogen-activated protein (MAP) kinase pathway, it now appears that Ste5 regulates at least one of them allosterically [23**]. A peptide containing a 29-residue minimal region of Ste5 stimulates the rate of Fus3 autophosphorylation by ~50-fold. The co-crystal structure of Fus3 in complex with the Ste5 peptide shows a bipartite mode of binding, with both N- and C-lobes of the kinase domain engaged by the peptide.

Superposition of peptide-bound and unbound structures of Fus3 shows a relative displacement between the N- and C-lobes, suggesting that Ste5 modulates Fus3 activity by coordinating an optimal orientation between the two lobes of the kinase (Figure 2). In going from the C-lobe to the N-lobe through a path that is distal to the active site, the topology of the Ste5 peptide bound to Fus3 is reminiscent of the way in which a C-terminal extension of PKA folds back onto the distal face of the kinase [2]. The C-terminal extension of PKA is thought to stabilize the active state and a similar mechanism may be one component of the activating effect of the Ste5 peptide on Fus3 [24] (Figure 2).
In another study concerning a MAP kinase cascade enzyme, TAK1 (a MAP kinase kinase kinase), the interactions between the kinase domain of TAK1 and a peptide segment of TAB1 (TAK1-binding protein 1) have been defined [25**]. The structure shows that a 36-residue TAB1 fragment docks onto a hydrophobic patch in the C-lobe. Just how this interaction increases the catalytic activity of TAK1 is unclear at present, but it is intriguing that the α helix formed by the TAK1-interacting segment of TAB1 inserts hydrophobic sidechains into a cavity at the base of the C-lobe of the kinase domain [25**], in a similar position to where a myristoyl group is bound to the structure of autoinhibited c-Ab1 [9].

**A symmetrical kinase domain dimer: PKR**

RNA-dependent protein kinase (PKR) shuts down protein translation by phosphorylating the translation initiation factor eIF2 [26]. The upstream signal to this event is the binding of double-stranded RNA to PKR. As for the majority of protein kinases, activation of PKR involves autophosphorylation of the activation loop [27]. Mutations in PKR that impair RNA binding also affect the ability of the kinase molecules to dimerize. These mutations also result in reduced PKR autophosphorylation and catalytic activity [28–31], suggesting a link between dimerization, autophosphorylation and substrate phosphorylation. Indeed, a recent study uncovered several mutations in the kinase domain that result in elevated kinase activity by promoting dimerization [32**].

The dimerization mechanism of PKR has been revealed by crystallizing the kinase domain of PKR in complex with its protein substrate, eIF2 [33**]. The PKR kinase domains in these structures form a symmetrical dimer with back-to-back interactions between the N-lobes of both kinases (Figure 3). Although the mutational analysis makes it clear that the formation of this dimer is important for the activation of PKR, the structure does not completely explain why this is the case. In this regard, it is most intriguing that a very similar mode of dimerization has been observed in the crystal structures of a group of protein kinases that are unrelated to PKR, the *Mycobacterium tuberculosis* kinases PknB and PknE [34**,35,36]. Again, the consequences of dimerization for the catalytic activity of the kinase domain are not entirely clear from these studies [34**,35], but the close similarity of the dimer interfaces of PKR and the *M. tuberculosis* kinases emphasizes the functional importance of this interaction. A key aspect of the interaction between kinases in these dimers is the engagement by one kinase domain of the region abutting helix-C in the N-lobe of the other kinase domain. Interactions in this region are critical for the regulation of many protein kinases, for example, the Src kinases [37,38] and G-protein-coupled receptor kinases (GRKs) [39**] (Figure 3).

The PKR structure is also very important because it provides the first view of a kinase domain recognizing an intact protein substrate. eIF2 docks onto a region of the kinase domain that is adjacent to the active site, next to helix-G in the C-lobe. The structure suggests an allosteric coupling mechanism between substrate docking to helix-G and the conformation of the activation loop. Helix-G has also been implicated recently as a docking site for the mutual recognition of two kinase domains during transphosphorylation of the activation loop of p21-activated kinases (PAKs) [40**,41]. The coupling of kinase activation to substrate recognition has also been recognized in other protein kinase families, such as Src [42] and Cdkks [43].

**EGF receptor: a case of molecular plagiarism**

Members of the epidermal growth factor (EGF) receptor family, important in many cancers [44], transduce a ligand-binding event outside the cell into an increase in kinase activity inside the cell. EGF receptor is a transmembrane protein possessing ligand-binding and kinase domains that are separated from each other by the plasma membrane. The C-terminal tail of EGF receptor contains autophosphorylation sites that recruit signaling molecules when phosphorylated.

