REVIEW ARTICLE

Signalling specificity of Ser/Thr protein kinases through docking-site-mediated interactions

Ricardo M. BIONDI^{*1,2} and Angel R. NEBREDÁ^{†2}

^{*}Division of Signal Transduction Therapy, School of Life Sciences, University of Dundee, Dundee DD1 5EH, U.K. and [†]European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Signal transduction pathways use protein kinases for the modification of protein function by phosphorylation. A major question in the field is how protein kinases achieve the specificity required to regulate multiple cellular functions. Here we review recent studies that illuminate the mechanisms used by three families of Ser/Thr protein kinases to achieve substrate specificity. These kinases rely on direct docking interactions with substrates, using sites distinct from the phospho-acceptor sequences. Docking interactions also contribute to the specificity and regulation of protein kinase activities. Mitogen-activated protein kinase (MAPK) family members can associate with and phosphorylate specific substrates by virtue of minor variations in their docking sequences. Interestingly, the same MAPK docking pocket that binds substrates also binds docking sequences of positive and negative MAPK regulators. In the case of glycogen synthase kinase 3 (GSK3), the presence of a phosphate-binding site allows docking of previously phosphorylated (primed) substrates; this docking

site is also required for the mechanism of GSK3 inhibition by phosphorylation. In contrast, non-primed substrates interact with a different region of GSK3. Phosphoinositide-dependent protein kinase-1 (PDK1) contains a hydrophobic pocket that interacts with a hydrophobic motif present in all known substrates, enabling their efficient phosphorylation. Binding of the substrate hydrophobic motifs to the pocket in the kinase domain activates PDK1 and other members of the AGC family of protein kinases. Finally, the analysis of protein kinase structures indicates that the sites used for docking substrates can also bind N- and C-terminal extensions to the kinase catalytic core and participate in the regulation of its activity.

Key words: docking, glycogen synthase kinase 3 (GSK3), mitogen-activated protein kinase (MAPK), phosphoinositide-dependent protein kinase-1 (PDK1), phosphorylation, protein kinase.

INTRODUCTION

Protein phosphorylation is ubiquitous and is the most common mechanism of protein function regulation known to date. In eukaryotes, phosphorylations are carried out by protein kinases, a family of evolutionarily related enzymes that transfer the terminal phosphate from ATP to a specific Ser, Thr or Tyr residue on protein substrates. Protein kinases represent about 2 % of the proteins encoded by eukaryotic genomes [1–4], and this makes them the third most common protein domain in the human genome [4].

As key signalling enzymes, protein kinases participate in the regulation of multiple cellular responses and have evolved two properties that are essential for their function: sensitive means of regulation and high specificity for substrates. Recently, protein kinases have emerged as major targets in drug development, totalling almost one-third of newly validated targets in the pharmaceutical industry [5]. It is expected that elucidation of the molecular mechanisms underlying regulation and specificity may prompt novel approaches for the pharmacological modulation of protein kinase activities. Examples of mechanisms that generate

specificity for protein kinases have been described [6], and include subcellular co-localization, interaction with substrates via a scaffolding protein, modular docking interactions and specific protein kinase–substrate docking mechanisms.

This review will focus on recent findings regarding the function and regulation of direct docking interactions with substrates in protein kinases from the mitogen-activated protein kinase (MAPK), glycogen synthase kinase 3 (GSK3) and phosphoinositide-dependent protein kinase-1 (PDK1) families (Figure 1). We also speculate on the importance of docking interactions in other protein kinases and the regulatory properties of docking sites.

SPECIFICITY OF SER/THR PROTEIN KINASES: HISTORICAL PERSPECTIVE

Early studies on cAMP-dependent protein kinase (PKA) and phosphorylase kinase revealed that the principal substrate specificity determinants for these kinases were ‘recognition

Abbreviations used: CD domain, common docking domain; CDK, cyclin-dependent kinase; D-domain, docking domain; DEF domain, domain characterized by sequence Phe-Xaa-Phe-Pro; ERK, extracellular-signal-regulated kinase; FRAT, frequently rearranged in advanced T-cell lymphomas; FRATide, FRAT residues 188–226; GSK3, glycogen synthase kinase 3; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MAPKAPK, MAPK-activated protein kinase; MAPKK, MAPK kinase; MKP, MAPK phosphatase; MEF, myocyte enhancer factor; PAK, p21-activated kinase; MEK, MAPK/ERK kinase; MKK, MAP kinase kinase; MNK, MAPK-interacting kinase; MSK, mitogen- and stress-activated protein kinase; PDE, phosphodiesterase; PDK1, phosphoinositide-dependent protein kinase-1; PH, pleckstrin homology; PI3K, phosphatidylinositol 3-kinase; PIF, PDK1-interacting fragment; PIP₃, phosphatidylinositol 3,4,5-trisphosphate; PKA, cAMP-dependent protein kinase; PKB, protein kinase B; PKC, protein kinase C; PRK2, PKC-related protein kinase 2; PTP, protein tyrosine phosphatase; RSK, p90 ribosomal S6 kinase; S6K, p70 S6 kinase; STEP, striatum-enriched phosphatase.

¹ Present address: PhosphoSites, Starterzentrum 3, 66421, Homburg, Germany.

² To whom correspondence should be addressed (e-mail r.m.biondi@phosphosites.com).

Figure 1 Schematic representation of signalling pathways

Protein kinases that are involved in direct docking interactions are indicated in yellow. GSK3 activity is inhibited by PKB phosphorylation. The other protein kinases are activated by the phosphorylations shown.

motifs', located in short segments of the primary sequence around the phosphorylation sites [7,8]. These were key discoveries that shaped most of the future studies on the substrate specificity of protein kinases, and the recognition motif concept has since been successfully applied to many protein kinases [9]. As a consequence, protein kinase phosphorylation sites are tabulated and synthetic peptides are commonly used as specific substrates for diverse protein kinases. Furthermore, the sole identification of the phosphorylated residue within a particular sequence has been, in many cases, an essential hint in the discovery of the physiological kinase that phosphorylates that site. Nevertheless, it is unlikely that 'recognition motifs' provide the specificity required *in vivo* for several reasons: (i) many protein kinases share *in vitro* 'recognition motifs', for example Ser/Thr-Pro sites for different MAPKs and cyclin-dependent kinases (CDKs), or Arg residues N-terminal to the phospho-acceptor for related kinases, including p90 ribosomal S6 kinase (RSK), PKA and protein kinase B (PKB); (ii) recognition motifs within proteins may not be physiologically phosphorylated; (iii) many protein kinases do not seem to possess a clear consensus 'recognition motif' within peptide substrates; and (iv) peptides do not always mimic the phosphorylation kinetics of the whole protein [10].

Direct docking interactions that facilitate efficient phosphorylation of some substrates by Ser/Thr protein kinases were first postulated in the transcription factor c-Jun, a substrate for the MAPK JNK (c-Jun N-terminal kinase). It was proposed that binding of the δ -domain of c-Jun to JNK2 increased the local concentration of the substrate and contributed to its efficient phosphorylation [11,12]. Phosphorylase kinase was also postulated to possess secondary interaction sites with its substrate glycogen phosphorylase [13]. In recent years, sequences that facilitate kinase-substrate interactions have been reported in substrates of several Ser/Thr protein kinases, suggesting that this may be a wide-spread specificity mechanism. In addition to the focus of this review, MAPK, GSK3 and PDK1, docking sites have also been identified in the Smad substrates of the transforming growth factor- β receptor [14,15] and on some CDK substrates (p107, p130, E2F1) and inhibitors (p27Kip1, p21Cip1) [16,17]. In particular, the docking interactions of CDK2, which are mediated by cyclin A, have been the subject of elegant structural studies [18,19].

Figure 2 Docking mechanisms in MAPK signalling

MAPKK contains an N-terminal extension from the catalytic core with a cluster of positively charged and hydrophobic amino acids (indicated by a blue oval). The stripped oval at the back of the catalytic domain of the MAPK represents the docking pocket where MAPKK docking sequences bind. Upon phosphorylation of the activation loop of MAPK, the complex MAPKK-MAPK dissociates and the active MAPK can interact with and phosphorylate available substrates. The same MAPK docking pocket (hatched oval) is also used to interact with docking sequences (blue ovals) of MAPK substrates and phosphatases that inactivate MAPK.

MAPK

MAPK pathways convert different extracellular stimuli into specific cellular responses via phosphorylation of particular groups of substrates. There are three major mammalian MAPK subfamilies: ERK (extracellular-signal-regulated kinase), JNK and p38. Each subfamily has different biological functions, but all MAPKs can phosphorylate substrates containing the minimal consensus sequence Ser/Thr-Pro. In recent years, several MAPK-recognition modules that are involved in the specific binding to MAPKs have been reported. Equivalent motifs are found in substrates (both nuclear and cytoplasmic), as well as in positive and negative regulators of MAPK activity [20,21]. Thus the docking interactions with MAPKs must be somehow sequentially organized (Figure 2). It is now becoming clear that these interaction motifs are crucial for efficient and specific signal transduction by MAPKs.

Docking of transcription factors to MAPK

The docking site of c-Jun, known as the δ -domain, is required for specific binding to JNK, and includes the sequence Lys-Xaa-Xaa-Arg/Lys-Xaa₄-Leu-Xaa-Leu, where Xaa is any amino acid [12]. Sequences almost identical with the δ -domain of Jun were later identified in many transcription factors, including members of the bZIP, ETS and MADS families that are regulated by ERK, JNK and/or p38 MAPKs (reviewed in [20]). In all of these cases, the kinase-docking domains (or D-domains) enhance substrate phosphorylation by MAPKs. D-domains are also believed to contribute to specificity determination, although some of them can be recognized by more than one class of MAPKs [22]. The importance of the D-domains was also emphasized by mutation of specific acidic residues in the MAPK that were predicted to interact with the Lys or Arg residues of the substrate docking sequences [23].

Interestingly, the Leu-Xaa-Leu motif, preceded by 3–5 basic residues, has been found in transcription factors phosphorylated by ERK and JNK MAPKs, whereas some p38 substrates, such as myocyte enhancer factor (MEF) 2A and MEF2C, contain three hydrophobic residues instead of a Leu-Xaa-Leu motif [20]. Thus small changes in the consensus docking sequence may contribute to substrate specificity by different MAPKs [24].

D-domains are generally located N-terminal to the transcription-activation domain, less than 100 amino acids upstream from the MAPK phosphorylation site, which is important for the activation of the transcription factor. Recently, the Pointed domain of a subset of ETS transcription factors has been shown to contain an ERK2 docking sequence that is located C-terminal to the MAPK phosphorylation site [25].

