Biochemical Society Transactions (2017) **45** 665–681
DOI: 10.1042/BST20160331

Review Article

The secret life of kinases: insights into non-catalytic signalling functions from pseudokinases

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Over the past decade, our understanding of the mechanisms by which pseudokinases, which comprise ~10% of the human and mouse kinomes, mediate signal transduction has advanced rapidly with increasing structural, biochemical, cellular and genetic studies. Pseudokinases are the catalytically defective counterparts of conventional, active protein kinases and have been attributed functions as protein interaction domains acting variously as allosteric modulators of conventional protein kinases and other enzymes, as regulators of protein trafficking or localisation, as hubs to nucleate assembly of signalling complexes, and as transmembrane effectors of such functions. Here, by categorising mammalian pseudokinases based on their known functions, we illustrate the mechanistic diversity among these proteins, which can be viewed as a window into understanding the non-catalytic functions that can be exerted by conventional protein kinases.

Introduction

Counterparts of active enzymes that lack key catalytic residues and are therefore predicted to possess defective catalytic activities, termed pseudoenzymes, are thought to comprise ~10% of proteomes throughout the kingdoms of life [1,2]. Much of the recent interest in these proteins has originated from studies of the catalytically defective cousins of kinases, called pseudokinases [3]; however, much remains unknown about pseudokinase function. Nonetheless, detailed studies of pseudokinases indicate that they serve functions in cell signalling as protein interaction modules that can allosterically regulate the activities of conventional protein kinases or other enzymes; regulate trafficking or cellular localisation of other proteins; and nucleate assembly of signalling complexes. As a result, it has become clear that these proteins are not merely bystanders in cell signalling, but rather perform crucial regulatory functions. Furthermore, the development of small molecules that modulate pseudokinase signalling functions may represent a novel avenue for therapeutic intervention in human diseases, including cancers [4].

With the growing interest in pseudokinase function, different approaches have emerged to group these proteins into different classes. Initially, pseudokinases were identified on the basis of deviations from the conventional Hanks et al. [5] VAI**K**, HR**D**, and **D**FG catalytic motifs [6], corresponding to the ATP-positioning (Lys, YAM**K**^{72}), catalytic loop (Asp, YR**D**^{166}), and Mg^{2+}-binding (Asp, **D**^{184}FG), motifs in the archetypal kinase, protein kinase A (PKA) [7]. Subsequently, Boudeau et al. [8] categorised pseudokinases into groups based on the catalytic motif(s) absent from their sequences. Subsequent classifications have focused on pseudokinase function, such as capacity to bind nucleotides, divalent cations, and nucleotides in the presence of divalent cations [9]. While the role of nucleotide binding in pseudokinase function remains an open question, an absence of detectable ATP binding would be incompatible with a pseudokinase exhibiting phosphoryl-transfer catalytic activity. Such a functional analysis lends itself to consideration of kinase-like domains, like those of receptor tyrosine kinase-like orphan receptor (ROR)1, ROR2, receptor-like tyrosine kinase (RYK), and BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B/BUBR1) that, based on the presence of

Received: 27 January 2017
Revised: 8 March 2017
Accepted: 9 March 2017

Version of Record published: 15 June 2017

Biochemical Society Transactions (2017) 45 665–681
DOI: 10.1042/BST20160331

conventional Hanks motifs, would be predicted to exhibit nucleotide binding, but experimentally fail to bind [9,10]. Surprisingly, the survey of nucleotide binding by 31 pseudokinases revealed that less than half were capable of nucleotide binding [9], supporting the notion that pseudokinases have evolved functions as protein interaction domains distinct from catalytic activities. From this perspective, we can use insights into protein interactions mediated by pseudokinases to explore the secret lives of conventional protein kinases as protein interaction modules [11]. Here, we have aimed to further classify mammalian pseudokinases based on their functions in cell signalling deduced by recent biophysical, biochemical, cellular, and genetic studies.

Pseudokinases as allosteric modulators of enzymatic activities

As part of the process of switching between an inactive and active state, protein kinases undergo dynamic structural changes typified by the assembly of hydrophobic regulatory (R-) and catalytic (C-) spines [12]. This ability to sample different conformations equips kinase (and kinase-like) domains to function allosterically to modulate the activity of other enzymes, and in particular other protein kinases. Owing to their catalytic deficiencies, pseudokinases have emerged as useful models to study the non-catalytic, allosteric properties of kinases in isolation. Many pseudokinases allosterically influence the activity of kinase domains within the same protein or within complexes with proteins (Figure 1). In addition, there are examples of pseudokinases modulating other classes of enzymes. In the latter class, this includes vaccinia-related kinase 3 (VRK3), which binds to and allosterically regulates the Erk phosphatase DUSP3/VHR [13–15], and PAN3 poly(A)-specific ribonuclease subunit (PAN3), which associates with the deadenylase PAN2 and is responsible for both promoting PAN2 catalytic activity and the recruitment of mRNA to the complex [16–19].

