REVIEW ARTICLE

SOcK, MiSTs, MASK and STicKs: the GCKIII (germinal centre kinase III) kinases and their heterologous protein–protein interactions

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The GCKIII (germinal centre kinase III) subfamily of the mammalian Ste20 (sterile 20)-like group of serine/threonine protein kinases comprises SOK1 (Ste20-like/oxidant-stress-response kinase 1), MST3 (mammalian Ste20-like kinase 3) and MST4. Initially, GCKIIIs were considered in the contexts of the regulation of mitogen-activated protein kinase cascades and apoptosis. More recently, their participation in multiprotein heterocomplexes has become apparent. In the present review, we discuss the structure and phosphorylation of GCKIIIs and then focus on their interactions with other proteins. GCKIIIs possess a highly-conserved, structured catalytic domain at the N-terminus and a less-well conserved C-terminal regulatory domain. GCKIIIs are activated by tonic autophosphorylation of a T-loop threonine residue and their phosphorylation is regulated primarily through protein serine/threonine phosphatases [especially PP2A (protein phosphatase 2A)]. The GCKIII regulatory domains are highly disorganized, but can interact with more structured proteins, particularly the CCM3 (cerebral cavernous malformation 3)/PDCD10 (programmed cell death 10) protein. We explore the role(s) of GCKIIIs (and CCM3/PDCD10) in STRIPAK (striatin-interacting phosphatase and kinase) complexes and their association with the *cis*-Golgi protein GOLGA2 (golgin A2; GM130). Recently, an interaction of GCKIIIs with MO25 has been identified. This exhibits similarities to the STRADα (STE20-related kinase adaptor α)–MO25 interaction (as in the LKB1–STRADα–MO25 heterotrimer) and, at least for MST3, the interaction may be enhanced by *cis*-autophosphorylation of its regulatory domain. In these various heterocomplexes, GCKIIIs associate with the Golgi apparatus, the centrosome and the nucleus, as well as with focal adhesions and cell junctions, and are probably involved in cell migration, polarity and proliferation. Finally, we consider the association of GCKIIIs with a number of human diseases, particularly cerebral cavernous malformations.

Key words: cytoskeleton, focal adhesion, germinal centre kinase III subfamily (GCKIII), Golgi, MO25, protein phosphatase 2A (PP2A), protein phosphorylation, STRAD (sterile 20-related kinase adaptor α), striatin.

INTRODUCTION

The mSte20-like [mammalian Ste20 (sterile 20)-like] or mSte20-related group of serine/threonine protein kinases is so-called because of their similarities (especially in their catalytic domains) to the Ste20 kinases of yeasts. The catalytic domains of yeast Ste20 kinases are also similar to the yeast Sps1 (sporulation-specific protein 1) kinases and this term is sometimes included in the mSte20-like kinase nomenclature. The mSte20-like kinases are subclassified into the GCK (germinal centre kinase) and PAK (p21-activated protein kinase) families [1,2]. The GCK family consists of eight subfamilies (GCKI–GCKVIII, a total of 22

kinases) and the PAK family comprises two subfamilies (PAKI and PAKII, a total of six kinases). In addition, there are two STRAD (Ste20-related adaptor kinase) pseudokinases (STRADα and STRADβ) which are most closely related to the GCKVIs, but which also show similarities with the GCKIIIs. The primary structure of the mSte20-like kinases contains a generic conserved protein kinase catalytic domain [3] and a variable regulatory domain. GCKs differ from PAKs in that the kinase domain lies towards the N-terminus with the regulatory domain towards the C-terminus, whereas the order is reversed in PAKs. In terms of the relative position of the kinase and regulatory domains, GCKs resemble yeast Sps1 kinases, whereas PAKs resemble the

yeast Ste20 kinases. In most mSte20-like kinases, the regulatory domains contain conserved or identifiable motifs (e.g. citron-homology or myosin head domains) and these motifs, along with the relative position of the catalytic domain, form the basis for assignment of the kinases to the individual subfamilies [1]. However, conservation of the regulatory domain is less apparent in GCKIIIs than in most other mSte20-like kinases. The phylogenetic relationships between the mSte20-like kinase subfamilies have been discussed in depth [1]. Whereas PAKs are orthologous (i.e. derived from a common ancestral gene) with yeast Ste20 kinases, Dan et al. [1] do not consider the GCKs to be orthologous with yeast Sps1 kinases because of the diverse nature of their regulatory domains (but then neither are they truly orthologous with yeast Ste20 kinases).

There are three members of the GCKIII subfamily (for additional reviews see [4,5]): SOK1 [Ste20-like/oxidant-stress-response kinase 1, also known as STK25 (serine/threonine kinase 25) and YSK1 (yeast Sps1/Ste20-related kinase 1)], MST3 [mSte20-like kinase 3, or STK24 (serine/threonine kinase 24)] and MST4 [also known as MASK (MST3 and SOK1-related kinase)]. In the present review, we will use the human GCKIIIs as the paradigms for GCKIII sequences and structures, although these features are conserved in other mammalian GCKIIIs. Although mSte20-like kinases in general [1,2] and GCKIIIs in particular (Table 1; [6–9]) have been considered in the context of regulation of MAPK (mitogen-activated protein kinase) cascades and of apoptosis, recent publications suggest that these are not their only or even their primary roles. In the present review, in addition to discussing the involvement of GCKIIIs in MAPK signalling and apoptosis, we will describe the more recent studies of GCKIIIs in biological processes.

IDENTIFICATION, CLONING AND TISSUE DISTRIBUTION OF GCKIIIs

All three human GCKIII genes consist of 12 exons and are ubiquitously expressed. In two GCKIIIs (*SOK1* and *MST4*), exon 1 encodes a 5′ untranslated region, but this is not the case for *MST3*. SOK1 was the first to be cloned, its mRNA encoding a 426-residue protein derived from exons 2–12 [10,11]. Northern blot analysis shows that a 2.2–2.3 kB SOK1 transcript is most highly expressed in the testis with lesser amounts in other tissues, whereas two transcripts (∼2.6 and 1.5 kB) are detectable in the stomach. *MST3* was cloned next and its mRNA encodes a 431-residue protein [MST3[(431)]] [12]. MST3[(431)] transcripts are most highly expressed in the heart, skeletal muscle and pancreas [12]. Unusually, MST3[(431)] exhibits a marked preference for Mn^{2+} over Mg^{2+} as its metal ion cofactor [12], although other divalent metal ions can act as a substitute [13]. *MST3[(431)]* mRNA contains a 42 nt open reading frame from exon 1 (encoding [NH2]MAHSPVQSGLPGMQ) spliced to exons 3–12. Subsequently, a brain-restricted 443-residue variant [MST3b[(443)]] was cloned [14]. MST3b[(443)] contains an alternative 78-nt open reading frame from exon 2 (encoding [NH2]MDSRAQLWGLALNKRRATLPHPGGST) spliced to exons 3–12 [14]. Disparities in the nomenclature for the two human MST3 isoforms are sometimes encountered. MST3b[(443)] was originally termed MST3b [14], although some databases list MST3[(431)] as MST3b and MST3b[(443)] as MST3a. In the present review, and for clarity, the nomenclature will include the length of the primary sequence to distinguish between the two isoforms except when it is unclear to which isoform a given article is referring.

Full-length *MST4* mRNA (variant 1) encodes a 416-residue protein [6,15,16]. It is most highly expressed in the placenta and in tissues of the immune system (spleen and thymus), followed by the prostate [6,15–17]. In addition to the variant 1 transcript, two alternatively spliced transcripts (variants 2 and 3) have been identified. Transcript variant 2 lacks protein kinase subdomains I–III (important in the binding and orientation of ATP) as well as subdomain IV [3], which are encoded by exon 3. The protein kinase domain of transcript variant 3 lacks the C-terminus of subdomain IX, subdomain X and the N-terminus of subdomain XI [3] encoded by exon 7. Thus any proteins encoded by transcript variants 2 or 3 are likely to be catalytically inactive. Indeed, this has been demonstrated empirically for the variant 3-encoded protein [15]. Nevertheless, proteins such as those encoded by the MST4 variant 2 and 3 transcripts could possibly be 'decoy' or inhibitory (dominant-negative) species. Interestingly, transcript variant 3 was the first MST4 transcript to be identified and this involved use of a yeast 2-hybrid screen with a modified c-Raf catalytic domain 'bait' [15]. This, however, appears to be a spurious (or serendipitous) interaction as no interactions of transfected MST4 variants 1 or 3 with transfected c-Raf could be detected [15].

PRIMARY AND SECONDARY STRUCTURE OF GCKIIIs

GCKIIIs are the shortest of the mSte20-like kinases [1], a fact attributable to the limited length of their C-terminal regulatory domains (Figures 1A and 1B). At the N-termini of GCKIIIs, a short variable sequence is followed by a highly conserved 279-residue sequence derived from exons 3–7 and the 5′ region of exon 8. This region encompasses the 251-residue catalytic domain (Figures 1A and 1B) which exhibits the features typical of protein kinases [2,3]. As shown by sequence alignment (Figure 1B), from the N-terminus, all GCKIII subgroup members possess an ATP-binding site [$\underline{G}X\underline{G}X(F)\underline{G}X_{16}\underline{K}$ in GCKIIIs] in subdomains I and II, the essential lysine residue being Lys[53] in MST3[(431)] and MST4 and Lys[49] in SOK1. Mutation of the essential lysine residue to an arginine renders GCKIIIs catalytically inactive [7,8,18]. The catalytic loop sequence is HRDIK (subdomain VIB) and the Mg^{2+}-co-ordinating sequence is DFG (subdomain VII). The T-loop threonine residue (phosphorylated during activation) is Thr[178] in MST3[(431)] and MST4, Thr[190] in MST3b[(443)], and Thr[174] in SOK1. Finally, the protein substrate-binding site is contained within a GTPPWMAPE motif in the P + 1 loop of subdomain VIII. Molecular modelling indicates that the GCKIII catalytic domains are highly ordered (Figure 2) and confirm the crystallographic studies [19,20]. Typical of the highly ordered general structure of protein kinase catalytic domains, GCKIII catalytic domains possess a smaller N-lobe and a larger C-lobe with the protein substrate-binding cleft lying between them.

The regulatory domain lies C-terminal to the catalytic domain. The 23 N-terminal residues of the regulatory domain are conserved (Figures 1A and 1B) and contain the putative bipartite NLS (nuclear localization sequence) [21]. This region of the proteins is also probably highly ordered (Figure 2). These conserved regions are followed by highly variable regions [134 residues in MST3[(431)] and MST3b[(443)], 133 residues in SOK1, and 119 residues in MST4], which are encoded by the 3′ region of exon 8 and exons 9–12. Molecular modelling of the variable C-termini of the GCKIII regulatory domains confirms that they are highly disordered (Figure 2). However, intrinsically disordered proteins can be induced to assume ordered conformations by interactions with suitable ordered macromolecular ligands. In the case of signalling proteins, such disordered proteins may act as 'hubs' in signalling networks through these interactions. Until recently (and in contrast to other GCKs), conserved or consensus motifs had not been recognized in
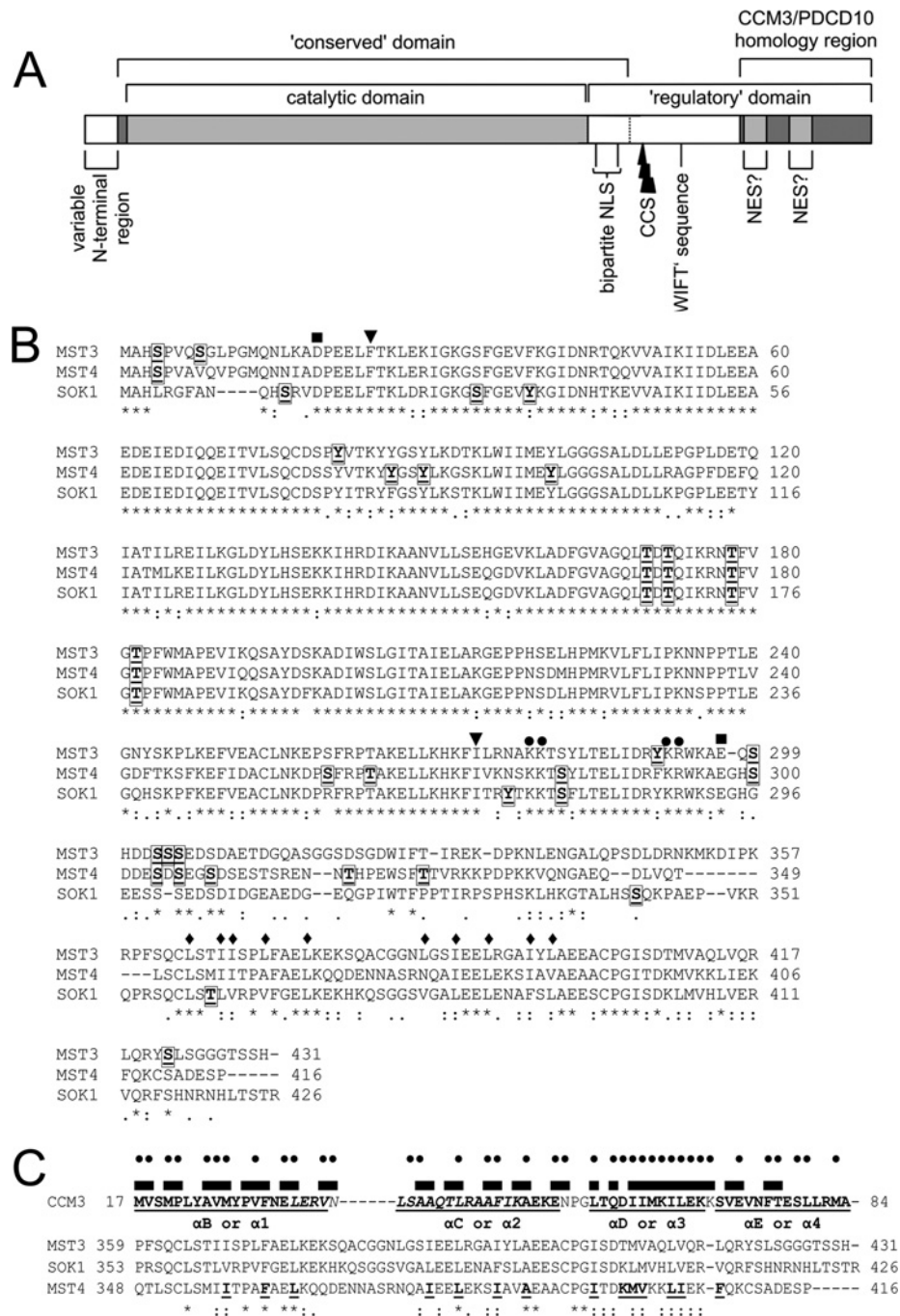
A

'conserved' domain

CCM3/PDCD10
homology region

catalytic domain — 'regulatory' domain

variable N-terminal region | bipartite NLS | CCS | 'WIFT' sequence | NES? | NES?

B

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MST3  MAHSPVQSGLPGMQNLKADPEELFTKLEKIGKGSFGEVFKGIDNRTQKVVAIKIIDLEEA  60
MST4  MAHSPVAVQVPGMQNNIADPEELFTKLERIGKGSFGEVFKGIDNRTQQVVAIKIIDLEEA  60
SOK1  MAHLRGFAN---QHSRVDPEELFTKLDRIGKGSFGEVYKGIDNHTKEVVAIKIIDLEEA   56
      ***    *:    .  :.*********::*****:********.*:: **********
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MST3  EDEIEDIQQEITVLSQCDSPYVTKYYGSYLKDTKLWIIMEYLGGGSALDLLEPGPLDETQ  120
MST4  EDEIEDIQQEITVLSQCDSSYVTKYYGSYLKGSKLWIIMEYLGGGSALDLLRAGPFDEFQ  120
SOK1  EDEIEDIQQEITVLSQCDSPYITRYFGSYLKSTKLWIIMEYLGGGSALDLLKPGPLEETY  116
      *******************  *::.**.****..********************  ::*
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MST3  IATILREILKGLDYLHSEKKIHRDIKAANVLLSEHGEVKLADFGVAGQLTDTQIKRNTFV  180
MST4  IATMLKEILKGLDYLHSEQGDVKLADFGVAGQLTDTQIKRNTFV                 180
SOK1  IATILREILKGLDYLHSERKIHRDIKAANVLLSEQGDVKLADFGVAGQLTDTQIKRNTFV  176
      ***:*:************** :****************
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MST3  GTPFWMAPEVIKQSAYDSKADIWSLGITAIELARGEPPHSELHPMKVLFLIPKNNPPTLE  240
MST4  GTPFWMAPEVIQQSAYDSKADIWSLGITAIELAKGEPPNSDMHPMRVLFLIPKNNPPTLV  240
SOK1  GTPFWMAPEVIKQSAYDFKADIWSLGITAIELAKGEPPNSDLHPMRVLFLIPKNSPPTLE  236
      ***********:***** :**************:****:*:: ***:****** ****
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MST3  GNYSKPLKEFVEACLNKEPSFRPTAKELLKHKFILRNAKKTSYLTELIDRYKRWKAE-QS  299
MST4  GDFTKSFKEFIDACLNKDPSFRPTAKELLKHKFIVKNSKKTSYLTELIDRFKRWKAEGHS  300
SOK1  GQHSKPFKEFVEACLNKDPRFRPTAKELLKHKFITRYTKKSFLTELIDRYKRWKSEGHG  296
      *: .:*.:***::****:* ***********   . :**:.********:****.*  .
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MST3  HDDSSSEDSDAETDGQASGGSDSGDWIFT-IREK-DPKNLENGALQPSDLDRNKMKDIPK  357
MST4  DDESDSEGSDSESTSREN--NTHPEWSFTTVRKKPDPKKVQNGAEQ--DLVQT-------  349
SOK1  EESS-SEDSDIDGEAEDG--EQGPIWTFPPTIRPSPHSKLHKGTALHSQKPAEP--VKR   351
      :.:.*  **.**  *        * *   . :::.:*:    .
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MST3  RPFSQCLSTIISPLFAELKEKSQACGGNLGSIEELRGAIYLAEEACPGISDTMVAQLVQR  417
MST4  ---LSCLSMIITPAFAELKQQDENNASRNQAIEELEKSIAVAEAACPGITDKMVKKLIEK  406
SOK1  QPRSQCLSTLVRPVFGELKEKHKQSGGSVGALEELENAFSLAEESCPGISDKLMVHLVER  411
        .*** :* *  *.***:*    ..  ::***  :. :  **:.****:* :  :*::
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MST3  LQRYSLSGGGTSSH-  431
MST4  FQKCSADESP-----  416
SOK1  VQRFSHNRNHLTSTR  426
      .*: *  .  .
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C