Receptor dimerization, or the formation of higher order oligomers, results in the activation of EGF receptor [45–47]. Unlike most protein kinases, the EGF receptor does not require activation loop phosphorylation [48–51] and thus the mechanism for the increase in activity upon ligand-induced dimerization has remained elusive. Recently, a re-examination of previously determined crystal structures [51,52], as well as the analysis of new ones, led to the discovery that an interface between two kinase domains that is seen in all crystal structures of active EGF receptor kinase domains provides an explanation for the mechanism of receptor activation by dimerization [53**].

In the active kinase crystal structures [51,53**], the crystal packing between kinase molecules includes an asymmetric C-lobe to N-lobe interaction between two kinase domains. The crystals also contain a symmetric interface that has been analyzed extensively [54], but does not seem to be critical for the activation process [53**]. The intriguing feature of the asymmetric arrangement is that it resembles the interactions seen between Cdk2 and its activator, cyclin A [4] (Figure 4). Sequence alignments show that residues at this interface are invariant among the three catalytically active EGF receptor family members, EGF receptor, HER2 and HER4, but are not conserved in the N-lobe face of the catalytically inactive ErbB3 [53**]. The formation of the cyclin-like N-lobe to C-lobe interface explains how ErbB3, despite being catalytically dead, can still activate other kinase domains by heterodimerization [55]. Disruption of this interface by mutagenesis results in the adoption by the kinase...
domain of an inactive structure that closely resembles those of Cdk2 and the Src kinases [53**]. This inactive Src/Cdk-like structure is also seen in the crystal structure of the EGF receptor kinase domain bound to the cancer drug tykerb [52].

Src/Cdk-like inactive conformations have recently been observed in several kinases, including GCN2 [56], c-Abl [57] and Snf1 [58,59**], a member of the AMP-activated kinase family [60]. Snf1 also forms a dimer, but, in contrast to the EGF receptor, the Snf1 dimer stabilizes the inactive rather than the active conformation [59**].

**Dimers within supramolecular assemblies: CaMK-II**

Ca^{2+}/calmodulin-dependent protein kinase II (CaMK-II) is a key player in processes underlying learning and memory, and also in other functions throughout the body [61]. The most remarkable property of this enzyme is its apparent ability to respond not only to the amplitude of intracellular Ca^{2+} spikes that occur during neuronal stimulation but also to their frequency [61,62]. CaMK-II is unique among protein kinases in that it forms stable dodecamers. Although it is not clear how CaMK-II can decode Ca^{2+} spike frequencies, the answer may lie in the supramolecular organization of the enzyme.
The domain structure of CaMK-II resembles that of other CaM-dependent kinases in that it has an N-terminal kinase domain followed by a regulatory region of approximately 40 residues. Unique to CaMK-II is the C-terminal association domain, which is responsible for oligomerization. Studies using electron microscopy have shown that CaMK-II forms two hexameric rings that are stacked one on top of the other [63–65]. A detailed view of the manner in which the two stacked rings are assembled has been provided by a crystal structure of the association domain of CaMK-II [53**].

Figure 4

Comparison of the mechanism of EGF receptor activation by dimerization with the Cdk2–cyclin A complex. In the EGF receptor asymmetric dimer, one kinase catalytic domain is activated by another kinase catalytic domain in a C-lobe to N-lobe interaction [53**]. This is analogous to the mechanism of activation of Cdk2 by cyclin A (PDB code 1FIN) [4].

Figure 5

Structure of the CaMK-II kinase domain and regulatory region. (a) The kinase catalytic domains within the dimer are shown in blue and green. Dimerization occurs through the regulatory regions, which form a coiled-coil strut. The regulatory regions contain the CaM-binding site, shown in red. The location of the major regulatory autophosphorylation site, Thr286, is shown for one of the monomers (PDB code 2BDW) [68**]. (b) Hypothetical reconstruction of the CaMK-II holoenzyme, based on the crystal structure and SAXS analysis [68**], showing a possible location of the dimer unit within the dodecameric holoenzyme.

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CaMK-II [66]. One curious fact is that the association domain on its own (without the kinase domain) forms rings that are sevenfold rather than sixfold symmetric [67].

The crystal structure of the autoinhibited kinase domain and regulatory region has been described recently [68••] (Figure 5). As seen in other CaM-dependent protein kinases [69,70], the regulatory region blocks the active site. CaM binding relieves the autoinhibition, releasing an intrinsically active kinase domain (i.e. phosphorylation of the activation loop of CaMK-II is not required for activity). The binding of CaM also exposes the major autophosphorylation site of CaMK-II, Thr286 (mouse αCaMK-II numbering), which is located in the regulatory region. Autophosphorylation of Thr286 renders the kinase constitutively active and no longer responsive to Ca2+/CaM. In this manner, CaMK-II retains a ‘memory’ of the previous calcium spike [61].