The Janus kinase (JAK) family are a well-studied example of pseudokinase modulation of a kinase domain within the same protein. JAKs are non-receptor tyrosine kinases involved in signalling downstream from cytokine receptors, and contain pseudokinase and canonical tyrosine kinase domains in tandem (reviewed in ref. [20]). Although the JAK2 pseudokinase domain has been reported to possess residual catalytic activity [21], the main function of this domain in all JAK family members is to associate with, and negatively regulate, the kinase domain. The tyrosine kinase domain of all JAKs displays constitutive activity when expressed independently, and this is suppressed on co-expression with the pseudokinase domain, either independently or in tandem [22–25]. Deletion of the pseudokinase domain alone leads to constitutive activity of the adjacent tyrosine kinase domain in JAK2 [23] and suppression of kinase activity in tyrosine kinase 2 (TYK2) [26], with contradictory reports in JAK3 [24,27]; however, in all these cases, there is a loss of responsiveness to upstream cytokine signalling. Similarly, impacts of disease-associated mutations within the pseudokinase domain of JAK family members are varied, with mutations causing activation of the tyrosine kinase domain common in haematological malignancies (reviewed in ref. [28]) and loss-of-function mutations presenting in immune deficiencies (reviewed in ref. [29]). The only reported multi-domain structure for the JAK pseudokinase–kinase tandem domains is for TYK2, which shows multiple interfaces between the N lobes and linker region of the two domains, 'locking' the kinase domain and preventing its activity, supporting an inhibitory mechanism [22]. While it is possible that this mechanism is conserved throughout the JAK family, an alternative idea has been proposed where the JAK1 and JAK2 pseudokinase domains might inhibit the activity of the tyrosine kinase domain from a receptor-associated JAK dimer *in trans* [30–32]. Despite being heavily studied, the exact mechanism by which the JAK pseudokinase domains suppress the activity of the tyrosine kinase domain remains an open question. Another protein that contains a tandem kinase and pseudokinase domain is eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4/GCN2), whose primary function is as a negative regulator of translation during amino acid starvation (reviewed in ref. [33]). In contrast with the JAKs, the pseudokinase domain of GCN2 positively regulates activity of the kinase domain, and is required for its catalytic activity, with mutation of conserved residues in the pseudokinase domain abrogating kinase function [34].

The STE20-related kinase adaptor (STRAD) pseudokinases allosterically regulate the kinase domain of a different protein. Both STRADA and STRADB bind to serine/threonine kinase 11 (LKB1/STK11), a key upstream regulator in AMPK signalling, with the assistance of the scaffold protein calcium-binding protein 39 (MO25/CAB39) [35–37]. The structure for the heterotrimeric complex of STRADA–LKB1–MO25 has been determined and shows multiple interactions between all three proteins that promote an active conformation of LKB1, including direct associations of the substrate-binding site and pseudoactivation loop of STRADA with the LKB1 kinase domain [38]. Calcium/calmodulin-dependent serine protein kinase (CASK), which acts as a

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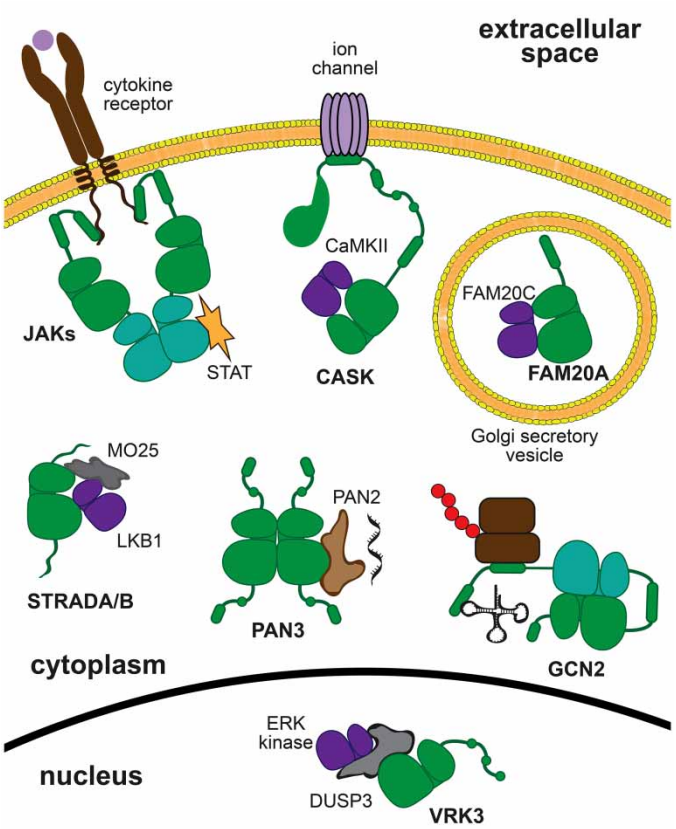

Figure 1. Pseudokinase as allosteric modulators.

JAKs act downstream from cytokine receptors to recruit and activate STAT transcription factors (represented as a star). The pseudokinase domain of JAKs generally negatively regulates the tyrosine kinase domain (blue) and is also important for signal transduction after activation. CASK associates with ion channels and other membrane complexes, and allosterically modulates activity of the active kinase CaMKII. FAM20A (green) controls both the activity and secretion of the kinase FAM20C (purple). STRADA and STRADB are both able to allosterically regulate the kinase activity of LKB1/STK11, with the help of the adapter protein MO25/CAB39. PAN3 associates with, and is important for activity of, the deadenylase PAN2. The pseudokinase domain of GCN2/EIF2AK4, a protein involved in control of translation, is required for the activity of its kinase domain. VRK3 associates with, and allosterically regulates, the Erk phosphatase DUSP3. The pseudokinase domains are drawn throughout in green; active kinase domains are shown in light blue (in proteins containing a pseudokinase domain) or dark purple.

scaffold for synaptic membrane proteins and ion channels and is proposed to have limited catalytic activity under cation-free conditions [39], associates with the regulatory region of calcium/calmodulin-dependent protein kinase II (CaMKII) and promotes inhibitory autophosphorylation of CaMKII in Ca^{2+}/CaM-free conditions [40–42]. CASK also associates with CaMKII in the presence of Ca^{2+}/CaM, allowing it phosphorylate substrates, but with lower efficiency than unbound CaMKII [40]. Last is the recently described Golgi-associated secretory pathway pseudokinase, FAM20A, a secretory protein that forms heterotetrameric complexes with the active kinase FAM20C [43,44]. This interaction promotes stability and secretion of FAM20C and increases FAM20C kinase activity in a manner consistent with allosteric regulation [43,45].