Analysis of gain-of-function mutations of the ETS transcription factor LIN-1, which is negatively regulated by MAPKs, has identified a new motif named the DEF domain. This domain, characterized by the sequence Phe-Xaa-Phe-Pro (where one of the Phe residues may be a Tyr), is also required for efficient phosphorylation by ERK [26]. DEF domain sequences, together with potential MAPK phosphorylation sites, have also been identified by database searches in GATA family transcription factors, MAPK phosphatases and other ERK regulators [26]. In the transcription factor Elk-1, the DEF and D-domains have been shown to direct phosphorylation of different Ser/Thr-Pro residues by ERK [27]. DEF domains have also been proposed to allow immediate early gene products, such as c-Fos, to translate differences in the kinetics of ERK activation into specific cellular responses [28].

It therefore appears that different types of MAPK recognition modules may provide unique functions by working either synergistically or competitively. When combined, DEF and D-domains may stabilize kinase–substrate binding, enhance kinase selectivity and improve efficiency of phosphorylation.

Docking of cytoplasmic MAPK substrates

D-domains specific for ERK have been found in substrates other than transcription factors, including protein kinases, protein phosphatases and cAMP phosphodiesterases (PDEs). The docking sites of PDE4D [29] and protein tyrosine phosphatases (PTPs) [30,31] resemble those found in transcription factors [26]. In particular, DEF domains have been shown to be functionally relevant for the phosphorylation of PDE4D3 and the KSR-1 (kinase suppressor of Ras-1) scaffold by ERK [27].

In contrast, docking sequences of MAPK-activated protein kinases (MAPKAPKs) contain the sequence Leu-Xaa-Xaa-Arg-Arg followed by several basic residues. This docking sequence is required for the efficient phosphorylation and activation of MAPKAPKs of the RSK family *in vitro* and in cells. When fused to another protein, it is sufficient for the stable and specific association with ERK [32,33]. Mutagenesis analysis indicates that the dissociation of the ERK–RSK complex is actually due to the phosphorylation of RSK. Interestingly, an N-terminally truncated RSK mutant can constitutively interact with ERK [34].

The sequence Leu-Xaa-Xaa-Arg-Arg may be also a relevant docking site in other ERK MAPKAPKs, such as MNK (MAPK-interacting kinase) and mitogen- and stress-activated protein kinase (MSK) [35–39]. Moreover, the same motif is present and required for ERK binding in the PTPs PTP-SL and STEP (striatum-enriched phosphatase) [30]. It is therefore possible that this class of MAPK docking site may allow the association of inactive ERK MAPKs with cytoplasmic targets. This would ensure the preferential phosphorylation of the

associated substrate, especially for those cases in which partial or transient levels of active MAPK are produced. Furthermore, it may be of particular importance when the targets themselves are protein kinases or phosphatases with the potential to modulate the intensity of the signal.

Although RSK family members are specifically activated by ERK, other MAPKAPKs are activated either by p38 (MAPKAPK-2 and -3) or by both ERK and p38 (MNK1 and MSK1). Where does the specificity p38/ERK come from in the D-domains of MAPKAPKs? The generation of chimaeric proteins has indicated that MAPK docking sites are located in the C-terminal tails of the MAPKAPKs. These sequences show similar specificity for ERK or p38 binding as the full-length proteins [40]. As mentioned above for transcription factors, the variability in the number and position of hydrophobic and basic residues within the docking sequences may determine specificity. The number of basic residues, in particular, makes an important contribution but does not appear to be the only specificity determinant [40,41].

What about MAPK substrates that lack D-domains? It is conceivable that new kinds of docking sequences remain to be identified. However, there may also be substrates that contain no MAPK-interaction modules other than the phospho-acceptor motif. MAPKs could promiscuously phosphorylate these proteins if their local concentration is high enough, for example by association with mutual scaffolding molecules.

D-domains in MAPK regulatory proteins

MAPK D-domains have been found in many MAPK regulatory proteins, including activating kinases, down-regulating phosphatases and scaffold proteins [21,42]. All of these proteins are likely to interact with the same residues of MAPK as the substrates discussed above. The binding site on the p38 α MAPK structure is shown in Figure 3(A) (box), and is represented as a striped oval at the back of the kinase domain in Figure 2.

The N-terminal region of MAPK kinases (MAPKKs) contain a docking site that is very similar in sequence to the D-domain of MAPK substrates [43]. In many cases, this docking site is known to be required for binding and activation of the MAPKs; both basic and hydrophobic residues are involved in MAPK recognition. However, the presence of the N-terminal docking site does not appear to be essential for MAPK activation and there is evidence that other docking mechanisms, in addition to the D-domain, might regulate MAPK–MAPKK interactions [44,45].

Cytosolic PTPs, such as PTP-SL and STEP [30,31], also contain functional MAPK docking sites that are similar to the D-domain. There are mechanistic differences, however, as PTPs associate with inactive MAPKs in the cytoplasm and the complex dissociates upon ERK activation and PTP phosphorylation. On the other hand, ERK interacts more efficiently with the phosphorylated form of transcription factors, such as Elk-1. Interestingly, the docking interaction between ERK and PTP-SL can be blocked by PKA-catalysed phosphorylation of Ser²³¹, within the kinase interaction motif of PTP-SL [46]. Phosphorylation of PTP-SL may interfere with down-regulation of ERK, according to the model presented in Figure 2.

Dual-specificity phosphatases that dephosphorylate both phospho-Thr and phospho-Tyr residues in MAPKs (termed MKPs) also contain a D-domain-like sequence in their non-catalytic N-terminus. In some cases, like MKP3, the D-domain has been shown to be involved in selectivity [47]. As mentioned for other MAPK interactors, the number and spacing of positively charged residues might account for specificity in MKP docking. Mutation of an N-terminal cluster of basic residues in MKP1

Figure 3 MAPK docking interactions with substrates, activators and regulators

The docking groove for MEF2A (1LEW) and MKK3b (1LEZ) on p38 α MAPK is equivalent to a site which in other kinases is occupied by extensions to the catalytic domain. **(A)** p38 MAPK structure is shown as ribbons and the co-crystallized peptide (docking site) backbone as sticks. The docking groove is shown bound to MEF2A and MKK3b (right panels). The involvement of the different secondary structure regions of the kinase is indicated in the p38 α MAPK–MEF2A peptide magnification. The red arrow indicates the region termed 'MAPK insert'. **(B)** A site equivalent to the p38 α MAPK docking groove is occupied by sequences C-terminal to the catalytic domain in the protein kinases PAK1 (1F3M) and CDK2 (1HCL). The numbers/letters in parentheses are Protein Data Bank codes for the structures used in the Figure.

has also suggested the existence of distinct binding determinants for different MAPKs [48]. In general, there is good correlation between binding of a MKP to a MAPK and its activity towards the MAPK, but there are also examples where MAPK–MKP interactions do not appear to ensure specificity [49,50].

In some cases, D-domains are not involved in the efficient and accurate phosphorylation of substrates, but serve to recruit MAPKs into signalling complexes. For example, the JNK scaffold JIP-1 (Janus kinase interacting protein-1) contains a D-domain [51]. Another example is JunB, which through its D-domain recruits JNK to phosphorylate its heterodimerization partner JunD [52]. Docking interactions have also been described between the MAPKKs MEK (MAPK/ERK kinase) 1/2 and MKK (MAP kinase kinase) 4 and their upstream activators Raf1 and MEKK1 (MEK kinase 1) respectively [53,54]. These interactions should prevent cross-talk and contribute to efficient and specific MAPK signalling.

MAPK specificity determinants that recognize docking motifs

Based on the generation of JNK1 and JNK2 chimaeras, it was proposed that the δ domain of Jun binds to a short surface-exposed segment in the large lobe of the JNK2 kinase domain [12].

One motif containing acidic residues, termed the common docking (CD) domain, has been identified in all MAPKs. It was suggested that this domain establishes electrostatic interactions with the basic residues of the D-domain of MAPK activators, substrates and regulators [23,55,56]. In addition, two Tyr residues close to the CD domain of ERK2 are also required for MEK1 docking [56,57]. In some cases, like the ERK1b spliced variant, mutation of the CD domain does not affect activation by MEK1, but the mutant protein shows reduced sensitivity to phosphatases [58]. The *Drosophila* ERK 'sevenmaker' allele is also mutated in the CD domain and has decreased sensitivity to dephosphorylation [59].

Other regions of MAPKs that have been implicated in specificity regulation include the Glu–Asp domain of p38 α , which is required for docking of MAPKAPs and MKPs [41], the activation loop of p38 β 2 [44] and N-terminal domains of ERK2 [60]. Mutations at distinct regions of the C-terminus of ERK2 and p38 α have also been shown to differentially affect their association with PTPs [61]. Recently, ERK2 point mutants have been identified that are selectively impaired in binding to the MAPKK MEK1 but not to MNK1, RSK or MKP3 [62]. The position of these mutations in the ERK structure suggests that a 30-amino-acid sequence, known as the MAPK insert [63] (Figure 3A), which is homologous to

an insert found in GSK3 and other kinases (see below), may be implicated in MAPK–MAPKK interaction.

GSK3

GSK3 has been known for more than 20 years as part of the insulin signalling pathway, and is still receiving a great deal of attention, due to its role in this and other disease-related signalling pathways. Recent reviews on GSK3 have been published both on general issues [64–66] and on its involvement in neurodegenerative disease, cell fate and tumorigenesis [67–69]. The importance of GSK3 is highlighted by its possible use as a drug target for the treatment of neurodegenerative diseases and diabetes [70,71].

GSK3 interaction with primed substrates of the insulin signalling pathway is linked to the mechanism of GSK3 inhibition by phosphorylation

Glycogen synthase is the terminal enzyme in the insulin signalling pathway that regulates glycogen synthesis. In response to insulin, GSK3 becomes inhibited, facilitating the dephosphorylation and activation of glycogen synthase [72] (Figure 1). It was subsequently found that GSK3 becomes inhibited upon insulin stimulation by PKB-mediated phosphorylation of an N-terminal Ser (Ser²¹ in GSK3 α and Ser⁹ in GSK3 β) [73], but the actual molecular mechanism of inhibition was not deciphered until recently.