The asymmetric unit of the crystal contains a dimer in which the regulatory regions of the two kinase domains form an antiparallel coiled-coil strut [68••]. This peculiar architecture is suggestive of two properties that may be important for enzyme function. First, a new layer of autoinhibition is added by having the regulatory region of one kinase domain make contact with the regulatory region of a second kinase domain, a feature that may be crucial to prevent background activation by transphosphorylation. Maintaining the dodecamer in a continuously repressed state is not an easy task considering that, in this setting, the local concentration of kinase domains is in the millimolar range [68••]. Second, such an arrangement can help explain the cooperative nature of Ca2+/CaM binding to the holoenzyme [68••,71]: if the dimer is the basic unit of the holoenzyme assembly, binding of the first CaM to one kinase domain would, in principle, free up its interaction with the regulatory region of the second kinase domain, making the CaM-binding site more accessible.

Conclusions

Recent structural studies on protein kinases have uncovered novel regulatory mechanisms involving protein–protein interactions. Some of these mechanisms exhibit striking similarity among functionally unrelated kinase families. The interaction between Ste5 and Fus3 bears some resemblance to the manner in which extra segments of PKA interact with the kinase domain. The dimerization of PKR is strikingly similar to that of bacterial kinases of the PknB family. The activation of the EGF receptor uses the same trick seen in the Cdk–cyclin complex, with the EGF receptor kinase domain serving as its own ‘cyclin’. It will be interesting to determine if the conserved kinase domain fold possesses a limited number of interfaces from which control of activity can be exerted.

Protein molecules in a crystal must necessarily interact with each other and crystal structures are usually replete with potential protein–protein interaction surfaces. Caution must therefore be employed in interpreting the biological significance of crystal packing interfaces [72]. Some of the studies discussed here demonstrate that the development of hypotheses based on crystal packing and confirmed by biochemical and biophysical techniques can greatly contribute to our understanding of protein kinase regulation.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

Allosteric regulation of protein kinases Pellicena and Kuriyan


The first study to describe in detail the intramolecular autophosphorylation of the activation loop of a protein kinase. The study presents evidence of a transitional intermediate with residue and inhibitor specificities that differ from those of the mature kinase.


The allosteric regulation of a kinase by a scaffolding protein is uncovered. Structural and biochemical studies help put forth a model for the mechanism of regulation. Yeast genetics is used to further explore the consequences of the allosteric activation of the kinase. This is the first detailed demonstration that scaffolds proteins can play an active role in signaling.


The crystal structure of a complex between a kinase domain fused to a segment of a protein known to stimulate autophosphorylation is presented.


A random mutagenesis screen of the PKR kinase domain results in the identification of a dimeric interface important for activation. Further mutational and biochemical analyses, together with the crystal structures described in [33], are combined to put forth a mechanism for PKR regulation.


Crystal structures of PKR show a back-to-back dimer important for kinase activation. Complexes between the dimer and its protein substrate, together with the studies described in [32], are used to present a model in which dimerization, activation loop phosphorylation and substrate recognition may function together.


The back-to-back dimer seen in PKR has also been described for PknE and other M. tuberculosis kinases, suggesting there may be a common mechanism for the activation of several protein kinase families.


This paper reports the crystal structure of GRK2 in complex with fully dissociated Galphaq and Gbetagamma. In the complex, Galphaq forms an effector-like interaction with GRK2.


A re-examination of PAK1 crystal structures, combined with NMR and mutational analyses, uncovers an interface that is used as a docking site in the transphosphorylation reaction of PAK2 kinase domains.


8 Catalysis and regulation


Crystallographic, biochemical and mutational analyses are combined to demonstrate that the activation of EGFR is accomplished by the formation of a catalytic domain asymmetric dimer. The C-lobe of one kinase domain interacts with the N-lobe of the dimer partner in a manner analogous to the activation of Cdk2 by cyclin A. The structure of the kinase C-lobe is, however, distinct from that of the cyclins.


The crystal structure of Snf1 reveals a kinase dimer with both catalytic domains in an inactive conformation. Dimer formation is also demonstrated in solution. The catalytic domains are found to resemble the inhibited form of Cdk2. The structure of Snf1 is also discussed in [58], but that study concluded that dimer formation in solution was unlikely.


The unexpected observation that the basic CaMKII-II unit is a catalytic domain dimer in which the regulatory regions of each form a coiled-coil strut is used to explain certain biochemical features of the enzyme. Together with other biophysical studies and small-angle X-ray scattering (SAXS) analysis of the holoenzyme, the dimer seen in the crystal structure is used to present a model for the structure of the holoenzyme.