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CCM3  17  MVSMPLYAVMYPVFNELERVN------LSAAQTLRAAFIKAEKENPGLTQDIIMKILEKKSVEVNFTESLLRMA-  84
                αB or α1              αC or α2            αD or α3           αE or α4
MST3  359  PFSQCLSTIISPLFAELKEKSQACGGNLGSIEELRGAIYLAEEACPGISDTMVAQLVQR-LQRYSLSGGGTSSH-  431
SOK1  353  PRSQCLSTLVRPVFGELKEKHKQSGGSVGALEELENAFSLAEESCPGISDKLMVHLVER-VQRFSHNRNHLTSTR  426
MST4  348  QTLSCLSMIITPAFAELKQQDENNASRNQAIEELEKSIAVAEAACPGITDKMVKKLIEK-FQKCSADESP-----  416
          *  :: * * **:.          :  *. *.  : **  **:::: ::  :::::
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Figure 1   Primary structure of human GCKIIIs

(**A**) Diagrammatic representation primary sequence features of GCKIIIs. The diagram depicts MST3[(431)] although it is generally applicable to all GCKIIIs. For details, see the text. CCS, empirically established or predicted highest probability caspase-cleavage sequence (see Table 2); 'WIFT' sequence, the tripeptide sequence equivalent to 'site D' of STRADα [WX(F/W)] [91] followed by the MST3[(431)](Thr[328]) regulatory domain phosphorylation site [18]. In MST4, the sequence is WSFT, whereas the equivalent site in SOK1 is WTFPPT (see **B**). However, it is not known whether the threonine residues C-terminal to site D in MST4 and SOK1 are phosphorylated. (**B**) Sequences of human MST3[(431)] (GenPept accession number NP_001027467), MST4 (GenPept accession number NP_057626) and SOK1 (GenPept accession number NP_001258906) were aligned using Clustal W2. MST3b[(443)] (not shown) is identical to MST3[(431)] apart from the N-terminus (see Figure 6). The boundaries of conserved domains are denoted by filled squares with the boundaries of the protein kinase catalytic domains (according to the Hanks and Hunter classification [3]) denoted by filled inverted triangles. The bipartite NLS residues are denoted by filled circles and the potential NES residues are shown by filled diamonds. Residues conserved between the human and/or rat and mouse that are reportedly phosphorylated (serine, threonine and tyrosine) (see PhosphoSitePlus at http://www.phosphosite.org) are shown superimposed on the human sequences, although not all have necessarily been detected in the human. These residues are boxed, emboldened and underlined. The kinases responsible for most of these phosphorylations are unknown though the serine residues lying at the C-terminus of the conserved sequence are in highly acidic environments of the type favoured by casein kinases 1 and 2. (**C**) Alignment of the N-terminal homodimerization domain of CCM3/PDCD10 with the C-terminal domains of GCKIIIs. The (slightly differing) residues reportedly involved in homodimerization of CCM3/PDCD10 are denoted by filled circles or filled squares [23,24]. The four α-helices of the CCM3/PDCD10 homodimerization domain [24] are underlined. {Note that the α1–α4 designations of the α-helices in [23,30] differ from those in [24] (αB–αE) though inspection of the sequences reveals that they are identical.} The residues in MST4 determined crystallographically as interacting with the homodimerization domain of CCM3/PDCD10 [30] are in bold and underlined. The 18-residue region in CCM3/PDCD10, deletion of which may result in cerebral cavernous malformations [82], is italicized. The residues in this region reportedly phosphorylated by SOK1 are Ser[39] and Thr[43] [22].
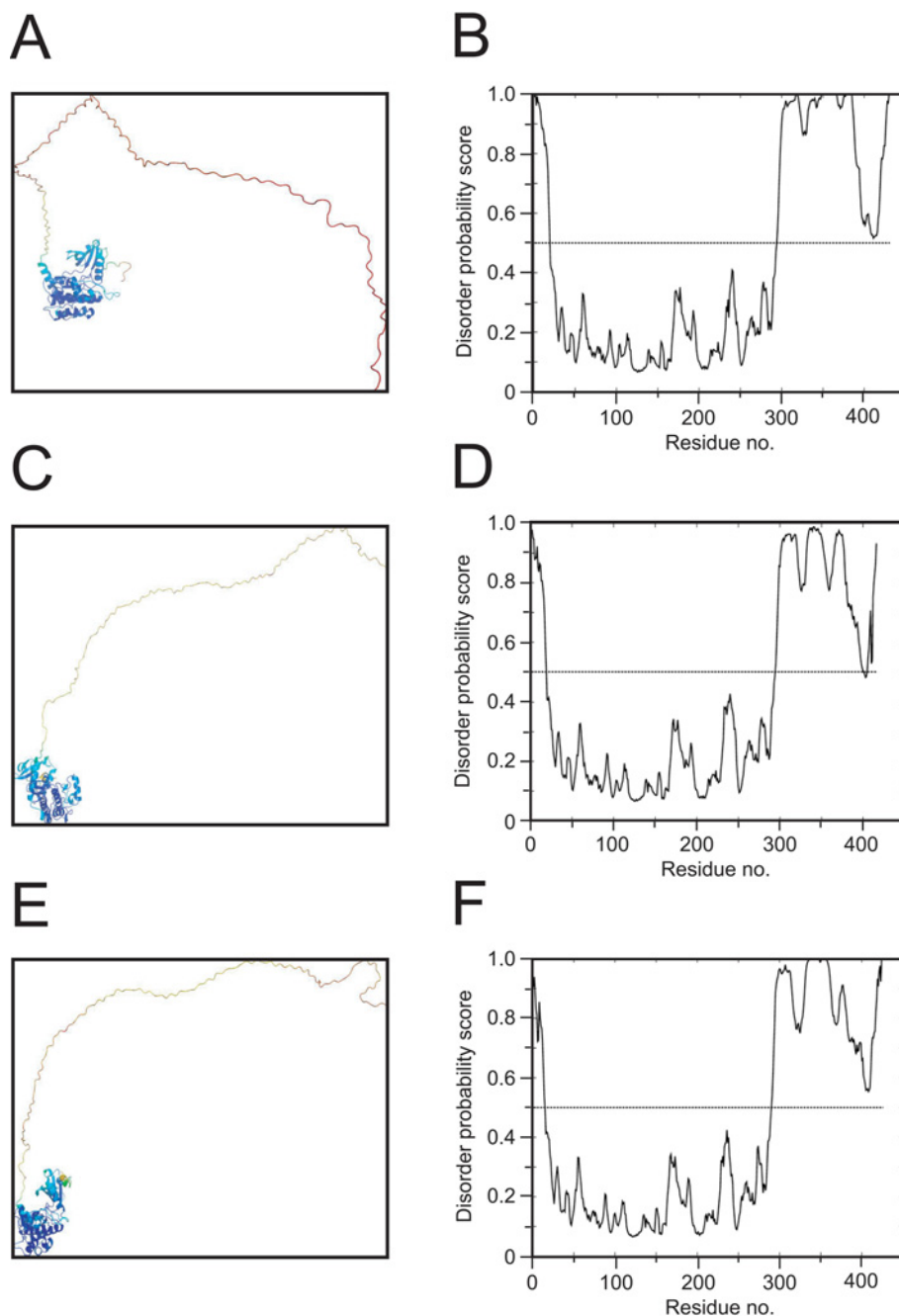
Figure 2   Modelling of human GCKIII structures

Representations of the three-dimensional models (**A**, **C** and **E**) and disorder probability plots (**B**, **D** and **F**) and for MST3[(431)] (GenPept accession number NP_001027467) (**A** and **B**), MST4 (GenPept accession number NP_057626) (**C** and **D**) and SOK1 (GenPept accession number NP_001258906) (**E** and **F**). Representations of the three-dimensional models were produced using IntFOLD-TS [145–147]. Colouring represents predicted local errors from blue (high accuracy) through to green, yellow and orange to red (low accuracy) using the ModFOLDclust2 model quality assessment method [148]. The intrinsically unstructured residues which have a high predicted error are shown in red. Each image was rendered using PyMOL (http://www.pymol.org). Disorder probability plots were produced using the DISOclust method [149] for the prediction of intrinsically unstructured regions. A disorder probability score of 0.5 is set as the transition value from order (<0.5) to disorder (>0.5). An interactive three-dimensional version of this Figure can be found at http://www.biochemj.org/bj/454/0013/bj4540013add.htm.

the regulatory domains of GCKIIIs. However, the C-termini of the GCKIII regulatory domains display similarity to the N-terminal homodimerization domain of a highly ordered 212-residue protein known as CCM3 (cerebral cavernous malformation 3)/ PDCD10 (programmed cell death 10) [22,23] (Figure 1C). CCM3/PDCD10 is so-called because of its potential relevance to these cerebral malformations and to cell survival or apoptosis. The CCM3/PDCD10 N-terminal α-helical region of approximately 70

residues [24,25] does indeed heterodimerize with the regulatory domains of all three GCKIIIs [23,26–30]. Furthermore, a recent study showed that essentially full-length CCM3/PDCD10 co-crystallizes with the C-terminus (residues 346–416) of MST4 [31], and even more recently the crystallographic structure of the heterodimer between CCM3/PDCD10 (residues 9–212) and the C-terminus of MST4 (residues 325–413) has been reported [30]. In this complex, MST4[(325–413)] assumes a

conformation containing three α-helices that interact with the α-helices 1–4 of CCM3/PDCD10 [30]. The interaction between the disordered regulatory domains of GCKIIIs (Figure 2) and the predominantly α-helical N-terminal domain of CCM3/PDCD10 [24,25,30] thus potentially represents an example of conformation being induced in a disordered protein by interaction with a highly ordered protein. Interestingly, the CCM3/PDCD10 interaction domains in GCKIIIs (Figure 1C) each encompass a region of decreased disorder from approximately residue 380 to the C-termini (Figure 2), and these might represent nucleation domains for structure with increased order.

ACTIVATION AND PHOSPHORYLATION OF GCKIIIs

Few stimuli and activators (or indeed inhibitors) of GCKIIIs have been identified. MST3[(431)] and MST4 have been reported to possess 'high basal activities' [12,15], although MST3 is clearly capable of being stimulated by *in vitro* autophosphorylation [12]. As its name implies, SOK1 is activated ($\sim$3-fold) by oxidative stress [10], and increased oxidative stress (in combination with Ca^{2+}) is instrumental in its activation during chemical anoxia [32]. Fidalgo et al. [33] demonstrated recently that all GCKIIIs are activated by H_2O_2, although there is not universal agreement here [12]. In some cells, serum or EGF (epidermal growth factor) may activate MST4 [16,17]. MST3b[(443)] (considered in greater detail below) and SOK1 are activated by nerve growth factors in neuronal tissues and cells, but, overall, there is little evidence that neurohumoral agonists activate GCKIIIs and even the effects of EGF on MST4 could be mediated by its ability to promote the formation of reactive oxygen species [34].

Rather than requiring a distinct upstream kinase, GCKIIIs are most likely activated by constitutive autophosphorylation of the T-loop threonine residue [6,7,10–12,14,15,35], and primary regulation of GCKIII activity therefore probably lies at the level of inhibition of the protein phosphatase(s) catalysing dephosphorylation or possibly by prevention of interactions between protein phosphatases and GCKIIIs (e.g. by spatial separation). Generally, the regulation of protein phosphatase activities is not as well understood as the regulation of protein kinase activities. The likeliest candidate phosphatase for dephosphorylation of GCKIIIs is PP2A (protein phosphatase 2A), and PP2A inhibitors, such as calyculin A and okadaic acid [both of which may also inhibit PP1 (protein phosphatase 1)], are non-physiological agonists which activate MST3[(431)] *in cellulo* [12,18]. Whether autophosphorylation occurs by a *trans* or a *cis* mechanism is unclear. From yeast 2-hybrid studies, there is evidence of the homodimerization of SOK1 and this is dependent on an intact regulatory domain [36]. Equally, limited homodimerization of MST4[(1–300)] (i.e. predominantly the 'conserved' domain of MST4 encompassing the catalytic domain; Figure 1B) has been detected in ultracentrifugation studies [20]. Although the two reports clearly differ in terms of the domains involved, homodimerization should facilitate *trans*-autophosphorylation. In addition to the T-loop threonine residue GCKIIIs are phosphorylated on further sites and, although there is little evidence that they directly or greatly affect activity, these phosphorylations may regulate additional functions of GCKIIIs, as discussed below.

MST3[(431)] is probably the best-studied of the GCKIIIs from the standpoint of their phosphorylation (Figure 3). For MST3[(431)], only threonine residues are detectably autophosphorylated [12]. The T-loop residue [MST3[(431)](Thr^{178})] was identified in *in vitro* assays using a model peptide substrate [residues 172–194 of MST3[(431)]] [35]. Mutation of MST3[(431)](Thr^{178}) to an alanine
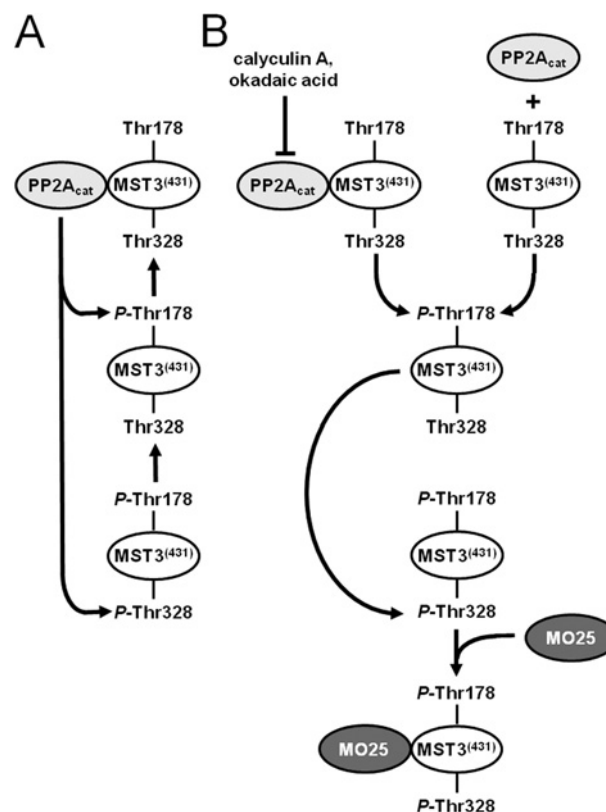

Figure 3   A scheme for the phosphorylation of MST3[(431)]

(**A**) MST3[(431)] is maintained in its dephosphorylated (inactive) state by $PP2A_{cat}$ with which it is in close proximity, possibly in STRIPAK complexes. $PP2A_{cat}$ thereby prevents any tonic basal autophosphorylation of MST3[(431)]. (**B**) When $PP2A_{cat}$ is inhibited by calyculin A or okadaic acid, or when it is spatially separated from MST3[(431)], the tonic low-level basal activity of MST3[(431)] results in autophosphorylation of Thr^{178}, thereby activating MST3[(431)]. This allows *cis*-autophosphorylation of the threonine residue immediately C-terminal to the STRADα site D sequence (Thr^{328} in MST3) by MST3[(431)](phospho-Thr^{178}) [MST3[(431)](P-Thr^{178})]. Dual-phosphorylation of MST3[(431)] increases its ability to bind MO25. However, the biological significance of the formation of the complex of MO25 with MST3[(431)](phospho-Thr^{178}/phospho-Thr^{328}) is not understood. Adapted from Fuller et al. [18].

residue prevents this phosphorylation. MST3[(431)](Thr^{182}) may also become phosphorylated during autophosphorylation though the function of this phosphorylation is less-well defined [37]. A second phosphopeptide was found to be present in tryptic digests of autophosphorylated MST3[(431)], and this provided the first evidence for one or more additional autophosphorylation sites [35]. Treatment of cells expressing an epitope- (FLAG) tagged MST3[(431)] with PP2A/PP1 inhibitors (calyculin A or okadaic acid) generates a form of activated phospho-FLAG–MST3[(431)] that migrates with a significantly reduced mobility ($\sim$58 kDa compared with $\sim$54 kDa) during SDS/PAGE [18,37]. Subsequent treatment of isolated 58 kDa FLAG–MST3[(431)] with the catalytic subunit of PP2A ($PP2A_{cat}$) results in reversion to the 54 kDa species [18]. Phosphorylation of Thr^{178} in FLAG–MST3[(431)] itself (i.e. in the absence of any additional phosphorylations) is minimally responsible for the large decrease in mobility induced by PP2A/PP1 inhibitors, but the reduction in FLAG–MST3[(431)] migration is abolished by mutation of Thr^{178} to an alanine residue. Thus the reduction in migration probably results from an additional *cis*-autophosphorylation site [*cis* because cells treated with calyculin A contain endogenous activated MST3[(431)] that should be capable of catalysing *trans*-autophosphorylation].

Table 1   A summary of the involvement of GCKIIIs in MAPK cascade signalling

ERK, extracellular-signal-regulated kinase; HEK, human embryonic kidney; JNK, c-Jun N-terminal kinase. shRNA, short hairpin RNA.