In glycogen synthase, as in other substrates downstream of insulin and growth factor signalling, GSK3 requires the previous phosphorylation of a Ser located four amino acids C-terminal to the actual phosphorylation site ($n + 4$); this was termed 'primed phosphorylation' [74]. Recent biochemical and structural work has revealed that docking of primed substrates is achieved by direct interaction with a specific phosphate-binding site within the catalytic core of GSK3 [75–77]. Interestingly, the molecular mechanism of GSK3 inhibition is also based on the occupancy of the same phosphate-binding site by a phosphorylated N-terminal Ser that may act as a pseudosubstrate [75,76]. As indicated in Figure 4(A), glycogen synthase is normally maintained in an inactive state by GSK3-mediated phosphorylation. Upon insulin stimulation, PKB phosphorylates the N-terminus of GSK3. This blocks the GSK3 phosphate-binding site, which in turn interferes with the docking (and phosphorylation) of glycogen synthase, facilitating its dephosphorylation and activation. Thus the GSK3 phosphate-binding site has a fundamental role both in binding primed (active) glycogen synthase and in the inhibition of GSK3 by insulin. As noted above, docking interactions can produce highly efficient concentration of substrates. It is therefore possible that phosphorylation of the N-terminal Ser may not inhibit the phosphorylation of substrates that possess high affinity for GSK3, for example by having additional docking sites.

GSK3 interactions with substrates of the Wnt signalling pathway

Apart from its role in the insulin signalling pathway, a pool of GSK3 forms part of multiprotein complexes that participate in the Wnt signalling pathway, phosphorylating at least two of its components, axin and β -catenin (Figure 1). Wnts constitute a family of secreted glycoproteins that are involved in embryonic development throughout the animal kingdom and have been also implicated in some forms of human cancers [78,79]. Multiprotein complex interactions of proteins involved in Wnt signalling have been extensively studied (reviewed in [80]). As shown in Figure 4(B), the key effector of this pathway is β -catenin, which upon phosphorylation by GSK3 is targeted for proteasome degra-

Figure 4 Docking mechanism of GSK3 and the mechanism of GSK3 inhibition by phosphorylation

(A) Non-phosphorylated GSK3 is fully active. Substrates that have a priming phosphate, such as glycogen synthase, bind to a specific GSK3 pocket and become phosphorylated on a Ser or a Thr located four amino acids N-terminal to the priming phosphate. Upon cell stimulation by insulin (or growth factors), PKB can phosphorylate a Ser located on the N-terminal tail of GSK3 and the tail is transformed into an inhibitory pseudosubstrate. The phospho-Ser occupies the same pocket as the priming phosphate of the substrate, blocking access to the active site. (B) A pool of GSK3 molecules form part of a high-molecular-mass complex that includes adenomatous polyposis coli (APC), axin and β -catenin (β -Cat). Upon cell stimulation by Wnt, FRAT competes with axin for the same binding site on GSK3 and the APC–axin– β -catenin–GSK3 complex dissociates, resulting in β -catenin dephosphorylation and stabilization, which stimulates gene expression. The phosphorylation of FRAT upon Wnt signalling is speculative.

ation. In response to Wnt signalling, GSK3 is inhibited and the non-phosphorylated (stable) β -catenin binds to transcription factors of the TCF (T-cell factor) family, which in turn activate the expression of Wnt target genes.

The Wnt signalling multiprotein complex includes GSK3 β , β -catenin, the tumour suppressor adenomatous polyposis coli (APC) and the scaffolding protein axin. Axin binds tightly to GSK3 and this interaction is thought to be responsible for bringing β -catenin to the complex, which allows its phosphorylation by GSK3 (Figure 4B). The physiological inhibition of GSK3 by Wnt signalling may be mediated by the displacement of axin from GSK3 β by another protein, termed FRAT (frequently rearranged in advanced T-cell lymphomas)/GBP (GSK3 binding protein) [81–83]. Thus FRAT may displace axin from GSK3 β by binding to an overlapping site, which results in the inhibition of β -catenin phosphorylation (Figure 4B). The axin/FRAT-docking site on GSK3 is distinct from the phosphate docking site used by primed substrates (Figure 5A). Interestingly, the pool of GSK3 that participates in Wnt signalling corresponds only to the β isoform and appears to be regulated neither by insulin nor by phosphorylation at N-terminal residues [84].

Modulation of GSK3 substrate phosphorylation by regulated docking interactions

Phosphorylation of GSK3 substrates does not appear to be mediated by activation of the kinase. Instead, substrates are phosphorylated when they are able to interact tightly with

Figure 5 GSK3 docking interactions with substrates

The GSK3 β structure depicts the phosphate binding site and FRATide/axin binding site. (A) Ribbon representation of GSK3 β with a phosphate bound to the phosphate binding site and FRATide (backbone represented as sticks) bound to the FRAT/axin binding site (1GNG). The right panel shows a magnification of the phosphate-binding site that interacts with 'primed substrates' of GSK3. The phosphate interactions are equivalent to those of the activation loop phosphorylation site in other protein kinases. GSK3 does not possess an analogous activation loop phosphorylation site, and phospho-Tyr²¹⁶ in the activation loop points outwards, similarly to phospho-Tyr¹⁸⁵ in MAPK (1CM8). The bottom panel shows a magnification of the GSK3 β -FRATide binding site. (B) The equivalent region in protein kinase CK2 (1DAW) interacts with a helix (red arrow) formed within a large insertion at the bottom of the large lobe of the catalytic domain. The numbers/letters in parentheses are Protein Data Bank codes for the structures used in the Figure.

GSK3. Thus axin and β -catenin phosphorylation is abolished by displacement of axin from GSK3 by FRAT. What regulates FRAT binding to GSK3 is not known. One possibility is that FRAT contains a second docking site [in addition to the one depicted in the GSK3/FRATide (FRAT residues 188–226) crystal structure], which may be regulated by phosphorylation and docks into the GSK3 phosphate docking site (Figure 4B). If this was the case, the physiological FRAT that binds GSK3 in response to Wnt signalling should be phosphorylated and able to inhibit GSK3 activity on primed substrates. This could explain the inhibition of GSK3 activity on primed peptide substrates observed upon Wnt stimulation.

The primed phosphorylations that have been most studied are from the insulin/growth factor signalling pathway (e.g. glycogen synthase; Figure 4A). More recently, it has also been suggested that β -catenin, a substrate of GSK3 in the Wnt signalling pathway, is phosphorylated in a site dependent on an (n + 4) priming phosphate [85]. This is consistent with the effect of a GSK3 mutant disrupted in the phosphate docking site (Arg⁹⁶ → Ala) on Wnt signalling [86]. However, the kinase responsible for the priming phosphorylation is casein kinase-1, which itself usually requires an (n – 4) priming phosphate. Thus phosphorylation of

Figure 6 Schematic representation of PDK1 and other AGC protein kinases

PDK1 and its substrates, members of the AGC kinase family, contain a phosphorylation site at the activation loop of the catalytic domain (black arrows, Thr³⁰⁸ in PKB α) which is necessary for activity. Most PDK1 substrates also have a hydrophobic motif phosphorylation site (red arrows, Ser⁴⁷³ in PKB α) within the motif Phe-Xaa-Xaa-Phe-Ser/Thr(P)-Tyr, but some have a negatively charged residue in that position (Asp in PRK2). The hydrophobic motif phosphorylation is essential for some substrates to dock to PDK1. PDK1 has a C-terminal PH domain but does not possess the hydrophobic motif. PKA has a truncated hydrophobic motif that lacks the phosphorylation site. S6K regulates its interaction with PDK1 via a C-terminal 'autoinhibitory domain', whereas PRK2 interaction with PDK1 is regulated by Rho binding. RSK requires prior docking to ERK in order to phosphorylate and activate its C-terminal domain, which in turn phosphorylates the hydrophobic motif. This phosphorylation allows docking to PDK1, which phosphorylates the activation loop of the N-terminal domain and enables full RSK activity.

this site could be dependent on the activity of either of both kinases or perhaps, more interestingly, on the exposure of the sites by other mechanisms. Thus β -catenin may be an example of a GSK3 substrate that relies on the combined effect of interaction with the scaffold protein axin and priming phosphate docking for efficient phosphorylation.

In summary, GSK3 activity is regulated by the displacement of docking interactions with its substrates. In one case, axin is displaced by FRAT, whereas most primed substrates are displaced by the phosphorylated N-terminus of GSK3.

PDK1

PDK1 is a key enzyme of the insulin/growth factor signalling pathway that lies downstream of phosphatidylinositol 3-kinase (PI3K) activation (Figure 1) [87,88]. PDK1 consists of a protein kinase catalytic core with a 75-amino-acid extension N-terminal to the catalytic core and a pleckstrin homology (PH) domain at the C-terminal end (Figure 6). PDK1 was originally isolated for its ability to phosphorylate the activation loop of PKB (Thr³⁰⁸ in PKB α) in the presence of phosphatidylinositol 3,4,5-trisphosphate (PIP₃), the product of PI3K [89,90]. PIP₃ binds the PH domain of both PDK1 and PKB. Thr³⁰⁸ phosphorylation is crucial for PKB activation and, together with Ser⁴⁷³, which is located at the C-terminus within a hydrophobic motif, these

residues are the two phosphorylation sites known to regulate PKB activity (Figure 6). The phosphorylation of Ser⁴⁷³ is not sufficient for the activation of PKB but stimulates the kinase activity of Thr³⁰⁸-phosphorylated PKB. Sites equivalent to the active site (Thr³⁰⁸) and the hydrophobic motif (Ser⁴⁷³) in PKB α are strikingly conserved, and are also important for activity of evolutionarily related protein kinases that are grouped within the AGC kinase family, to which PDK1 also belongs (Figure 6). PDK1 can also phosphorylate the activation loop of several other AGC kinases, including RSK, PKA, p70 S6 kinase (S6K), serum- and glucocorticoid-induced kinase (SGK), protein kinase C (PKC) isoforms, PKC-related protein kinase 2 (PRK2) and MSK1 [91,92]. Studies using PDK1-knockout cells have shown that this enzyme phosphorylates most of these protein kinases, except for PKA and MSK1 [93,94].

PDK1 is thought to be a constitutively active kinase that may use distinct mechanisms for the phosphorylation of different substrates inside cells. Accordingly, many studies have found no increase in the activity of PDK1 immunoprecipitated from cells in which the phosphorylations of endogenous PDK1 substrates were stimulated. It should be noted, however, that there is *in vitro* evidence suggesting the activation of PDK1 upon binding to its substrates [95,96] (see below). A small change in the *in vitro* PDK1 activity towards PKB has also been reported in some studies [97,98]. Nevertheless, the work of several groups strongly suggests that docking interactions are very important specificity determinants for PDK1.

