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PCTK Proteins: The Forgotten Brain Kinases?

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PCTK · PCTAIRE · Kinase · Brain · Neuron · Phosphorylation

Abstract
PCTAIRE kinases (PCTKs) are highly conserved serine/threonine kinases that are closely related to cyclin-dependent kinases. They are enriched in post-mitotic neurons of adult brains, suggesting they might perform important neuron-specific functions independent of the cell cycle. So far, the biological functions of PCTKs in the brain have been largely neglected and remain to be discovered. This review summarises preliminary investigations into the expression and characterisation of PCTK kinase activity, providing a basis for further investigations. In particular, it identifies three key areas of priority for further research: (1) do PCTKs require activating phosphorylation by an upstream kinase or binding to an essential co-factor for maximal kinase activity; (2) what are the physiological substrates of PCTKs, and (3) what is the functional effect of PCTK kinase activity in primary neurons?

PCTK Genes and Proteins

PCTK genes are conserved in eukaryotes, from *Dictyostelium* slime moulds, parasitic trypanosomes and nematode worms to fish, birds, reptiles and mammals. The human PCTK1 gene is located on the X chromosome (Xp11.3–p11.23), while PCTK2 is located on chromosome 12 (12q23.1) and PCTK3 on chromosome 1 (1q31–q32). Interestingly, while many alleles on one of the X chromosomes in females are inactivated by CpG methylation, PCTK1 is actively transcribed from both alleles (there is no PCTK1 gene on the Y chromosome) [1]. This phenomenon might be restricted to humans, since one of the two PCTK1 alleles in female mice is methylated in the 5′ untranslated region, inhibiting transcription of that allele [1]. It has not been reported if escape from X-linked inactivation of the second PCTK1 allele results in increased expression of PCTK1 in human females versus males.

Two mRNA transcripts are generated from PCTK1, 2 and 3 genes [2, 3]. For PCTK1, isoform 1 (2.4 kb) is the most predominant and encodes for a protein of 496 amino acids and 56 kDa. Isoform 2 (3.3 kb) uses an alternative exon in the 5′ untranslated region and 5′ coding region, and encodes for a protein with a slightly longer N-terminal domain (503 amino acids). Two mRNA transcripts of 4.4 and 4.7 kb have been detected for PCTK2, although only a single protein isoform of 523 amino acids and 60 kDa is generated. A 3.1-kb transcript is predominant for PCTK3, encoding a protein of 472 amino acids and 54 kDa, although a longer PCTK3b transcript encodes a protein with an additional 30 residues in the N-terminal domain [3]. Western blotting of brain homogenates using antibodies that recognise PCTK1 detect a doublet at approximate M_r 60,000–62,000 and 65,000–

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68,000 [4, 5], while only single bands for PCTK2 [6, 7] and PCTK3 [8] have been detected.

PCTK proteins are comprised of 3 separate domains: a central kinase domain that is highly conserved between the isoforms (~80% identity) and displays high similarity with other CMGC kinases [2, 6], plus N-terminal and C-terminal domains that are more unique to the PCTKs and distinguish each isoform. The N-terminal domains are longer than those of Cdks and have already been shown to mediate several protein–protein interactions (see below). The C-terminal domains are considerably shorter and their function is completely unknown. The C-terminal domain of PCTK3 contains a PDZ domain that is not found in the other isoforms, although it is not yet known if or what proteins bind to this region. The kinase domains of human PCTK1, 2 and 3 display high sequence identity with the kinase domain of human Cdk2 (53, 51 and 52%, respectively), as well as other Cdk members [2]. They contain all of the features necessary for an active kinase domain, including the VALK motif in subdomain II that orientates ATP in the active site, the HRD motif in subdomain VIb in which the aspartic acid is the catalytic residue for phosphotransfer, and the DFG motif in subdomain VII that coordinates Mg^{2+} ions and ATP in the ATP binding cleft (fig. 1). Therefore, it is highly likely that the PCTKs are active kinases and not inactive pseudo-kinases. In support of this, several studies have observed kinase activity of PCTK isoforms isolated from mammalian cells using generic substrates, such as myelin basic protein (MBP) and histone H1 in in vitro kinase assays (discussed below). Crystal structures have not yet been generated for any PCTK isoform. These would greatly improve our understanding of the mechanisms of PCTK kinase activity, as they have done for other CMGC kinase family members (e.g. Cdk2, Cdk5, GSK3).

Does the Expression Pattern of PCTK Indicate Post-Mitotic Function?

PCTK1 mRNA is widely expressed in mammalian tissues [2], as well as many cell lines of different tissue origins [9]. PCTK2 and 3 transcripts are also expressed in several cell lines, but at lower levels than PCTK1 [10]. PCTK3 mRNA is expressed in many, but not all tissues, with highest expression in the heart and brain. PCTK2, on the other hand, is almost completely restricted to the brain and lung [2]. Similar observations were made at the protein level, whereby PCTK1 is detectable in many tissues, but is highest in the brain and testis [4, 6]. One report also detected high PCTK1 protein expression in the heart and kidney [6]. PCTK3 protein is only detectable in the brain and testis, while PCTK2 protein was restricted to the brain only [6]. During development, PCTK1 and 2 mRNA transcripts peaked at P7–15 in the rat brain and decreased thereafter, while PCTK3 mRNA peaked after P15 [6]. Within the brain, PCTK1, 2 and 3 transcripts were detectable in all areas. For PCTK1 and 2, highest expression was detected in the hippocampus and olfactory bulb [6], while PCTK3 mRNA expression was highest in the substantia nigra, thalamus and spinal chord of mice [3]. PCTK1 protein was also ubiquitously expressed throughout the brain, with the highest relative expression detected in layer II of the pyriform cortex, the pyramidal layer of the hippocampus (especially in CA3) and Purkinje cells of the cerebellum [4, 11, 12]. In the neocortex, PCTK1 protein was high in pyramidal neurons of layers II–III and V–VI [4, 11]. PCTK2 protein expression was highest in pyramidal cells of the cerebral cortex (particularly layer V), but was absent from the Purkinje cells of the cerebellum [7]. PCTK1 protein was relatively highly expressed in terminally differentiated cells of the brain and testis [4], while in primary co-cultured cells, PCTK2 protein was detected in neurons but not astrocytes [6]. Together with the temporal peak of expression of PCTK mRNAs post-natally and localisation to neuronal layers of the mature brain, these observations indicate the PCTK isoforms are relatively more abundant in post-mitotic cells.