Transmembrane pseudokinases

Nearly a quarter of known pseudokinases contains a transmembrane domain and acts as receptors themselves or modulators of membrane receptor function (Figure 2). Of these, the largest subgroup are the receptor guanylyl cyclases (RGCs), whose pseudokinase domain sits in the cytoplasmic portion between their transmembrane domain and a functional guanylyl cyclase domain (reviewed in ref. [46]). The RGCs all form homodimers and,

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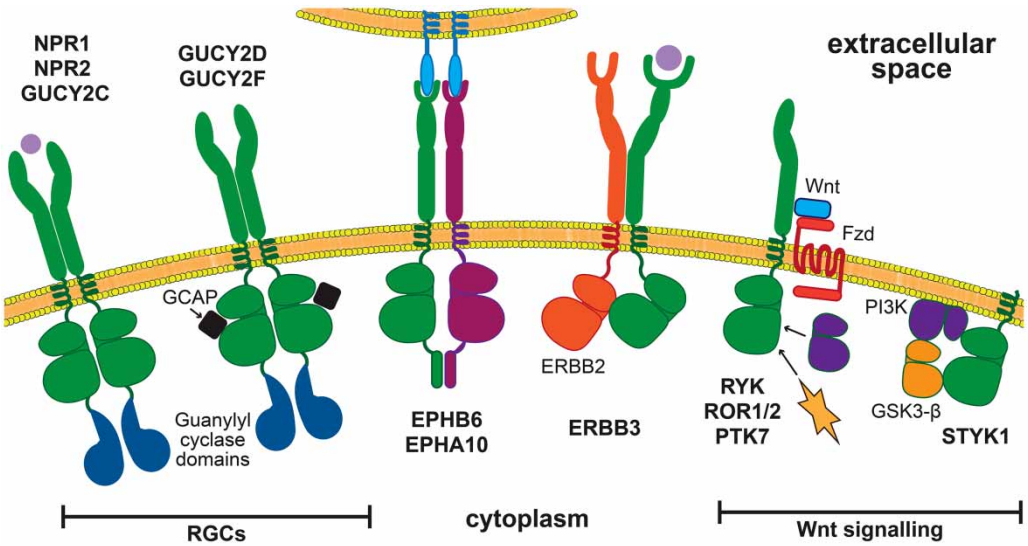

Figure 2. Transmembrane pseudokinases.

The pseudokinase domains of RGCs are responsible for controlling the activity of their guanylyl cyclase domains (shown in dark blue). NRP1, NRP2, and GUCY2C all bind extracellular ligands; however, the retinal RGCs (GUCY2D and GUCY2F) are activated by the binding of guanylyl cyclase-activating proteins (GCAPs; shown in black) to their pseudokinase domains. Both EPHB6 and EPHA10 are able to bind ephrins. The functional role of EPHA10 is unclear, but EPHB6 can form heterodimeric associations with other EPH receptors and influence signalling pathways. ERBB3 can associate with, and allosterically regulate, other ERBB family members, and can act as a scaffold for other signalling components. Additionally, RYK, ROR1, ROR2, and PTK7 all bind non-canonical Wnt ligands, and can also recruit other proteins, such as kinases (shown in purple) or transcription factors (shown as a star), to these complexes. They can also form interactions with many other RTKs. STYK1 is believed to act as a scaffold to facilitate the phosphorylation of GSK3-β by PI3Ks. Pseudokinase domains are drawn throughout in green; kinase domains are drawn in purple or orange.

upon ligand stimulation, the association of the pseudokinase domains enables activation of the guanylyl cyclase domains through a proposed allosteric mechanism (reviewed in ref. [47]). The pseudokinase domains of this receptor family also bind ATP, which has been found to be important for their function [48–51].

The RGCs all have a similar basic function, which is to restore cellular levels of cGMP; however, the stimulus that initiates this varies. Natriuretic peptide receptor 1 and 2 (NPR1/ANPa and NPR2/ANPb) are both receptors for natriuretic peptide hormones and are involved in cellular processes such as control of blood pressure and heart function [52–54]. Guanylate cyclase 2C (GUCY2C/HSER) is preferentially expressed in the gut and the best-described ligand is an *Escherichia coli* enterotoxin (reviewed in ref. [55]). The two retinal specific RGCs, guanylate cyclase 2D and 2E (GUCY2D and GUCY2F), have no known extracellular ligand and instead are stimulated by the binding of activating proteins to the pseudokinase domain [56–58]. Interestingly, chimeric proteins in which the pseudokinase domains of GUCY2D and GUCY2F were swapped retained the characteristics of the originating pseudokinase domains, highlighting its importance for both protein activity and specificity [56].

The next sub-group of pseudokinases responds to the binding of Wnt ligands. This includes ROR1 and the better-studied ROR2, which both bind the non-canonical Wnt ligand Wnt5a [59,60], with ROR2 also known to bind other canonical and non-canonical Wnt ligands [61]. While these proteins are unlikely to possess catalytic activity because they do not detectably bind nucleotides [9], they function in Wnt signalling and act as scaffolds to recruit other interacting partners, including Src and glycogen synthase kinase 3 (GSK3) [62–64]. ROR2 is known to form homodimers or heterodimers with ROR1, and associates with Fzd-receptors, which can induce their internalisation [60,65]. ROR1 is predominantly expressed during development and is proposed to function as a signalling hub by associating with various receptor tyrosine kinases (RTKs) [66,67] and as a switch between pro-apoptotic p38 signalling and pro-survival Src-dependent protein kinase B (PKB/AKT) activation [68].

The pseudokinase RYK is another important Wnt5a-binding protein, and RYK knockout mice have many phenotypic similarities to Wnt5a knockout mice [69–72]. In addition, RYK can bind other Wnt ligands and has a role in Wnt-induced neural outgrowth [73,74]. Another Wnt receptor is protein tyrosine kinase 7 (PTK7/CCK4), a Wnt4 co-receptor that promotes non-canonical Wnt signalling by binding Dishevelled and receptor for activated C kinase 1 (RACK1) through an interaction with the pseudokinase domain [75–77]. In addition, the pseudokinase domain acts as a scaffold to bind and stabilise β-catenin in the absence of canonical Wnt signalling [78]. Although it does not contain an ectodomain, the membrane-bound pseudokinase, serine/threonine/tyrosine kinase 1 (STYK1/NOK/SuRTK106), acts as a scaffold to promote the phosphorylation of GSK3-β, an important Wnt kinase, as well as interacts with and promotes phosphorylation of PKB/AKT and modulates MAPK signalling pathways [79,80]. It is proposed that STYK1 influences both of these pathways by enabling the interaction of these kinases with upstream PI3Ks [79].