PDK1 specificity

The analysis of the sequences surrounding PDK1 phosphorylated residues indicates that the possible 'recognition motif' should be located C-terminal to the phosphorylation site. This region bears significant similarity among different substrates (Figure 6). Peptides based on the activation loop of protein kinase substrates of PDK1 are useful to measure intrinsic PDK1 activity [95,98,99]. However, these peptides are very poor substrates [95,100], indicating that they lack determinants found in the native proteins [100].

Early studies found that PDK1 can form stable complexes with some of its substrates, including PKCs and S6K [101–103]. Moreover, the C-terminal 76 amino acids of PRK2 were found to bind PDK1 in a yeast two-hybrid screen [104]. This peptide, termed PDK1-interacting fragment (PIF), has homology with residues present at the C-terminus of most AGC kinases (Figure 6). Initially, it was suggested that binding of PIF might allow PDK1 to phosphorylate Ser⁴⁷³ of PKB [104]. An alternative possibility is that the interaction of PIF with PKB [105,106] accounts for the observed phosphorylation of Ser⁴⁷³ *in vitro*. Consistent with the latter possibility, the mutant PDK1-Leu¹⁵⁵ → Glu (which does not bind PIF) can only phosphorylate Ser⁴⁷³ of PKB upon addition of PIF (R. Biondi and D. Alessi, unpublished work). PRK2 and PDK1 can also interact inside cells (see below).

The hydrophobic and the Asp residues within the motif Phe-Xaa-Xaa-Phe-Asp-Tyr in PIF were found to be critical for the binding to PDK1. The hydrophobic motif of AGC kinases is likely to interact with their own catalytic core, in a similar way to that of PKA (Figure 7A). This has recently been supported by the determination of the structure of active PKB [107]. Thus binding to PDK1 through the hydrophobic motif sequence may be subjected to competition between inter- and intra-molecular interactions [108,109] (Figure 7B).

Figure 7 PDK1 docking interactions and the mechanism of activation of AGC kinases

(A) PDK1 and AGC kinases are activated by interaction of a hydrophobic motif with the 'PIF pocket' on the protein kinase catalytic core. In contrast with other AGC kinases, PDK1 lacks the C-terminal hydrophobic motif. (B) The docking of substrates to PDK1 is similar to the PDK1 activation mechanism. When a substrate containing a phosphorylated hydrophobic motif (at the C-terminal extension to the catalytic core) binds to the PDK1 PIF pocket, PDK1 is activated and phosphorylates the activation loop of the substrate. This phosphorylation favours an active conformation of the substrate, which is further stabilized by the intramolecular interaction with its own phosphorylated hydrophobic motif. PDK1 may be lost from the complex when the substrate sequesters the hydrophobic docking sequence by interaction with its own pocket.

PDK1 docking with substrates is a requirement for efficient phosphorylation

Fusion of the hydrophobic motif of PRK2 (PIF) to a particular peptide makes a much better substrate for PDK1 than the same peptide alone [95], suggesting that the interaction between PDK1 and its substrates is of functional significance. Similarly, RSK2 phosphorylation by PDK1 requires the prior phosphorylation of RSK2 at the hydrophobic motif site that promotes PDK1 docking [96]. Interestingly, RSK2 mutants that do not contain the hydrophobic motif docking sequences are not phosphorylated *in vivo* on the activation loop unless they are co-expressed with PDK1. These results suggest that increasing the concentration of the two proteins may be sufficient to trigger their interaction *in vivo* [96].

Studies with other AGC family kinases further support the theory that activation loop phosphorylation of different PDK1 substrates is ultimately modulated by distinct regulatory docking interactions [96,109–112]. Additional variations come from the participation of other domains in the PDK1 substrates. For example, phosphorylation of C-terminal residues in S6K may expose the hydrophobic motif to make it available for interaction with PDK1 [111,113,114]. A similar mechanism may operate in the case of PRK2, which requires prior binding of the small GTP-binding protein Rho for interaction with PDK1 [112].

Figure 7(B) shows a simplified model of how the PIF-like hydrophobic motif of PDK1 substrates can regulate their interaction with, and phosphorylation by, PDK1. In contrast, PKB activation does not seem to rely on this type of docking mechanism to gain proximity to PDK1 [111].

Figure 8 PDK1-substrate docking interaction

The docking pocket in PDK1 is equivalent to a site that, in other AGC kinases, binds a hydrophobic motif located in the C-terminal extension to the catalytic core. The hydrophobic motif Phe-Xaa-Xaa-Phe of PDK1 substrates binds to the PDK1 docking pocket in a manner equivalent to the binding of the PKA hydrophobic motif to its own pocket. **(A)** The ribbon structure of PKA (1ATP), indicating N-terminal (N-T) or C-terminal (C-T) extensions to the catalytic domain, is shown. The Phe of the hydrophobic motif and the activation loop phosphorylation site (pThr¹⁹⁷) interacting with His⁸⁶ from α C-helix are shown as sticks. The right panel shows a magnification of the PKA pocket where Phe residues bind. **(B)** The PDK1 crystal structure (1H1M), depicting a hydrophobic pocket equivalent to that in PKA, and a phosphate-docking site that interacts with the hydrophobic motif of PDK1 substrates are shown. Sticks represent residues from α B- and α C-helices, as well as from β 4 and β 5 that have been implicated in binding and activation of PDK1. The numbers/letters in parentheses are Protein Data Bank codes for the structures used in the Figure.

PKB: a peculiar PDK1 substrate

PKB is different from the rest of the PDK1 substrates in that it possesses a PH domain (Figure 6) that, similar to PDK1, can bind PIP₃ lipids at the plasma membrane. Both PKB and PDK1 translocate to specific regions in the plasma membrane where PIP₃ is generated as a consequence of PI3K activity [115,116]. There is also evidence that the binding of PIP₃ to PKB is sufficient to produce a conformational change that is required for PDK1 phosphorylation. In fact, PKB is the only substrate of PDK1 that requires the presence of PIP₃ to be efficiently phosphorylated by PDK1. Moreover, PKB phosphorylation *in vivo* takes place seconds after PI3K stimulation and production of PIP₃, while the phosphorylation of other PI3K-dependent substrates, such as S6K, is maximal 15–40 min later [111]. Furthermore, PIF overexpression does not affect PKB activation, but inhibits the phosphorylation and activation of other PDK1 substrates [110,111,117]. Mutagenesis analysis also supports that, in contrast with other PDK1 substrates, PKB does not appear to rely on the hydrophobic motif docking interaction for phosphorylation. However, the situation appears to be different for PKB mutants lacking the PH domain, which may require docking with the PDK1 PIF binding pocket, similar to other substrates of PDK1 [111,118].

In conclusion, the mechanism of PKB phosphorylation appears to rely less on the hydrophobic motif-PIF pocket interaction, perhaps to facilitate phosphorylation only when PKB and PDK1 co-localize with PIP₃ at the plasma membrane.

Structural analysis of the PDK1 PIF pocket that docks the hydrophobic motif of substrates

In contrast with other PDK1 substrates, PKA only possesses a truncated hydrophobic motif (Phe-Xaa-Xaa-Phe), where the second Phe corresponds to the C-terminus of the protein. In the PKA crystal structure, the Phe residues occupy a deep hydrophobic pocket in the small lobe of the catalytic domain [119] (Figure 8A). Biochemical studies demonstrated that these two Phe residues are important for PKA activity [120,121]. Molecular modelling of PDK1, based on PKA, allowed the biochemical characterization of the PDK1 pocket where PIF binds [95]. Recently, the crystal structure of PDK1 was solved and the details of the 'PIF pocket' revealed (see below).

Participation of the PIF pocket in PDK1 activation

In order to study the effect of docking sequences on the intrinsic kinase activity, it is necessary to use a substrate that does not require a docking mechanism for interaction. For PDK1, kinase assays were developed with either a peptide substrate based on the activation loop of PKB [95,98], or a truncated RSK2 [96]. These assays revealed that peptides encompassing the hydrophobic motifs increase PDK1 intrinsic activity 4–6-fold. Interestingly, mutation of Leu¹⁵⁵ or Lys¹¹⁵ in the PIF-binding pocket increases the activity of PDK1 4-fold. Other mutants, such as Ile¹¹⁹ → Ala and Gln¹⁵⁰ → Ala, having decreased affinity for PIF, are fully activated only at higher concentrations of PIF [95].

The mechanism of activation of PDK1 has recently been shown to be similar to that of other AGC kinases [106,122,123]. Structural studies suggest that the mechanism of PKB activation involves the stabilization of the α -C helix by binding of the phosphorylated hydrophobic motif to the 'PIF pocket' site on PKB [106,107]. In addition, the interaction of an Asp from the PKB C-terminal extension with the Arg³ in substrates [107] could suggest a secondary mechanism of activation.

In summary, the docking of PDK1 substrates is coupled to the activation of PDK1 via a mechanism similar to the intramolecular activation of AGC kinases. PDK1 does not possess the hydrophobic motif itself, but can complement its protein kinase catalytic core using the hydrophobic motif of AGC kinases (Figure 7).

WHERE DO DOCKING SEQUENCES DOCK?

Protein kinase structures

PKA was the first protein kinase to be crystallized and its structure has been a hallmark for the whole protein kinase family [119]. Protein kinases consist of an N-terminal small lobe and a C-terminal large lobe that give the catalytic core a characteristic 'bean-like' structure. ATP binds between the two lobes, directing the γ -phosphate outwards while the adenine ring lies deep in the cleft between the two lobes. The substrate binding site for peptides was structurally depicted by co-crystallization of PKA with its pseudo-substrate inhibitor PKI [124]. It was later confirmed that other crystallized peptide substrates bind to protein kinases in an extended conformation [125].

Protein kinases usually possess both N-terminal and C-terminal extensions from the kinase catalytic core. In many cases, these regions fold back into the catalytic core, forming important specific interactions. In PKA, N- and C-terminal extensions from the catalytic core promote stability or activity [126–128]. Alternatively, these extensions may promote inhibitory interactions by acting as pseudosubstrates [129].