At the subcellular level, PCTK1 was mostly found in the cytoplasm of the cell bodies and proximal processes, but not in axons of post-mitotic neurons in tissue sections of rat brain [4]. When ectopically expressed in several cell lines, PCTK1 and 3 were consistently cytoplasmic and excluded from the nucleus [8, 9], although one report showed PCTK1 protein localised to nucleoli within Purkinje cells of the cerebellum and pyramidal neurons of the hippocampus [12]. Biochemical fractionation of the adult rat brain showed that PCTK1 was present in all fractions obtained, including synaptosomal fractions (the nucleus was not tested) [5]. The subcellular localisation of the PCTK2 and 3 isoforms is less well studied, with only one group showing that PCTK2 was insoluble in buffer containing 1% NP-40 and remained in the particulate fraction [6]. This group later showed that it might localise to mitochondria in COS7 cells [7]. Graeser et al. [13] showed that PCTK1 induced neurite outgrowth in Neuro-2A cells when localised to the cytoplasm, but not when artificially sequestered to the nucleus by introduction of a nuclear localisation sequence. These observa-

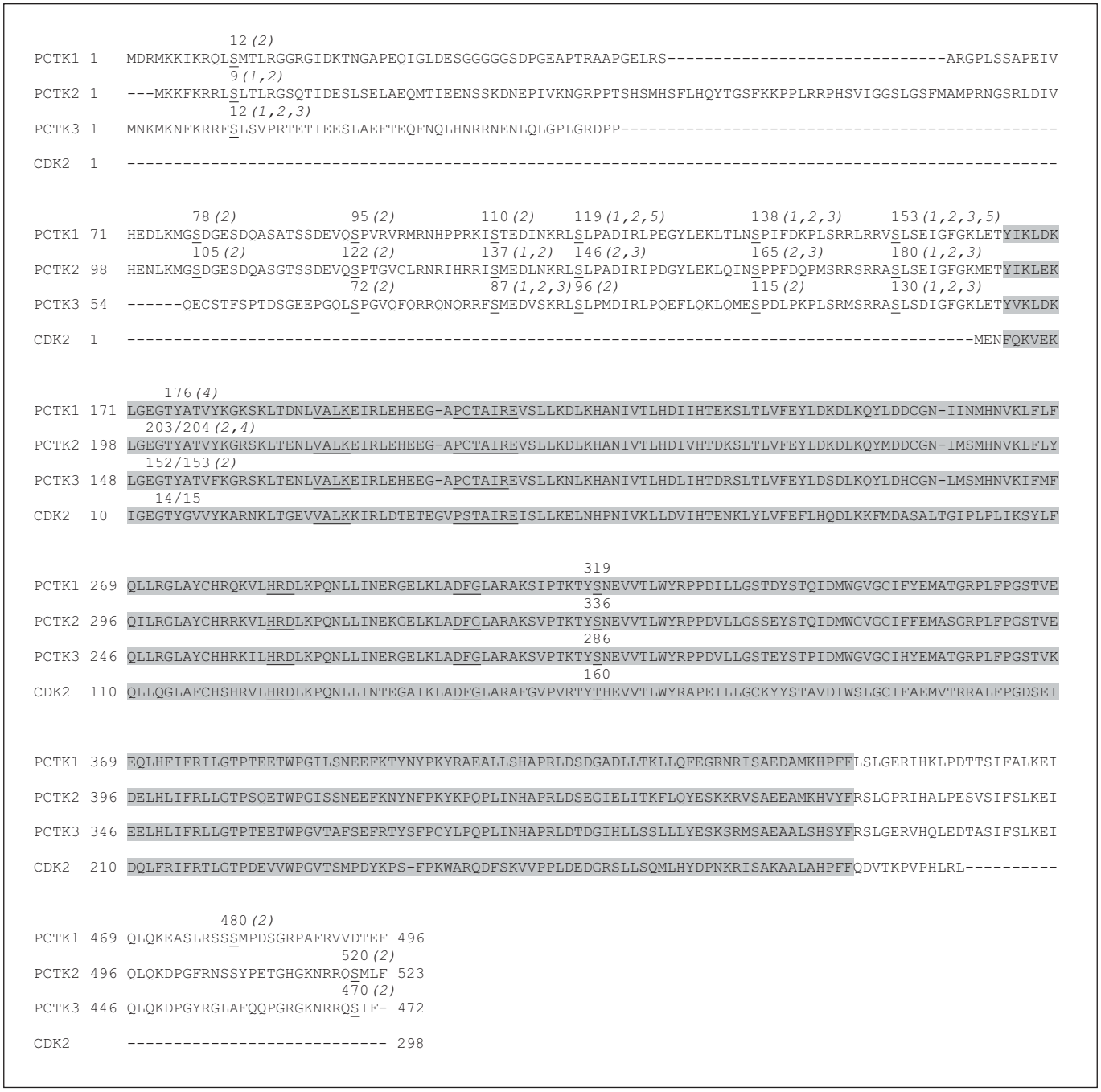

Fig. 1. Sequence alignment of PCTK1, 2 and 3 with Cdk2. The primary amino acid sequences of full-length human PCTK1, 2 and 3 (predominant isoforms), as well as human Cdk2 were aligned using the ClustalW program (Kyoto University Bioinformatics Centre; http://align.genome.jp). The central kinase domains are highlighted in grey. Conserved catalytic residues of the kinase domains are underlined, such as the VALK motif in subdomain II that orientates ATP in the active site, the HRD motif in subdomain VIb that catalyzes phosphotransfer, and the DFG motif in subdomain VII that coordinates Mg^{2+} ions and ATP in the ATP-binding cleft. The characteristic PCTAIRE/PSTAIRE motifs are also underlined. Phosphorylation sites are numbered and underlined. Numbers in brackets refer to the phosphoproteomic studies that report phosphorylation at each site; 1 = Wissing et al. [26]; 2 = Daub et al. [27]; 3 = Dephoure et al. [28]; 4 = Rikova et al. [29]; 5 = Olsen et al. [30]. Note that while Thr160 in Cdk2 is phosphorylated and regulates Cdk2 kinase activity, phosphorylation of PCTKs at the equivalent sites has not yet been detected.

tions suggest that PCTKs are predominantly cytoplasmic, but one isoform at least might also reside in cytoskeletal fractions that are insoluble in cell lysis buffers containing mild detergents.

Are PCTKs Involved in Regulation (or Are They Regulated) by the Cell Cycle?

PCTKs are most closely related in primary sequence to Cdks, which are key regulators of the cell cycle. Therefore, it is reasonable to predict that PCTKs might also be involved in regulation of the cell cycle. Some reports have supported this hypothesis by showing that in Hs68 human fibroblast cells, endogenous PCTK1 activity was low during G1 and G1-S phases of the cell cycle, but increased during S and G2 phases [9]. This correlated with decreased tyrosine phosphorylation on PCTK1 during S and G2 phases of the cell cycle, although the phosphotyrosine residues were not specified [9]. The kinase domains of PCTKs contain residues equivalent to Thr14 and Tyr15 of Cdk1 (e.g. T195, Y196 in PCTK1) that are conserved in many Cdks and can be phosphorylated at different stages of the cell cycle, regulating the activity of the kinase [14]. Phosphorylation at one or both of these sites on PCTK isoforms has been reported in phosphoproteomic studies (fig. 1), although it is not yet known which kinases might target these sites or if they regulate PCTK activity in vitro or in vivo. Elsewhere, adenoviral-mediated expression of PCTK3 in