GCKIII	Cell type	Result	Reference(s)
MST3[(431)]	Unclear	Overexpression does not activate ERK1/2, JNKs or p38MAPKs, and expression of inhibitory MST3[(431)](K53R) does not inhibit their agonist-stimulated activation	[12]
MST3[(431)]	HEK-293	Overexpression of MST3[(431)] activates ERK1/2, but not JNKs or p38MAPKs	[14]
MST3b[(443)]	HEK-293	Overexpression of MST3b[(443)] does not activate MAPKs, and this may result from its phosphorylation at Thr[18] by cAMP-dependent PKA	[14]
MST3b[(443)]	DRG neurons	Depletion with shRNA reduces ERK1/2 activation	[114]
MST3	HCT116	MST3-deficient cells are resistant to H_2O_2-induced cell death because JNKs are more readily activated	[144]
MST3	Trophoblasts	H_2O_2- or hypoxia-induced expression of MST3 protein prevented by inhibition of JNKs	[41,123]
MST3	HeLa	Reduced staurosporine-stimulated JNK activation in MST3-deficient cells, implying MST3 may activate JNKs	[124]
SOK1	COS1, COS7, NIH 3T3 and others	Overexpression does not activate MAPKs	[10,11]
SOK1	HeLa or PC-3 (?)	In cells exposed to H_2O_2, overexpression in combination with CCM3/PDCD10 increases ERK1/2 activity, whereas depletion of SOK1 and CCM3/PDCD10 decreases ERK1/2 activity. No effects on JNKs or p38MAPK	[9]
MST4	HEK-293	Overexpression does not activate MAPKs and expression of inhibitory MST4(K53R) does not inhibit their agonist-stimulated activation	[15]
MST4	Phoenix (a HEK-293T line)	Overexpression of MST4, but not MST4(K53R), activates ERK1 and/or ERK2. MST4 does not activate JNKs or p38MAPK	[16]
MST4	HeLa and PC-3	Overexpression of MST4 activates ERK1/2 as measured in an Elk1-regulated luciferase reporter assay, as does 'constitutively-activated' MST4 (no details of construct provided) in combination with CCM3/PDCD10	[27]

This additional phosphorylation site has now been identified as MST3[(431)](Thr[328]) [18], and a scheme for MST3[(431)] phosphorylation is shown in Figure 3(B). Phosphorylation of MST3[(431)](Thr[328]) further requires residues 341–431 suggestive of an essential internal docking or other protein–protein interaction domain in the C-terminus. Phosphorylation of MST3[(431)](Thr[328]) is not required for MST3[(431)] activity since the activation profiles of wild-type MST3[(431)] and MST3[(431)](T328A) following treatment of cells with calyculin A are indistinguishable [18]. However, it does affect the ability of MST3[(431)] to interact with other proteins [18], as described below. Interestingly, MST3 is activated in colonic adenoma and adenocarcinoma and here it migrates significantly more slowly during SDS/PAGE than in the normal colon [38], suggesting that the sequential phosphorylation of MST3[(431)](Thr[178]) and MST3[(431)](Thr[328]) may occur during the development of these malignancies.

Whether a phosphorylation pathway similar to that proposed for MST3 (Figure 3B) is equally applicable to SOK1 and MST4 is not yet known, but, from the point of T-loop phosphorylation, the schemes in Figure 3 almost certainly apply. However, the T-loop threonine residues in SOK1 and MST4 were probably misidentified initially [10,15]. Thus, for example, Qian et al. [15] suggested that the site(s) of autophosphorylation lies in the C-terminal MST4[(310–416)] region because MST4[(2–309)] does not autophosphorylate. This is not convincing evidence because MST4[(310–416)] may be essential for autophosphorylation even though it does not contain the phosphorylated residue(s). Further work indicated that, analogously to MST3[(431)], T-loop autophosphorylations of SOK1(Thr[174]) and MST4(Thr[178]) are responsible for activation [8,36].

In addition to the autophosphorylations described, cAMP-dependent PKA (protein kinase A) phosphorylates Thr[18] in MST3b[(443)] at a PKA-consensus phosphorylation sequence [14]. This phosphorylation may affect its signalling properties (Table 1). In protein array experiments, the Parkinson's disease-associated kinase LRRK2 (leucine-rich repeat kinase 2) reportedly phosphorylates SOK1 and MST3, although the

increase in phosphorylation over background is relatively small (50%) and no details of the residues that are phosphorylated are given [39]. Numerous other phosphorylations of GCKIIIs have been identified in global proteomics screens (Figure 1B), although these have not always been biochemically validated and the protein kinases potentially responsible have only been identified by bioinformatics approaches (Figure 1B, see the legend for details). Clearly, whereas there is evidence for additional phosphorylation sites in GCKIIIs, further work is required to identify the sites and to clarify whether they have any physiological significance.

FUNCTIONAL ROLES OF GCKIIIs

GCKIIIs are associated with and implicated in a diverse array of cellular responses. In addition to autophosphorylation, GCKIIIs are capable of phosphorylating a number of extrinsic proteins. As pointed out by Delpire [2], GCKs generally are not 'basophilic' protein kinases (i.e. kinases that prefer an arginine residue at the −2 or −5 position in the substrate relative to the phosphorylated serine/threonine residue) because they lack one of two required acidic residues in their catalytic domains. This has been demonstrated experimentally for MST4 [40]. Although the catalytic domains of GCKIIIs are very similar, there are probably differences in substrate specificity, and substrate preferences are presumably dictated by the regulatory domains. By analogy with yeasts, early work (reviewed in [1]) suggested that mSte20-like kinases may act as upstream regulators of MAPK cascades. However, the situation is far from resolved. As summarized in Table 1, there are reports that GCKIIIs activate [possibly by acting as MAP4Ks (MAPK kinase kinase kinases)] or do not activate MAPKs, and it is difficult to come to any consistent conclusions. MAPKs have even been placed upstream of MST3 [41]. GCKIIIs are also associated with the stimulation of apoptosis (as described below), although these effects may be secondary to their influence on other fundamental processes, such as cell

migration and proliferation. More recently, it appears to us that an emerging common denominator in GCKIII-regulated responses is the close association of GCKIIIs with different microtubular networks facilitated by a variety of protein–protein interactions. Through these interactions, GCKIIIs (often in conjunction with CCM3/PDCD10) participate in cell migration and proliferation and perversely in cell protection or apoptosis (see, for example, [10,27,29,30,33,41]).

Association of GCKIIIs with the Golgi and centrosome complex

The integrity of the Golgi apparatus relies on a microtubular network. In many cells, the Golgi apparatus is closely associated with the centrosome [42,43], a major MTOC (microtubule-organizing centre) present in many (but not all) cells from which microtubules emanate and explore the cytoplasm, and both the Golgi and the centrosome are associated with the nucleus in quiescent cells [44]. During cell migration, the perinuclear Golgi realigns from a random orientation to face the direction of migration [44–46]. Several studies place GCKIIIs nearby the Golgi apparatus where they interact directly (but possibly in association with CCM3/PDCD10) with the Golgi-associated protein GOLGA2 (golgin A2; Figure 4A), which is also known as GM130 [29,36,47]. Additionally, through interaction with CCM3/PDCD10, GCKIIIs interact indirectly with striatins (mediated by linking through CCM3/PDCD10) and are 'core' components of the STRIPAK (striatin-interacting phosphatase and kinase) complexes (Figures 4B and 4C) [47]. We elaborate on these points below.

GOLGA2

As mentioned above, SOK1 and MST4 associate with the Golgi apparatus via GOLGA2 [36]. GOLGA2 is a *cis* Golgi protein (i.e. located on the entry face for vesicular material destined for exocytosis) that facilitates the fusion of vesicles with the Golgi [48]. The central domain of GOLGA2 forms a putative coiled-coil structure (indicative of a rod-like conformation) and non-coil regions at the N- and C-termini anchor GOLGA2 to the Golgi via GRASP65 (Golgi reassembly stacking protein 1, 65 kDa) [48]. The coiled-coil structure of GOLGA2 interacts with residues 270–302 of SOK1 (encompassing the conserved N-terminus of the regulatory domain of SOK1) and increases SOK1 activity [36]. However, it is not clear whether increased SOK1 activity is required to enhance this association [36,49]. MST4 also interacts with GOLGA2 [36]. Initially, MST3 was thought not to associate with GOLGA2 [36] which is surprising given the high degree of conservation of the SOK1[(270–302)] region in the other GCKIIIs (Figure 1B), but subsequent studies demonstrated that (overexpressed) MST3[(431)] does interact with it, an interaction that is decreased when MST3[(431)] is activated [18].

Depletion of SOK1 or MST4 results in dispersal of the Golgi apparatus, indicating that these GCKIIIs are required for Golgi integrity [36]. Because the integrity of the Golgi relies on an intact microtubular network, the effect of loss of GCKIIIs on Golgi dispersion could result from an indirect effect on the microtubular network. The effect of SOK1 on Golgi integrity may be mediated by 14-3-3ζ which localizes to the Golgi and which SOK1 (but not MST4 nor MST3) phosphorylates at Ser[58] [36,50]. Phosphorylation of 14-3-3ζ may trigger cell migration and associated events (e.g. changes in Golgi polarity and cell adhesion) [36] and may prevent Golgi dispersion [29]. However, confusingly, phosphorylation of 14-3-3ζ(Ser[58])

under conditions of oxidative stress leads to activation of the pro-apoptotic protein kinase, ASK1 [apoptosis signal-regulating kinase 1; also known as MAP3K5 (MAPK kinase kinase 5)] [50].

It is therefore difficult to reconcile the events of the maintenance of Golgi integrity and cell migration with the stimulation of apoptosis. Zhou et al. [50] have proposed that oxidative stress releases SOK1 from the Golgi, thereby stimulating apoptosis. Conversely, Fidalgo et al. [33] have proposed that the oxidative stress-driven release of MST4 from the Golgi is cytoprotective. These two disparate schemes are difficult to rationalize unless the biological roles of SOK1 and MST4 are very different. Even then, if there were simultaneous release of both kinases, the cytotoxicity of SOK1 would counteract the cytoprotection afforded by MST4. Possibly coincidentally, Scansite (http://scansite.mit.edu; [50b]) suggests that phosphorylation of MST3b[(443)](Thr[18]) by PKA [14] creates a potential 14-3-3ζ/δ-binding site. This may indicate an additional connection between GCKIIIs and 14-3-3 proteins, although there is no experimental evidence that 14-3-3ζ/δ binding to phospho-MST3b[(443)](Thr[18]) occurs.

STRIPAK complexes

GCKIIIs associate with striatin scaffolding proteins and are components of multiprotein assemblies known as 'striatin modules' or STRIPAK complexes (Figures 4B and 4C) [47,51–54]. Interestingly, GCKIVs {MINK1 (misshapen-like kinase 1), MAP4K4 and TNIK [TRAF2 (tumour necrosis factor receptor-associated factor 2)- and NCK-interacting kinase] (see below)} have also been identified as potential STRIPAK complex components [55]. Many proteins present in STRIPAK complexes are evolutionarily conserved, with orthologues expressed in yeasts [56] suggesting that they are of universal importance. Striatins and other STRIPAK complex components become phosphorylated following exposure of cells to okadaic acid [57], suggesting that STRIPAK complex-associated protein kinases (possibly GCKs) regulate STRIPAK complex structure and function [37]. 'Core' STRIPAK complex components, in addition to GCKIIIs and striatins, include CCM3/PDCD10, MOB (Mps one binder) 4/phocein and PP2A (Figures 4B and 4C). However, other proteins are also associated with the complexes, though possibly more loosely and possibly in a mutually exclusive manner.

STRIPAK complex components: striatins. The first of the three striatins to be identified was SG2NA [S/G₂ nuclear autoantigen or STRN3 (striatin, calmodulin-binding protein 3) [58] of which there are two well-characterized isoforms (the 713 residue α isoform and the 797 residue β isoform). Despite its name, SG2NA is a membrane-bound cytoplasmic protein [59]. STRN itself (named thus because it is highly expressed in brain striatum) was identified as a 780-residue protein concentrated in dendritic spines [59,60], and the third striatin identified was zinedin or STRN4 [61]. Striatins possess a caveolin-binding domain (residues 53–63 in STRN), a coiled-coil domain (residues 70–166 in STRN) which is important in the homo-oligomerization of striatins and a calmodulin-binding domain (residues 149–166 in STRN), followed by a C-terminal region of putatively eight WD40 repeats [59,60]. STRN and zinedin are most highly expressed in nervous tissue (with detectable expression in lung and spleen), whereas SG2NA is more widely expressed [61]. Assuming tissue expression is not a restricting factor, the oligomerization of striatins potentially leads to the formation of large STRIPAK complexes composed of multiple different striatins and GCKIIIs.
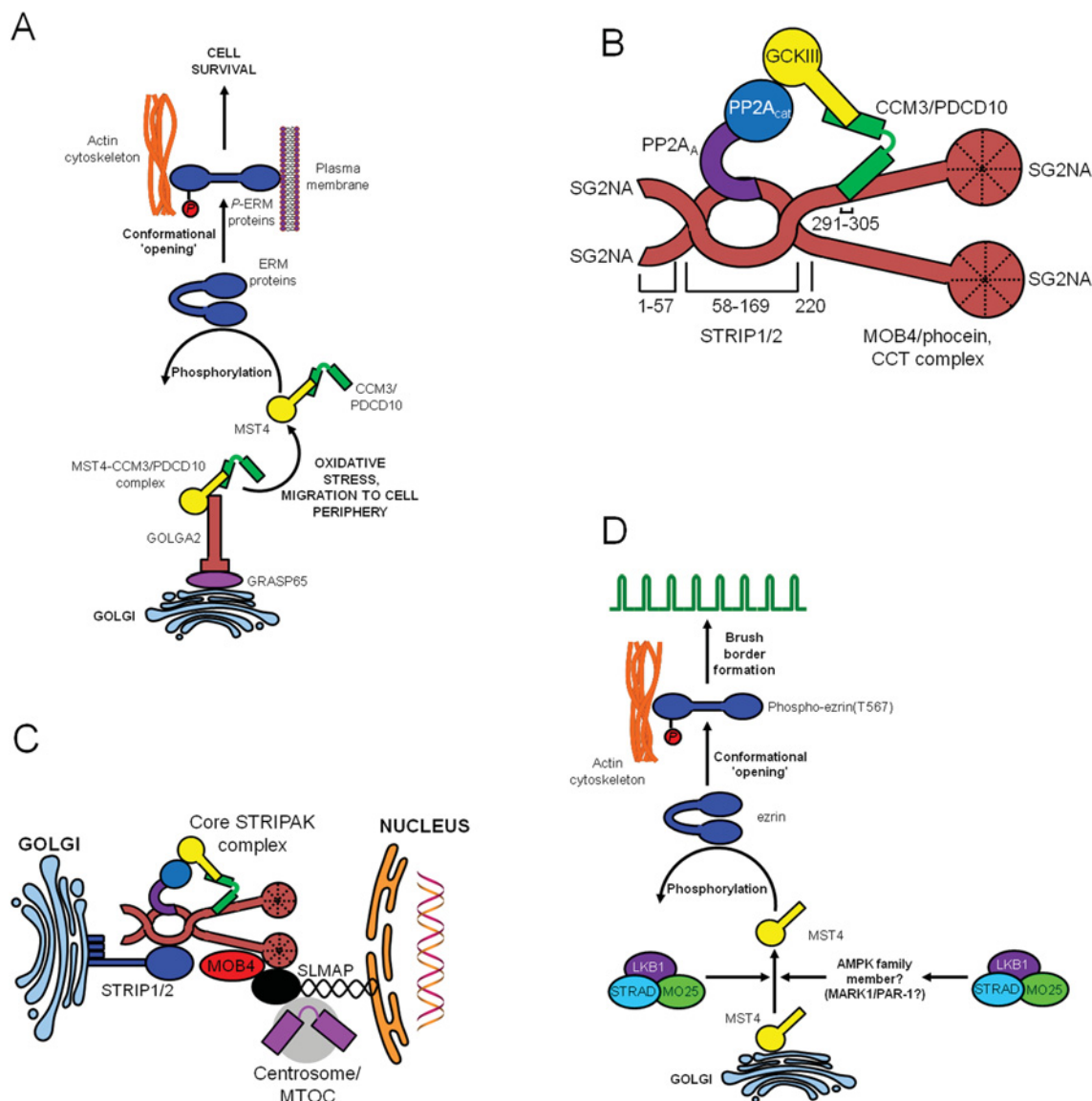
Figure 4   Heterotropic interactions of GCKIIIs

(**A**) Phosphorylation of ERM proteins by MST4 and cytoprotection. Oxidative stress dissociates the MST4–CCM3/PDCD10 dimer which migrates to the cell periphery. During this process, MST4 becomes activated and phosphorylates ERM proteins on a threonine residue in their C-terminal region. This promotes an 'open' conformation that allows them to bracket the plasma membrane to the F-actin cytoskeleton, thereby promoting cytoprotection. Adapted from Fidalgo et al. [33]. (**B**) The 'core' STRIPAK complex. 'Core' STRIPAK complex components are taken to be the striatins (probably as oligomers), PP2A$_A$, PP2A$_{cat}$, CCM3/PDCD10 and the GCKIIIs. SG2NAα is the striatin depicted. Residues 69–79 represent the caveolin-binding domain, residues 86–183 represent the coiled-coil domain and residues 167–183 are the calmodulin-interacting domain. The eight putative WD40 repeats (depicted as a 'spoked wheel') lie in the C-terminal region of the protein. In the 'core' STRIPAK complex, only PP2A$_A$ and the FAT domain of CCM3/PDCD10 interact directly with the striatins which homo-oligomerize via their coiled-coil domains. The interaction of SG2NA with PP2A$_A$ requires the caveolin-binding domain and some of the coiled-coil domain of SG2NA, whereas CCM3/PDCD10 interacts with a more C-terminal region. MOB4/phocein also interacts (whether directly or indirectly) with the more C-terminal regions of the striatins, and the WD40 repeats are at least partially responsible for this. However, residues 58–328 may also be involved in the interaction to some extent. Adapted from Kean et al. [47]. Although SG2NA is shown, essentially the same findings apply to STRN [37] and presumably zinedin. (**C**) Interaction of the 'core' STRIPAK complex with the Golgi apparatus, the MTOC or centrosome, and the nuclear membrane. STRIP1/2 bind to the coiled-coil regions of striatins and couple the 'core' STRIPAK complex to the Golgi, whereas the SLMAP interact with the more C-terminal regions of striatins and couple the 'core' STRIPAK complex to the nuclear membrane. These interactions with subcellular organelles may vary with the stages of the cell cycle. Adapted from Frost et al. [56]. (**D**) Involvement of the LKB1–STRAD–MO25 heterotrimer in ezrin phosphorylation and epithelial brush border formation. In some way not understood [but possibly involving an AMPK family member (MARK1/PAR-1c)], the LKB1–STRAD–MO25 heterotrimer stimulates release of MST4 from the Golgi and hence stimulates ezrin phosphorylation. The interaction of the plasma membrane with the actin cytoskeleton is thereby stabilized, and this results in epithelial cell polarization and brush border formation. Adapted from ten Klooster et al. [90]. An animated version of this Figure can be found at http://www.biochemj.org/bj/454/0013/bj4540013add.htm.