Structural analysis of protein kinase sites involved in docking interactions

MAPK

Recently the structural basis for docking interactions in MAPKs has started to be elucidated by the co-crystallization of p38 α MAPK with the D-domains of the substrate MEF2A and the activating kinase MKK3b [130]. This work confirmed that both domains bind to the same docking groove in p38 α , with Ile¹¹⁶ and Gln¹²⁰ playing a key role in the interaction with the hydrophobic motif of the D-domain (Figure 3A). Similar docking grooves exist in other MAPK family members, but the p38 α residues in contact with the docking peptides are not always conserved, suggesting that small differences in the structure of the groove may contribute to MAPK specificity. Unexpectedly (based on previous mutagenesis studies), no interactions between the acidic residues of the MAPK CD domain and basic residues of the substrate/activator D-domain were observed in the p38 α crystal structures. This might be due to the use of non-phosphorylated (inactive) p38 α and docking site peptides (instead of the whole proteins) for the crystallographic study. The MAPK Glu-Asp site also does not participate in these interactions although it is located close to the docking groove. Interestingly, binding of the two D-domain peptides induces conformational changes in the p38 α MAPK, which are different in the case of MEF2A and MKK3b,

suggesting that the docking interactions may also regulate MAPK activity [130].

The interaction of p38 α with both peptide substrates is mainly hydrophobic, with an important participation of the residues Leu-Arg-Val and Leu-Arg-Ile. As other docking sequences are known to be hydrophobic, the question of whether the same docking pocket is used for other docking sequences arises. Mutations within the hydrophobic pocket of MAPK family members may reveal whether this is the case.

GSK3

Structural insight into the phosphorylation-mediated docking interaction between GSK3 and primed substrates has recently been obtained [76,77]. In the GSK3 structures, the phosphate-binding site is depicted by a sulphonate moiety from the Hepes buffer, or by a sulphate group present in the crystallization conditions. The phosphate binding site of GSK3 is defined by residues Arg⁹⁶, Arg¹⁸⁰ and Lys²⁰⁵ (Figure 5). These residues are structurally equivalent to the residues defining the activation loop phosphate-binding site in a number of other protein kinases, such as PKA, PDK1 and ERK2 (reviewed in [131,132]). This finding raises the possibility that docking of primed substrates could activate GSK3. In this case, a short phosphopeptide that specifically interacts with the docking pocket (phosphate-binding site) would be expected to promote GSK3 activity on a non-primed substrate. However, experimental evidence to support this point is lacking [75]. Recently, crystals of active GSK3 have also been formed in the absence of sulphonate/phosphate moieties, arguing that these are not indispensable for the active conformation to be adopted [133]. The elucidation of the possible role of the priming phosphate in GSK3 activation may require the use of shorter phosphopeptides, or chemical compounds that specifically interact with the phosphate-binding site without interfering with the catalytic binding site.

Recent biochemical work has also characterized the axin and FRAT binding sites on GSK3 [134,135]. Furthermore, the crystal structure of GSK3 β bound to a polypeptide derived from FRAT residues 188–226 (FRATide) has been solved [133]. The FRATide binding site is located at the front of the large kinase lobe, in a position that does not block the substrate-binding site (Figure 5). This may explain why FRATide does not block the phosphorylation of primed peptide substrates. FRATide bound to GSK3 has a helix-loop-helix structure, where the helices are amphipathic and the main interactions are of a hydrophobic nature. The C-terminal helix of FRATide induces a shift in a GSK3 large loop and exposes the hydrophobic groove where the C-terminal helix binds. This loop is absent in most protein kinases, but has structural similarity to an insert found in CDKs and MAPKs (MAPK insert). It appears that the axin binding site on GSK3 probably overlaps with that for FRATide. Its proximity to the active site may actually explain how axin acts as a scaffold protein promoting β -catenin phosphorylation.

PDK1

The details of PDK1 substrate docking have been revealed by solving the crystal structure of ATP-bound PDK1 (Figure 8). The binding of the hydrophobic motif Phe-Xaa-Xaa-Phe to the so-called PIF pocket was previously modelled, based on the structure of PKA, which has itself the homologous pocket occupied by the two C-terminal Phe residues from its own polypeptide (see above). The hydrophobic PIF pocket is thus located between β 4– β 5 and α -C helix A, with a side formed by the short α -B helix (which

in non-AGC kinases forms a loop instead of a helix) (Figure 8). The phosphate binding site that interacts with the phosphorylated hydrophobic motif of substrates is clearly depicted by an ordered sulphate ion situated next to the PIF pocket of PDK1 and has potential specific interactions with Lys⁷⁶, Arg¹³¹, Thr¹⁴⁸ and Gln¹⁵⁰. Interestingly, modelling had also revealed that Arg¹³¹ and possibly Lys⁷⁶ could be responsible of this interaction [122]. The structure of PDK1 reveals that the PIF pocket is strikingly similar to the pockets in the active structures of PKA (Figure 8) and PKB [107], which bind their own C-terminal hydrophobic motifs. Thus the PDK1 study provides information on the structure of the 'active' conformation that AGC kinases may adopt upon binding of their phosphorylated hydrophobic motif to the pocket. The inactive conformation may be similar to that of a PKB mutant lacking the C-terminal hydrophobic motif [106].

Based on the different biochemical responses obtained with some PDK1 mutations, it is expected that the mode of interaction between PDK1 and each hydrophobic motif sequence of substrates will have specific characteristics. Moreover, it seems likely that each protein kinase substrate may have particular interactions with PDK1, besides its PIF pocket interaction site and the activation loop phosphorylation site (as described above). The specific and common interaction sites will only be fully revealed by resolving the structure of PDK1 bound to the different protein kinase substrates.

Substrate docking sequences complement regions of the protein kinase catalytic core

A revision of available structures of Ser/Thr protein kinases shows that regions similar to those used for docking substrates in MAPK, GSK3 and PDK1 are also complemented by regions outside of the catalytic core in other protein kinases.

The docking sequences from MKK3b and MEF2a bind p38 α MAPK in a manner strikingly similar to the interactions found between the C-terminal regions of CDK2 and PAK (p21-activated kinase) 1 with its own catalytic cores (Figure 3). Interestingly, PAK1 and CDKs are phylogenetically close to the MAPK family. Moreover, there is also a partial overlap between part of the MAPK interaction site with docking sequences and the C-terminal extensions to the catalytic core of PKA, PDK1 and GSK3.

In GSK3, the phosphate docking site used by primed substrates is homologous with the activation loop phosphate-binding site in many other kinases (Figure 5) [131,132]. One of the α -helices within FRATide binds to GSK3 in a manner that is similar to the binding of a non-conserved α -helix originating from an insertion (equivalent to the MAPK insert) on the large lobe of the protein kinase CK2 catalytic domain (Figure 5).

Several protein kinases complement themselves in the equivalent site to the PIF-binding pocket in PDK1. For example, the N-terminal residue Trp¹⁰ complements an equivalent groove in calmodulin kinase-2. In MAPK family members, the site is complemented with C-terminal extensions to the catalytic core. Interestingly, the groove between α -C-helix and β -4- β -5, equivalent to the site used by PIF interaction with PDK1, was recently suggested as the site of interaction for a non-ATP competitive drug against the MAPKK MEK1 [136]. The CDK2-cyclin interaction region occupies a large area, which also includes the same groove between α -C-helix and β -4- β -5 (Figure 8). Binding of cyclins is known to be the first event in the activation of CDKs [137]. Interestingly, viral cyclins show specific interactions that are very different from the *bona fide* cyclins. However, one core region which binds between α -C-helix and β -4- β -5 is conserved [138].

In summary, structural analysis of protein kinases suggests that interactions of the catalytic core with N-terminal or C-terminal extensions may participate in the regulation of kinase activity. Alternatively, equivalent sites seem to be used for docking of substrates to protein kinases.

CONCLUDING REMARKS

Amino acid sequences surrounding the phospho-acceptor motifs have been extensively studied, and have shown to be important determinants for the substrate specificity of many protein kinases. In the last few years, however, it has become increasingly obvious that docking interactions, without any significant requirement for specific phosphorylation motifs, are a major mechanism for substrate recognition in Ser/Thr protein kinases, such as CDK, MAPK, GSK3 and PDK1. It is therefore to be expected that the importance of direct docking interactions may be recognized throughout the whole family of Ser/Thr protein kinases in the future.

In the docking interactions reviewed here, a linear sequence of amino acids are binding to the catalytic domain of a protein kinase. This may just reflect that 'docking sequences' are more easily identified than interactions where a tertiary structure site might be involved. Interestingly, docking interactions in PDK1 and GSK3 are regulated by phosphorylation. Docking interactions with MAPKs may be also regulated by phosphorylation, although the precise mechanisms involved remain to be elucidated. Thus the regulation of docking interactions by phosphorylation allows an important additional level of control to these signalling pathways.

The analysis of sites in kinase structures where docking sequences bind supports the idea that protein kinases may require 'complementation' of their catalytic domains for stability and/or activity. In some cases, interacting partners, such as substrates, may provide the complementary regions. Further work will be required to verify whether docking interactions could be used as targets for non-ATP competitive drugs against protein kinases. Compounds interfering with docking sites could potentially be useful as substrate selective protein kinase drugs.

We thank Dario Alessi, Emma Black, Ana Cuenda, Morten Frodin, Anne-Claude Gavin and Almudena Porras for critical reading of this review. We are also grateful to B. Bax (GlaxoSmithKline) for the GSK3-FRATide co-ordinates.