some glioma cell lines increased the proportion of cells in S and G2/M phase of the cell cycle (decrease in G0/1 phase cells), leading to inhibited proliferation and induction of cell death [15]. In contrast, several other studies suggest that PCTK function is not associated with the cell cycle. For example, transfection of Neuro-2A cells with PCTK1 did not affect cycle progression and there is no change in PCTK expression during cycling of several other cell types [9, 13, 16]. In an early study, Cdc28-deficient yeast that has defective cell cycle progression (Cdc28 is the yeast homologue of mammalian Cdk1) was rescued by expression of Cdks but not PCTKs [2], so it does not appear to be functionally interchangeable. Also as mentioned above, the expression pattern of PCTKs in non-cycling, post-mitotic cells of the brain and testes predicts important non-cell cycle-related functions. Therefore, at present it is reasonable to assume that PCTKs function independently of the cell cycle (at least in the adult brain), although it is premature to completely rule out a role with the cell cycle in other tissues and in other stages of development.

Does PCTK Require a Co-Factor for Maximal Kinase Activity?

Exogenous expression of PCTK1 and PCTK2 in a variety of mammalian cell lines and isolated by immunoprecipitation phosphorylates the generic kinase substrates MBP and histone H1 in vitro (MBP better than histone H1) [4, 9, 13, 17, 18], while PCTK3 exhibits no kinase activity at all [8]. PCTK1 expressed in bacteria is also inactive [13]. However, PCTK1 (but not PCTK3) isolated from cell lines or insect cells exhibits increased kinase activity following pre-incubation with a testes or fibroblast homogenate [4, 8, 9]. Meanwhile, the activity of PCTK1 and PCTK2 isolated from brain and testes homogenates is reduced upon washing with 500 mM NaCl [4, 6], which also shifts the localisation of PCTK2 from the particulate to the soluble cytosolic fraction [7]. These observations suggest that a salt-labile co-factor is necessary for full activity of PCTK proteins. In support of this, the majority of PCTK1 elutes in a broad peak at approximately 50–110 kDa on gel exclusion fractionation of a mouse brain homogenate, with the highest levels of PCTK1 activity (using MBP as a substrate) in fractions 80–110 kDa [13], suggesting the existence of a low-molecular-weight PCTK-binding protein required for its activity.

As stated earlier, PCTKs are most closely related to Cdks. All members of the Cdk family contain a highly conserved PSTAIRE motif, which mediates binding to co-activating proteins called cyclins. The activity of Cdks varies throughout different stages of the cell cycle, in large part due to transcriptional control and availability of their respective cyclins. A PSTAIRE motif in the kinase domain of Cdks is necessary for binding to the cyclin proteins. This interaction is essential for maximum Cdk kinase activity, since it induces a conformational change in the Cdk that causes rearrangement of the T-loop and the ATP/substrate-binding cleft, resulting in activation of kinase activity [19]. PCTK proteins have retained this important protein–protein interaction motif, although the serine residue has been exchanged for a cysteine. Other members of the CMGC family that do not require binding of co-factors for maximal kinase activity and are active as monomers do not contain a PSTAIRE (or related) motif (e.g. GSK3, p38, JNK1, MAPK, HIPK2, DYRK1a). Furthermore, the primary sequence of the activation loop of PCTKs is more closely related to Cdks than the monomeric CMGC family members mentioned above. Therefore, it is likely that the PCTAIRE motif in PCTKs is important for interactions with a salt-labile co-

