

# Emerging roles of pseudokinases

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**Kinases control virtually all aspects of biology. Forty-eight human proteins have a kinase-like domain that lacks at least one of the conserved catalytic residues; these proteins are therefore predicted to be inactive and have been termed pseudokinases. Here, we describe exciting work suggesting that pseudokinases, despite lacking the ability to phosphorylate substrates, are still pivotal in regulating diverse cellular processes. We review evidence that the pseudokinase STRAD controls the function of the tumour suppressor kinase LKB1 and that a single amino acid substitution within the pseudokinase domain of the tyrosine kinase JAK2 leads to several malignant myeloproliferative disorders. We also discuss the emerging functions of other pseudokinases, including HER3 (also called ErbB3), EphB6, CCK4 (also called PTK7), KSR, Trb3, GCN2, TRRAP, ILK and CASK.**

## Introduction

Protein kinases regulate the function of a large fraction of cellular proteins by catalyzing the covalent attachment of phosphate onto Ser, Thr and Tyr residues in target proteins. In recent years, genome-wide analyses have led to the complete description of the protein kinase complement of several eukaryotic organisms, including human [1], mouse [2], *Caenorhabditis elegans* [3], *Dictyostelium* [4] and yeast [5,6]. These studies have revealed that 2–3% of all eukaryotic genes encode proteins containing a kinase domain. Unexpectedly, ~10% of these proteins lack one or more of the conserved amino acids in the kinase domain that are required for catalytic activity; they are therefore predicted to be catalytically inactive. Sequence analysis of these 'pseudokinases' indicates that they lack at least one of three motifs in the catalytic domain that are essential for catalysis. The three motifs are: the Val-Ala-Ile-Lys (VAIK) motif in subdomain II of the kinase domain, in which the lysine residue interacts with the  $\alpha$  and  $\beta$  phosphates of ATP, anchoring and orienting the ATP; the His-Arg-Asp (HRD) motif in subdomain VIb, in which the aspartic acid is the catalytic residue, functioning as a base acceptor to achieve proton transfer; and the Asp-Phe-Gly (DFG) motif in subdomain VII, in which the aspartic acid binds the  $Mg^{2+}$  ions that coordinate the  $\beta$  and  $\gamma$  phosphates of ATP in the ATP-binding cleft.

## An inventory of human pseudokinases

Out of 518 protein kinases encoded by the human genome (the 'kinome'), 48 have been classified as pseudokinases [1].

The location of these proteins on the phylogenetic tree of kinases is illustrated in Figure 1 [1]. Pseudokinases are scattered throughout the distinct protein kinase subfamilies, suggesting that they have evolved from diverse active kinases. Twenty-eight pseudokinases have homologues in mouse, worms, flies and yeast that lack the equivalent catalytic residues [1,2]. We have classified the human pseudokinases into seven groups (A to G) according to which of the three motifs in their pseudokinase domain they lack (Table 1). The amino acid sequence of each pseudokinase and a description of missing conserved residues are reported in the landmark study by Manning and colleagues [1].

Figure 2 shows a comparison of the domain structures of all human pseudokinases. Several, such as isoforms of STRAD, Trb, NRBP, SgK495, Slob, VRK3 and SuRTK106 (see Glossary), have a simple structure consisting essentially of only a pseudokinase domain. Others, such as isoforms of ANP, CASK, CCK4 (also called PTK7), EphB6, EphA10 and TRRAP, are part of much larger multi-domain proteins and are likely to have functions independent of their pseudokinase domain (Figure 2). Intriguingly, the four Janus tyrosine kinases (JAK1, JAK2, JAK3 and Tyk2) and the serine/threonine kinase GCN2 have a pseudokinase domain and a functional kinase domain within the same polypeptide; the implications of this particular structure are discussed in more detail below.

Not all kinase domains that lack essential catalytic residues are inactive. For example, the four isoforms of the kinase WNK were originally classified as 'unusual' as they lacked the invariant catalytic lysine residue in the VAIK motif in subdomain II of the catalytic domain [7]. However, WNK1 is catalytically active and structural analysis of its catalytic domain demonstrates that a lysine residue in subdomain I substitutes for the missing lysine residue in subdomain II [8]. Similarly, the kinase PRPK, despite lacking the lysine residue equivalent to that missing in the WNK isoforms, is catalytically active [9]. It is possible that a lysine residue in a AVIK motif that is conserved in all eukaryotic and archaeal PRPK homologues (Lys60 in human PRPK) substitutes for the canonical lysine residue (G. Manning, personal communication).