REFERENCES

- Hunter, T. and Plowman, G. D. (1997) The protein kinases of budding yeast: six score and more. *Trends Biochem. Sci.* **22**, 18–22
- Plowman, G. D., Sudarsanam, S., Bingham, J., Whyte, D. and Hunter, T. (1999) The protein kinases of *Caenorhabditis elegans*: a model for signal transduction in multicellular organisms. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13603–13610
- Morrison, D. K., Murakami, M. S. and Cleghon, V. (2000) Protein kinases and phosphatases in the *Drosophila* genome. *J. Cell Biol.* **150**, F57–F62
- Manning, G., Whyte, D. B., Martinez, R., Hunter, T. and Sudarsanam, S. (2002) The protein kinase complement of the human genome. *Science* **298**, 1912–1934
- Cohen, P. (2002) Protein kinases – the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.* **1**, 309–315
- Pawson, T. and Nash, P. (2000) Protein–protein interactions define specificity in signal transduction. *Genes Dev.* **14**, 1027–1047
- Kemp, B. E., Bylund, D. B., Huang, T. S. and Krebs, E. G. (1975) Substrate specificity of the cyclic AMP-dependent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3448–3452
- Zetterqvist, O., Ragnarsson, U., Humble, E., Berglund, L. and Engstrom, L. (1976) The minimum substrate of cyclic AMP-stimulated protein kinase, as studied by synthetic peptides representing the phosphorylatable site of pyruvate kinase (type L) of rat liver. *Biochem. Biophys. Res. Commun.* **70**, 696–703
- Pinna, L. A. and Ruzzene, M. (1996) How do protein kinases recognize their substrates? *Biochim. Biophys. Acta* **1314**, 191–225

- 10 Kemp, B. E. and Pearson, R. B. (1991) Design and use of peptide substrates for protein kinases. *Methods Enzymol.* **200**, 121–134
- 11 Adler, V., Franklin, C. C. and Kraft, A. S. (1992) Phorbol esters stimulate the phosphorylation of c-Jun but not v-Jun: regulation by the N-terminal δ domain. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5341–5345
- 12 Kallunki, T., Su, B., Tsigelny, I., Sluss, H. K., Derijard, B., Moore, G., Davis, R. and Karin, M. (1994) JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation. *Genes Dev.* **8**, 2996–3007
- 13 Lowe, E. D., Noble, M. E., Skamni, V. T., Oikonomakos, N. G., Owen, D. J. and Johnson, L. N. (1997) The crystal structure of a phosphorylase kinase peptide substrate complex: kinase substrate recognition. *EMBO J.* **16**, 6646–6658
- 14 Chen, Y. G., Hata, A., Lo, R. S., Wotton, D., Shi, Y., Pavletich, N. and Massague, J. (1998) Determinants of specificity in TGF- β signal transduction. *Genes Dev.* **12**, 2144–2152
- 15 Lo, R. S., Chen, Y. G., Shi, Y., Pavletich, N. P. and Massague, J. (1998) The L3 loop: a structural motif determining specific interactions between SMAD proteins and TGF- β receptors. *EMBO J.* **17**, 996–1005
- 16 Adams, P. D., Sellers, W. R., Sharma, S. K., Wu, A. D., Nalin, C. M. and Kaeling, W. G. (1996) Identification of a cyclin-cdk2 recognition motif present in substrates and p21-like cyclin-dependent kinase inhibitors. *Mol. Cell. Biol.* **16**, 6623–6633
- 17 Schulman, B. A., Lindstrom, D. L. and Harlow, E. (1998) Substrate recruitment to cyclin-dependent kinase 2 by a multipurpose docking site on cyclin A. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 10453–10458
- 18 Brown, N. R., Noble, M. E., Endicott, J. A. and Johnson, L. N. (1999) The structural basis for specificity of substrate and recruitment peptides for cyclin-dependent kinases. *Nat. Cell Biol.* **1**, 438–443
- 19 Lowe, E. D., Tews, I., Cheng, K. Y., Brown, N. R., Gul, S., Noble, M. E., Gamblin, S. J. and Johnson, L. N. (2002) Specificity determinants of recruitment peptides bound to phospho-CDK2/cyclin A. *Biochemistry* **41**, 15625–15634
- 20 Sharrocks, A. D., Yang, S. H. and Galanis, A. (2000) Docking domains and substrate-specificity determination for MAP kinases. *Trends Biochem. Sci.* **25**, 448–453
- 21 Tanoue, T. and Nishida, E. (2002) Docking interactions in the mitogen-activated protein kinase cascades. *Pharmacol. Ther.* **93**, 193–202
- 22 Yang, S.-H., Whitmarsh, A. J., Davis, R. J. and Sharrocks, A. D. (1998) Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1. *EMBO J.* **17**, 1740–1749
- 23 Tanoue, T., Adachi, M., Moriguchi, T. and Nishida, E. (2000) A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat. Cell Biol.* **2**, 110–116
- 24 Barsyte-Lovejoy, D., Galanis, A. and Sharrocks, A. D. (2002) Specificity determinants in MAPK signaling to transcription factors. *J. Biol. Chem.* **277**, 9896–9903
- 25 Seidel, J. J. and Graves, B. J. (2002) An ERK2 docking site in the Pointed domain distinguishes a subset of ETS transcription factors. *Genes Dev.* **16**, 127–137
- 26 Jacobs, D., Glossip, D., Xing, H., Muslin, A. J. and Kornfeld, K. (1999) Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev.* **13**, 163–175
- 27 Fantz, D. A., Jacobs, D., Glossip, D. and Kornfeld, K. (2001) Docking sites on substrate proteins direct extracellular signal-regulated kinase to phosphorylate specific residues. *J. Biol. Chem.* **276**, 27256–27265
- 28 Murphy, L. O., Smith, S., Chen, R. H., Finger, D. C. and Blenis, J. (2002) Molecular interpretation of ERK signal duration by immediate early gene products. *Nat. Cell Biol.* **4**, 556–564
- 29 MacKenzie, S. J., Baillie, G. S., McPhee, I., Bolger, G. B. and Houslay, M. D. (2000) ERK2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases. The involvement of COOH-terminal docking sites and NH2-terminal UCR regions. *J. Biol. Chem.* **275**, 16609–16617
- 30 Pulido, R., Zuniga, A. and Ullrich, A. (1998) PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif. *EMBO J.* **17**, 7337–7350
- 31 Zuniga, A., Torres, J., Ubeda, J. and Pulido, R. (1999) Interaction of mitogen-activated protein kinases with the kinase interaction motif of the tyrosine phosphatase PTP-SL provides substrate specificity and retains ERK2 in the cytoplasm. *J. Biol. Chem.* **274**, 21900–21907
- 32 Gavin, A. C. and Nebreda, A. R. (1999) A MAP kinase docking site is required for phosphorylation and activation of p90^{rsk}/MAPKAP kinase-1. *Curr. Biol.* **9**, 281–284
- 33 Smith, J. A., Poteet-Smith, C. E., Malarkey, K. and Sturgill, T. W. (1999) Identification of an extracellular signal-regulated kinase (ERK) docking site in ribosomal S6 kinase, a sequence critical for activation by ERK *in vivo*. *J. Biol. Chem.* **274**, 2893–2898
- 34 Gavin, A. C., Ni Ainle, A., Chierici, E., Jones, M. and Nebreda, A. R. (1999) A p90(rsk) mutant constitutively interacting with MAP kinase uncouples MAP kinase from p34(cdc2)/cyclin B activation in *Xenopus* oocytes. *Mol. Biol. Cell* **10**, 2971–2986
- 35 Deak, M., Clifton, A. D., Lucocq, J. M. and Alessi, D. R. (1998) Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *EMBO J.* **17**, 4426–4441
- 36 Fukunaga, R. and Hunter, T. (1997) MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J.* **16**, 1921–1933
- 37 Waskiewicz, A. J., Flynn, A., Proud, C. G. and Cooper, J. A. (1997) Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J.* **16**, 1909–1920
- 38 Pierrat, B., Correia, J. S., Mary, J. L., Tomas-Zuber, M. and Lesslauer, W. (1998) RSK-B, a novel ribosomal S6 kinase family member, is a CREB kinase under dominant control of p38 α mitogen-activated protein kinase (p38 α MAPK). *J. Biol. Chem.* **273**, 29661–29671
- 39 New, L., Zhao, M., Li, Y., Bassett, W. W., Feng, Y., Ludwig, S., Padova, F. D., Gram, H. and Han, J. (1999) Cloning and characterization of RLPK, a novel RSK-related protein kinase. *J. Biol. Chem.* **274**, 1026–1032
- 40 Smith, J. A., Poteet-Smith, C. E., Lannigan, D. A., Freed, T. A., Zoltoski, A. J. and Sturgill, T. W. (2000) Creation of a stress-activated p90 ribosomal S6 kinase. The carboxyl-terminal tail of the MAPK-activated protein kinases dictates the signal transduction pathway in which they function. *J. Biol. Chem.* **275**, 31588–31593
- 41 Tanoue, T., Maeda, R., Adachi, M. and Nishida, E. (2001) Identification of a docking groove on ERK and p38 MAP kinases that regulates the specificity of docking interactions. *EMBO J.* **20**, 466–479
- 42 Enslin, H. and Davis, R. J. (2001) Regulation of MAP kinases by docking domains. *Biol. Cell* **93**, 5–14
- 43 Bardwell, L. and Thorner, J. (1996) A conserved motif at the amino termini of MEKs might mediate high-affinity interaction with the cognate MAPKs. *Trends Biochem. Sci.* **21**, 373–374
- 44 Enslin, H., Branchio, D. M. and Davis, R. J. (2000) Molecular determinants that mediate selective activation of p38 MAP kinase isoforms. *EMBO J.* **19**, 1301–1311
- 45 Alonso, G., Ambrosino, C., Jones, M. and Nebreda, A. R. (2000) Differential activation of p38 mitogen-activated protein kinase isoforms depending on signal strength. *J. Biol. Chem.* **275**, 40641–40648
- 46 Blanco-Aparicio, C., Torres, J. and Pulido, R. (1999) A novel regulatory mechanism of MAP kinases activation and nuclear translocation mediated by PKA and the PTP-SL tyrosine phosphatase. *J. Cell Biol.* **147**, 1129–1136
- 47 Muda, M., Theodosiou, A., Gillieron, C., Smith, A., Chabert, C., Camps, M., Boschert, U., Rodrigues, N., Davies, K., Ashworth, A. and Arkinstall, S. (1998) The mitogen-activated protein kinase phosphatase-3 N-terminal noncatalytic region is responsible for tight substrate binding and enzymatic specificity. *J. Biol. Chem.* **273**, 9323–9329
- 48 Slack, D. N., Seternes, O. M., Gabrielsen, M. and Keyse, S. M. (2001) Distinct binding determinants for ERK2/p38 α and JNK map kinases mediate catalytic activation and substrate selectivity of map kinase phosphatase-1. *J. Biol. Chem.* **276**, 16491–16500
- 49 Chen, P., Hutter, D., Yang, X., Gorospe, M., Davis, R. J. and Liu, Y. (2001) Discordance between the binding affinity of mitogen-activated protein kinase subfamily members for MAP kinase phosphatase-2 and their ability to activate the phosphatase catalytically. *J. Biol. Chem.* **276**, 29440–29449
- 50 Masuda, K., Shima, H., Watanabe, M. and Kikuchi, K. (2001) MKP-7, a novel mitogen-activated protein kinase phosphatase, functions as a shuttle protein. *J. Biol. Chem.* **276**, 39002–39011
- 51 Whitmarsh, A. J. and Davis, R. J. (1998) Structural organization of MAP-kinase signaling modules by scaffold proteins in yeast and mammals. *Trends Biochem. Sci.* **23**, 481–485
- 52 Kallunki, T., Deng, T., Hibi, M. and Karin, M. (1996) c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. *Cell* **87**, 929–939
- 53 Catling, A. D., Schaeffer, H. J., Reuter, C. W., Reddy, G. R. and Weber, M. J. (1995) A proline-rich sequence unique to MEK1 and MEK2 is required for raf binding and regulates MEK function. *Mol. Cell. Biol.* **15**, 5214–5225
- 54 Xia, Y., Wu, Z., Su, B., Murray, B. and Karin, M. (1998) JNKK1 organizes a MAP kinase module through specific and sequential interactions with upstream and downstream components mediated by its amino-terminal extension. *Genes Dev.* **12**, 3369–3381
- 55 Camps, M., Nichols, A., Gillieron, C., Antonsson, B., Muda, M., Chabert, C., Boschert, U. and Arkinstall, S. (1998) Catalytic activation of the phosphatase MKP-3 by ERK2 mitogen-activated protein kinase. *Science* **280**, 1262–1265
- 56 Rubinfeld, H., Hancock, T. and Seger, R. (1999) Identification of a cytoplasmic-retention sequence in ERK2. *J. Biol. Chem.* **274**, 30349–30352
- 57 Xu, B., Stippes, S., Robinson, F. L. and Cobb, M. H. (2001) Hydrophobic as well as charged residues in both MEK1 and ERK2 are important for their proper docking. *J. Biol. Chem.* **276**, 26509–26515
- 58 Yung, Y., Yao, Z., Aebersold, D. M., Hancock, T. and Seger, R. (2001) Altered regulation of ERK1b by MEK1 and PTP-SL and modified Elk1 phosphorylation by ERK1b are caused by abrogation of the regulatory C-terminal sequence of ERKs. *J. Biol. Chem.* **276**, 35280–35289
- 59 Bott, C. M., Thorncroft, S. G. and Marshall, C. J. (1994) The sevenmaker gain-of-function mutation in p42 MAP kinase leads to enhanced signalling and reduced sensitivity to dual specificity phosphatase action. *FEBS Lett.* **352**, 201–205