factor, although its identity is not yet known. PCTK1 is not able to interact with cyclins D1, E, A, B1, B2, G, F or suc1 in co-IP assays [9, 11]. Also, a putative activating co-factor was detected in rat brain homogenates, which do not normally express cyclin proteins [6]. The serine residue within the PSTAIRE motif of Cdk1 and Cdk2 makes contact with cyclin proteins [19, 20]. It is possible that replacement of the serine for a cysteine serves to prevent PCTKs binding to cyclins, and at the same time provides a unique binding site for another unidentified PCTK co-factor. It would be interesting to exchange the cysteine for a serine residue within the PCTAIRE motif of PCTKs to see if they become activated by cyclin co-factors and the cell cycle, and vice versa for Cdks.

PCTK1, but not PCTK2, has been reported to bind to p35, which is an activating co-factor for Cdk5 [18]; however, binding of p35 did not affect PCTK1 activity in vitro [18, 21]. Some other PCTK-interacting proteins have been identified (see below), however, none of these directly affect PCTK kinase activities. When bacterially expressed PCTK1 was incubated with brain or testes homogenates, no stoichiometric binding partners were observed [13]. It is possible that the co-factor is insoluble in TX-100/NP-40-based buffers, as reported for the majority of endogenous PCTK2 in cultured neurons [6]. Alternatively, binding to membranous organelles or cytoskeletal elements may be required for PCTK kinase activity.

Another possible explanation is that the N-terminal domains of PCTK isoforms serve as co-factors for their adjacent kinase domains. The closely-related Cdks are relatively short proteins, restricted to a complete kinase domain only (no N- or C-terminal domains). The Cdks rely on binding to co-factors (cyclins) for structural rearrangement of the active site to achieve maximum kinase activity. Other kinases contain extended N- or C-terminal domains that form intramolecular interactions with the adjacent kinase domain to influence its activity. For example, the N-terminal region of GSK3 is phosphorylated by PKB and other AGC kinases upon growth factor stimulation [22, 23] and the resultant phosphoserine residue binds to a pocket on the kinase domain, inhibiting its activity [24]. Human PCTKs possess extended N-terminal domains (164, 191, 141 residues for PCTK1, 2, 3, respectively). Deletion of the entire N-terminal domain of PCTK1 (leaving the kinase and C-terminal domains only) abolished kinase activity against MBP in vitro [13]. This supports the hypothesis that the N-terminal domain acts as a co-factor for the adjacent kinase domain. Furthermore, phosphorylation of the N-terminal domain by PKA (an AGC kinase) inhibits PCTK1 activity [13], similar to GSK3. Together, these observations suggest that the N-terminal domain of PCTK1 might fold back onto the kinase domain to stabilise an active conformation and that phosphorylation of the N-terminal domain may disturb this interaction, inhibiting its kinase activity. However, if this is the case, it is difficult to explain why incubation of full-length PCTK1 with brain/testes homogenates increased its kinase activity (in the presence of phosphatase inhibitors), or why washing immunoprecipitates of PCTK1 with 500 mM NaCl reduced its activity. Identification of the co-activating protein/domain or subcellular structure responsible for maximal activation of PCTK proteins is a priority, since future investigations into the mechanisms of activity and biological function require a fully active and regulatable PCTK complex.

It is interesting to note that the first appearance of an N-terminal domain on PCTK proteins during evolution occurs in the phylum Platyhelminthes (e.g. flatworms) and is conserved in all higher organisms, including worms, insects, birds, reptiles, fish and mammals. PCTK isoforms in lower organisms, such as kinetoplasts (*Leishmania* and *Trypanosoma* parasites), mycetozoans *(Dictyostelium)* and cnidarians (*Hydra*, sea anemones) are shorter in length, display high sequence identity with the kinase domain of human PCTKs, retain the characteristic PCTAIRE motif, but do *not* contain N-terminal domains. This correlates with the first appearance of a centralised and complex nervous system, supporting a role for PCTKs (or at least their N-terminal domains) in the coordination/regulation of the central nervous system.

Does Phosphorylation of PCTK Regulate Its Kinase Activity?

Phosphatase treatment of PCTK1 isolated from testes and brain using calf intestine phosphatase caused faster migration on SDS-PAGE for essentially all of the detectable PCTK1 protein [4], suggesting that it might be stoichiometrically phosphorylated. If so, a likely site of stoichiometric phosphorylation is on the activation T loop of the kinase domain. This site is commonly phosphorylated on CMGC kinases, either constitutively (e.g. GSK, DYRK) or inducibly (e.g. Erk, JNK, p38). The prototypic TEY motif in the activation loop of Erk1 and Erk2 is inducibly phosphorylated at the threonine and tyrosine residues by MEK, which induces a conformation of the active site that is conducive for maximal kinase activity (similar for JNK and p38). This phosphorylation is reversible and is efficiently dephosphorylated by DUSP