STRIPAK complex components: CCM3/PDCD10. There are three CCM proteins (CCM1, CCM2 and CCM3/PDCD10), so-called because mutations in any one of the three can result in cerebral cavernous malformations, relatively-common diseases (prevalence as high as 0.5%) that are characterized by collections of thin-walled capillaries prone to

leakage and rupture [22,28,62–67]. CCM2 {malcavernin or OSM [osmosensing scaffold for MAP3K3 (MAPK kinase kinase 3)]} is a scaffold protein that interacts with CCM1 [or KRIT1 (K-Rev interaction trapped 1)] and CCM3/PDCD10 [28,68]. In addition to its interaction with CCM2, CCM3/PDCD10 is a core member of STRIPAK complexes. CCM3/PDCD10 is a highly ordered

212-residue protein which is predominantly α-helical [24,25] and which contains an N-terminal homodimerization domain that (as mentioned above) also heterodimerizes with GCKIIIs [23,26–29]. This N-terminal domain is coupled by a linker to the α-helices in the C-terminal FAT (focal adhesion targeting) domain which links CCM3/PDCD10 to focal adhesions by binding to paxillin (see below) [69]. In addition, the FAT domain facilitates the interaction of CCM3/PDCD10 with CCM2 [24] and is required for interaction with striatins (see below) [47], suggesting that CCM3/PDCD10 could recruit GCKIIIs to a variety of protein complexes (STRIPAK complexes, complexes containing CCM2, paxillin etc.) by acting as a 'bridge' or 'bracket'.

STRIPAK complex components: MOB4/phocein. MOB4/phocein [other aliases include MOB3, MOBKL3 (MOB kinase activator-like 3) and PREI3 (pre-implantation protein 3)] was identified as a widely expressed striatin-interacting protein with similarity to MOB1-like proteins [57,70]. In neurons, like striatins, MOB4/phocein is expressed only in the somato-dendritic region. In HeLa cells, MOB4/phocein and SG2NA co-localize to the Golgi region, and brefeldin A (an inhibitor of Arf small G-proteins and of protein transport from the endoplasmic reticulum to the Golgi apparatus) disrupts this co-localization with dispersal of SG2NA and MOB4/phocein throughout the cell [70].

STRIPAK complex components: PP2A. PP2A is a heterotrimer consisting of one of two different 'A' scaffold proteins (PP2A$_A$), one of two catalytic PP2A$_{cat}$ subunits and one of a number of regulatory B subunits which supposedly determine substrate specificity [71–73]. There are three well-established 'B' subunit families (B, B' and B''), and striatins probably represent a fourth (B''') family [51]. Whether striatins (particularly SG2NA) always associate with PP2A$_A$ and PP2A$_{cat}$ and whether they act as true regulatory PP2A$_B$ subunits are controversial topics. Alternatively, STRNs may themselves be regulated by their interaction with PP2A. One role for PP2A in STRIPAK complexes is to restrain the autophosphorylation and catalytic activity of GCKIIIs, and a reduction in the association of PP2A with striatins leads to activation of GCKIIIs [37].

STRIPAK complex components: additional proteins. Proteomics studies have identified additional STRIPAK complex components, including STRIPs (striatin-interacting proteins) 1 and 2 [also known as FAM40A (family with sequence similarity 40 A) and FAM40B respectively] and SLMAP (sarcolemmal membrane-associated protein) [52–54]. Other more loosely associated STRIPAK complex components (which may participate in STRIPAK complexes in a mutually exclusive manner) include CTTNBP2 (cortactin-binding protein 2), CTTNBP2NL (CTTNBP2 N-terminal-like), TRAF3IP3 (tumour necrosis factor receptor-associated factor 3-interacting protein 3) a c-Jun N-terminal kinase activating modulator, a complex of SIKE (suppressor of IκB kinase ε) and FGFR1OP2 (fibroblast growth factor receptor 1 oncogene partner 2), and the CCT [chaperonin-containing TCP (T-complex protein)] complex [also known as the TRiC (TCP1-containing ring complex)] [47,52–54]. The roles of these proteins in STRIPAK complexes and their influence on the functions of the complexes are not well understood.

STRIPAK complex structure

Detailed analyses of deletion mutants of STRN or SG2NA indicate that, in terms of STRIPAK complex assembly (Figure 4B), the N-terminal residues (1–45 in STRN and 1–

57 in SG2NA) are dispensable [37,47,54]. The caveolin-binding domain and at least some of the coiled-coil domain towards the N-terminus are required for the oligomerization of striatins and for interaction with PP2A$_A$, PP2A$_{cat}$ and STRIP1/2. However, only PP2A$_A$ interacts directly with striatins. MOB4/phocein, GCKIIIs, CCM3/PDCD10 and the CCT complex interact mainly with more C-terminal regions of the striatins. Of these, only CCM3/PDCD10, through its C-terminal FAT domain, appears to interact directly with striatins [47]. CCM3/PDCD10 thus acts as a bridge between striatins and GCKIIIs which, as mentioned above, interact through their regulatory domains with the N-terminal region of CCM3/PDCD10 [23].

Interactions between STRIPAK complexes and the Golgi?

CCM3/PDCD10 represents a third component of the GCKIII–GOLGA2 complex, with SOK1 acting as a bridge between CCM3/PDCD10 and GOLGA2 [29]. Depletion of CCM3/PDCD10 causes Golgi disassembly, impaired cell migration and degradation of GCKIIIs through ubiquitinylation and proteasomal degradation [29]. The effects on the Golgi are reminiscent of the effects of depletion of SOK1 or MST4 [29] and raise the question of the relationship between STRIPAK complexes and the GOLGA2–GCKIII–CCM3/PDCD10 complex.

MST4 clearly associates with GOLGA2 [36]. Although proteomics studies identify tubulins and other Golgi proteins in the immunoprecipitates of overexpressed GOLGA2, such studies fail to detect any STRIPAK complex-associated proteins other than MST4 [47]. These data suggest that the GCKIII-containing STRIPAK complexes and GCKIII-containing GOLGA2 complexes are separate. However, STRIPAK complexes localize to the Golgi via STRIPs and may link the Golgi with the centrosomes and nuclear membrane [56]. Depletion of CCM3/PDCD10 reduces the interaction of MST4 with striatins, but does not decrease the interaction between MST4 and GOLGA2 in the Golgi [47]. These data are consistent with MST4 forming a bridge from GOLGA2 to CCM3/PDCD10 and CCM3/PDCD10 forming a bridge from striatins to MST4 [47]. Thus MST4, in association with CCM3/PDCD10, may shuttle between the protein complexes. MST4 is not concentrated solely nearby the Golgi and is also present in a punctate non-Golgi cytoplasmic localization [47], but it is not clear if the cytoplasmic MST4 is STRIPAK complex-based. Nevertheless, these subcellular localization studies further suggest that MST4 operates in different compartments. In apparent contradiction with the effects of CCM3/PDCD10 depletion, striatin depletion causes a loss of MST4 from the Golgi apparatus and dispersal into the cytoplasm [47]. However, this could be explained by MST4 and/or CCM3/PDCD10 associating with additional protein complexes other than STRIPAK complexes or GOLGA2.

Overall, the data indicate that MST4 (and potentially other GCKIIIs) operate within multiple protein complexes. In association with STRIPAK complexes, GCKIII activities are restrained because of the proximity to STRIPAK complex-associated PP2A (Figure 4B) [37]. Though not definitively established, it is probable that recruitment of GCKIIIs to other protein complexes, such as with GOLGA2, results in their activation by autophosphorylation given that they should no longer be associated with PP2A (Figure 4A). The question then arises as to what are the consequences of GCKIII activation? Interaction of GCKIIIs with the Golgi apparatus may be important in the regulation of cell migration when the normally randomly oriented perinuclear Golgi apparatus reorganizes to face the

direction of cell migration [45]. Depletion of CCM3/PDCD10 reduces the repositioning of Golgi in cell-migration assays, whereas depletion of striatins enhances it [47]. Assuming that the localization of MST4 and the ability of the Golgi to reposition are interconnected, the interpretation is that binding of striatins, MST4 and presumably STRIPAK complexes to the Golgi reduces repositioning.

GCKIIIs AT THE PLASMA MEMBRANE

At the plasma membrane GCKIIIs are associated with interactions with the extracellular matrix, being recruited to focal adhesions and integrin complexes via paxillin. They are also associated with the ERM (ezrin, radixin, moesin) proteins that organize the cortical actin cytoskeleton intracellularly. Given that co-ordinated management of both structures is necessary for cell migration and polarity, it is perhaps not surprising that GCKIIIs are associated with such events.

GCKIIIs and paxillin

The scaffolding protein paxillin is a component of focal adhesions and is involved in the regulation of cell motility when, as part of the focal adhesion complex, it becomes concentrated at the leading edges of cells [74,75]. The C-terminus of paxillin possesses four LIM (an acronym of the three gene products Lin-11, Isl-1 and Mec-3) domains that are required for focal adhesion targeting. The N-terminus contains the majority of the signalling elements of paxillin with five LD domains (leucine–aspartate repeat domains) to which a number of different proteins bind. There are also various serine, threonine and tyrosine residue phosphorylation sites that modulate binding of proteins to paxillin [74]. Tyrosine residue phosphorylation of paxillin is modulated by the protein tyrosine phosphatase PTPN12 (protein tyrosine phosphatase, non-receptor type 12 or PTP-PEST). Well-established binding partners for paxillin include the NCK–PAK–PIX (PAK-interacting exchange factor)–GIT (G-protein-coupled receptor kinase interacting ArfGAP) complex [76], which is intimately involved with the activity of Rac1, a Rho family small G-protein which modulates the actin cytoskeleton.

CCM3/PDCD10 has been identified as a binding partner for paxillin, interacting via its FAT domain with three of the paxillin LD domains [69]. The interactions between CCM3/PDCD10 and GCKIIIs [23,26,28,29], and between CCM3/PDCD10 and paxillin [69], suggests that GCKIIIs could be recruited directly to focal adhesions, although (to our knowledge) this has not been demonstrated experimentally. Overexpression of MST3[431] inhibits cell migration and invasion and is associated with an increase in the phosphorylation at Tyr[31] and Tyr[118] of paxillin [35]. In contrast, depletion of MST3 or overexpression of dominant-negative MST3[431](T178A) enhances cell migration and reduces tyrosine residue phosphorylation of paxillin. The effect of MST3[431] on paxillin is probably indirect and mediated by PTPN12 which is inhibited by phosphorylation of Ser[39] [77]. Thus, Lu et al. [35] proposed a scheme where MST3[431] inhibits cell mobility by inhibiting PTPN12, thereby increasing the tyrosine residue phosphorylation status of paxillin. SOK1 and MST4 also influence cell migration/invasion, although their effects have been attributed to the phosphorylation of 14-3-3ζ [36] and/or increased tyrosine residue phosphorylation of FAK (focal adhesion kinase) [78].

MST4 and the ERM axis

ERM proteins couple plasma membranes and their component proteins (e.g. transmembrane receptors) to the cortical F-actin (filamentous actin) cytoskeleton and are therefore important in regulating cell morphology during events such as migration, mitosis and differentiation [79,80]. In the open conformation, ERM proteins act as 'brackets' binding to phosphatidylinositol (4,5)-bisphosphate in the plasma membrane via their N-terminal FERM domains and to F-actin via their C-terminal binding sites. The open conformation is adopted on phosphorylation of a threonine residue in the C-terminal region (Thr[567] in ezrin, Thr[564] in radixin and Thr[558] in moesin), and various protein kinases, including MST4, phosphorylate these sites [33].

CCM3 is also named PDCD10 because of its involvement in apoptosis, though it appears that it can be either cytoprotective or cytotoxic. In the context of oxidative stress, CCM3/PDCD10 is up-regulated by H_2O_2 and protects against apoptosis [33]. This protection is mediated via the phosphorylation of ERM proteins by MST4 (in this particular instance, alone amongst GCKIIIs), and requires CCM3/PDCD10 [33]. Interestingly, oxidative stress promotes the redistribution of MST4 from the Golgi to the periphery where it co-localizes with phosphorylated ERM proteins. This relocalization also requires CCM3/PDCD10. These data lead to the conclusion that CCM3/PDCD10 enables MST4 to traffic from the Golgi to the plasma membrane where it can phosphorylate ERM proteins, resulting presumably in more stable interactions between the plasma membrane and the actin cytoskeleton (Figures 4A and 4D). MST3[431] may also phosphorylate ERM proteins since depletion of MST3 reduces ERM protein phosphorylation following oxidative stress [33]. However, Fidalgo et al. [33] suggest that the pro-apoptotic effects of the conserved domain of cleaved MST3[431] [i.e. MST3[431](1–313)] [7] outweigh any cytoprotection that might be afforded by MST3[431]-mediated ERM protein phosphorylation.

ERM proteins play a particularly important role in maintaining epithelial or endothelial cell junctions [79,80]. Loss of endothelial junctional integrity or lack of junction formation could account for some of the features associated with cerebral cavernous malformations (i.e. the collections of thin-walled capillaries prone to leakage and rupture). Consistent with a role for GCKIIIs and CCM3/PDCD10 in regulating ERM proteins, depletion of SOK1 and/or CCM3/PDCD10 in human microvascular endothelial cell monolayers reduces the phosphorylation of moesin [62]. The electrical resistance across the monolayers is decreased, indicative of junction dysfunction. Conversely, MST3 or MST4 increase the phosphorylation of moesin in endothelial cells and enhance junction integrity. Furthermore, in a three-dimensional system using collagen matrices populated with human umbilical vein endothelial cells, depletion of CCM3/PDCD10 also prevents lumen formation consistent with it being required for the integrity of intercellular junctions [81]. Although depletion of individual GCKIIIs does not affect lumen formation, combinatorial knockdown of SOK1 and MST3 or SOK1 and MST4, but not of MST3 and MST4, reduces lumen formation. Thus GCKIIIs are likely to play an important role in mammalian vascularization by acting at the adherens junctions.

Further information on the *in vivo* roles of GCKIIIs can be gleaned from simpler models such as zebrafish. This system was used to establish that SOK1 phosphorylates the N-terminus of zebrafish or human CCM3/PDCD10 on Ser[39] and Thr[43] [22,28]. An in-frame 18-residue deletion encompassing this region impairs the interaction of CCM3/PDCD10 with GCKIIIs, but predictably not with CCM1 or CCM2 [22,62], and this deletion can result in cerebral cavernous malformations in humans [82]. In

zebrafish, the equivalent deletion causes cardiac malformations characterized by a dilated thin-walled heart, suggesting that the interaction of CCM3/PDCD10 with GCKIIIs is important in cardiac development [22,62]. Depletion of zebrafish GCKIII orthologues produces cardiac malformations, although there is some disagreement about their precise nature possibly because of technical considerations [62,63]. Similarly, as expected, depletion of CCM3/PDCD10 causes malformations in the zebrafish cranial vasculature and these can be rescued by expression of a zebrafish SOK1 orthologue [63]. Overall, the conclusion from these data is that GCKIIIs act downstream from CCM3/PDCD10 to regulate vascular development in zebrafish (and probably other organisms).

GCKIIIs AND ASSOCIATION WITH LKB1–STRAD–MO25

MO25 is an 'armadillo-repeat' protein (compare with β-catenin) that has been highly conserved through evolution [83,84]. There are two mammalian isoforms, MO25α and MO25β. Confusingly, their gene names are *CAB39* (Ca^{2+}-binding protein 39, MO25α) and *CAB39L* (CAB39-like, MO25β), the CAB39 nomenclature originating from the putative Ca^{2+}-binding domains identified in the primary sequences [83]. MO25 forms a heterotrimer with LKB1 and one of the two STRAD pseudokinases to form a protein complex which regulates the activity of AMPK (AMP-activated protein kinase; the so-called 'cellular fuel gauge') and other protein kinases of the AMPK group [85–87]. The crystallographic structures of the LKB1–MO25–STRAD heterotrimer and the MO25–STRAD dimer indicate that surface-exposed STRADα residues form contacts with MO25 (Figure 5) [88,89]. In STRADα, the residues that have been examined in depth are Glu^{105} (site A), Ile^{138} (site B), Phe^{185} (site C), and Trp^{429} and Phe^{431} in the C-terminal tripeptide Trp–Glu–Phe (site D) which generically may be Trp–Xaa–(Phe/Trp) [WX(F/W)] (Figure 5). Sites A–C form contacts with the 'concave' face of MO25 and site D forms a contact with its C-terminal 'convex' face. Mutation of the residues of sites A–D reduces the ability of STRADα to bind to MO25 [89].