Haspin was originally reported to lack the conserved magnesium-binding DFG motif in subdomain VII of the catalytic domain, but recently another motif, Asp-Tyr-Thr-Leu-Ser (DYTLS), has been suggested to substitute for this key motif [10] and there is biochemical evidence that haspin might function as an active kinase [11,12]. There has also been some debate as to whether titin (TTN), a protein that has over 26 000 residues, is catalytically

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## Glossary

### Pseudokinase abbreviations

**ANP:** atrionatriuretic peptide receptor (guanylate cyclase)  
**CAK1:** Cyclin-dependent kinase-activating kinase 1  
**CASK:** calcium/calmodulin-dependent serine kinase  
**CCK4:** colon carcinoma kinase 4  
**CYGF:** guanylate cyclase 2D (GUCY2D)  
**CYGF:** guanylate cyclase 2F (GUCY2F)  
**Drl:** derailed  
**Eph:** erythropoietin-producing hepatocyte (Eph); ephrin receptor  
**ErbB:** v-erb-b2 erythroblastic leukemia viral oncogene homolog  
**GCN2:** general control non-derepressible 2  
**HER:** human epidermal growth factor receptor  
**HSE:** heat stable enterotoxin receptor (GUCY2C; guanylate cyclase 2C)  
**ILK:** integrin-linked kinase  
**IRAK:** interleukin-1 receptor-associated kinase  
**JAK:** Janus kinase  
**JH:** JAK homology domains  
**JNK:** c-Jun N-terminal kinase  
**KSR:** kinase suppressor of Ras  
**MLKL:** mixed lineage kinase-like  
**NRBP:** nuclear receptor binding protein  
**OTK:** off-track kinase  
**PIKK:** phosphatidylinositol 3-kinase-related kinase  
**PRPK:** p53-related protein kinase  
**PSKH:** protein serine kinase H  
**PTK7:** protein tyrosine kinase 7  
**RSKL:** ribosomal protein S6 kinase-like  
**Ryk:** receptor-like tyrosine kinase  
**Sgk:** sugen kinase  
**STRAD:** STE20-related adaptor protein  
**SuRTK106:** serine threonine tyrosine kinase 1 (STYK1)  
**TBCK:** tubulin-binding cofactor kinase  
**TRRAP:** transactivation-transformation domain-associated protein  
**Trb:** Tribbles homolog

**TTN:** giant muscle filament protein titin  
**ULK:** UNC (uncoordinated)-51-like kinase  
**VACAMKL:** vesicle-associated CaM kinase-like  
**VRK:** vaccinia-related kinase

### Other abbreviations

**ATF:** activating transcription factor  
**ATM:** ataxia-telangiectasia mutated kinase  
**CDC:** cell division cycle  
**CHOP:** C/EBP homologous protein  
**COP1:** constitutive photomorphogenic protein-1  
**DNA-PK:** DNA-dependent protein kinase  
**EGFR:** epidermal growth factor receptor  
**ERK:** extracellular signal-regulated kinase  
**FERM:** band F ezrin-radixin-moesin homology domains  
**HEAT:** Huntington, elongation factor 3, PR65/A, TOR domain  
**IFN- $\gamma$ :** interferon- $\gamma$   
**IL:** interleukin  
**LIM:** Lin-11, Isl-1, Mec-3 homology domains  
**MAPK:** mitogen-activated protein kinase  
**MEK:** MAPK/ERK kinase  
**MO25:** mouse protein 25  
**mTOR:** mammalian target of rapamycin  
**PDZ:** PSD-95 (post-synaptic density protein 95), discs large protein, ZO-1 (zonula occludens protein 1) homology domains  
**PI3-K:** phosphatidylinositol 3-kinase  
**PKC:** protein kinase C  
**PTB:** phosphotyrosine binding domain  
**RTK:** receptor protein-tyrosine kinase  
**SAM:** sterile alpha motif  
**SH:** Src homology  
**STAT:** signal transducers and activators of transcription  
**WNK:** with-no-K(Lys) kinase  
**ZAP-70:** zeta-chain-associated protein kinase-70

active. All vertebrate homologues of titin contain a glutamic acid residue instead of an aspartic acid at the DFG motif in subdomain VII, whereas this residue is an aspartic acid in invertebrate titin-like kinases. The 3D structure of the catalytic domain of titin reveals that it is an active kinase that is maintained in an auto-inhibited conformation and is activated by phosphorylation of a tyrosine residue in the kinase domain and through interaction with  $\text{Ca}^{2+}$  and calmodulin [13,14].

Similarly, there is uncertainty about whether the Wnt receptor tyrosine kinase Ryk, which regulates axon guidance, is a pseudokinase. Mammalian Ryk and its *Drosophila* homologue Derailed (Drl), rather than having a DFG motif in subdomain VII, have Asp-Asn-Ala (DNA; human) or Asp-Ser-Ala (DSA; *Drosophila*). Mutation of the DNA motif in Ryk to DFG reportedly restores catalytic activity [15]. Furthermore, mutation of the invariant lysine residue in subdomain II of Drl does not affect its ability to drive axon guidance in *Drosophila*, indicating that Ryk/Drl kinases do not need intrinsic kinase activity to regulate axon guidance [16]. As Ryk and Drl still have the crucial Asp residue in subdomain VII, and thus have the potential to phosphorylate substrates, we and others [1] have not classified them as pseudokinases.