phosphatases. Under basal conditions, the stoichiometry of phosphorylation at the TEY motif is low, hence low basal kinase activity for the Erks. In GSK3 and DYRK, the equivalent regions contain constitutively phosphorylated tyrosine residues (2 in DYRK, 1 in GSK3), conferring high stoichiometric phosphorylation at this site (approaching 100%) and high basal kinase activity. Cdk2 contains a THE motif, whereby the threonine residue is inducibly phosphorylated by CAK and CCRK at certain phases of the cell cycle, while the negatively charged glutamate residue may mimic the phosphotyrosine residue in Erk, GSK3 and DYRK. In similar fashion, Cdk5 has an SAE motif in place of TEY, so the glutamate may mimic the phosphotyrosine; however, phosphorylation of the serine residue, in this case, is not required for maximal kinase activity [25]. A similar motif (SNE) is highly conserved in the PCTKs, and although several phosphoproteomic studies have identified many phosphorylation sites on all 3 PCTK isoforms [26–30], none have detected phosphorylation at this serine residue. Although this does not prove that the serine is not normally phosphorylated, it might indicate that phosphorylation of the T loop is not required for PCTK kinase activity (like Cdk5 but unlike most other CMGC kinases). On the other hand, mutation of Ser319 on the T loop of PCTK1 to a non-phosphorylatable alanine caused reduced phosphorylation of MBP in vitro [13]. Therefore, it remains to be determined if the T loop in PCTKs is phosphorylated, whether it is catalysed by autophosphorylation or another upstream kinase, and whether phosphorylation of the T loop is required for maximal kinase activity.

Other phosphorylation sites on PCTKs have been detected in large-scale phosphoproteomic studies that display overlapping patterns in the 3 isoforms (fig. 1) [26–30]. Of note are several phosphoserines in the N-terminal domain that lie within RRXS motifs that are targeted by members of the AGC family of kinases, especially PKA. Purified PKA can phosphorylate recombinant PCTK1 in vitro at several of these sites in the N-terminal region [13]. Interestingly, phosphorylation by PKA reduces the kinase activity of wild-type PCTK1 against MBP, but not non-phosphorylatable mutants of PCTK1, although this was in comparison to untreated wild-type PCTK1 that had relatively low kinase activity [13]. Nevertheless, this observation suggests that PKA might directly regulate the activity of PCTK1. A different phosphosite in the N-terminal domain of PCTK1, Ser95, is reported to be phosphorylated by Cdk5, increasing PCTK1 activity [18]. Serine 95 is contained within an optimal Cdk5 consensus sequence (SPXR), and while the equivalent serine residues in PCTK2 and 3 are also found to be phosphorylated in a large-scale phosphoproteomic study [27], the sequences surrounding this residue in PCTK2 and 3 have a non-charged glycine or valine residue, respectively, in place of Arg98, which would be expected to prevent phosphorylation by Cdk5. In addition, PCTK1 can be phosphorylated on tyrosine residues, mostly during S and G2 phases of the cell cycle, although the exact sites of phosphorylation have not been determined [9]. Further studies into regulatory phosphorylation sites on the PCTK proteins will help to identify signalling pathways that directly regulate PCTK kinase activity and function.

What Is the Substrate Specificity of PCTKs?

Many protein kinases will only phosphorylate proteins at sites that lie within a certain primary amino acid sequence, termed a phosphorylation consensus sequence. This provides specificity for phosphorylation of one particular substrate by one particular kinase. For example, Cdk5 and other Cdks prefer to phosphorylate proline-directed serine or threonine residues with a basic residue (lysine/arginine/histidine) at the +3 position (e.g. S/TPXK/R/H, where X represents any amino acid) [31]. So far, the phosphorylation consensus sequence for PCTKs is unknown. Since nearly all CMGC kinases are proline-directed serine/threonine kinases, it is likely that the PCTKs will be the same. However, other features of an optimal phosphorylation consensus sequence have not yet been determined. The only likely physiological substrate of a PCTK reported so far is *N*-ethylmaleimide-sensitive fusion protein (NSF), a vesicular transport protein that regulates protein exocytosis (see below) [5]. PCTK1 phosphorylates NSF at Ser569, which lies within an optimal consensus sequence for Cdks (SPXR). This suggests that PCTKs may share similar substrate specificity determinants as the Cdks. Having said that, PCTKs phosphorylate the generic substrate MBP at a faster rate than histone H1 in vitro [13], which is opposite to the Cdk's preference. Both substrates contain many proline-directed serine/threonine residues; however, histone H1 also contains many basic residues C-terminal to the phosphosites, which is optimal for phosphorylation by Cdks. Therefore, other features of an optimal phosphorylation consensus sequence for PCTKs will be determined as more physiological substrates are identified. This knowledge will help to validate proposed candidates as physiological substrates of the PCTKs, as well as predict novel potential targets.

Table 1. List of binding proteins for PCTKs

Name	PCTK-binding partner	Method of identification	Expression pattern	PCTK-binding region	Effect on PCTK kinase activity	Biological function of interaction	References
14-3-3	PCTK1 *(PCTK2, 3 ND)*	Co-IP	Ubiquitous	N-terminal domain, PKA-mediated phosphosites	None	Regulates subcellular localisation of signalling proteins	Le Bouffant et al. [11], 1998 Graeser et al. [13], 2002
Trap	PCTK1, 2 but not PCTK3	Yeast 2-hybrid, Co-IP	Brain, testes	N-terminal domain	None	Possibly a scaffold for signalling proteins	Hirose et al. [7], 2000
p35	PCTK1, but not PCTK2 *(PCTK3 ND)*	Yeast 2-hybrid, Co-IP	Brain	ND	None	Activating co-factor for Cdk5	Cheng et al. [18], 2002
NSF	PCTK1 *(PCTK2, 3 ND)*	Yeast 2-hybrid, Co-IP	Ubiquitous	ND	None	Exocytosis of membrane vesicles	Liu et al. [5], 2006
Sec23p	PCTK1, 3 *(PCTK2 ND)*	Yeast 2-hybrid, Co-IP	Ubiquitous	ND	None	Export of secretory proteins from the ER to the Golgi	Palmer et al. [10], 2005
p11	PCTK1 *(PCTK2, 3 ND)*	Co-IP	Ubiquitous	Requires full-length protein	None	Trafficking of transmembrane and signalling proteins to the plasma membrane	Le Bouffant et al. [11], 1998

ND = Not determined.