The remaining membrane pseudokinases are proposed to influence receptor signalling through their interaction with transmembrane kinases of the same family. Erb-b2 receptor tyrosine kinase 3 (ERBB3/HER3) is a well-studied pseudokinase long known to form homodimers and heterodimers with other EGFR/ErbB family members [81–83]. In heterodimers, ERBB3/HER3 allosterically activates the partner kinase to enable downstream signalling through stabilisation of the N-lobe of the active kinase by interaction with the ERBB3/HER3 C-lobe Space required between the two entities [84–86]. Although ERBB3/HER3 has been found to possess residual catalytic activity [87], mutations that ablate this do not affect signalling (reviewed in ref. [88]), implying that its kinase activity has limited biological relevance. Moreover, active kinases within the HER3/ErbB3 family have also been shown to allosterically regulate other family members and themselves [86]. The idea that a pseudokinase or conventional kinase can equally function as an allosteric modulator ('activator') of a 'receiver' kinase's activity is highly illustrative of the lessons one can learn about allostery among conventional kinases from detailed studies of the pseudokinases. Finally, there are two pseudokinase members in the Eph family of receptors: EPH receptor B6 (EPHB6) and EPHA10. Although little is known about the function of EPHA10, EPHB6 can form heterodimers with, and is phosphorylated by, many other Eph receptors, and acts as a negative regulator of JNK signalling [89–92]. Additionally, EPHB6 strongly associates with the Src family kinase Fyn, as well as the oncogenic E3 ligase c-Cbl [89,90,92].

Pseudokinase signalling hubs

In addition to the JAK kinase family, there are many other cytoplasmic pseudokinases that are integral for modulating signals through receptor complexes (Figure 3). The kinase suppressor of Ras 1 and 2 (KSR1/2) proteins are excellent examples of this and are capable of acting as inducers or inhibitors of Ras/MAPK signalling, depending on the cellular context and KSR expression levels [93,94]. They act downstream from multiple RTKs and, although there has been some debate over whether they possess catalytic activity [95–97], their best-studied role is as a scaffold to mediate interactions between MEK and Raf kinases [97–100]. Raf kinases, like the EGFR/ErbB family of kinases, also form homo- and heterodimeric units, where one partner acts as a non-catalytic activator of the partner ('receiver') protein's kinase activity. In this case, both the conventional protein kinase Raf and the catalytically defective KSR1 can heterodimerise with Raf proteins to promote their kinase activity [101,102], again asserting that non-catalytic functions (the 'secret lives') of conventional enzymes can be revealed by detailed studies of their dead counterparts. Both KSR1 and KSR2 have multiple documented interacting partners and form large protein complexes stabilised by many chaperone proteins [103,104].

Signalling through nuclear factor kappa B (NF-κB) downstream from Toll-like receptor ligands, interleukin 1, and transforming growth factor β (TGF-β) is controlled by the interleukin 1 receptor-associated kinase (IRAK) family (reviewed in ref. [105]). Although IRAK1 and IRAK4 are conventional (active) protein kinases, both IRAK2 and IRAK3 are classified as pseudokinases. IRAK2 is a positive regulator of NF-κB pro-inflammatory signalling by promoting TRAF6 polyubiquitylation [106–108], and mutations within the pseudokinase domain can either enhance or suppress the magnitude of this signalling response [109–111]. IRAK3 binds to and antagonises other IRAK family members, acting as a switch to initiate suppression of the inflammatory response through MEKK3-driven NF-κB signalling [112–114]. Also involved in TGF-β signalling is pseudopodium-enriched atypical kinase 1 (PEAK1/SgK269), which enables a switch from canonical SMAD2/3 signalling to non-canonical Src-MAPK signalling pathways [115]. In addition, SgK269 acts as a scaffold between Src and ERBB2/HER2, enabling the Src-dependent phosphorylation of ERBB2/HER2, thereby promoting its activation [116].