A yeast 2-hybrid screen with MO25 as 'bait' identified MST4 as a major interactant and MST3 as a minor interactant [90]. STRADα sites A–D are conserved in GCKIIIs (Figure 5) (and indeed in GCKVIs), though the STRADα site B Ile^{138} is conservatively replaced by a leucine residue in GCKIIIs and valine in GCKVIs [91]. Sites A–C lie in the catalytic domains of the GCKIIIs and site D lies in their regulatory domains. MO25 binds to and activates GCKIIIs and GCKVIs, and individual mutation of the residues in sites A–D prevents activation indicating that all sites are required [91]. As mentioned above, MST3$^{(431)}$ is phosphorylated on Thr^{328} [18]. This residue lies immediately C-terminal to the STRADα site D-like WX(F/W) sequence [WIF in MST3$^{(431)}$] (Figures 1A and 1B) [89]. Phosphorylation of MST3$^{(431)}$(Thr^{328}) does not affect its kinase activity directly, but it enhances the interaction of MST3$^{(431)}$ with MO25α/β [18]. Mutation of Thr^{328} to an alanine residue in MST3$^{(431)}$ prevents the interaction with MO25 [18]. Recent evidence suggests that the interaction of MO25 with the (slightly C-terminally extended) catalytic domain of MST3$^{(431)}$ [MST3$^{(431)}$(19–289), in which the T-loop Thr^{178} is presumably unphosphorylated] stabilizes the catalytic domain in a closed active conformation even in the absence of ATP [92]. The functional consequences of the interaction remain unclear because binding of MO25 to MST3$^{(431)}$ is enhanced by *cis*-autophosphorylation of Thr^{328} in MST3$^{(431)}$ following phosphorylation of Thr^{178}, and phosphorylation of Thr^{178} alone
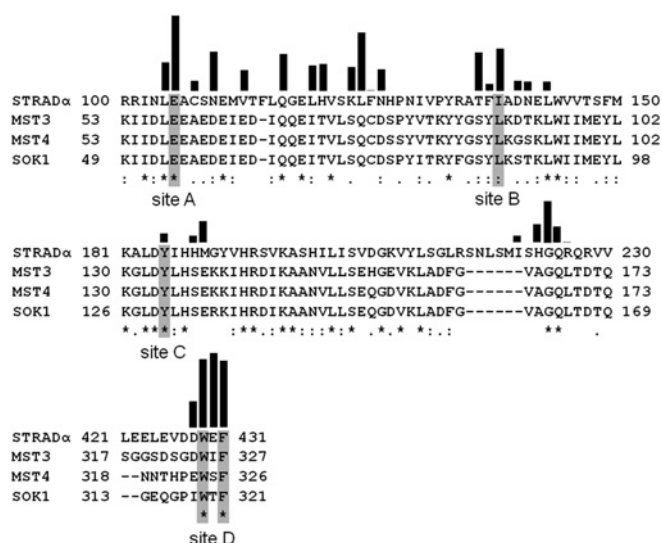
Figure 5   Conservation of STRADα and GCKIII sequences

Human STRADα (GenPept accession NP_001003787) was aligned with human MST3$^{(431)}$ (GenPept accession NP_001027467), MST4 (GenPept accession NP_057626) and SOK1 (GenPept accession NP_001258906) using Clustal W2. The heights of the bars indicate the contact areas between STRADα and MO25 normalized for the molecular masses of the participating amino acids [89]. Four conserved sites of interaction of STRADα with GCKIIIs [Glu^{105}, Ile^{138}, Tyr^{185} and Trp^{429}–(Glu^{430})–Phe^{431}, sites A–D respectively] are denoted by grey shading [91]. Three of the GCKIII sites are identical to the STRADα sites and one (Ile^{138}) is conservatively replaced by a leucine residue in the GCKIIIs. These residues also participate in the interaction of STRADα with MO25 [89]. Other sites in STRADα involved in its interaction with MO25 (Leu^{104}, Gln^{116}, Leu^{143}, Gly^{224} and Gln^{225}) are identical or are conservatively substituted (Asn^{109}, Leu^{119}, Asn^{126} and Thr^{136}).

(i.e. in the absence of phosphorylation of Thr^{328} or MO25 binding) is apparently sufficient to stimulate the kinase activity MST3$^{(431)}$ fully [18]. Notably, two GCKIIIs have a threonine residue immediately C-terminal to the conserved STRADα site D-like WX(F/W) sequence, the extended conserved sequences being MST3$^{(431)}$(WIFT^{328}IR) and MST4(WSFT^{327}T^{328}VR), whereas SOK1 possesses a threonine at the $+3$ position relative to the WX(F/W) sequence (WTFPPT^{324}IR). It will therefore be important to examine the binding of MO25 to inactive or activated SOK1 and MST4, and roles of the relevant threonine residues [SOK1(Thr^{324}) and MST4(Thr$^{327/328}$)] in MO25 binding. In addition, a serine residue lies immediately C-terminal to the WX(F/W) site D sequence in the two GCKVI members, OXSR1 (oxidative-stress responsive 1) and SPAK (Ste20/Sps1-related proline-alanine-rich protein kinase), raising the possibility of phosphorylation-dependent interaction with MO25 [18], and participation of MO25 in signalling through GCKVIs has been documented recently [93].

The LKB1–STRAD–MO25 complex is an important regulator of epithelial and neuronal cell polarity [94–96]. This may involve activation of a member of the AMPK group, MARK1 [MAP (microtubule-associated protein)/microtubule affinity-regulating kinase 1 (also known as PAR-1c)], a protein kinase that is specifically associated with microtubule dynamics and cell polarity and which phosphorylates MAPs [96,97]. Activation of LKB1 by increased STRAD expression in intestinal epithelial cells results in cytoskeletal reorganization and leads to the polarization of the cells into apical and basolateral membranes, thereby promoting brush border formation [94,95]. This polarization requires MO25 [90]. MST4 may play a role in regulating cell polarity since there is simultaneous migration of MST4 from the Golgi to the plasma membrane where it

phosphorylates the regulatory Thr[567] in ezrin, increasing the interactions of ezrin with the actin cytoskeleton. Furthermore, brush border formation is prevented by dominant-negative MST4(T178A) [90]. ten Klooster et al. [90] propose a scheme whereby MST4 lies downstream from the LKB1–STRAD–MO25 complex, though whether an AMPK family kinase lies between LKB1–STRAD–MO25 and MST4 is unclear (Figure 4D). A key question relates to the mechanism(s) associated with MST4 activation (by phosphorylation of Thr[178]) since this is presumably a prerequisite for the phosphorylation of ezrin (assuming that ezrin is phosphorylated by MST4 itself). Here, dissociation of MST4 from the Golgi and relocation to the plasma membrane may, in itself, be sufficient to activate MST4. As indicated above, removal of MST4 from the vicinity of PP2A (in STRIPAK complexes?) presumably permits the activation of GCKIIIs by autophosphorylation. A second question relates to the mechanism(s) associated with MST4 migration from the Golgi to the periphery. Recent studies indicate that apical membrane organization involves LKB1–STRAD–MO25-mediated redistribution of phosphatidylinositol (4,5)-bisphosphate to the membrane that will form the apical surface where it is hydrolysed by phospholipase D1 [98]. This results in a local increase in phosphatidic acid that triggers a signalling cascade involving the GEF (guanine-nucleotide-exchange factor) PDZ-GEF (PDZ domain-containing GEF), activation of the Ras family small G-protein Rap2A and its effector, the GCKIV TNIK [99]. Of possible relevance is that, as mentioned, TNIK may also be a component of STRIPAK complexes [55]. TNIK activation favours the redistribution of MST4 from the Golgi to the surface destined to become the apical membrane. A third question relates to the involvement of MO25 in the process given that STRAD and MST4 each interact with MO25 in a similar manner, suggesting that their associations with MO25 are mutually exclusive [91] (although MO25 could presumably exist simultaneously in two or more separate complexes). However, a scenario in which active MST4 sequesters MO25 away from LKB1–STRAD to decrease signalling through LKB1 could be proposed. Equally, the formation of a MST4–MO25 complex may increase MST4 signalling, perhaps by transferring it to a different location in the cell.

The LKB1–STRAD–MO25 heterotrimer and PAR-1c/MARK also participate in the neuronal polarization into axons and somatodendrites [96], a process that involves SOK1 and GOLGA2 [49]. The data indicate that LKB1 signals via SOK1 to initiate Golgi condensation and axonal specification. Thus depletion of either LKB1 or SOK1 inhibits axonal specification and this is rescued by overexpression of SOK1. However, in contrast with the experiments on the polarity of epithelial cells [90], either wild-type SOK1 or inactive SOK1(K49R) rescue the phenotype, suggesting that SOK1 activity itself is dispensable. This might indicate that SOK1 functions as a scaffold linking LKB1–MO25 signalling to GOLGA2. Conversely, overexpression of SOK1 results in initiation of a multiple axon phenotype. Depletion of GOLGA2 decreases axon initiation, but this is not rescued by overexpression of SOK1, suggesting that GOLGA2 lies downstream from SOK1 and is indicative of a sequential LKB1/SOK1/GOLGA2 pathway. As in other systems, depletion of any of these three proteins causes dispersion of the Golgi which may be an underlying cause of the loss of axonal specification.

GCKIIIs AND NT (NEUROTROPHIN) RECEPTORS

The effects of NTs [NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor), and NT-3 and NT-4/5] are mediated through a group of four receptors which comprise three receptor protein tyrosine kinases [TrkA (tyrosine kinase receptor)/NTRK1 (neurotrophic tyrosine kinase receptor), TrkB/NTRK2 and TrkC/NTRK3] and a member of the tumour necrosis factor receptor family [p75[NTR]/NGFR (NGF receptor)] [100,101]. NGF was identified over 50 years ago and its effects are well-established with, for example, NGF promoting neurite outgrowth and terminal differentiation in PC12 cells [102,103]. However, although NTs and their receptors promote cell survival in the nervous system, they paradoxically induce the death of cells of a neural origin in some circumstances. From the point of view of the present review, it is interesting that some of the effects of NGF and the other NTs may be mediated by signalling through MST3b[(443)].

MST3b[(443)] and NTs

In the mid-1980s, a NGF-activated protein kinase of 25 or 45 kDa molecular mass was identified in PC12 cells that was referred to as PKN (protein kinase N) [104,105]. To avoid confusion with the protein kinase C-related kinase now known as PKN1 [106], we will refer to the NGF-activated kinase as PK-NGF. PK-NGF was eventually purified as a 45 kDa monomer [107] and is a serine/threonine protein kinase that is equally active at a metal ion concentration of 10 mM Mn^{2+} {compare with MST3[(431)] [12]} or 10 mM Mg^{2+} [105]. PK-NGF is at least partially associated with NGF receptors [108,109]. Purine derivatives (e.g. 6-thioguanine) inhibit PK-NGF in addition to inhibiting neurite outgrowth and differentiation in PC12 cells [110,111], suggesting that PK-NGF is required for axonal specification. PK-NGF may also be activated by a variety of stimuli (peptide growth factors, phorbol esters and cell-permeating cAMP derivatives) [112].

As discussed above, MST3b[(443)] expression is restricted to the brain and other neuronal cells with expression being particularly high in the hippocampus, cerebral cortex and hypothalamus [14]. The biological functions of MST3b[(443)] remained unclear until its probable identity with PK-NGF was revealed by Irwin et al. [113] who purified a 445-residue protein from the bovine brain. The sequence of this protein is largely identical to human MST3b[(443)] (Figure 6). Consistent with studies of PK-NGF, MST3b[(443)] is activated by NGF in PC12 cells and its activation is prevented by 6-thioguanine [113]. Further studies of neurite outgrowth in PC12 cells stimulated with NGF and rat embryonic forebrain neurons treated with BDNF confirmed that MST3b[(443)] represents a protein kinase intimately involved in this response. Other studies implicate MST3b[(443)] in axon outgrowth in rat retinal ganglion cells and dorsal root ganglion neurons [114], suggesting that it is required universally for neurite outgrowth. Furthermore, silencing MST3b[(443)] in vivo attenuates peripheral nerve regeneration.

The importance of MST3b[(443)] in axon growth raises the question of whether it may be manipulated therapeutically to promote neuronal regeneration following, for example, stroke. It is well-established that inosine promotes neurite outgrowth in various neuronal cell types [115,116]. Interestingly, inosine reverses the effects of 6-thioguanine or NGF on neurite outgrowth and on MST3b[(443)] in PC12 cells [113] and the therapeutic utility of inosine to promote nerve regeneration following stroke is now under examination [117]. Here, in combination with other factors or 'environmental enrichment', inosine enhances rat forelimb use after experimental stroke [117].

Coupling of GCKIIIs to TrkA receptors

Although CCM3/PDCD10 is clearly established as a protein that interacts with GCKIIIs, CCM2 (the scaffold to which
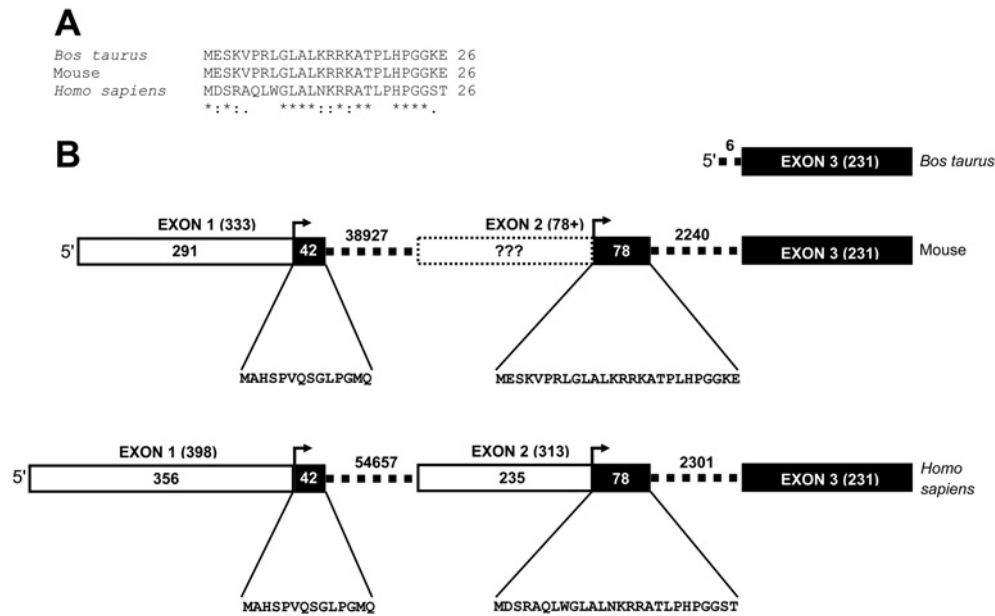
Figure 6   N-terminal sequences of MST3b[(443)] protein and 5′ organization of *MST3* genes

(**A**) N-terminal sequences of MST3b[(445)] of the cow as described by Irwin et al. [113] (the protein is 445 residues long), the putative 26-mer N-terminal sequence of mouse MST3b[(443)] found by translating the mouse MST3 (*Stk24*) gene (NCBI Reference Sequence NC_000080.6) and the human N-terminal 26-mer sequence described by Zhou et al. [14]. Note the identities of the cow and mouse sequences, and the conservation of Thr[18] (phosphorylated by PKA [14]). The cow and mouse sequences show 100% coverage and 62–85% conservation in rodents, dogs, cats and the giant panda, whereas the human sequence show 100% coverage and 96–100% identity in primates. (**B**) The 5′ organization of the MST3 (*Stk24/STK24*) genes of the cow, mouse and human. The numbers of bases in each region are indicated. Sequences that are both transcribed and translated are indicated by filled boxes, 5′ untranslated regions are indicated by open boxes and introns are indicated by thick dashed lines. Translational start sites are indicated by arrows. The putative 5′ untranslated region of mouse exon 2 is indicated by a box enclose by dots. The cow gene (GenBank® accession number AC_000169.1) is apparently incomplete and starts six bases 5′ to the translated sequence of exon 3. The predicted 26-mer amino acid sequence translated from the putative exon 2 of the mouse MST3 (*Stk24*) gene was obtained by translating the mouse MST3 (*Stk24*) gene (NCBI Reference Sequence NC_000080.6). The 5′ organization of the human MST3 (*STK24*) gene (NCBI Reference Sequence NC_000013.10) is established. MST3[(431)] is obtained by splicing exon 1 to exon 3, whereas brain-restricted MST3b[(443)] is obtained by splicing exon 2 to exon 3.

CCM3/PDCD10 and CCM1 bind) may also play a role in GCKIII regulation. CCM2 contains an N-terminal PTB (phosphotyrosine-binding) domain (recently crystallized [118]) and a C-terminal Karet domain [119,120]. CCM3/PDCD10 (through its FAT domain) heterodimerizes with CCM2 via the CCM2 PTB domain [24], but CCM2 binds to TrkA through this same domain to promote cell death [119]. Proteomics screening suggests that the Karet domain of CCM2 can interact with all GCKIIIs, although a direct interaction was confirmed only for SOK1 and MST3[(431)] [it is not clear whether CCM2 is interacting with MST3[(431)] or MST3b[(443)] in the proteomics screen] [121]. Activation of SOK1 [i.e. phosphorylation of SOK1(Thr[174])] by NGF in medulloblastoma cells increases association with CCM2 and TrkA, and SOK1 phosphorylates CCM2, particularly at Ser[384] in the Karet domain. Only SOK1 (not other GCKIIIs) is implicated in NGF-induced TrkA-mediated CCM2-dependent cell death in medulloblastoma cells [121]. These findings are clinically-relevant in that neuroblastoma regression correlates positively with expression of TrkA (particularly in paediatric patients) [120,122] and with expression of CCM2 and SOK1 [121].