What Is the Biological Function of PCTKs?

Probably the most direct way of determining the biological function of the PCTKs is to generate knockout or transgenic animal models; however, these are not yet available. Another approach is to catalogue their specific substrates, since this is the primary function of a protein kinase. To date, only one substrate has been reported for PCTK1 (NSF), and none for PCTK2 or PCTK3. This work will identify molecules, pathways and cellular processes directly regulated by PCTK function, as well as delineate the mechanisms by which PCTKs exert their effects. Also, it is difficult and possibly even misleading to study pathways and stimuli that regulate PCTK activity without using phosphorylation of a bona fide PCTK substrate as a direct measure of PCTK activity.

An additional approach to characterising the function of PCTKs is to identify proteins that physically interact with them. Several reports have used the yeast 2-hybrid technique to identify novel interacting partners of PCTK1, which has provided clues to the function of this kinase (table 1).

Vesicular Transport and Exocytosis

A yeast 2-hybrid analysis revealed that PCTK1 can interact with NSF, which was confirmed by co-immunoprecipitation of the proteins from adult rat brain [5]. NSF is a hexameric cytoplasmic protein that is recruited to the plasma membrane, where it binds to SNAP-SNARE complexes to regulate exocytosis events, including fusion of synaptic vesicles with the pre-synaptic membrane for neurotransmitter release. Phosphorylation of NSF at Ser569 by PCTK1 reduces oligomerisation of the protein, thus disrupting exocytosis. Growth hormone (GH) release by PC12 cells (a measure of exocytosis) was promoted by expression of the non-phosphorylatable mutant NSF-S569A. Similarly, expression of a kinase-dead form of PCTK1 increased GH release, while wild-type PCTK1 attenuated GH release. Other studies have also implicated PCTK1 as a regulator of vesicular trafficking and exocytosis. For example, another yeast 2-hybrid study showed that PCTK1 and 3 (PCTK2 not tested) interact with the Sec23p subunit of the COPII complex, which is important for export of secretory cargo (transmembrane and secreted proteins) from the ER to the plasma membrane [10]. PCTK kinase activity was not required for the interaction with Sec23p and there was no evidence that Sec23p was

phosphorylated by PCTK1. Expression of a kinase-dead version of PCTK1 or siRNA-mediated knockdown of expression caused disruption of the ER-Golgi secretory pathway in HeLa cells [10]. Elsewhere, an siRNA screen identified PCTK1 as a potential regulator of glucose uptake in adipocytes by regulating transport of the cell surface receptors Glut1 and Glut4 [32]. Together, these observations suggest that PCTKs might be important regulators of protein secretion and translocation of transmembrane receptor proteins at the cell surface. Therefore, it will be interesting to investigate if PCTKs regulate neurotransmission in the brain by regulating exocytosis of neurotransmitters at the synapse and/or cell surface expression of neurotransmitter receptors.

Neurite Outgrowth

PCTK activity has been implicated in the negative regulation of neurite outgrowth, whereby transfection of Neuro-2A neuroblastoma cells with wild-type PCTK1 induced neurite outgrowth in about 5% of transfected cells, while the kinase-dead form (K194R) induced outgrowth in 10% of transfected cells [13]. Treatment with forskolin to activate PKA proportionally doubled neurite outgrowth in cells transfected with YFP (control), wild-type PCTK1 and PCTK1-K194R. Meanwhile, transfection with a PCTK-S119/153A mutant, which cannot be phosphorylated at these sites by PKA and hence has higher basal activity, completely blocked neurite outgrowth. This suggests that PKA induces neurite outgrowth by phosphorylating and inhibiting PCTK1 kinase activity. Accordingly, in forskolin-treated and untreated cells, the highest neurite outgrowth was observed in cells transfected with kinase-dead PCTK1-K194R. However, combination of the kinase-dead mutation (K194R) with the PKA phosphosite mutants (S119A or S119A/S153A) reduced outgrowth to levels comparable to YFP-transfected control cells. The similar induction of neurite outgrowth by forskolin in cells expressing either WT or kinase-dead PCTK suggests that PKA can also regulate neurite outgrowth independent of PCTK1 kinase activity. This could be PCTK independent or alternatively might involve PKA phosphorylation of Ser119, generating a 14-3-3-binding site that sequesters PCTK1 away from its physiological targets [13]. Three isoforms of 14-3-3 proteins (γ, θ and ξ) have been identified as PCTK1-interacting proteins [11, 13, 33]. In addition, immobilised 14-3-3 has been used to purify active PCTK1 from rat cerebellum [11]. Binding of 14-3-3 to PCTK1 does not affect its activity in vitro when using MBP as a substrate [13]. A common function of 14-3-3 proteins is to sequester signalling proteins in the cytoplasm and out of the nucleus. PCTK1 is predominantly cytoplasmic, so it will be interesting to see if 14-3-3 proteins influence this localisation.

Other PCTK-Binding Proteins

Yeast 2-hybrid experiments have shown that PCTK2 (and PCTK1 but not PCTK3) is part of a complex that includes Trap and Cables (also called ik3-1). Trap is a large protein that is predominantly expressed in the brain and testes, and is localised to mitochondria [7] and/or ribonucleoprotein complexes [34]. Meanwhile, Cables was identified as an adaptor molecule linking the non-receptor tyrosine kinase c-abl with Cdk5 in neurons [35]. Cables and PCTK2 do not interact directly, although they can be immunoprecipitated within the same complex [17]. Both Cables and PCTK2 bind to Tudor repeats 4 and 5 near the C-terminus of Trap, although it is not yet clear if they compete for the same binding site [7, 17]. Neither Trap nor Cables affect the kinase activity of PCTK2 in vitro using MBP or histone H1 as a substrate [7, 17]. In a separate yeast 2-hybrid study, PCTK1 (but not PCTK2) was found to directly interact with the Cdk5 co-factor p35 [18]. The authors suggest that phosphorylation of PCTK1 at Ser95 by Cdk5/p35 increased PCTK1 kinase activity. Since Cables can bind to Cdk5/p35, it will be interesting to see if they are also components of the PCTK/Trap/cables complex. If so, it is tempting to speculate that Trap might be a scaffolding protein that facilitates phosphorylation and regulation of PCTK activity by upstream kinases, such as Cdk5, or vice versa.