It should be noted that protein kinases are well known to have functional roles that are independent of their ability to phosphorylate substrates. The human EGF receptor tyrosine kinase (pseudokinase) HER3 (also called ErbB3) and its homologues in dog and zebrafish lack the catalytic aspartic acid residue in their HRD motifs and are inactive. Curiously, mouse HER3 has an aspartic acid residue in its HRD motif but is still inactive, suggesting

that it lacks other residues that are required for kinase activity [2]. Attempts to convert human HER3 to an active kinase by reinstating the HRD motif and other potentially lacking residues have failed to restore activity [17].

It is possible that some of the 48 pseudokinases listed in Figures 1 and 2, many of which have not been studied, might turn out to have catalytic activity when analyzed. Caution should be taken when expressing a pseudokinase in mammalian cells and assessing whether it has catalytic activity. As discussed below, several pseudokinases form complexes with active protein kinases that might be responsible for any activity measured with pseudokinases purified from eukaryotic cells. Here, we review the roles of some of the better studied pseudokinases.

### Pseudokinases similar to receptor tyrosine kinases

Receptor protein-tyrosine kinases (RTKs) are transmembrane proteins made up of an extracellular ligand-binding domain and an intracellular tyrosine kinase domain. RTKs dimerize upon ligand binding, which results in the intermolecular autophosphorylation and activation of their cytoplasmic domains. This, in turn, leads to the recruitment of phosphotyrosine-binding signalling components to the plasma membrane and triggers the activation of signalling cascades that regulate diverse cellular processes, including proliferation, survival, apoptosis and migration [18]. Gain-of-function mutations of several RTKs are frequently observed in cancer [19]. Four RTKs are classified as pseudokinases: the EGF receptor family member HER3, the ephrin receptors EphA10 and EphB6 and the orphan receptor CCK4, which is overexpressed in several cancers.





**Table 1. Classification of pseudokinases into seven groups based on the catalytic motifs that they lack**

Group	A	B	C	D	E	F	G
Motifs missing	DFG	HRD	DFG, HRD	VAIK	DFG, VAIK	HRD, VAIK	DFG, HRD, VAIK
Pseudokinases	CASK	ANP $\alpha$	ILK	KSR1	ULK4	GCN2	EphA10
	CCK4	ANP $\beta$	IRAK2	KSR2	RSKL1		EphB6
	SgK223	CYGD	MLKL		RSKL2		NRBP1
	SgK269	CYGF	SgK307				NRBP2
	SgK495	HER3	STRAD $\beta$				SCYL1
	SuRTK106	HSER					SCYL2
	Trb1	JAK1					SCYL3
	Trb2	JAK2					SgK196
	Trb3	JAK3					SgK424
		PSKH2					Slob
		SgK071					STRAD $\alpha$
		SgK396					TBCK
		TYK2					TRRAP
		VACAMKL					VRK3

### HER3

The pseudokinase HER3 is one of the four members of the EGF receptor family, which also includes the active tyrosine kinases HER1 (also called EGFR or ErbB1), HER2 (also called Neu or ErbB2) and HER4 (also called ErbB4; Figure 1). The interaction of EGF family RTKs with their ligands induces their clustering and dimerization, triggering the activation of signalling cascades that promote cell proliferation and survival. Overexpression and activating mutations of all four EGF receptors are found in human cancers [20], and HER1 and HER2 are targets of approved anticancer therapeutics [21]. Upon binding to its ligand neuregulin, HER3 forms a heterodimeric complex with the active kinase HER2. This results in the inactive HER3 pseudokinase domain interacting with the functional tyrosine kinase domain of HER2 and stimulating the autophosphorylation and activation of the HER2 tyrosine kinase domain [22,23] (Figure 3a). The neuregulin-induced interaction of HER3 with HER2 stimulates signalling cascades, such as the PI3-kinase and ERK signalling pathways, that tumour cells rely upon for their development, proliferation and survival [22]. Interestingly, although HER2 has a functional kinase domain, it cannot interact with the neuregulin ligand and is therefore dependent upon heterodimerization with the neuregulin-binding HER3 to become activated. A recent study has elegantly shown that, in the absence of ligand, HER2 is maintained in an inhibited conformation that is converted into an active conformation following ligand-induced heterodimerization with HER3 through an allosteric mechanism, as summarized in Figure 3a [24].