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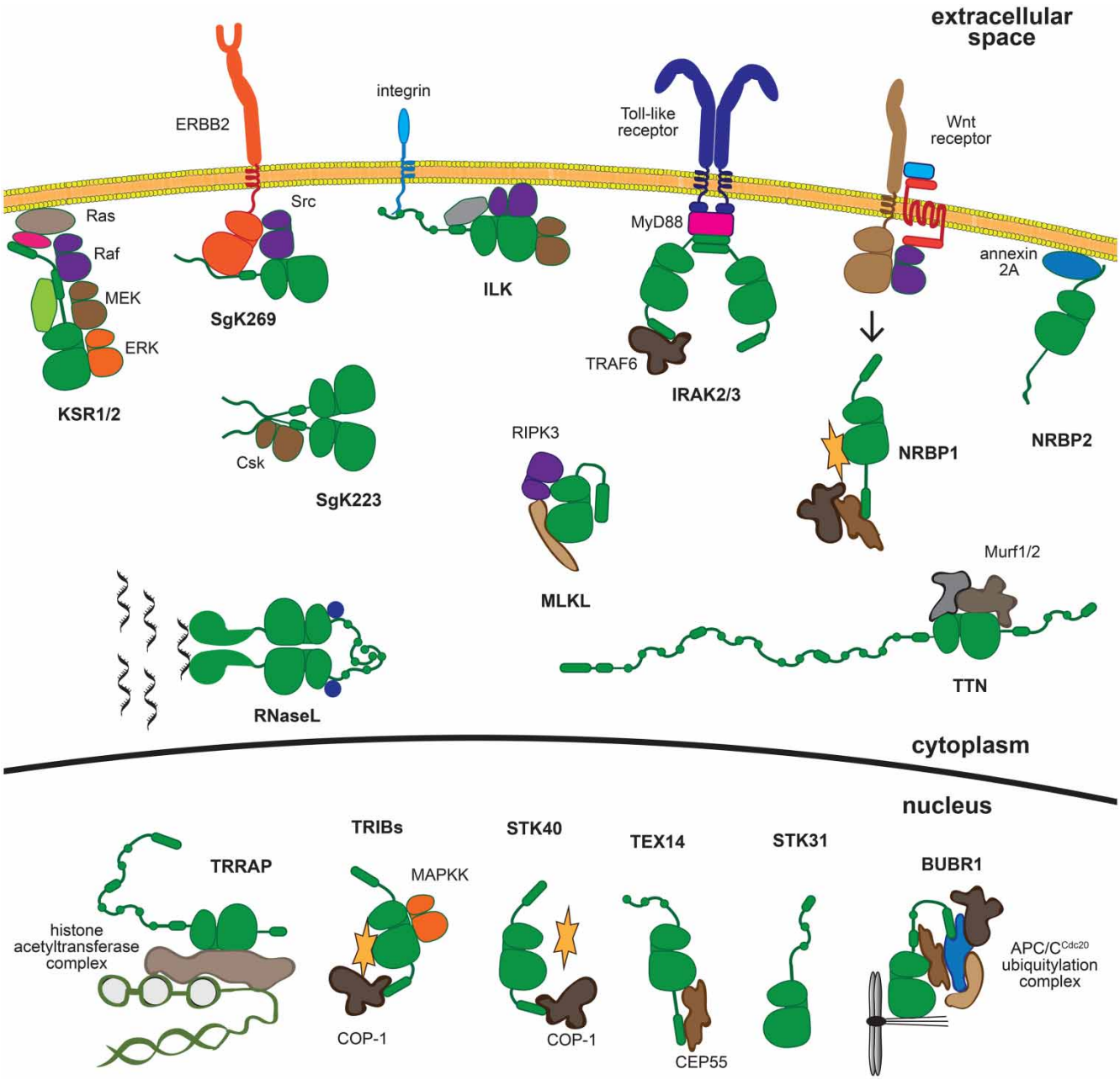

Figure 3. Pseudokinases involved in signalling in the cytoplasm and nucleus.

Many pseudokinases are important for signalling downstream from receptor complexes: KSR1 or KSR2, PEAK1/SgK269, ILK, IRAK2, and IRAK3. NRBP1 is a small adapter protein that is predicted to have roles in many cellular compartments, including scaffolding ubiquitin ligase complexes to negatively regulate Wnt signalling. The lesser-known relative, NRBP2, is believed to influence PKB/AKT signalling through an association with annexin 2A at membranes. Several pseudokinases located in the cytoplasm perform various protein interaction functions: MLKL, RNaseL, PRAG1/SgK223, and TTN. The MLKL pseudokinase domain is thought to restrain the cell-killing activity of its N-terminal domain until phosphorylated by RIPK3, when membrane translocation and permeabilisation ensue. The RNaseL pseudokinase domain mediates dimerisation, which enables the decay of cytoplasmic RNA. TTN is a large polypeptide found in muscle sarcomeres, whose pseudokinase domain recruits the E3 ligases Murf1 and Murf2. In the nucleus, TRRAP scaffolds the histone acetyltransferase complex assembly, and the TRIBs assemble complexes between E3 ligases, such as COP-1, and its various transcription factor substrates (depicted as a star). STK40 also binds COP-1 and affects transcription factor function; however, direct binding to transcription factors has not been shown. TEX14 and STK31 have also been implicated in cell cycle control. Finally, BUBR1/BUB1B is an important scaffold for the APC/C^{Cdc20} ubiquitylation complex and acts as a mechanosensor to modulate kinetochore/microtubule tension. Pseudokinase domains are drawn throughout in green; active kinases are drawn in purple, orange, or brown.

Integrin-linked kinase (ILK), whose primary role is as a scaffold to recruit key proteins involved in integrin function and dynamics [117,118], is also important for enabling the activation of other key signalling kinases, including GSK3, PKB/AKT, and ERK1/2 [119–121]. Some of these interactions were proposed to involve catalytic activity of ILK; however structural, biochemical, and genetic studies indicate that ILK is unlikely to possess catalytic activity, with mutations originally used to validate ILK kinase activity likely to affect protein stability [121–123]. Another pseudokinase is nuclear receptor-binding protein 1 (NRBP1), an adapter protein that contains a putative Src homology 2 and nuclear receptor-binding domains, nuclear import and export sequences [124]. The most well-documented role of NRBP1 is as a negative regulator downstream from Wnt-Ras signalling, where it is believed to act as a scaffold for ubiquitin ligase complex assembly [125,126]. Although little is known about the related pseudokinase NRBP2, recent data suggest a role in PKB/AKT signalling through an interaction with annexin A2 [127].

In the nucleus, we find the Tribbles pseudokinase (TRIB) family (Figure 3). TRIB2 has been shown to exhibit some catalytic activity in the absence of cations [128]; however, like NRBP1, these pseudokinases are primarily adapter proteins which mediate a range of different intracellular functions [129]. First, they bind to other kinases through their pseudokinase domain, such as members of the MAPKK family and PKB/AKT, modulating their phosphorylation and activation [130–132], with these interactions occurring in both cytoplasmic and nuclear compartments [133]. Secondly, they act as scaffolds to bring together various transcription factors, co-activators, and cell cycle modulators with E3 ligases such as COP-1, thus acting as regulators of transcription and cell cycle control [134–139]. Serine/threonine kinase 40 (STK40/SgK495), which is distantly related to the Tribbles proteins, has also been shown to interact with COP-1 [140]. Furthermore, it has been implicated in the regulation of various transcription factors, has a predominantly nuclear localisation, and more recently, has been implicated in the regulation of histone deacetylases (HDACs) [141–143].