p11 (S100A10) is a member of the large S100 family of small acidic proteins that has been found to interact with PCTK1 [11]. This protein is widely expressed throughout the body, including the brain. It is localised to the cytoplasm and peripheral cell membrane, where it forms homo- and heterodimers and mediates trafficking of transmembrane and signalling proteins to the plasma membrane, such as annexin II and several neuronal ion channels [for review, see 36]. It is not yet clear if p11 influences the subcellular localisation or the kinase activity of PCTK1, nor if it can bind to PCTK2 or PCTK3.

PCTKs and Neurological Disorders

Increased expression of PCTK3 has been reported in human post-mortem brain tissue from Alzheimer's disease patients compared to age-matched controls using Western blot analysis [8]. PCTK3 was also detected in

paired helical filaments isolated from Alzheimer's disease tissue. Co-transfection of PCTK3 and Tau in CHO cells caused hyperphosphorylation of Tau at Thr231 and Ser235, but not at Ser202, Ser396/Ser404. Strangely, two different kinase-dead mutants of PCTK3 had the same effect, suggesting that Tau is not a direct substrate of PCTK3 and that any effect PCTK3 has upon Tau phosphorylation is indirect. This is surprising, since many other closely related members of the CMGC family of kinases have been reported to directly phosphorylate Tau (e.g. cdc2, Cdk5, GSK3, DYRK, JNK, p38, Erk). Perhaps further investigations will clarify the potential involvement of PCTK3 (or any PCTK isoform) in Alzheimer's disease. Elsewhere, PCTK3 has been reported to be upregulated in rats administered venlafaxine and fluoxetine, two medications commonly used to treat depression [37]. Together, these studies represent the first steps towards elucidating a potential involvement of PCTKs in neurological disorders. As more research tools become available (e.g. antibodies, substrates for assays, knockout animals, etc.), these investigations may become more extensive.

Conclusions

PCTKs are brain-enriched, serine/threonine protein kinases whose biological function is almost completely uncharacterised. This is despite the fact that PCTKs are related (in primary structure at least) to the well-characterised Cdk family. Preliminary observations suggest important roles for PCTKs in mammalian brains and possibly even in some disease states. Of immediate interest is clarification of the regulation of PCTK kinase activity, the elucidation of specific physiological substrates for each PCTK isoform, and the subsequent understanding of the physiological roles of PCTKs. So far, the small number of preliminary studies have implicated PCTKs in protein exocytosis/cell surface expression and neurite outgrowth. However, it should be noted that most studies have been performed in immortalised cell lines that are constitutively proliferating. To date, none have been performed in post-mitotic primary neurons that are a major site of expression of the PCTKs. Therefore, it will be important for future studies to investigate the function of PCTKs in a more appropriate model system (e.g. cultured primary neurons). Ultimately, the generation of PCTK-knockout mice will yield valuable information about the biological function of each isoform. Downstream of that, knock-in mutants that prevent phosphorylation of specific PCTK substrates will help to elucidate the targets of PCTKs that mediate its biological effects. At that point, it is likely that PCTKs will become as well known as their more famous relatives.

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References

1 Carrel L, Clemson CM, Dunn JM, Miller AP, Hunt PA, Lawrence JB, et al: X inactivation analysis and DNA methylation studies of the ubiquitin activating enzyme E1 and PCTAIRE-1 genes in human and mouse. Hum Mol Genet 1996;5:391–401.

2 Meyerson M, Enders GH, Wu CL, Su LK, Gorka C, Nelson C, et al: A family of human cdc2-related protein kinases. EMBO J 1992; 11:2909–2917.

3 Herskovits AZ, Davies P: Cloning and expression analysis of two novel PCTAIRE 3 transcripts from human brain. Gene 2004; 328:59–67.

4 Besset V, Rhee K, Wolgemuth DJ: The cellular distribution and kinase activity of the Cdk family member Pctaire1 in the adult mouse brain and testis suggest functions in differentiation. Cell Growth Differ 1999;10: 173–181.

5 Liu Y, Cheng K, Gong K, Fu AK, Ip NY: Pctaire1 phosphorylates N-ethylmaleimide-sensitive fusion protein: implications in the regulation of its hexamerization and exocytosis. J Biol Chem 2006;281:9852–9858.

6 Hirose T, Tamaru T, Okumura N, Nagai K, Okada M: PCTAIRE 2, a Cdc2-related serine/threonine kinase, is predominantly expressed in terminally differentiated neurons. Eur J Biochem 1997;249:481–488.

7 Hirose T, Kawabuchi M, Tamaru T, Okumura N, Nagai K, Okada M: Identification of tudor repeat associator with PCTAIRE 2 (Trap). A novel protein that interacts with the N-terminal domain of PCTAIRE 2 in rat brain. Eur J Biochem 2000;267:2113–2121.

8 Herskovits AZ, Davies P: The regulation of tau phosphorylation by PCTAIRE 3: implications for the pathogenesis of Alzheimer's disease. Neurobiol Dis 2006;23:398–408.

9 Charrasse S, Carena I, Hagmann J, Woods-Cook K, Ferrari S: PCTAIRE-1: characterization, subcellular distribution, and cell cycle-dependent kinase activity. Cell Growth Differ 1999;10:611–620.