THE ROLE(S) OF GCKIIIs IN APOPTOSIS

GCKIIIs have been associated with the induction of apoptosis [6–8,41,123]. Most of the mechanisms proposed involve caspase-dependent cleavage of GCKIIIs, followed by translocation of a fragment consisting mainly of the GCKIII catalytic domain to the nucleus and induction of apoptosis through the mitochondrial

death pathway. However, Lin et al. [124] have also proposed the existence of an additional MST3-regulated caspase-independent pathway which involves reduced mitochondrial integrity and the nuclear translocation of apoptosis-inducing factor and endonuclease G.

MST3[(431)] is cleaved *in vitro* by recombinant caspases 3, 7 or 8 into fragments of ∼35 kDa (N-terminal fragment) and ∼15 kDa (C-terminal fragment) [7]. Consistent with this, incubation of MST3[(431)] with apoptotic Jurkat cell extracts produces a 35 kDa fragment (in a caspase-dependent manner) and endogenous MST3[(431)] is also cleaved and detected as a 15 kDa band on Western blots with antibodies against the C-terminus of MST3[(431)] in apoptotic Jurkat cells. The cleavage site lies C-terminal to Asp[313] in MST3[(431)] (see Figures 1A and 1B). Caspase-dependent cleavage of proteins may reveal myristoylation sequences which are potentially important in regulating apoptosis [125]. Cleavage of human MST3[(431)] creates one such myristoylation acceptor site on Gly[314] of MST3[(431)](314–431) [126]. Though potentially important in ensuring that MST3[(431)](314–431) associates with membranes, thereby separating it spatially from MST3[(431)](1–313), the myristoylation site is not conserved (e.g. the glycine residue is replaced by serine in the rat and cow), suggesting that myristoylation of MST3[(431)](314–431) is not of universal significance.

Like MST3, SOK1 is cleaved during cell death (induced by chemical anoxia) to generate a 35 kDa N-terminal fragment which translocates from the Golgi to the nucleus [8]. However, Asp→Asn scanning of the putative cleavage region [SOK1(300–340)] failed to identify the caspase cleavage site(s). Notably, the role of SOK1 in apoptosis appears to be distinct from its role in

Table 2   Potential caspase cleavage sequence in GCKIIIs

GCKIII sequences of humans were examined using the Cascleave programme (http://sunflower.kuicr.kyoto-u.ac.jp/~sjn/Cascleave/) [127]. The aspartic acid residue N-terminal to the potential cleavage site is indicated with an asterisk. For comparison, the score for the *bona fide* caspase cleavage site in human poly(ADP-ribose) polymerase 1 (D^{211}EVD*GVDE^{218}) [150] is 1.289.

MST3$^{(431)}$		SOK1		MST4	
Cleavage sequence	Score	Cleavage sequence	Score	Cleavage sequence	Score
S^{75}QCD*SPYV^{82}	0.898	S^{71}QCD*SPYI^{78}	0.898	S^{75}QCD*SSYV^{82}	0.796
A^{310}ETD*GQAS^{317}	1.009	S^{304}DID*GEAE^{311}	0.612	D^{302}ESD*SEGS^{309}	1.051
D^{321}SGD*WIFT^{328}	0.765	E^{309}AED*GEQG^{316}	0.703		

Golgi integrity. *In vitro* approaches to identify MST4 cleavage have proved equivocal. Although Huang et al. [7] could not detect cleavage of MST4 by recombinant caspases 3, 7 or 8 *in vitro*, Dan et al. [6] showed that isolated (N-terminally-tagged) MST4 can be cleaved by exogenous caspase 3 potentially at a DESD (residues 302–305) caspase-recognition motif. Inexplicably, cleavage of MST4 by caspase 3 was incomplete (perhaps <10%) even after prolonged incubation.

The *Cascleave* program (http://sunflower.kuicr.kyoto-u.ac.jp/~sjn/Cascleave/) [127] indicates that the sequences in MST3$^{(431)}$ and MST4 are most likely to be cleaved by caspases that lie in the N-terminal regions of the regulatory domains [C-terminal to Asp^{313} in MST3$^{(431)}$ and Asp^{305} in MST4] (Table 2). SOK1(Asp^{307}) and SOK1(Asp^{312}) represent less-favoured cleavage sites at analogous regulatory domain positions (Table 2). The conclusion is that all GCKIIIs might be cleaved by caspases in the N-terminal region of their regulatory domains, although experimental evidence of this is sometimes lacking. All human GCKIIIs have predicted possible cleavage sites C-terminal to a SQCD sequence in the (highly ordered hence possibly inaccessible) catalytic domain (Table 2), though there is no experimental evidence to indicate that cleavage occurs here. However, it should be borne in mind that (though clearly important) caspases are not the only proteases activated during cell death and other proteases (e.g. calpains [128]) might participate in GCKIII cleavage.

Full-length MST3$^{(431)}$ is predominantly cytoplasmic, but caspase cleavage reveals a bipartite NLS in the C-terminal region of MST3$^{(431)}$(1–313) (Figures 1A and 1B) that could account for constitutive localization to the nucleus [7,18,21]. In HeLa cells, leptomycin B [an inhibitor of Crm1 (chromosome region maintenance 1 protein homologue)-mediated nuclear export] induces nuclear localization of MST3$^{(431)}$ suggesting that the regulatory domain contains a NES(s) [nuclear export signal(s)] [21]. MST3$^{(431)}$ contains putative NES sequences rich in hydrophobic aliphatic residues (leucine, isoleucine and valine) at residues 364–375 and 386–396 (or 398?) (Figures 1A and 1B) and empirical studies indicate that one NES sequence(s) lies between residues 335–386. No studies of potential caspase cleavage of MST3b$^{(443)}$ have been published though, by analogy with MST3$^{(431)}$, cleavage and nuclear translocation are theoretically possible. The SOK1 catalytic domain translocates from the Golgi to the nucleus following caspase-dependent cleavage and unmasking of a bipartite NLS (Figures 1A and 1B) as with MST3$^{(431)}$ [8]. Although MST4 contains the same bipartite NLS and the two NES sequences as MST3$^{(431)}$ and SOK1 (Figure 1B), whether it is genuinely cleaved by caspases is debatable, as noted above [6,7]. However, cleavage of MST4 by proteases other than caspases may be sufficient to reveal the bipartite NLS resulting in nuclear localization as detected by Lee et al. [21].

Whether the cleavage of GCKIIIs during apoptosis results in its activation may depend on the cell type. In cardiac myocytes, overexpressed MST3$^{(431)}$(1–313) (i.e. equivalent to the caspase-cleaved form) does not exhibit increased basal activity, is not activated [i.e. there is no increase in autophosphorylation of the T-loop threonine residue which only occurs when cells expressing MST3$^{(431)}$(1–313) are treated with calyculin A] and, in isolation, it does not promote apoptosis [18]. This may be because cardiac myocytes possess a vast array of cytoprotective mechanisms. However, in other cells, MST3$^{(431)}$(1–313) is more active than full-length MST3$^{(431)}$ in immunokinase assays, an effect the authors suggest could result from loss of an autoinhibitory sequence rather than T-loop phosphorylation [7]. Catalytically inactive MST3$^{(431)}$(K53R) or truncated MST3$^{(431)}$(1–313)(K53R) do not promote apoptosis suggesting that protein kinase activity is required, and the overexpression of MST3$^{(431)}$(1–313) alone is sufficient to induce apoptotic changes [7]. SOK1 is the only other GCKIII to be studied with respect to whether or not kinase activity is required for its effects on apoptosis. The decreases in cell viability produced by wild-type SOK1 and by catalytically inactive full-length SOK1 [SOK1(K49R) or SOK1(T174A)] are not significantly different, although all are cleaved during apoptosis and enter the nucleus [8]. In contrast, and confusingly, inactivating mutations of truncated SOK1(1–331) decrease its ability to induce apoptosis. Overall, the conclusion is that GCKIIIs may promote apoptosis, but many of the experiments use overexpressed nuclear-targeted (C-terminally-deleted) GCKIIIs and non-physiological nuclear localization of an overexpressed protein could non-specifically stimulate apoptosis. Hence, matters are far from settled.

GCKIIIs AND CELL-CYCLE PROGRESSION

ERK1/2 (extracellular-signal-regulated kinase 1/2) are intimately involved in cell-cycle progression and cell proliferation. Thus activation of ERK1/2 (and other MAPKs) by GCKIIIs (Table 1) was initially considered in this context. Indeed, the overexpression of MST4 was originally reported to activate ERK1/2 and increase cell proliferation [16]. Furthermore, MST4 is associated with anchorage-independent growth and proliferation of prostate cancer cells [17]. More recently, CCM3/PDCD10 together with MST4 were shown to promote proliferation by enhancing ERK1/2 signalling [27]. However, given the evidence linking MST4 to the regulation of the Golgi and plasma membrane-associated events (e.g. those involving paxillin and the ERM axis), it is possible that the effects on ERK1/2 signalling and cell proliferation could be indirect.

MST3$^{(431)}$ is also associated with cell-cycle progression via the phosphorylation of the AGC kinases NDR1 [nuclear Dbf2 (dumbbell-former 2)-related 1] and NDR2 (orthologues of the conserved yeast Dbf2 kinases, hence their name) [129]. Phosphorylation by MST3$^{(431)}$ at Thr^{444} and Thr^{442} in the C-terminal hydrophobic domains of NDR1 and NDR2 respectively, stimulates NDR1/2 activity by increasing autophosphorylation

of the T-loop residues NDR1(Ser[281]) and NDR2(Ser[282]). Interestingly, as pointed out by Mehellou et al. [92], the sequences surrounding the sites in NDR1 and NDR2 which are phosphorylated by MST3[(431)] show a similarity to that surrounding MST3(Thr[328]), the site in MST3 that is *cis*-autophosphorylated following its activation by phosphorylation of MST3(Thr[178]) [18]. Depending on the alignment chosen, the consensus sequence is either DW(aHy)FX$_{0/3}$T*(Hy)(Basic) or Φ(aHy)X$_{0/1}\Phi$T*(Hy)(Basic), where aHy is an aliphatic hydrophobic residue (isoleucine, leucine or valine), T* is the threonine residue phosphorylated, Hy is any hydrophobic residue, Basic is arginine or lysine, and Φ is any aromatic residue (phenylalanine, tyrosine or tryptophan). Binding of MOB1A to the N-termini of NDR1/2 further increases their activities. MOB proteins ($\sim$200–240 residues in length in the human) are highly conserved and, in yeasts, they activate the protein kinase Mps1p (monopolar spindle 1; involved in mitosis). In spite of their nomenclature, NDRs are present in both the nucleus and cytoplasm (especially in the perinuclear region) in mammalian cells [130–132]. They are involved in the regulation of centrosome duplication [133] and G_1/S progression by controlling the stability of the cyclin-dependent kinase inhibitor p21[CIP1] [134]. Phosphorylation of NDR1 and NDR2 by MST3[(431)] could represent another route by which GCKIIIs regulate cell-cycle progression.

ASSOCIATION OF GCKIIIs WITH HUMAN DISEASES

The intimate association of GCKIIIs with CCM proteins strongly suggests that they are likely to be involved in the cerebral cavernous malformation diseases. Although there is, as yet, no direct evidence for this, it is interesting that genetic studies in mice indicate that expression of SOK1 correlates with expression of CCM1, CCM2 and CCM3 [135]. The involvement of GCKIIIs in cell migration and polarity, together with the regulation of the Golgi apparatus and centrosome-mediated microtubular organization, further suggests that they could be involved in various additional human diseases. In particular, SOK1 promotes the phosphorylation of the brain microtubule-binding protein Tau [136], hyperphosphorylation of which is associated with a number of neurodegenerative disorders. Thus Tau is hyperphosphorylated in Dab1-deleted C57BL6 or C57BL6/129SV mice and depletion of SOK1 reduces Tau phosphorylation in embryonic neurons derived from these animals. The Dab1/reelin pathway may oppose the LKB1/SOK1/GOLGA2 signalling pathway [49]. However, SOK1 may not phosphorylate Tau directly given that overexpression of SOK1 itself does not affect the degree of Tau phosphorylation.

In the current era, genetic profiling is proving useful in identifying candidate genes for diseases and in identifying associated non-causative markers of diseases. In humans the *SOK1* locus is located at chromosome 2q37.3, a region directly associated with two diseases involving defects in bone and Ca^{2+} metabolism, namely Albright hereditary osteodystrophy and pseudopseudohypoparathyroidism [137–140]. Whether or not mutations in SOK1 are causative of either disease remains to be established. Studies of different mouse strains suggest that SOK1 expression is associated with expression of distal targets and may thus affect susceptibility to diseases [135]. Furthermore, the murine *Stk25* gene (i.e. the gene encoding SOK1) is one of two neighbouring genes [the other being *Farp2* (FERM, RhoGEF and pleckstrin domain protein 2)] linked to plasma HDL (high-density lipoprotein) concentrations [141], raised levels of which are associated with cardiovascular protection. A single 'neutral' ('synonymous') base change in the triplet encoding SOK1(Thr[320]) may cause increased expression of SOK1 protein and a non-synonymous change in FARP2 protein may alter protein folding. Both mutations are associated with elevated plasma HDL. However, the *Stk25*/*STK25* and *Farp2*/*FARP2* genes lie head-to-tail in both the murine and human genomes, and a complicating consideration is that, in the human genome, a microRNA (miR3133) is embedded within an intron towards the 3′ end of *FARP2* [142].

A link between SOK1 and glucose intolerance in skeletal muscle has also emerged [143]. Depletion of SOK1 from L6 myoblasts increases the expression of uncoupling protein 1 and hence increased lipid oxidation. At the same time, expression of proteins important in muscle glucose uptake and metabolism [GLUT (glucose transporter) 1, GLUT4 and hexokinase], also increases. Correlated with these findings, higher expression of SOK1 occurs in skeletal muscle from human Type 2 diabetics, suggesting that depletion or inhibition of SOK1 might improve glucose tolerance and utilization. Finally, MST4 is expressed in the prostate and ectopic overexpression of MST4 [but not of inactive MST4(K53E)] in prostate cancer cell lines results in peripheral actin staining (suggestive of formation of lamellipodia), increased anchorage-independent growth and increased tumorigenicity [17]. Given the association of GCKIII expression with various clinical conditions, selective inhibition of GCKIIIs by small molecules could be potentially therapeutically useful, as well as assisting in deciphering the functional code of the GCKIIIs. However, unfortunately, no clearly selective inhibitors of GCKIIIs have yet been discovered.

REFERENCES

1 Dan, I., Watanabe, N. M. and Kusumi, A. (2001) The Ste20 group kinases as regulators of MAP kinase cascades. Trends Cell Biol. **11**, 220–230

2 Delpire, E. (2009) The mammalian family of sterile 20p-like protein kinases. Pflügers Arch. **458**, 953–967

3 Hanks, S. K. and Hunter, T. (1995) The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J. **9**, 576–596

4 Pombo, C. M., Force, T., Kyriakis, J., Nogueira, E., Fidalgo, M. and Zalvide, J. (2007) The GCK II and III subfamilies of the STE20 group kinases. Front. Biosci. **12**, 850–859

5 Ling, P., Lu, T.-J., Yuan, C.-J. and Lai, M.-D. (2008) Biosignaling of mammalian Ste20-related kinases. Cell. Signalling **20**, 1237–1247

6 Dan, I., Ong, S.-E., Watanabe, N. M., Blagoev, B., Nielsen, M. M., Kajikawa, E., Kristiansen, T. Z., Mann, M. and Pandey, A. (2002) Cloning of MASK, a novel member of the mammalian germinal center kinase III subfamily, with apoptosis-inducing properties. J. Biol. Chem. **277**, 5929–5939

7 Huang, C.-Y. F., Wu, Y.-M., Hsu, C.-Y., Lee, W.-S., Lai, M.-D., Lu, T.-J., Huang, C.-L., Leu, T.-H., Shih, H.-M., Fang, H.-I. et al. (2002) Caspase activation of mammalian Sterile 20-like kinase 3 (Mst3). Nuclear translocation and induction of apoptosis. J. Biol. Chem. **277**, 34367–34374

8 Nogueira, A., Fidalgo, M., Molnar, A., Kyriakis, J., Force, T., Zalvide, J. and Pombo, C. M. (2008) SOK1 translocates from the Golgi to the nucleus upon chemical anoxia and induces apoptotic cell death. J. Biol. Chem. **283**, 16248–16258

9 Zhang, H., Ma, X., Deng, X., Chen, Y., Mo, X., Zhang, Y., Zhao, H. and Ma, D. (2012) PDCD10 interacts with STK25 to accelerate cell apoptosis under oxidative stress. Front. Biosci. **17**, 2295–2305

10 Pombo, C. M., Bonventre, J. V., Molnar, A., Kyriakis, J. and Force, T. (1996) Activation of a human Ste20-like kinase by oxidant stress defines a novel stress response pathway. EMBO J. **15**, 4537–4546

11 Osada, S., Izawa, M., Saito, R., Mizuno, K., Suzuki, A., Hirai, S. and Ohno, S. (1997) YSK1, a novel mammalian protein kinase structurally related to Ste20 and SPS1, but is not involved in the known MAPK pathways. Oncogene **14**, 2047–2057

12 Schinkmann, K. and Blenis, J. (1997) Cloning and characterization of a human STE20-like protein kinase with unusual cofactor requirements. J. Biol. Chem. **272**, 28695–28703

13 Lu, T.-J., Huang, C.-Y. F., Yuan, C.-J., Lee, Y.-C., Leu, T.-H., Chang, W.-C., Lu, T.-L., Jeng, W.-Y. and Lai, M.-D. (2005) Zinc ion acts as a cofactor for serine/threonine kinase MST3 and has a distinct role in autophosphorylation of MST3. J. Inorg. Biochem. **99**, 1306–1313

14 Zhou, T.-H., Ling, K., Guo, J., Zhou, H., Wu, Y.-L., Jing, Q., Ma, L. and Pei, G. (2000) Identification of a human brain-specific isoform of mammalian STE20-like kinase 3 that is regulated by cAMP-dependent protein kinase. J. Biol. Chem. **275**, 2513–2519