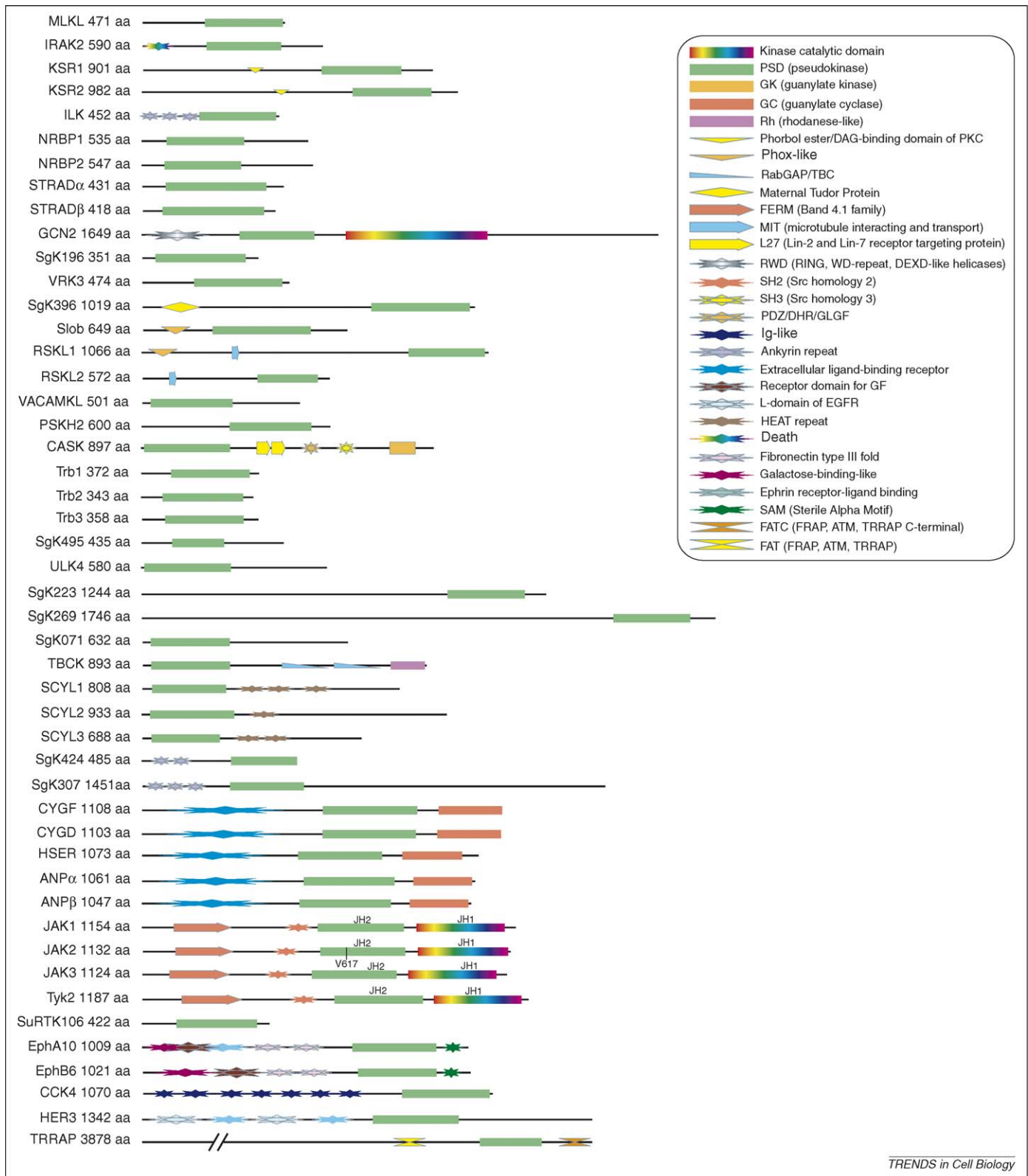
### EphB6

Erythropoietin-producing hepatocyte (Eph) kinases are the largest family of receptor tyrosine kinases. Their ligands, called ephrins, are located on the surfaces of adjacent cells. These kinases guide cell pattern formation during tissue and organ development [25]. Of the 14 members of the Eph family, two (EphA10 and EphB6) are predicted pseudokinases. Although little is known about EphA10, EphB6 has been shown not to have intrinsic tyrosine kinase activity and to be predominantly expressed in T cells [26]. Mice lacking EphB6 develop normally but have defects in T cell function, such as compromised

proliferation and secretion of lymphokines (IL-2, IL-4 and IFN- $\gamma$ ), thus indicating a role for EphB6 in T cell development in the immune system [27]. EphB6, despite lacking tyrosine kinase activity, can still trigger activation of the tyrosine kinase ZAP-70 upon ephrin binding [27]. Mechanistically, it is not known how this occurs, and it has not been tested whether EphB6-induced phosphorylation of ZAP-70 requires the pseudokinase domain. By analogy with HER3, it is possible that EphB6 exerts its effects by forming heterodimers with other Eph receptor kinases, such as EphB1, which has been reported to interact with EphB6 [28], and/or other signalling components.