10 Palmer KJ, Konkel JE, Stephens DJ: PCTAIRE protein kinases interact directly with the COPII complex and modulate secretory cargo transport. J Cell Sci 2005;118: 3839–3847.

11 Le Bouffant F, Capdevielle J, Guillemot JC, Sladeczek F: Characterization of brain PCTAIRE-1 kinase immunoreactivity and its interactions with p11 and 14-3-3 proteins. Eur J Biochem 1998;257:112–120.

12 Le Bouffant F, Le Minter P, Traiffort E, Ruat M, Sladeczek F: Multiple subcellular localizations of PCTAIRE-1 in brain. Mol Cell Neurosci 2000;16:388–395.

13 Graeser R, Gannon J, Poon RY, Dubois T, Aitken A, Hunt T: Regulation of the CDK-related protein kinase PCTAIRE-1 and its possible role in neurite outgrowth in Neuro-2A cells. J Cell Sci 2002;115:3479–3490.
14 Malumbres M, Barbacid M: Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 2009;9:153–166.
15 Naumann U, Huang H, Wolburg H, Wischhusen J, Weit S, Ohgaki H, et al: PCTAIRE3: a putative mediator of growth arrest and death induced by CTS-1, a dominant-positive p53-derived synthetic tumor suppressor, in human malignant glioma cells. Cancer Gene Ther 2006;13:469–478.
16 Whitfield ML, Sherlock G, Saldanha AJ, Murray JI, Ball CA, Alexander KE, et al: Identification of genes periodically expressed in the human cell cycle and their expression in tumors. Mol Biol Cell 2002;13:1977–2000.
17 Yamochi T, Nishimoto I, Okuda T, Matsuoka M: ik3-1/Cables is associated with Trap and Pctaire2. Biochem Biophys Res Commun 2001;286:1045–1050.
18 Cheng K, Li Z, Fu WY, Wang JH, Fu AK, Ip NY: Pctaire1 interacts with p35 and is a novel substrate for Cdk5/p35. J Biol Chem 2002;277:31988–31993.
19 Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massague J, et al: Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. Nature 1995;376:313–320.
20 McGrath CF, Pattabiraman N, Kellogg GE, Lemcke T, Kunick C, Sausville EA, et al: Homology model of the CDK1/cyclin B complex. J Biomol Struct Dyn 2005;22:493–502.
21 Tsai LH, Delalle I, Caviness VS Jr, Chae T, Harlow E: p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. Nature 1994;371:419–423.
22 Sutherland C, Cohen P: The alpha-isoform of glycogen synthase kinase-3 from rabbit skeletal muscle is inactivated by p70 S6 kinase or MAP kinase-activated protein kinase-1 in vitro. FEBS Lett 1994;338:37–42.
23 Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA: Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995;378:785–789.
24 Frame S, Cohen P, Biondi RM: A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation. Mol Cell 2001;7:1321–1327.
25 Tarricone C, Dhavan R, Peng J, Areces LB, Tsai LH, Musacchio A: Structure and regulation of the CDK5-p25(nck5a) complex. Mol Cell 2001;8:657–669.
26 Wissing J, Jansch L, Nimtz M, Dieterich G, Hornberger R, Keri G, et al: Proteomics analysis of protein kinases by target class-selective prefractionation and tandem mass spectrometry. Mol Cell Proteomics 2007;6:537–547.
27 Daub H, Olsen JV, Bairlein M, Gnad F, Oppermann FS, Korner R, et al: Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol Cell 2008;31:438–448.
28 Dephoure N, Zhou C, Villen J, Beausoleil SA, Bakalarski CE, Elledge SJ, et al: A quantitative atlas of mitotic phosphorylation. Proc Natl Acad Sci USA 2008;105:10762–10767.
29 Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, et al: Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190–1203.
30 Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, et al: Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell 2006;127:635–648.
31 Songyang Z, Lu KP, Kwon YT, Tsai LH, Filhol O, Cochet C, et al: A structural basis for substrate specificities of protein Ser/Thr kinases: primary sequence preference of casein kinases I and II, NIMA, phosphorylase kinase, calmodulin-dependent kinase II, CDK5, and Erk1. Mol Cell Biol 1996;16:6486–6493.
32 Tang X, Guilherme A, Chakladar A, Powelka AM, Konda S, Virbasius JV, et al: An RNA interference-based screen identifies MAP4K4/NIK as a negative regulator of PPARgamma, adipogenesis, and insulin-responsive hexose transport. Proc Natl Acad Sci USA 2006;103:2087–2092.
33 Sladeczek F, Camonis JH, Burnol AF, Le Bouffant F: The Cdk-like protein PCTAIRE-1 from mouse brain associates with p11 and 14-3-3 proteins. Mol Gen Genet 1997;254:571–577.
34 Hosokawa M, Shoji M, Kitamura K, Tanaka T, Noce T, Chuma S, et al: Tudor-related proteins TDRD1/MTR-1, TDRD6 and TDRD7/TRAP: domain composition, intracellular localization, and function in male germ cells in mice. Dev Biol 2007;301:38–52.
35 Zukerberg LR, Patrick GN, Nikolic M, Humbert S, Wu CL, Lanier LM, et al: Cables links Cdk5 and c-Abl and facilitates Cdk5 tyrosine phosphorylation, kinase upregulation, and neurite outgrowth. Neuron 2000;26:633–646.
36 Svenningsson P, Greengard P: p11 (S100A10) – an inducible adaptor protein that modulates neuronal functions. Curr Opin Pharmacol 2007;7:27–32.
37 Khawaja X, Xu J, Liang JJ, Barrett JE: Proteomic analysis of protein changes developing in rat hippocampus after chronic antidepressant treatment: implications for depressive disorders and future therapies. J Neurosci Res 2004;75:451–460.