15 Qian, Z., Lin, C., Espinosa, R., LeBeau, M. and Rosner, M. R. (2001) Cloning and characterization of MST4, a novel Ste20-like kinase. J. Biol. Chem. **276**, 22439–22445

16 Lin, J.-L., Chen, H.-C., Fang, H.-I., Robinson, D., Kung, H.-J. and Shih, H.-M. (2001) MST4, a new Ste20-related kinase that mediates cell growth and transformation via modulating ERK pathway. Oncogene **20**, 6559–6569

17 Sung, V., Luo, W., Qian, D., Lee, I., Jallal, B. and Gishizky, M. (2003) The Ste20 kinase MST4 plays a role in prostate cancer progression. Cancer Res. **63**, 3356–3363

18 Fuller, S. J., McGuffin, L. J., Marshall, A. K., Giraldo, A., Pikkaraanen, S., Clerk, A. and Sugden, P. H. (2012) A novel, non-canonical mechanism of regulation of MST3 (mammalian Sterile20-related kinase 3). Biochem. J. **442**, 595–610

19 Ko, T.-P., Jeng, W.-Y., Liu, C.-I., Lai, M.-D., Wu, C.-I., Chang, W.-J., Shr, H.-L., Lu, T.-J. and Wang, A. H. J. (2010) Structures of human MST3 kinase in complex with adenine, ADP and Mn^{2+}. Acta Crystallogr., Sect. D: Biol. Crystallogr. **66**, 145–154

20 Record, C. J., Chaikuad, A., Rellos, P., Das, S., Pike, A. C. W., Fedorov, O., Marsden, B. D., Knapp, S. and Lee, W. H. (2010) Structural comparison of human mammalian Ste20-like kinases. PLoS ONE **5**, e11905

21 Lee, W.-S., Hsu, C.-Y., Wang, P.-L., Huang, C.-Y. F., Chang, C.-H. and Yuan, C.-J. (2004) Identification and characterization of the nuclear import and export signals of the mammalian Ste20-like protein kinase 3. FEBS Lett. **572**, 41–45

22 Voss, K., Stahl, S., Hogan, B. M., Reinders, J., Schleider, E., Schulte-Merker, S. and Felbor, U. (2009) Functional analyses of human and zebrafish 18-amino acid in-frame deletion pave the way for domain mapping of the cerebral cavernous malformation 3 protein. Hum. Mutat. **30**, 1003–1011

23 Ceccarelli, D. F., Laister, R. C., Mulligan, V. K., Kean, M. J., Goudreault, M., Scott, I. C., Derry, W. B., Chakrabartty, A., Gingras, A.-C. and Sicheri, F. (2011) CCM3/PDCD10 heterodimerizes with germinal center kinase III (GCKIII) proteins using a mechanism analogous to CCM3 homodimerization. J. Biol. Chem. **286**, 25056–25064

24 Li, X., Zhang, R., Zhang, H., He, Y., Ji, W., Min, W. and Boggon, T. J. (2010) Crystal structure of CCM3, a cerebral cavernous malformation protein critical for vascular integrity. J. Biol. Chem. **285**, 24099–24107

25 Ding, J., Wang, X., Li, D.-F., Hu, Y., Zhang, Y. and Wang, D.-C. (2010) Crystal structure of human programmed cell death 10 complexed with inositol-(1,3,4,5)-tetrakisphosphate: a novel adaptor protein involved in human cerebral cavernous malformation. Biochem. Biophys. Res. Commun. **399**, 587–592

26 Rual, J.-F., Venkatesan, K., Hao, T., Hirozane-Kishikawa, T., Dricot, A., Li, N., Berriz, G. F., Gibbons, F. D., Dreze, M., Ayivi-Guedehoussou, N. et al. (2005) Towards a proteome-scale map of the human protein–protein interaction network. Nature **437**, 1173–1178

27 Ma, X., Zhao, H., Shan, J., Long, F., Chen, Y., Chen, Y., Zhang, Y., Han, X. and Ma, D. (2007) PDCD10 interacts with Ste20-related kinase MST4 to promote cell growth and transformation via modulation of the ERK pathway. Mol. Biol. Cell **18**, 1965–1978

28 Voss, K., Stahl, S., Schleider, E., Ullrich, S., Nickel, J., Mueller, T. D. and Felbor, U. (2007) CCM3 interacts with CCM2 indicating common pathogenesis for cerebral cavernous malformations. Neurogenetics **8**, 249–256

29 Fidalgo, M., Fraile, M., Pires, A., Force, T., Pombo, C. and Zalvide, J. (2010) CCM3/PDCD10 stabilizes GCKIII proteins to promote Golgi assembly and cell orientation. J. Cell Sci. **123**, 1274–1284

30 Zhang, M., Dong, L., Shi, Z., Jiao, S., Zhang, Z., Zhang, W., Liu, G., Chen, C., Feng, M., Hao, Q. et al. (2013) Structural mechanism of CCM3 heterodimerization with GCKIII kinases. Structure **21**, 680–688

31 Xu, X., Wang, X., Ding, J. and Wang, D.-C. (2012) Crystallization and preliminary crystallographic studies of CCM3 in complex with the C-terminal domain of MST4. Acta Crystallogr., Sect. F: Struct. Biol. Crystal. Commun. **68**, 760–763

32 Pombo, C. M., Tsujita, T., Kyriakis, J. M., Bonventre, J. V. and Force, T. (1997) Activation of the Ste20-like oxidant stress response kinase-1 during the initial stages of chemical anoxia-induced necrotic cell death. Requirement for dual inputs of oxidant stress and increased cytosolic [Ca^{2+}]. J. Biol. Chem. **272**, 29372–29379

33 Fidalgo, M., Guerrero, A., Fraile, M., Iglesias, C., Pombo, C. M. and Zalvide, J. (2012) Adaptor protein cerebral cavernous malformation 3 (CCM3) mediates phosphorylation of the cytoskeletal proteins ezrin/radixin/moesin by mammalian Ste20-4 to protect cells from oxidative stress. J. Biol. Chem. **287**, 11556–11565

34 Bae, Y. S., Kang, S. W., Seo, M. S., Baines, I. C., Tekle, E., Chock, P. B. and Rhee, S. G. (1997) Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF-receptor-mediated tyrosine phosphorylation. J. Biol. Chem. **272**, 217–221

35 Lu, T.-J., Lai, W.-Y., Huang, C.-Y. F., Hsieh, W.-J., Yu, J.-S., Hsieh, Y.-J., Chang, W.-T., Leu, T.-H., Chang, W.-C., Chuang, W.-J. et al. (2006) Inhibition of cell migration by autophosphorylated mammalian Sterile 20-like kinase 3 (MST3) involves paxillin and protein-tyrosine phosphatase-PEST. J. Biol. Chem. **281**, 38405–38417

36 Preisinger, C., Short, B., De Corte, V., Bruyneel, E., Haas, A., Kopajtich, R., Gettemans, J. and Barr, F. A. (2004) YSK1 is activated by the Golgi matrix protein GM130 and plays a role in cell migration through its substrate 14-3-3ζ. J. Cell Biol. **164**, 1009–1020

37 Gordon, J., Hwang, J., Carrier, K. J., Jones, C. A., Kern, Q. L., Moreno, C. S., Karas, R. H. and Pallas, D. C. (2011) Protein phosphatase 2a (PP2A) binds within the oligomerization domain of striatin and regulates the phosphorylation and activation of the mammalian Ste20-like kinase Mst3. BMC Biochem. **12**, 54

38 Hennig, E. E., Mikula, M., Rubel, T., Dadlez, M. and Ostrowski, J. (2012) Comparative kinome analysis to identify putative colon tumor biomarkers. J. Mol. Med. **90**, 447–456

39 Zach, S., Felk, S. and Gillardon, F. (2010) Signal transduction protein array analysis links LRRK2 to Ste20 kinases and PKCζ that modulate neuronal plasticity. PLoS ONE **5**, e13191

40 Zhu, G., Fujii, K., Liu, Y., Codrea, V., Herrero, J. and Shaw, S. (2005) A single pair of acidic residues in the kinase major groove mediates strong substrate preference for P-2 or P-5 arginine in the AGC, CAMK, and STE kinase families. J. Biol. Chem. **280**, 36372–36379

41 Wu, H.-Y., Lin, C.-Y., Chen, T.-C., Pan, S.-T. and Yuan, C.-J. (2011) Mammalian Ste20-like protein kinase 3 plays a role in hypoxia-induced apoptosis of trophoblast cell line 3A-sub-E. Int. J. Biochem. Cell Biol. **43**, 742–750

42 Bornens, M. (2012) The centrosome in cells and organisms. Science **335**, 422–266

43 Bettencourt-Dias, M. (2013) Q&A: who needs a centrosome? BMC Biol. **11**, 28

44 de Forges, H., Bouissou, A. and Perez, F. (2012) Interplay between microtubule dynamics and intracellular organization. Int. J. Biochem. Cell Biol. **44**, 266–274

45 Etienne-Manneville, S. and Hall, A. (2001) Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ. Cell **106**, 489–498

46 Millarte, V. and Farhan, H. (2012) The Golgi in cell migration: regulation by signal transduction and its implications for cancer cell metastasis. Sci. World J. **2012**, 498278

47 Kean, M. J., Ceccarelli, D. F., Goudreault, M., Sanches, M., Tate, S., Larsen, B., Gibson, L. C. D., Derry, W. B., Scott, I. C., Pelletier, L. et al. (2011) Structure–function analysis of core STRIPAK proteins: a signaling complex implicated in Golgi polarization. J. Biol. Chem. **286**, 25065–25075

48 Nakamura, N. (2010) Emerging new roles of GM130, a cis-Golgi matrix protein, in higher order cell functions. J. Pharmacol. Sci. **112**, 255–264

49 Matsuki, T., Matthews, R. T., Cooper, J. A., van der Brug, M. P., Cookson, M. R., Hardy, J. A., Olson, E. C. and Howell, B. W. (2010) Reelin and STK25 have opposing roles in neuronal polarization and dendritic Golgi deployment. Cell **143**, 826–836

50 Zhou, J., Shao, Z., Kerkela, R., Ichijo, H., Muslin, A. J., Pombo, C. and Force, T. (2009) Serine 58 of 14-3-3ζ is a molecular switch regulating ASK1 and oxidant stress-induced cell death. Mol. Cell. Biol. **29**, 4167–4176

50b Obenauer, J. C., Cantley, L. C. and Yaffe, M. B. (2003) Scansite 2.0: proteome-wide prediction of cell signaling interactions using short sequence motifs. Nucleic Acids Res. **31**, 3635–3641

51 Moreno, C. S., Park, S., Nelson, K., Ashby, D., Hubalek, F., Lane, W. S. and Pallas, D. C. (2000) WD40 repeat proteins striatin and S/G$_2$ nuclear autoantigen are members of a novel family of calmodulin-binding proteins that associate with protein phosphatase 2A. J. Biol. Chem. **275**, 5257–5263

52 Ewing, R. M., Chu, P., Elisma, F., Li, H., Taylor, P., Climie, S., McBroom-Cerajewski, L., Robinson, M. D., O'Connor, L., Li, M. et al. (2007) Large-scale mapping of human protein–protein interactions by mass spectrometry. Mol. Syst. Biol. **3**, 89

53 Glatter, T., Wepf, A., Aebersold, R. and Gstaiger, M. (2009) An integrated workflow for charting the human interaction proteome: insights into the PP2A system. Mol. Syst. Biol. **5**, 237

54 Goudreault, M., D'Ambrosio, L. M., Kean, M. J., Mullin, M. J., Larsen, B. G., Sanchez, A., Chaudhry, S., Chen, G. I., Sicheri, F., Nesvizhskii, A. I. et al. (2009) A PP2A phosphatase high density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to the cerebral cavernous malformation 3 (CCM3) protein. Mol. Cell. Proteomics **8**, 157–171

55 Hyodo, T., Ito, S., Hasegawa, H., Asano, E., Maeda, M., Urano, T., Takahashi, M., Hamaguchi, M. and Senga, T. (2012) Misshapen-like kinase 1 (MINK1) is a novel component of striatin-interacting phosphatase and kinase (STRIPAK) and is required for the completion of cytokinesis. J. Biol. Chem. **287**, 25019–25029

56 Frost, A., Elgort, M. G., Brandman, O., Ives, C., Collins, S. R., Miller-Vedam, L., Weibezahn, J., Hein, M. Y., Poser, I., Mann, M. et al. (2012) Functional repurposing revealed by comparing S. pombe and S. cerevisiae genetic interactions. Cell **149**, 1339–1352

57 Moreno, C. S., Lane, W. S. and Pallas, D. C. (2001) A mammalian homolog of yeast MOB1 is both a member and a putative substrate of striatin family-protein phosphatase 2A complexes. J. Biol. Chem. **276**, 24253–24260

58   Muro, Y., Chan, E. K. L., Landberg, G. and Tan, E. M. (1995) A cell-cycle nuclear autoantigen containing WD-40 motifs expressed mainly in S and G_2 phase cells. Biochem. Biophys. Res. Commun. **207**, 1029–1037

59   Benoist, M., Gaillard, S. and Castets, F. (2006) The striatin family: a new signaling platform in dendritic spines. J. Physiol. **99**, 146–153

60   Castets, F., Bartoli, M., Barnier, J. V., Baillat, G., Salin, P., Moqrich, A., Bourgeois, J.-P., Denizot, F., Rougon, G., Calothy, G. and Monneron, A. (1996) A novel calmodulin-binding protein, belonging to the WD-repeat family, is localized in dendrites of a subset of CNS neurons. J. Cell Biol. **134**, 1051–1062

61   Castets, F., Rakitina, T., Gaillard, S., Moqrich, A., Mattei, M.-G. and Monneron, A. (2000) Zinedin, SG2NA, and striatin are calmodulin-binding, WD repeat proteins principally expressed in the brain. J. Biol. Chem. **275**, 19970–19977

62   Zheng, X., Xu, C., Di Lorenzo, A., Kleaveland, B., Zou, Z., Seiler, C., Chen, M., Cheng, L., Xiao, J., He, J. et al. (2010) CCM3 signaling through sterile 20-like kinases plays an essential role during zebrafish cardiovascular development and cerebral cavernous malformations. J. Clin. Invest. **120**, 2795–2804

63   Yoruk, B., Gillers, B. S., Chi, N. C. and Scott, I. C. (2012) Ccm3 functions in a manner distinct from Ccm1 and Ccm2 in a zebrafish model of CCM vascular disease. Dev. Biol. **362**, 121–131

64   Riant, F., Bergametti, F., Ayrignac, X., Boulday, G. and Tournier-Lasserve, E. (2010) Recent insights into cerebral cavernous malformations: the molecular genetics of CCM. FEBS J. **277**, 1070–1075

65   Chan, A. C., Li, D. Y., Berg, M. J. and Whitehead, K. J. (2010) Recent insights into cerebral cavernous malformations: animal models of CCM and the human phenotype. FEBS J. **277**, 1076–1083

66   Faurobert, E. and Albiges-Rizo, C. (2010) Recent insights into cerebral cavernous malformations: a complex jigsaw puzzle under construction. FEBS J. **277**, 1084–1096

67   Cavalcanti, D. D., Kalani, M. Y. S., Martirosyan, N. L., Eales, J., Spetzler, R. F. and Preul, M. C. (2012) Cerebral cavernous malformations: from genes to proteins to disease. J. Neurosurg. **116**, 122–132

68   Zawistowski, J. S., Stalheim, L., Uhlik, M. T., Abell, A. N., Ancrile, B. B., Johnson, G. L. and Marchuk, D. A. (2005) CCM1 and CCM2 protein interactions in cell signaling: implications for cerebral cavernous malformations pathogenesis. Hum. Mol. Genet. **14**, 2521–2531

69   Li, X., Ji, W., Zhang, R., Folta-Stogniew, E., Min, W. and Boggon, T. J. (2011) Molecular recognition of leucine–aspartate repeat (LD) motifs by the focal adhesion targeting homology domain of cerebral cavernous malformation 3 (CCM3). J. Biol. Chem. **286**, 26138–26147

70   Baillat, G., Moqrich, A., Castets, F., Baude, A., Bailly, Y., Benmerah, A. and Monneron, A. (2001) Molecular cloning and characterization of phocein, a protein found from the Golgi complex to dendritic spines. Mol. Biol. Cell **12**, 663–673

71   Virshup, D. M. and Shenolikar, S. (2009) From promiscuity to precision: protein phosphatases get a makeover. Mol. Cell **33**, 537–545

72   Shi, Y. (2009) Serine/threonine phosphatases: mechanism through structure. Cell **139**, 468–484

73   Slupe, A. M., Merrill, R. A. and Strack, S. (2011) Determinants for substrate specificity of protein phosphatase 2A. Enzyme Res. **2011**, 398751

74   Deakin, N. O. and Turner, C. E. (2008) Paxillin comes of age. J. Cell Sci. **121**, 2435–2444

75   Harburger, D. S. and Calderwood, D. A. (2009) Integrin signalling at a glance. J. Cell Sci. **122**, 159–163

76   Zhao, Z. S., Manser, E., Loo, T. H. and Lim, L. (2000) Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. Mol. Cell. Biol. **20**, 6354–6363

77   Garton, A. J. and Tonks, N. K. (1994) PTP-PEST: a protein tyrosine phosphatase regulated by serine phosphorylation. EMBO J. **13**, 3763–3771

78   Chen, X.-D. and Cho, C.-Y. (2011) Downregulation of SOK1 promotes the migration of MCF-7 cells. Biochem. Biophys. Res. Commun. **407**, 389–392

79   Fehon, R. G., McClatchey, A. I. and Bretscher, A. (2010) Organizing the cell cortex: the role of ERM proteins. Nat. Rev. Mol. Cell Biol. **11**, 276–287