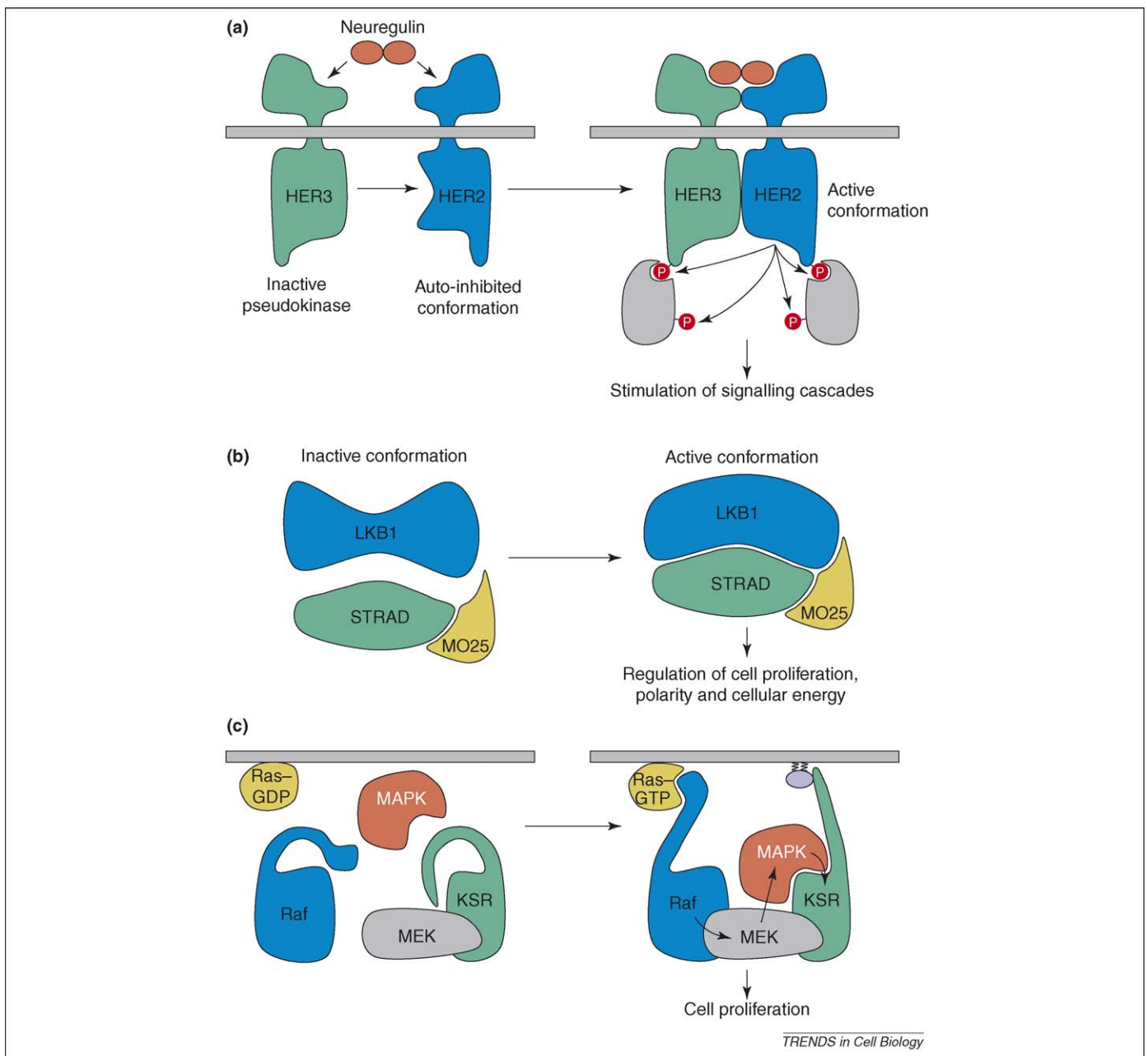
### CCK4

The pseudokinase CCK4 was originally identified as a protein overexpressed in several cancer cell lines, including melanoma [29] and colon cancer [30] lines. It is made up of a signal peptide, seven immunoglobulin domains, a single transmembrane region and a C-terminal domain with homology to tyrosine kinases. The ligand for CCK4 has not yet been identified. Mouse embryos expressing a truncated form of CCK4 consisting of only the first 114 residues (which does not include the pseudokinase domain) die perinatally, with profound defects in neural tube closure and orientation of the stereociliary bundles in the inner ear, indicating that this pseudokinase is involved in the regulation of polarity within planes of epithelial cells [31]. The specific role of the pseudokinase domain of CCK4 in this process has not been established. The *Drosophila* homologue of CCK4, OTK, has been implicated in plexin signalling during axon guidance [32]; it is not known whether the mammalian homologue has a similar role. To learn more about CCK4, it will be important to define whether it interacts with other receptor tyrosine kinases and/or signalling components. Detailed sequence analysis of the CCK4 pseudokinase domain has also shown that it is highly conserved throughout evolution and is predicted to lack activity in all species examined, including organisms such as *Hydra*, which occupies a near-basal position in the metazoan radiation [33]. This indicates that, despite lacking activity and a known role, the precise pseudokinase domain structure and the nature of the residues in this domain are nevertheless likely to have crucial roles.