In addition to the Tribbles proteins, there are several other pseudokinases that are principally nuclear localized, which are, either involved in cell cycle control or transcriptional control (Figure 3). BUBR1/BUB1B is an important component of the mitotic checkpoint complex and also modulates the activity of the APC/C^{Cdc20} ubiquitylation complex [144–148]. In addition to acting as a scaffold between multiple proteins in these complexes, BUBR1 also acts as a mechanosensor between kinetochores and microtubules [149,150], a function that would be tempting to attribute to the dynamic properties of the kinase-like domain. Both testes expressed 14 (TEX14/SgK307) and serine/threonine kinase 31 (STK31/SgK396) are also involved in cell cycle control [151,152], and although it is known that TEX14 binds to, and probably sequesters, centrosomal protein 55 (CEP55) [153,154], the mechanisms behind the actions of STK31 are not well defined. Finally, rounding out the nuclear pseudokinases is the transformation/transcription domain-associated protein (TRRAP), which is a very large and important scaffold protein for histone acetyltransferase complex assembly [155–159].

At the cell surface, there is a group of pseudokinases involved in control of ciliary and flagellar function (Figure 4). The NME/NM23 family members (NMEs) do not contain conventional protein kinase domains, but contain one or more nucleoside diphosphate kinase (NDPK) domains (reviewed in ref. [160]). The primary role of NDPKs is in replenishment of nucleoside triphosphates; however, this domain also possesses histidine kinase activity (reviewed in ref. [161]). The human NME/NM23 family includes three proteins with pseudo-NDPK domains: NME5, NME7, and NME8 (reviewed in ref. [162]). NME7 contains two NDPK domains, and although domain A has been shown to be catalytically active, domain B does not have detectable phosphotransfer activity [163]. NME7 is associated with cilia and has an important role in microtubule organisation and nucleation through mediating interactions in the gamma tubulin ring complex, where the pseudokinase domain is essential for binding of NME7 to this complex [163,164]. NME5 and NME8 are both highly expressed in the testes [165,166], and although the specific functions of NME5 are not well known, NME8, which contains three NDPK domains, is believed to form part of the outer dynein arm in the flagellum and the cilium [167,168], and defects in this gene are associated with primary ciliary dyskinesia [169]. Additionally, NME8 is implicated in protection against oxidative stress in spermatozoa [170], presumably through its thioredoxin domain, which exhibits increased activity when expressed without the pseudokinase domains [166]. Finally, there are two pseudokinases associated with specialist cilia: retinitis pigmentosa 2 (RP2), which also contains an NDPK domain and is important for regulating the length of photoreceptor sensory cilia [171–173], and unc-51-like kinase 4 (ULK4), which plays an important scaffolding role for the cilia involved in the circulation of cerebrospinal fluid [174]. Both of these pseudokinases have additional roles in regulating the activity of GTPases that modulate assembly of larger protein complexes (Figure 4) [175,176].

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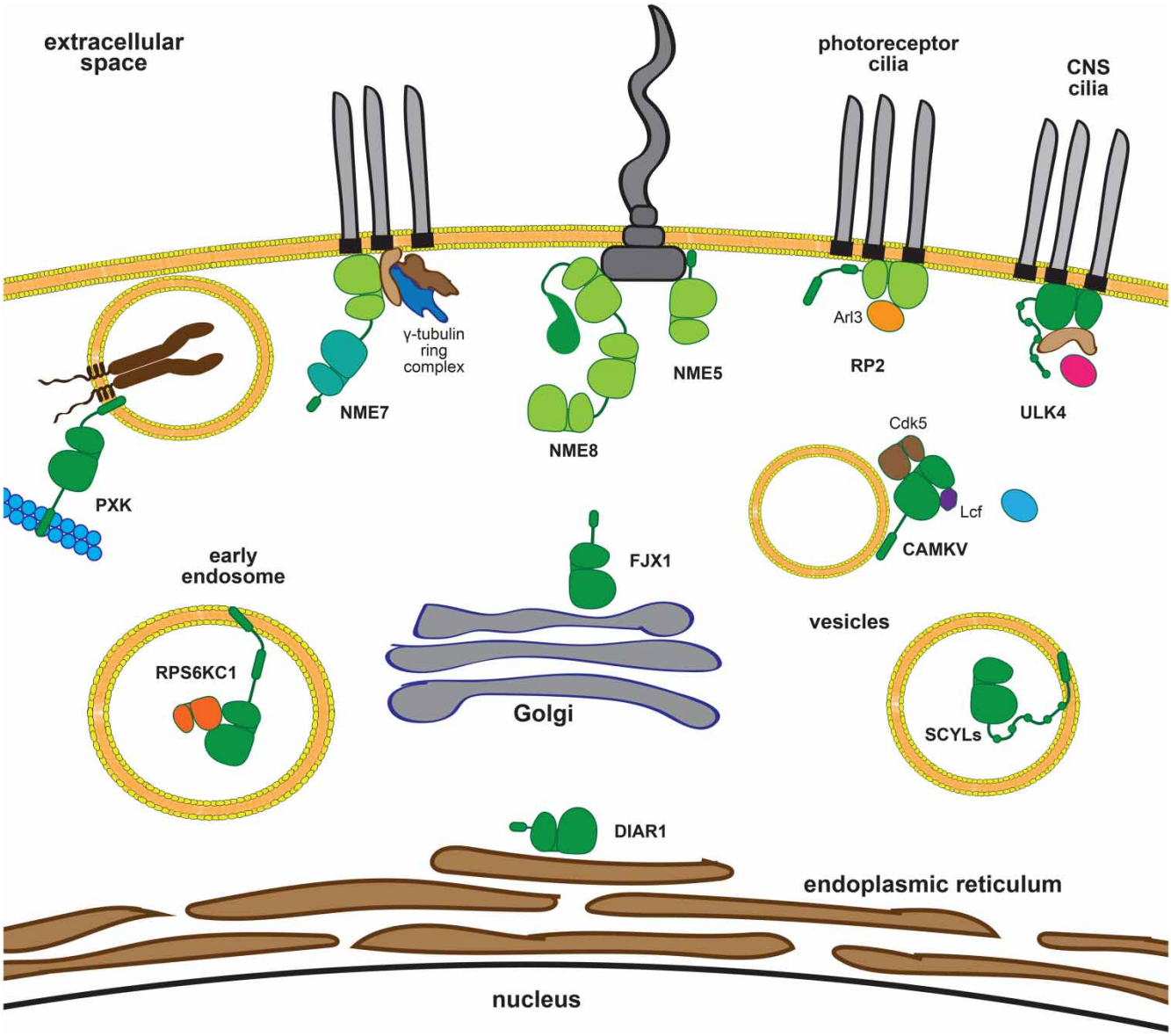

Figure 4. Pseudokinases involved in cellular trafficking and maintenance of cilia/flagella.