80   Neisch, A. L. and Fehon, R. G. (2011) Ezrin, radixin and moesin: key regulators of membrane–cortex interactions and signaling. Curr. Opin. Cell Biol. **23**, 377–382

81   Chan, A. C., Drakos, S. G., Ruiz, O. E., Smith, A. C. H., Gibson, C. C., Ling, J., Passi, S. F., Stratman, A. N., Sacharidou, A., Revelo, M. P. et al. (2011) Mutations in 2 distinct genetic pathways result in cerebral cavernous malformations in mice. J. Clin. Invest. **121**, 1871–1881

82   Bergametti, F., Denier, C., Labauge, P., Arnoult, M., Boetto, S., Clanet, M., Coubes, P., Echenne, B., Ibrahim, R., Irthum, B. et al. (2005) Mutations within the programmed cell death 10 gene cause cerebral cavernous malformations. Am. J. Hum. Genet. **76**, 42–51

83   Miyamoto, H., Matsushiro, A. and Nozaki, M. (1993) Molecular cloning of a novel mRNA sequence expressed in cleavage stage mouse embryos. Mol. Reprod. Dev. **34**, 1–7

84   Nozaki, M., Onishi, Y., Togashi, S. and Miyamoto, H. (1996) Molecular characterization of the *Drosophila Mo25* gene, which is conserved among *Drosophila*, mouse, and yeast. DNA Cell Biol. **15**, 505–509

85   Lizcano, J. M., Göransson, O., Toth, R., Deak, M., Morrice, N. A., Boudeau, J., Hawley, S. A., Udd, L., Mäkelä, T. P., Hardie, D. G. and Alessi, D. R. (2004) LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J. **23**, 833–843

86   Alessi, D. R., Sakamoto, K. and Bayascas, J. R. (2006) LKB1-dependent signaling pathways. Annu. Rev. Biochem. **75**, 137–163

87   Hardie, D. G., Ross, F. A. and Hawley, S. A. (2012) AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat. Rev. Mol. Cell Biol. **13**, 251–262

88   Zeqiraj, E., Filippi, B. M., Deak, M., Alessi, D. R. and van Aalten, D. M. F. (2009) Structure of the LKB1–STRAD–MO25 complex reveals an allosteric mechanism of kinase activation. Science **326**, 1707–1711

89   Zeqiraj, E., Filippi, B. M., Goldie, S., Navratilova, I., Boudeau, J., Deak, M., Alessi, D. R. and van Aalten, D. M. F. (2009) ATP and MO25α regulate the conformational state of the STRADα pseudokinase and activation of the LKB1 tumour suppressor. PLoS Biol. **7**, e1000126

90   ten Klooster, J. P., Jansen, M., Yuan, J., Oorschot, V., Begthel, H., Di Giacomo, V., Colland, F., de Koning, J., Maurice, M. M., Hornbeck, P. and Clevers, H. (2009) Mst4 and ezrin induce brush borders downstream of the Lkb1/Strad/Mo25 polarization complex. Dev. Cell **16**, 551–562

91   Filippi, B. M., de los Heros, P., Mehellou, Y., Navratilova, I., Gourlay, R., Deak, M., Plater, L., Toth, R., Zeqiraj, E. and Alessi, D. R. (2011) MO25 is a master regulator of SPAK/OSR1 and MST3/MST4/YSK1 protein kinases. EMBO J. **30**, 1730–1741

92   Mehellou, Y., Alessi, D. R., Macartney, T. J., Szklarz, M., Knapp, S. and Elkins, J. M. (2013) Structural insights into the activation of MST3 by MO25. Biochem. Biophys. Res. Commun. **431**, 604–609

93   Gagnon, K. B., Rios, K. and Delpire, E. (2011) Functional insights into the activation mechanism of Ste20-related kinases. Cell. Physiol. Biochem. **28**, 1219–1230

94   Baas, A. F., Smit, L. and Clevers, H. (2004) LKB1 tumor suppressor protein: PARtaker in cell polarity. Trends Cell Biol. **14**, 312–319

95   Baas, A. F., Kuipers, J., van der Wel, N. N., Batlle, E., Koerten, H. K., Peters, P. J. and Clevers, H. C. (2004) Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. Cell **116**, 457–466

96   Shelly, M. and Poo, M.-M. (2011) Role of LKB1-SAD/MARK pathway in neuronal polarization. Develop. Neurobiol. **71**, 508–527

97   Hayashi, K., Suzuki, A. and Ohno, S. (2012) PAR-1/MARK: a kinase essential for maintaining the dynamic state of microtubules. Cell Struct. Funct. **37**, 21–25

98   Gloerich, M., ten Klooster, J. P., Vliem, M. J., Koorman, T., Zwartkruis, F. J., Clevers, H. and Bos, J. L. (2012) Rap2A links intestinal cell polarity to brush border formation. Nat. Cell Biol. **14**, 793–801

99   Taira, K., Umikawa, M., Takei, K., Myagmar, B.-E., Shinzato, M., Machida, N., Uezato, H., Nonaka, S. and Kariya, K. (2004) The Traf2- and Nck-interacting kinase as a putative effector of Rap2 to regulate actin cytoskeleton. J. Biol. Chem. **279**, 49488–49496

100 Huang, E. J. and Reichardt, L. F. (2003) Trk receptors: roles in neuronal signal transduction. Annu. Rev. Biochem. **72**, 609–642

101 Schecterson, L. C. and Bothwell, M. (2010) Neurotrophin receptors: old friends with new partners. Develop. Neurobiol. **70**, 332–338

102 Tischler, A. S. and Greene, L. A. (1975) Nerve growth factor-induced process formation by cultured rat pheochromocytoma cells. Nature **258**, 341–342

103 Greene, L. A. and Tischler, A. S. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. U.S.A. **73**, 2424–2428

104 Blenis, J. and Erikson, R. L. (1986) Regulation of protein kinase activities in PC12 pheochromocytoma cells. EMBO J. **5**, 3441–3447

105 Rowland, E. A., Müller, T. H., Goldstein, M. and Greene, L. A. (1987) Cell-free detection and characterization of a novel nerve growth factor-activated protein kinase in PC12 cells. J. Biol. Chem. **262**, 7504–7513

106 Mukai, H. (2003) The structure and function of PKN, a protein kinase having a catalytic domain homologous to that of PKC. J. Biochem. **133**, 17–27

107 Volonté, C. and Greene, L. A. (1992) Nerve growth factor-activated protein kinase N. Characterization and rapid near homogeneity purification by nucleotide affinity-exchange chromatography. J. Biol. Chem. **267**, 21663–21670

108 Volonté, C., Ross, A. H. and Greene, L. A. (1993) Association of a purine-analogue-sensitive protein kinase activity with p75 nerve growth factor receptors. Mol. Biol. Cell **4**, 71–78

109 Volonté, C., Loeb, D. M. and Greene, L. A. (1993) A purine analog-sensitive protein kinase activity associates with Trk nerve growth factor receptors. J. Neurochem. **61**, 664–672

110 Volonté, C., Rukenstein, A., Loeb, D. M. and Greene, L. A. (1989) Differential inhibition of nerve growth factor responses by purine analogues: correlation with inhibition of a nerve growth factor-activated protein kinase. J. Cell Biol. **109**, 2395–2403

111 Volonté, C. and Greene, L. A. (1990) Induction of ornithine decarboxylase by nerve growth factor in PC12 cells: dissection by purine analogues. J. Biol. Chem. **265**, 11050–11055

112 Rowland-Gagné, E. and Greene, L. A. (1990) Multiple pathways of N-kinase activation in PC12 cells. J. Neurochem. **54**, 423–433

113 Irwin, N., Li, Y.-M., O'Toole, J. E. and Benowitz, L. I. (2006) Mst3b, a purine-sensitive Ste20-like protein kinase, regulates axon outgrowth. Proc. Natl. Acad. Sci. U.S.A. **103**, 18320–18325

114 Lorber, B., Howe, M. L., Benowitz, L. I. and Irwin, N. (2009) Mst3b, an Ste20-like kinase, regulates axon regeneration in mature CNS and PNS pathways. Nat. Neurosci. **12**, 1407–1414

115 Benowitz, L. I., Jing, Y., Tabibiazar, R., Jo, S. A., Petrausch, B., Stuermer, C. A. O., Rosenberg, P. A. and Irwin, N. (1998) Axon outgrowth is regulated by an intracellular purine-sensitive mechanism in retinal ganglion cells. J. Biol. Chem. **273**, 29626–29634

116 Petrausch, B., Tabibiazar, R., Roser, T., Jing, Y., Goldman, D., Stuermer, C. A. O., Irwin, N. and Benowitz, L. I. (2000) A purine-sensitive pathway regulates multiple genes involved in axon regeneration in goldfish retinal ganglion cells. J. Neurosci. **20**, 8031–8041

117 Zai, L., Ferrari, C., Dice, C., Subbaiah, S., Havton, L. A., Coppola, G., Geschwind, D., Irwin, N., Huebner, E., Strittmatter, S. M. and Benowitz, L. I. (2011) Inosine augments the effects of a Nogo receptor blocker and of environmental enrichment to restore skilled forelimb use after stroke. J. Neurosci. **31**, 5977–5988

118 Wang, X., Ding, J. and Wang, D. (2012) Crystallization and preliminary X-ray analysis of the C-terminal domain of CCM2, part of a novel adaptor protein involved in cerebral cavernous malformations. Acta Crystallogr., Sect. F: Struct. Biol. Crystal. Commun. **68**, 683–686

119 Harel, L., Costa, B., Tcherpakov, M., Zapatka, M., Oberthuer, A., Hansford, L. M., Vojvodic, M., Levy, Z., Chen, Z. Y., Lee, F. S. et al. (2009) CCM2 mediates death signaling by the TrkA receptor tyrosine kinase. Neuron **63**, 585–591

120 Harel, L., Costa, B. and Fainzilber, M. (2010) On the death Trk. Develop. Neurobiol. **70**, 298–303

121 Costa, B., Kean, M. J., Ast, V., Knight, J. D. R., Mett, A., Levy, Z., Ceccarelli, D. F., Gonzalez Badillo, B., Eils, R., König, R. et al. (2012) STK25 protein mediates TrkA and CCM2 protein-dependent death in pediatric tumor cells of neural origin. J. Biol. Chem. **287**, 29285–29289

122 Brodeur, G. M., Minturn, J. E., Ho, R., Simpson, A. M., Iyer, R., Varela, C. R., Light, J. E., Kolla, V. and Evans, A. E. (2009) Trk receptor expression and inhibition in neuroblastomas. Clin. Cancer Res. **15**, 3244–3250

123 Wu, H.-Y., Lin, C.-Y., Lin, T.-Y., Chen, T.-C. and Yuan, C.-J. (2008) Mammalian Ste20-like protein kinase 3 mediates trophoblast apoptosis in spontaneous delivery. Apoptosis **13**, 283–294

124 Lin, C.-Y., Wu, H.-Y., Wang, P.-L. and Yuan, C.-J. (2010) Mammalian Ste20-like protein kinase 3 induces a caspase-independent apoptotic pathway. Int. J. Biochem. Cell Biol. **42**, 98–105

125 Martin, D. D. O., Beauchamp, E. and Berthiaume, L. G. (2011) Post-translational myristoylation: fat matters in cellular life and death. Biochimie **93**, 18–31

126 Martin, D. D. O., Vilas, G. L., Prescher, J. A., Rajaiah, G., Falck, J. R., Bertozzi, C. R. and Berthiaume, L. G. (2008) Rapid detection, discovery, and identification of post-translationally myristoylated proteins during apoptosis using a bio-orthogonal azidomyristate analog. FASEB J. **22**, 797–806

127 Song, J., Tan, H., Shen, H., Mahmood, K., Boyd, S. E., Webb, G. I., Akutsu, T. and Whisstock, J. C. (2010) Cascleave: towards more accurate prediction of caspase substrate cleavage sites. Bioinformatics **26**, 752–760

128 Squier, M. K. T., Miller, A. C. K., Malkinson, A. M. and Cohen, J. J. (1994) Calpain activation in apoptosis. J. Cell Physiol. **159**, 229–237

129 Stegert, M. R., Hergovich, A., Tamaskovic, R., Bichsel, S. J. and Hemmings, B. A. (2005) Regulation of NDR protein kinase by hydrophobic motif phosphorylation mediated by the mammalian Ste20-like kinase MST3. Mol. Cell. Biol. **25**, 11019–11029

130 Stegert, M. R., Tamaskovic, R., Bichsel, S. J., Hergovich, A. and Hemmings, B. A. (2004) Regulation of NDR2 protein kinase by multi-site phosphorylation and the S100B calcium-binding protein. J. Biol. Chem. **279**, 23806–23812

131 Devroe, E., Erdjument-Bromage, H., Tempst, P. and Silver, P. A. (2004) Human Mob proteins regulate the NDR1 and NDR2 serine-threonine kinases. J. Biol. Chem. **279**, 24444–24451

132 Fuller, S. J., Pikkarainen, S., Tham, E. L., Cullingford, T. E., Molkentin, J. D., Cornils, H., Hergovich, A., Hemmings, B. A., Clerk, A. and Sugden, P. H. (2008) Nuclear Dbf2-related protein kinases (NDRs) in isolated cardiac myocytes and the myocardium: activation by cellular stresses and by phosphoprotein serine-/threonine phosphatase inhibitors. Cell. Signalling **20**, 1564–1577

133 Hergovich, A., Lamla, S., Nigg, E. A. and Hemmings, B. A. (2007) Centrosome-associated NDR kinase regulates centrosome duplication. Mol. Cell **25**, 625–634

134 Cornils, H., Kohler, R. S., Hergovich, A. and Hemmings, B. A. (2011) Human NDR kinases control G$_1$/S cell cycle transition by directly regulating p21 stability. Mol. Cell. Biol. **31**, 1382–1395

135 Ciobanu, D. C., Lu, L., Mozhui, K., Wang, X., Jagalur, M., Morris, J. A., Taylor, W. L., Dietz, K., Simon, P. and Williams, R. W. (2010) Detection, validation, and downstream analysis of allelic variation in gene expression. Genetics **184**, 119–128

136 Matsuki, T., Zaka, M., Guerreiro, R., van der Brug, M. P., Cooper, J. A., Cookson, M. R., Hardy, J. A. and Howell, B. W. (2012) Identification of *Stk25* as a genetic modifier of Tau phosphorylation in *Dab1*-mutant mice. PLoS ONE **7**, e31152

137 Phelan, M. C., Rogers, R. C., Clarkson, K. B., Bowyer, F. P., Levine, M. A., Estabrooks, L. L., Severson, M. C. and Dobyns, W. B. (1995) Albright hereditary osteodystrophy and del(2) (q37.3) in four unrelated individuals. Am. J. Med. Genet. **58**, 1–7

138 Davids, M. S., Crawford, E., Weremowicz, S., Morton, C. C., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Phelan, M. C., Comb, M. J. and Melnick, M. B. (2001) *STK25* is a candidate gene for pseudopseudohypoparathyroidism. Genomics **77**, 2–4

139 Shrimpton, A. E., Braddock, B. R., Thomson, L. L., Stein, C. K. and Hoo, J. J. (2004) Molecular delineation of deletions on 2q37.3 in three cases with an Albright hereditary osteodystrophy-like phenotype. Clin. Genet. **66**, 537–544

140 Chaabouni, M., Le Merrer, M., Raoul, O., Prieur, M., De Blois, M. C., Philippe, A., Vekemans, M. and Romana, S. P. (2006) Molecular cytogenetic analysis of five 2q37 deletions: refining the brachydactyly candidate region. Eur. J. Med. Genet. **49**, 255–263

141 Su, Z., Cox, A., Shen, Y., Stylianou, I. M. and Paigen, B. (2009) *Farp2* and *Stk25* are candidate genes for the HDL cholesterol locus on mouse chromosome 1. Arterioscler. Thromb. Vasc. Biol. **29**, 107–113

142 Stark, M. S., Tyagi, S., Nancarrow, D. J., Boyle, G. M., Cook, A. L., Whiteman, D. C., Parsons, P. G., Schmidt, C., Sturm, R. A. and Hayward, N. K. (2010) Characterization of the melanoma miRNAome by deep sequencing. PLoS ONE **5**, e9685

143 Nerstedt, A., Cansby, E., Andersson, C. X., Laakso, M., Stančáková, A., Blüher, M., Smith, U. and Mahlapuu, M. (2012) Serine/threonine protein kinase 25 (STK25): a novel negative regulator of lipid and glucose metabolism in rodent and human skeletal muscle. Diabetologia **55**, 1797–1807

144 Chen, C. B., Ng, J. K., Choo, P. H., Wu, W. and Porter, A. G. (2009) Mammalian sterile 20-like kinase 3 (MST3) mediates oxidative stress-induced cell death by modulation JNK activation. Biosci. Rep. **29**, 405–415

145 Roche, D. B., Buenavista, M. T., Tetchner, S. J. and McGuffin, L. J. (2011) The IntFOLD server: an integrated web resource for protein fold recognition, 3D model quality assessment, intrinsic disorder prediction, domain prediction and ligand binding site prediction. Nucleic Acids Res. **39**, W171–W176

146 McGuffin, L. J. and Roche, D. B. (2011) Automated tertiary structure prediction with accurate local model quality assessment using the IntFOLD-TS method. Proteins **79** (Suppl. 10), 137–146

147 Buenavista, M. T., Roche, D. B. and McGuffin, L. J. (2012) Improvement of 3D protein models using multiple templates guided by single-template model quality assessment. Bioinformatics **28**, 1851–1857

148 McGuffin, L. J. (2008) Intrinsic disorder prediction from the analysis of multiple protein fold recognition models. Bioinformatics **24**, 1798–1804

149 McGuffin, L. J. and Roche, D. B. (2010) Rapid model quality assessment for protein structure predictions using the comparison of multiple models without structural alignments. Bioinformatics **26**, 182–188

150 Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G. and Earnshaw, W. C. (1994) Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. Nature **371**, 346–347