**Figure 2.** Comparison of the domain structures of human pseudokinases. The figure summarizes the results obtained by interrogating InterPro using default parameters [70] with the full-length pseudokinase protein sequences. InterPro integrates the major databases of protein families, domains and functional site signatures, such as SUPERFAMILY, Pfam, PRINTS and PROSITE. Results were manually inspected and drawn to scale with gff2ps [71]. The major groups of accessory domains include catalytic activity (boxes); lipid-binding domains (down-arrows); domains that link to GTPase (wedges); RNA-binding domains (diamonds); targeting domains (right-arrows); protein-protein interaction domains (stars); and intracellular sensor domains (pairs of triangles).





**Figure 3.** Schematic models for the functions of HER3, STRAD and KSR. **(a)** In the absence of ligand, the catalytically active receptor HER2 is maintained in an inactive auto-inhibited monomeric conformation. Binding of the ligand neuregulin to HER3 induces formation of a heterodimeric complex between HER3 and HER2, in which the HER3 pseudokinase domain allosterically activates the functional tyrosine kinase domain of HER2. This results in HER2 phosphorylating itself as well as HER3, leading to recruitment of proteins containing SH2 and PTB domains to the receptor complexes, which are subsequently phosphorylated by the activated receptor. This leads to the activation of downstream signalling pathways that stimulate cell proliferation and survival. **(b)** The catalytic activity of the tumour suppressor kinase LKB1 is increased over 100-fold following the interaction of its catalytic domain with the pseudokinase domains of STRAD $\alpha$  and STRAD $\beta$ . The scaffolding proteins MO25 $\alpha$  and MO25 $\beta$  stabilize the LKB1–STRAD interaction by interacting with the C-terminal residues of STRAD proteins and an as-yet unidentified region of LKB1. **(c)** In quiescent cells, inactive GDP-bound Ras is located at the plasma membrane, while inactive MEK associated to the pseudokinase domain of KSR and inactive Raf are located in the cytoplasm. In response to extracellular signals, Ras binds to GTP, which induces translocation of Raf to the plasma membrane where it becomes activated. Upon Ras activation, KSR also translocates to the plasma membrane via its cysteine-rich zinc-finger domain, bringing inactive MEK into close proximity with its upstream activator Raf. MEK is consequently phosphorylated and activated by Raf. The membrane localization of KSR exposes MAPK docking sites, resulting in MAPK binding to KSR and being phosphorylated by activated MEK. KSR also serves as a substrate for MAP kinases.

### The roles of the pseudokinases STRAD, JAK and GCN2 in the regulation of the activity of functional kinases

A picture is emerging in which several pseudokinases function similarly to HER3, binding directly to the kinase domain of functional protein kinases and thereby controlling their activity.

### STRAD $\alpha$ and STRAD $\beta$

The pseudokinases STRAD $\alpha$  and STRAD $\beta$ , also called STK5 and STK6, share a high degree of sequence similarity to the STE20 family of protein kinases. These enzymes were originally thought to be active upstream kinases that triggered the activation of the p38 and JNK

MAP kinase pathways [34], before it was realized that they lack the DFG motif and the aspartic acid residue within the HRD motif, and they were shown experimentally to lack catalytic activity [35]. Recent work has shown that STRAD $\alpha$  and STRAD $\beta$  form heterotrimeric complexes with isoforms of the scaffolding protein MO25 and the tumour suppressor protein kinase LKB1, which regulates cell proliferation, polarity and cellular energy levels [36]. Unlike most kinases, LKB1 is not activated by phosphorylation of its activation loop by an upstream kinase, but is instead activated upon binding to STRAD proteins [35,37]. The pseudokinase domain of the STRAD proteins binds directly to the kinase domain of LKB1, enhancing its catalytic activity over 100-fold, and also functions to anchor LKB1 in the cytosol [35,36]. The molecular mechanism by which the STRAD proteins activate LKB1 has not yet been elucidated, but it is possible that the LKB1–STRAD interaction leads to a conformational change of LKB1 that stabilizes it in an active conformation (Figure 3b). Isoforms of MO25 interact with the C-terminal residues of STRAD and stabilize the interaction between LKB1 and the STRAD proteins [38]. Interestingly, several single amino acid substitution mutants of LKB1 isolated from human cancers have lost the ability to interact with STRAD $\alpha$  and are unable to induce a G1 cell cycle arrest when overexpressed in cells, emphasizing the importance of the STRAD–LKB1 interaction [35,37].

Curiously, STRAD $\alpha$  interacts with ATP and ADP with high affinity ( $K_d \sim 30\text{--}100 \mu\text{M}$ ) [37]. The functional significance of this is unclear, as mutations that prevent STRAD from binding ATP do not affect its ability to activate LKB1 or induce its cytoplasmic localization [37]. It has been speculated that the STRAD proteins evolved from an active protein kinase that perhaps once controlled LKB1 [37]. However, there is no evidence as yet to support this idea, as all vertebrate and sea urchin homologues of STRAD also lack catalytic residues. Moreover, attempts at restoring the catalytic activity of STRAD $\alpha$  by mutating residues back to those found in active kinases failed to reactivate STRAD $\alpha$ , suggesting that other residues are lacking in STRAD $\alpha$  that prevent it from phosphorylating substrates [37].