- 60 Eblen, S. T., Catling, A. D., Assanah, M. C. and Weber, M. J. (2001) Biochemical and biological functions of the N-terminal, noncatalytic domain of extracellular signal-regulated kinase 2. *Mol. Cell. Biol.* **21**, 249–259
- 61 Tarrega, C., Blanco-Aparicio, C., Munoz, J. J. and Pulido, R. (2002) Two clusters of residues at the docking groove of mitogen-activated protein kinases differentially mediate their functional interaction with the tyrosine phosphatases PTP-SL and STEP. *J. Biol. Chem.* **277**, 2629–2636
- 62 Robinson, F. L., Whitehurst, A. W., Raman, M. and Cobb, M. H. (2002) Identification of novel point mutations in ERK2 that selectively disrupt binding to MEK1. *J. Biol. Chem.* **277**, 14844–14852
- 63 Zhang, F., Strand, A., Robbins, D., Cobb, M. H. and Goldsmith, E. J. (1994) Atomic structure of the MAP kinase ERK2 at 2.3 Å resolution. *Nature* **367**, 704–711
- 64 Frame, S. and Cohen, P. (2001) GSK3 takes centre stage more than 20 years after its discovery. *Biochem. J.* **359**, 1–16
- 65 Ali, A., Hoefflich, K. P. and Woodgett, J. R. (2001) Glycogen synthase kinase-3: properties, functions, and regulation. *Chem. Rev.* **101**, 2527–2540
- 66 Grimes, C. A. and Jope, R. S. (2001) The multifaceted roles of glycogen synthase kinase 3 β in cellular signaling. *Prog. Neurobiol.* **65**, 391–426
- 67 Kaytor, M. D. and Orr, H. T. (2002) The GSK3 β signaling cascade and neurodegenerative disease. *Curr. Opin. Neurobiol.* **12**, 275–278
- 68 Kim, L. and Kimmell, A. R. (2000) GSK3, a master switch regulating cell-fate specification and tumorigenesis. *Curr. Opin. Genet. Dev.* **10**, 508–514
- 69 Manoukian, A. S. and Woodgett, J. R. (2002) Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. *Adv. Cancer Res.* **84**, 203–229
- 70 Coghlan, M. P., Culbert, A. A., Cross, D. A., Corcoran, S. L., Yates, J. W., Pearce, N. J., Rausch, O. L., Murphy, G. J., Carter, P. S., Roxbee Cox, L. et al. (2000) Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem. Biol.* **7**, 793–803
- 71 Cross, D. A., Culbert, A. A., Chalmers, K. A., Facci, L., Skaper, S. D. and Reith, A. D. (2001) Selective small-molecule inhibitors of glycogen synthase kinase-3 activity protect primary neurones from death. *J. Neurochem.* **77**, 94–102
- 72 Welsh, G. I. and Proud, C. G. (1993) Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. *Biochem. J.* **294**, 625–629
- 73 Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M. and Hemmings, B. A. (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378**, 785–789
- 74 Fiol, C. J., Mahrenholz, A. M., Wang, Y., Roeske, R. W. and Roach, P. J. (1987) Formation of protein kinase recognition sites by covalent modification of the substrate. Molecular mechanism for the synergistic action of casein kinase II and glycogen synthase kinase 3. *J. Biol. Chem.* **262**, 14042–14048
- 75 Frame, S., Cohen, P. and Biondi, R. M. (2001) A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation. *Mol. Cell* **7**, 1321–1327
- 76 Dajani, R., Fraser, E., Roe, S. M., Young, N., Good, V., Dale, T. C. and Pearl, L. H. (2001) Crystal structure of glycogen synthase kinase 3 β : structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell* **105**, 721–732
- 77 ter Haar, E., Coll, J. T., Austen, D. A., Hsiao, H. M., Swenson, L. and Jain, J. (2001) Structure of GSK3 β reveals a primed phosphorylation mechanism. *Nat. Struct. Biol.* **8**, 593–596
- 78 Moon, R. T., Bowerman, B., Boutros, M. and Perrimon, N. (2002) The promise and perils of Wnt signaling through β -catenin. *Science* **296**, 1644–1646
- 79 Polakis, P. (2001) More than one way to skin a catenin. *Cell* **105**, 563–566
- 80 Seidensticker, M. J. and Behrens, J. (2000) Biochemical interactions in the wnt pathway. *Biochim. Biophys. Acta* **1495**, 168–182
- 81 Farr, III, G. H., Ferkey, D. M., Yost, C., Pierce, S. B., Weaver, C. and Kimelman, D. (2000) Interaction among GSK-3, GBP, axin, and APC in *Xenopus* axis specification. *J. Cell Biol.* **148**, 691–702
- 82 Li, L., Yuan, H., Weaver, C. D., Mao, J., Farr, III, G. H., Sussman, D. J., Jonkers, J., Kimelman, D. and Wu, D. (1999) Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. *EMBO J.* **18**, 4233–4240
- 83 Behrens, J., Jerchow, B. A., Wurtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kuhl, M., Wedlich, D. and Birchmeier, W. (1998) Functional interaction of an axin homolog, conductin, with β -catenin, APC, and GSK3 β . *Science* **280**, 596–599
- 84 Ding, V. W., Chen, R. H. and McCormick, F. (2000) Differential regulation of glycogen synthase kinase 3 β by insulin and Wnt signaling. *J. Biol. Chem.* **275**, 32475–32481
- 85 Hagen, T. and Vidal-Puig, A. (2002) Characterisation of the phosphorylation of β -catenin at the GSK-3 priming site Ser45. *Biochem. Biophys. Res. Commun.* **294**, 324–328
- 86 Hagen, T., Di Daniel, E., Culbert, A. A. and Reith, A. D. (2002) Expression and characterization of GSK-3 mutants and their effect on β -catenin phosphorylation in intact cells. *J. Biol. Chem.* **277**, 23330–23335
- 87 Toker, A. and Newton, A. C. (2000) Cellular signaling: pivoting around PDK-1. *Cell* **103**, 185–188
- 88 Vanhaesebroeck, B. and Alessi, D. R. (2000) The PI3K-PDK1 connection: more than just a road to PKB. *Biochem. J.* **346**, 561–576
- 89 Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F. and Hawkins, P. T. (1997) Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. *Science* **277**, 567–570
- 90 Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R., Reese, C. B. and Cohen, P. (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B α . *Curr. Biol.* **7**, 261–269
- 91 Leslie, N. R., Biondi, R. M. and Alessi, D. R. (2001) Phosphoinositide-regulated kinases and phosphoinositide phosphatases. *Chem. Rev.* **101**, 2365–2380
- 92 Moore, M. J., Kanter, J. R., Jones, K. C. and Taylor, S. S. (2002) Phosphorylation of the catalytic subunit of protein kinase A. Autophosphorylation versus phosphorylation by phosphoinositide-dependent kinase-1. *J. Biol. Chem.* **277**, 47878–47884
- 93 Flynn, P., Wongdaggar, M., Zavar, M., Dean, N. M. and Stokoe, D. (2000) Inhibition of PDK-1 activity causes a reduction in cell proliferation and survival. *Curr. Biol.* **10**, 1439–1442
- 94 Williams, M. R., Arthur, J. S., Balendran, A., van der Kaay, J., Poli, V., Cohen, P. and Alessi, D. R. (2000) The role of 3-phosphoinositide-dependent protein kinase 1 in activating AGC kinases defined in embryonic stem cells. *Curr. Biol.* **10**, 439–448
- 95 Biondi, R. M., Cheung, P. C., Casamayor, A., Deak, M., Currie, R. A. and Alessi, D. R. (2000) Identification of a pocket in the PDK1 kinase domain that interacts with PIF and the C-terminal residues of PKA. *EMBO J.* **19**, 979–988
- 96 Frodin, M., Jensen, C. J., Merienne, K. and Gammeltoft, S. (2000) A phosphoserine-regulated docking site in the protein kinase RSK2 that recruits and activates PDK1. *EMBO J.* **19**, 2924–2934
- 97 Chen, H., Nystrom, F. H., Dong, L. Q., Li, Y., Song, S., Liu, F. and Quon, M. J. (2001) Insulin stimulates increased catalytic activity of phosphoinositide-dependent kinase-1 by a phosphorylation-dependent mechanism. *Biochemistry* **40**, 11851–11859
- 98 Park, J., Hill, M. M., Hess, D., Brazil, D. P., Hofsteenge, J. and Hemmings, B. A. (2001) Identification of tyrosine phosphorylation sites on 3-phosphoinositide-dependent protein kinase-1 and their role in regulating kinase activity. *J. Biol. Chem.* **276**, 37459–37471
- 99 Dong, L. Q., Zhang, R. B., Langlais, P., He, H., Clark, M., Zhu, L. and Liu, F. (1999) Primary structure, tissue distribution, and expression of mouse phosphoinositide-dependent protein kinase-1, a protein kinase that phosphorylates and activates protein kinase C ζ . *J. Biol. Chem.* **274**, 8117–8122
- 100 Belham, C., Wu, S. and Avruch, J. (1999) Intracellular signalling: PDK1 – a kinase at the hub of things. *Curr. Biol.* **9**, R93–R96
- 101 Dutil, E. M., Toker, A. and Newton, A. C. (1998) Regulation of conventional protein kinase C isozymes by phosphoinositide-dependent kinase 1 (PDK-1). *Curr. Biol.* **8**, 1366–1375
- 102 Romanelli, A., Martin, K. A., Toker, A. and Blenis, J. (1999) p70 S6 kinase is regulated by protein kinase C ζ and participates in a phosphoinositide 3-kinase-regulated signalling complex. *Mol. Cell. Biol.* **19**, 2921–2928
- 103 Le Good, J. A., Ziegler, W. H., Parekh, D. B., Alessi, D. R., Cohen, P. and Parker, P. J. (1998) Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science* **281**, 2042–2045
- 104 Balendran, A., Casamayor, A., Deak, M., Paterson, A., Gaffney, P., Currie, R., Downes, C. P. and Alessi, D. R. (1999) PDK1 acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2. *Curr. Biol.* **9**, 393–404
- 105 Koh, H., Lee, K. H., Kim, D., Kim, S., Kim, J. W. and Chung, J. (2000) Inhibition of Akt and its anti-apoptotic activities by tumor necrosis factor-induced protein kinase C-related kinase 2 (PRK2) cleavage. *J. Biol. Chem.* **275**, 34451–34458
- 106 Yang, J., Cron, P., Thompson, V., Good, V. M., Hess, D., Hemmings, B. A. and Barford, D. (2002) Molecular mechanism for the regulation of protein kinase B/Akt by hydrophobic motif phosphorylation. *Mol. Cell* **9**, 1227–1240
- 107 Yang, J., Cron, P., Good, V. M., Thompson, V., Hemmings, B. A. and Barford, D. (2002) Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. *Nat. Struct. Biol.* **9**, 940–949
- 108 Gao, T., Toker, A. and Newton, A. C. (2001) The carboxyl terminus of protein kinase C provides a switch to regulate its interaction with the phosphoinositide-dependent kinase, PDK-1. *J. Biol. Chem.* **276**, 19588–19596
- 109 Newton, A. C. (2001) Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chem. Rev.* **101**, 2353–2364
- 110 Balendran, A., Biondi, R. M., Cheung, P. C., Casamayor, A., Deak, M. and Alessi, D. R. (2000) A 3-phosphoinositide-dependent protein kinase-1 (PDK1) docking site is required for the phosphorylation of protein kinase C ζ (PKC ζ) and PKC-related kinase 2 by PDK1. *J. Biol. Chem.* **275**, 20806–20813
- 111 Biondi, R. M., Kieloch, A., Currie, R. A., Deak, M. and Alessi, D. R. (2001) The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB. *EMBO J.* **20**, 4380–4390