The NME proteins all possess pseudo-NDPK domains (light green) rather than conventional protein kinase domains, which act as scaffolds with cilia (NME7, RP2) or flagella (NME8, NME5). NME7 also has an active NDPK domain (shown in light blue). Similar to RP2, which associates with Arl GTPases, the pseudokinase ULK4 associates with cilia and influences GTPase activity through the binding of regulatory proteins. PXK is a pseudokinase associated with receptor internalisation and is believed to facilitate interactions between endosomes and actin. RPS6KC1 is proposed to act as an adapter to recruit many proteins, including kinases (shown in orange), to early endosomes. FJX1 and CXorf36/DIAR1 are poorly understood, but are thought to be localised to the Golgi and endoplasmic reticulum, respectively. CAMKV is a vesicle-associated pseudokinase involved in dendrite physiology at synapses, while SCYLs are a group of vesicle-associated pseudokinases thought to be involved with intracellular transport. Pseudokinase domains are drawn throughout in dark green.

Another subgroup of pseudokinases comprises those associated with intracellular trafficking (Figure 4), including the SCY1-like pseudokinase (SCYLs) [177]. Although the mechanisms involved in SCYL3-mediated transport are unclear [178], both SCYL1 and SCYL2 are known to play a scaffolding role in vesicle formation, with SCYL1 involved in formation of COPI-coated vesicles important in Golgi to ER retrograde transport [179,180], and SCYL2 is associated with the clathrin-coated vesicles involved in receptor mediated endocytosis

[181–183]. CaM kinase-like vesicle associated (CAMKV/VACAMKL) is a vesicle-associated pseudokinase, which until recently had no known function; however, a recent study shows it to be a substrate of Cdk5 and is important for regulation of Rho GTPases at synapses through binding of the guanine nucleotide exchange factor Lcf [184]. Associated with early endosomes by virtue of a PX domain is ribosomal protein S6 kinase C1 (RPS6KC1/RPK118/RSKL1), whose pseudokinase domain has been shown to bind many proteins and recruit them to the endosomal compartment [185,186]. PX domain-containing serine/threonine kinase like (PXK/Slob) is another pseudokinase associated with receptor internalisation and is believed to bind membranes at the C-terminal and actin at the N-terminal [187,188]. The mechanism behind PXK's function is not well defined; however, it is tempting to speculate that the pseudokinase domain may act as a mechanical switch to facilitate movement of receptor complexes inside the cell. To round out the intracellular traffickers, there are the less well-known chromosome X open reading frame 36 (CXorf36/DIA1R), which has been proposed to localise to secretory compartments [189], and FJX1, a secretory protein belonging to the newly categorised FAM20 kinase family, which is implicated in dendrite elongation [190].

Finally, there are pseudokinases that participate in intracellular signalling hubs predominantly in the cytoplasm (Figure 3). This includes the giant polypeptide titin (TTN), whose best known role is as a member of muscle fibre sarcomeres. The N-terminal kinase-like domain of TTN interacts with the zinc finger protein Nbr1, and mutations within the pseudokinase domain that interrupt this interaction are linked to hereditary muscle disease [191]. The TTN pseudokinase domain also recruits the E3 ligases Murf1 and Murf2 [191,192], and is also proposed to act as a mechanosensor, similar to BUBR1 [193]. Ribonuclease L (RNaseL) is a protein whose primary function is to cleave ssRNA through its C-terminal RNase domain, whose activity relies on dimerisation and essential cofactor binding by the central pseudokinase domain and N-terminal Ankyrin repeat domain [194–197]. Also in this group is PEAK1-related kinase-activating pseudokinase 1 (PRAG1/SgK223), which assembles into heteromeric complexes with PEAK1/SgK169 [198], and binds to and sequesters the Src family inhibitory kinase Csk in the cytoplasm to potentiate Src activation [199]. In addition, PRAG1/SgK223 promotes Rho GTPase activity, which is enhanced by the binding of the GTPase Rnd2 to the PRAG1/SgK223 pseudokinase domain [200]. Last, but not least, is the pseudokinase mixed lineage kinase domain-like (MLKL), which acts as the most downstream known effector in the necroptotic cell death pathway [201,202]. The killer domain of MLKL is its N-terminal four helical bundle (4HB) domain; however, in the absence of upstream stimuli, this domain is proposed to be held in check by interactions with the C-terminal pseudokinase domain [203,204]. The pseudokinase domain serves as a receiver of an upstream signal in the form of phosphorylation by RIPK3, whereupon MLKL leaves the cytoplasmic compartment and translocates to membranes to initiate cell death by an unclear mechanism [205].