### JAKs

The Janus tyrosine kinases (JAKs) are associated with cell surface cytokine receptors and are activated upon binding of cytokines to these receptors. Activated JAKs phosphorylate the cytoplasmic domain of the receptor, creating recruitment sites for signalling proteins, such as STATs (signal transducers and activators of transcription). STATs are phosphorylated by JAKs, which results in their dimerization and translocation to the nucleus where they regulate target gene expression. The JAK isoforms (JAK1, JAK2, JAK3 and Tyk2) have a C-terminal functional catalytic domain (JH1) that is preceded by a pseudokinase (JH2) domain as well as an N-terminus comprising a FERM domain (Figure 2). The JH2 pseudokinase domain binds directly to the JH1 catalytic domain and regulates its activity [39,40].

Mutagenesis and molecular modelling analysis suggest that one role of the JH2 domain is to keep the kinase

domain inactive in the absence of cytokines [41,42]. In support of this idea, analysis of *Drosophila* JAK identified a point mutation (E696K) within the JH2 pseudokinase domain that activated the enzyme [39]. Striking recent findings in humans have identified an amino acid substitution (V617F) in the JH2 pseudokinase domain of JAK2 in most patients with polycythemia vera and in a significant number of cases of essential thrombocythemia as well as chronic idiopathic myelofibrosis [43]. These malignant myeloproliferative disorders are characterized by the clonal overproduction of relatively normally differentiated hematopoietic lineages. The V617F substitution leads to constitutive activation of JAK2 and downstream effector signalling pathways including the STAT5 transcription pathway and the PI3-kinase and ERK signalling networks, which induce inappropriate cytokine-independent proliferation of cells [43]. Interestingly, the homologous mutations in JAK1 (V658F) and Tyk2 (V678F) also lead to the constitutive activation of these kinases, suggesting a common mechanism by which the JH2 pseudokinase domains control the catalytic activity of these enzymes [44]. By contrast, the homologous M592F mutation in JAK3 does not lead to activation, suggesting that JAK3 is regulated differently from other JAK isoforms [44]. The molecular mechanism by which mutations within the JH2 pseudokinase domain of JAKs activate these enzymes is unknown. It is possible that these mutations disrupt an auto-inhibitory interaction between the JH2 and JH1 domains, or induce an activating JH2–JH1 interaction, or this might even convert the JH2 domain into an active tyrosine kinase domain. Despite intensive effort, no crystallographic structure of the JH1–JH2 domains fragment of any JAK isoform has been reported; this would be a prerequisite to defining how the JH2 pseudokinase domain exerts its influence on JAK catalytic activity.

### GCN2

Phosphorylation of translation initiation factor 2- $\alpha$  (eIF2- $\alpha$ ) is an important mechanism for down-regulating protein synthesis in response to nutrient starvation conditions. The kinase GCN2 is one of the eIF2- $\alpha$  kinases that are activated by nutrient deprivation. Like JAKs, GCN2 has a pseudokinase domain N-terminal to its catalytic domain (Figure 2). The pseudokinase domain of GCN2 interacts with the catalytic domain and inhibits its catalytic activity under non-starvation conditions [45]. This interaction has also been proposed to have a role in activating GCN2 under starvation conditions [46]. Further work is required to define the mechanism by which the interaction between the pseudokinase and active kinase domains of GCN2 controls its catalytic activity.

### Pseudokinases that have scaffolding roles

Another emerging picture is that many pseudokinases have scaffolding roles and participate in the assembly of multi-protein complexes, as many pseudokinases, such as KSR, TRRAP, the Trb isoforms, ILK and CASK, contain protein–protein interaction domains in addition to their pseudokinase domain.

### KSR

KSR was originally identified as a suppressor of an activated Ras phenotype in *Drosophila* and *C. elegans*. Although there has been some debate as to whether KSR1 or the related protein KSR2 have an active catalytic domain, the bulk of the evidence indicates that they are devoid of activity [47,48]. KSR1 and KSR2 function as scaffolding proteins, interacting with the Raf, MEK and ERK protein kinases to form a membrane-localized regulatory complex that coordinates signal propagation through the ERK pathway, which is crucial in Ras signal transduction [47,48] (Figure 3c). The pseudokinase domains of KSR1 and KSR2 are related to the catalytic domain of Raf-1, which interacts with KSR1 and KSR2, but the functional significance of this and how the KSR pseudokinase domain exerts its influence on recruitment and activation of components of the ERK signalling pathway is not known.