- 112 Flynn, P., Mellor, H., Casamassima, A. and Parker, P. J. (2000) Rho GTPase control of protein kinase C-related protein kinase activation by 3-phosphoinositide-dependent protein kinase. *J. Biol. Chem.* **275**, 11064–11070
- 113 Weng, Q. P., Andrabi, K., Kozlowski, M. T., Grove, J. R. and Avruch, J. (1995) Multiple independent inputs are required for activation of the p70 S6 kinase. *Mol. Cell. Biol.* **15**, 2333–2340
- 114 Dennis, P. B., Pullen, N., Pearson, R. B., Kozma, S. C. and Thomas, G. (1998) Phosphorylation sites in the autoinhibitory domain participate in p70(s6k) activation loop phosphorylation. *J. Biol. Chem.* **273**, 14845–14852
- 115 Anderson, K. E., Coadwell, J., Stephens, L. R. and Hawkins, P. T. (1998) Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. *Curr. Biol.* **8**, 684–691
- 116 Filippa, N., Sable, C. L., Hemmings, B. A. and Van Obberghen, E. (2000) Effect of phosphoinositide-dependent kinase 1 on protein kinase B translocation and its subsequent activation. *Mol. Cell. Biol.* **20**, 5712–5721
- 117 Balendran, A., Currie, R., Armstrong, C. G., Avruch, J. and Alessi, D. R. (1999) Evidence that 3-phosphoinositide-dependent protein kinase-1 mediates phosphorylation of p70 S6 kinase *in vivo* at Thr-412 as well as Thr-252. *J. Biol. Chem.* **274**, 37400–37406
- 118 Scheid, M. P., Marignani, P. A. and Woodgett, J. R. (2002) Multiple phosphoinositide 3-kinase-dependent steps in activation of protein kinase B. *Mol. Cell. Biol.* **22**, 6247–6260
- 119 Knighton, D. R., Zheng, J. H., Ten Eyck, L. F., Ashford, V. A., Xuong, N. H., Taylor, S. S. and Sowadski, J. M. (1991) Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* **253**, 407–414
- 120 Batkin, M., Schwartz, I. and Shaltiel, S. (2000) Snapping of the carboxyl terminal tail of the catalytic subunit of PKA onto its core: characterization of the sites by mutagenesis. *Biochemistry* **39**, 5366–5373
- 121 Etcheberry, L. C., Van Bemmelen, M. X., Anjard, C., Traincard, F., Assemet, K., Reymond, C. and Veron, M. (1997) The catalytic subunit of *Dictyostelium* cAMP-dependent protein kinase – role of the N-terminal domain and of the C-terminal residues in catalytic activity and stability. *Eur. J. Biochem.* **248**, 820–826
- 122 Frodin, M., Antal, T. L., Dummiller, B. A., Jensen, C. J., Deak, M., Gammeltoft, S. and Biondi, R. M. (2002) A phosphoserine/threonine-binding pocket in AGC kinases and PDK1 mediates activation by hydrophobic motif phosphorylation. *EMBO J.* **21**, 5396–5407
- 123 Pearl, L. H. and Barford, D. (2002) Regulation of protein kinases in insulin, growth factor and Wnt signalling. *Curr. Opin. Struct. Biol.* **12**, 761–767
- 124 Knighton, D. R., Xuong, N. H., Taylor, S. S. and Sowadski, J. M. (1991) Crystallization studies of cAMP-dependent protein kinase. Co-crystals of the catalytic subunit with a 20 amino acid residue peptide inhibitor and MgATP diffract to 3.0 Å resolution. *J. Mol. Biol.* **220**, 217–220
- 125 Johnson, L. N., Lowe, E. D., Noble, M. E. and Owen, D. J. (1998) The structural basis for substrate recognition and control by protein kinases. *FEBS Lett.* **430**, 1–11
- 126 Veron, M., Radzio-Andzelm, E., Tsigelny, I., Ten Eyck, L. F. and Taylor, S. S. (1993) A conserved helix motif complements the protein kinase core. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10618–10622
- 127 Herberg, F. W., Zimmermann, B., McGlone, M. and Taylor, S. S. (1997) Importance of the A-helix of the catalytic subunit of cAMP-dependent protein kinase for stability and for orienting subdomains at the cleft interface. *Protein Sci.* **6**, 569–579
- 128 Dammann, H., Traincard, F., Anjard, C., van Bemmelen, M. X., Reymond, C. and Veron, M. (1998) Functional analysis of the catalytic subunit of *Dictyostelium* PKA *in vivo*. *Mech. Dev.* **72**, 149–157
- 129 Kemp, B. E., Parker, M. W., Hu, S., Tiganis, T. and House, C. (1994) Substrate and pseudosubstrate interactions with protein kinases: determinants of specificity. *Trends Biochem. Sci.* **19**, 440–444
- 130 Chang, C. I., Xu, B. E., Akella, R., Cobb, M. H. and Goldsmith, E. J. (2002) Crystal structures of MAP kinase p38 complexed to the docking sites on its nuclear substrate MEF2A and activator MKK3b. *Mol. Cell* **9**, 1241–1249
- 131 Johnson, L. N., Noble, M. E. and Owen, D. J. (1996) Active and inactive protein kinases: structural basis for regulation. *Cell* **85**, 149–158
- 132 Huse, M. and Kuriyan, J. (2002) The conformational plasticity of protein kinases. *Cell* **109**, 275–282
- 133 Bax, B., Carter, P. S., Lewis, C., Guy, A. R., Bridges, A., Tanner, R., Pettman, G., Mannix, C., Culbert, A. A., Brown, M. J. et al. (2001) The structure of phosphorylated GSK-3 β complexed with a peptide, FRATide, that inhibits β -catenin phosphorylation. *Structure* **9**, 1143–1152
- 134 Fraser, E., Young, N., Dajani, R., Franca-Koh, J., Ryves, J., Williams, R. S., Yeo, M., Webster, M. T., Richardson, C., Smalley, M. J. et al. (2002) Identification of the Axin and Frat binding region of glycogen synthase kinase-3. *J. Biol. Chem.* **277**, 2176–2185
- 135 Ferkey, D. M. and Kimelman, D. (2002) Glycogen synthase kinase-3 β mutagenesis identifies a common binding domain for GBP and Axin. *J. Biol. Chem.* **277**, 16147–16152
- 136 Delaney, A. M., Printen, J. A., Chen, H., Fauman, E. B. and Dudley, D. T. (2002) Identification of a novel mitogen-activated protein kinase kinase activation domain recognized by the inhibitor PD 184352. *Mol. Cell. Biol.* **22**, 7593–7602
- 137 Jeffrey, P. D., Russo, A. A., Polyak, K., Gibbs, E., Hurwitz, J., Massague, J. and Pavletich, N. P. (1995) Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. *Nature (London)* **376**, 313–320
- 138 Card, G. L., Knowles, P., Laman, H., Jones, N. and McDonald, N. Q. (2000) Crystal structure of a γ -herpesvirus cyclin-cdk complex. *EMBO J.* **19**, 2877–2888

Received 21 October 2002/20 February 2003; accepted 25 February 2003

Published as BJ Immediate Publication 25 February 2003, DOI 10.1042/BJ20021641