Conclusions

Based on known functions in allosteric enzyme regulation, regulation of cellular localisation/trafficking and as hubs for the assembly of signalling complexes, we have sought to functionally categorise pseudokinases. Much remains to be learned about additional functions of pseudokinases in signalling and the prevalence of pseudokinases serving a combination of these functions. For example, one of the best characterised pseudokinases, MLKL, is illustrative of the functional pleiotropy that can be served by pseudokinase domains, because this domain acts as a molecular switch [203], a suppressor of the 4HB executioner domain [204], and a protein interaction domain to recruit downstream effectors [206], such as the co-chaperones Cdc37-HSP90 [207]. It remains of enormous interest to determine whether regulation of the MLKL 4HB domain by its adjacent pseudokinase domain is illustrative of more widespread phenomena where pseudoenzyme domains have evolved functions as regulators of neighbouring domains *in cis*. This theme is evident among the tandem pseudokinase–kinase domains of the JAK family and GCN2, in addition to the tandem pseudokinase–guanylyl cyclase domains of the RGC family. While we have a detailed understanding of a subset of pseudokinase domain-containing proteins, there are five pseudokinases that are yet to be ascribed functions that we have been unable to categorise based on our present understanding. Ribosomal protein S6 kinase-like 1 (RPS6KL1), which is related to RPS6KC1, but lacks the phosphoinositide domain and thus any likely membrane localisation, has been proposed as important for cell survival in two separate RNAi studies, but the underlying mechanism is unknown [208,209]. The pseudokinase serine/threonine kinase-like domain-containing 1 (STLK1) has been identified as a strong candidate in screening studies for involvement in tamoxifen resistance and influenza virus replication [210,211], but its function remains enigmatic. TBC1 domain-containing kinase (TBCK1) has been implicated in mTOR signalling but, besides possessing a putative GTPase-activating domain, there is little

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DOI: 10.1042/BST20160331

known about its mechanism of action [212]. Selenoprotein O (SELENOO/SELO) is a mitochondrial selenoprotein with a possible role in chondrocyte differentiation and proliferation [213–215], although the role of SELO's pseudokinase domain is currently unclear. Finally, protein serine kinase H2 (PSKH2) is a pseudokinase of unknown function that has been lost from the mouse and rat kinomes, and is the counterpart of the conventional kinase PSKH1 [216]. Detailed mechanistic and biological studies will play an important role in deducing the precise functions of these poorly understood pseudokinases and will almost certainly reveal unexpected functions of others. We also expect that future studies will illuminate the extent to which the diverse signalling functions of pseudokinases provide a window into the non-catalytic mechanisms mediated by conventional protein kinases.

Abbreviations

4HB, N-terminal four helical bundle; BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B (also known as BUBR1); CAB39, calcium binding protein 39 (also known as MO25); CaMKII, calcium/calmodulin-dependent protein kinase II; CAMKV, CaM kinase-like vesicle associated (also known as VACAMKL); CASK, calcium/calmodulin-dependent serine protein kinase; CEP55, centrosomal protein 55; CXorf36, chromosome X open reading frame 36 (also known as DIA1R); EGFR/ErbB, epidermal growth factor receptor; EIF2AK4, eukaryotic translation initiation factor 2 alpha kinase 4 (also known as GCN2); EPHB6, EPH receptor B6; ER, Endoplasmic reticulum; ERBB3, erb-b2 receptor tyrosine kinase 3 (also known as HER3); ERK, extracellular signal-regulated kinase (also known as MAPK); FAM20A, Golgi-associated secretory pathway pseudokinase; GSK3, glycogen synthase kinase 3; GUCY2C, guanylate cyclase 2C (also known as HSER); GUCY2D, guanylate cyclase 2D (also known as CYGD); GUCY2F, guanylate cyclase 2D (also known as CYGF); ILK, integrin-linked kinase; IRAK, interleukin 1 receptor-associated kinase; JAK, Janus kinase; KSR, kinase suppressor of Ras; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK Kinase; MLKL, mixed lineage kinase domain-like; NDPK, nucleoside diphosphate kinase; NF-κB, nuclear factor kappa B; NME, NME/NM23 family member; NPR1, natriuretic peptide receptor 1 (also known as ANPa); NPR2, natriuretic peptide receptor 2 (also known as ANPb); NRBP, nuclear receptor-binding protein; PAN3, PAN3 poly(A)-specific ribonuclease subunit; PEAK1, pseudopodium-enriched atypical kinase 1 (also known as SgK269); PKB, protein kinase B (also known as AKT); PRAG1, PEAK- related kinase activating pseudokinase 1 (also known as SgK223 or Pragmin); PSKH2, protein serine kinase H2; PTK7, protein tyrosine kinase 7 (also known as CCK4); PXK, PX domain-containing serine/threonine kinase like (also known as Slob); RGC, receptor guanylyl cyclase; RNaseL, ribonuclease L; ROR, receptor tyrosine kinase-like orphan receptor; RPS6KC1, ribosomal protein S6 kinase C1 (also known as RPK118 or RSKL1); RPS6KL1, ribosomal protein S6 kinase-like 1 (also known as RSKL2); RP2, retinitis pigmentosa 2; RTKs, receptor tyrosine kinases; RYK, receptor-like tyrosine kinase; SCYL, SCY1-like pseudokinase; SELENOO, selenoprotein O (also known as SELO); STAT, signal transducer and activator of transcription; STK11, serine/threonine kinase 11 (also known as LKB1); STK31, serine/threonine kinase 31 (also known as SgK396); STK40, serine/threonine kinase 40; STRAD, STE20-related kinase adaptor; STYK1, serine/threonine/tyrosine kinase 1 (also known as NOK or SuRTK106); TEX14, testis expressed 14 (also known as SgK307); TGF-β, transforming growth factor β; TRIB, Tribbles pseudokinase; TRRAP, transformation/transcription domain-associated protein; TTN, titin; TYK2, tyrosine kinase 2; ULK4, unc-51-like kinase 4; VRK3, vaccinia-related kinase 3.

Funding

A.V.J. is supported by an Australian Government Research Training Program Scholarship and J.M.M. is supported by the National Health and Medical Research Council of Australia (NHMRC) fellowship [1105754]. Our work reviewed herein was supported by NHMRC Project grants [1057905, 1067289, 1124735, and 1124737], NHMRC IRIISS 9000220, and Victorian Government Operational Infrastructure Support.

Acknowledgements

We thank our colleagues in the field, including those at the Pseudoenzymes-focused meeting in Liverpool, for stimulating discussions. We apologise to those whose work we were unable to cite due to space considerations.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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