### TRRAP

A single member of the atypical protein kinase PIKK subfamily, TRRAP, which is homologous to the DNA-damage repair kinases ATM and DNA-PK, has an inactive C-terminal pseudokinase domain that lacks the catalytic residues in its orthologues in all eukaryotic organisms [49]. Like other PIKK subfamily members, TRRAP is a large protein (~400 kDa). TRRAP and its yeast homologue Tra1 function as essential components of most histone acetyltransferase complexes, which have crucial roles in regulating chromatin remodelling and gene expression [50]. TRRAP also associates with several transcription factors, including c-Myc and E2F1 [50], and controls repair of double-strand DNA breaks [51]. Genetic disruption of TRRAP in mice resulted in early embryonic lethality and defects in cell cycle progression [52]. The importance of the pseudokinase domain of TRRAP in regulating its functions has not been addressed. It would be important to define whether the pseudokinase domain of TRRAP is required for the regulation of chromatin remodelling and gene expression, or whether it has other roles.

### Trb3

The three isoforms of Trb are short proteins that are the mammalian orthologues of *Drosophila* Tribbles, a protein that inhibits mitosis early in development. Tribbles binds to the CDC25 homolog String, a key regulator of cell division and DNA damage repair, and promotes its ubiquitination and proteasome-mediated degradation [53]. All three mammalian Trb isoforms as well as Tribbles lack catalytic residues. Trb3 is highly expressed in some human lung, colon and breast tumours [54], and it has been suggested to interact with numerous proteins, including the transcription factors CHOP and ATF4, thereby inhibiting their transcriptional activity [55,56]. A recent study has shown that the expression of Trb3 is increased under fasting conditions in the adipose tissue, where it functions to inhibit lipid synthesis by stimulating the degradation of the rate-limiting enzyme of fatty acid synthesis, acetyl-coenzyme A, through an interaction with the COP1 E3 ubiquitin ligase [57]. Trb3 has also been reported to suppress insulin signal transduction by inhibiting the activity

of the protein kinase Akt [58,59]. However, a recent study has disputed the role of Trb3 in regulating Akt [60]. Despite this analysis, no clear molecular picture of how Trb isoforms work or function as scaffolds has emerged.

### ILK

The Integrin-linked (pseudo)kinase (ILK) was first identified in a yeast two-hybrid screen as a protein that interacted with the cytoplasmic tail of  $\beta$ 1-integrin. Although ILK lacks the HRD and DFG motifs in its pseudokinase domain, there has been considerable debate as to whether it has catalytic activity, following a report that it could phosphorylate Akt at one of its activating residues (Ser473) [61]. However, the phosphorylation of Akt at Ser473 is now generally accepted to be regulated by the protein kinase mTOR [62], rather than ILK. Consistent with this, phosphorylation of Akt at Ser473 is unaffected in ILK-deficient fibroblasts [63] and chondrocytes [64]. Supporting the notion that ILK is a pseudokinase, we have also not been able to demonstrate that ILK can phosphorylate any substrate tested, including Akt or itself (D.R.A., unpublished). The N-terminal domain of ILK contains ankyrin repeats that interact with LIM domain-containing adaptor proteins of the PINCH family. ILK also interacts through its C-terminal region with parvin to form a ternary complex termed IPP. The assembly of the IPP complex has been implicated in the control of many aspects of cell behaviour and morphology, including actin-cytoskeleton dynamics, cell adhesion, spreading, migration, polarity and cell-cell contact (reviewed in [65]). IPP exerts its effects by forming a scaffolding platform, linking integrins at the surface of cells with the actin cytoskeleton and other signalling pathways. The precise role of the pseudokinase domain of ILK in regulating these functions has not been defined. Loss of ILK in mice results in early embryonic lethality (at embryonic day E5.5–E6.5) [63].

### CASK

The pseudokinase CASK has an N-terminal pseudokinase domain with similarity to the calcium/calmodulin-dependent protein kinase domains, an inactive C-terminal guanylate kinase domain, as well as several PDZ domains and two SH3 domains. CASK forms complexes with proteins regulating presynaptic and postsynaptic processes [66,67]. Consistent with this, genetic studies indicated that *Drosophila* CAKI (or CMG), a homologue of human CASK, has an essential role in controlling the release of neurotransmitter vesicles at synapses [68]. CASK also interacts with Tbr-1, a T-box transcription factor that is involved in forebrain development [69].

### Conclusions and perspectives

Although much remains to be learnt about the intricate functions of pseudokinases, the initial studies outlined here suggest that, despite lacking catalytic activity, these proteins are not uninteresting and are likely to have important roles in regulating diverse fundamental processes relevant to understanding human disease. To our knowledge, no 3D structure of any pseudokinase has been reported, so in future it will be important to attempt structural analysis of these proteins. This will be



particularly crucial for defining the mechanism by which the HER3, STRAD, JAKs and GCN2 pseudokinase domains regulate the activity of functional protein kinase domains. It will also be important to undertake systematic screens to identify protein-binding partners of pseudokinases, as a key role of some of these proteins is clearly to participate in multi-protein complexes. It would be fascinating to understand why pseudokinases evolved and whether it would be possible to introduce mutations that restore catalytic activity to pseudokinases to explore their ancestral roles. It would also be interesting to undertake more detailed sequence analysis of homologues of pseudokinases in primitive eukaryotic species, to determine whether any of these proteins evolved from active protein kinases.

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