The FEBS Journal

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

Pseudophosphatase MK-STYX: the atypical member of the MAP kinase phosphatases

Shantá D. Hinton

Department of Biology, Integrated Science Center, William & Mary, Williamsburg, VA, USA

Keywords
dual-specificity phosphatase; MAPK phosphatase; MAPK phosphoserine/threonine/tyrosine-binding protein; mitogen-activated protein kinase; phosphoserine/threonine/tyrosine-interacting protein; protein tyrosine phosphatase; pseudoenzyme; pseudophosphatase

Correspondence
S. D. Hinton, Department of Biology, Integrated Science Center, William & Mary, 540 Landrum Drive, Williamsburg, VA 23185, USA
Tel: +1 757 221 2587
E-mail: sdhinton@wm.edu

(Received 18 December 2019, revised 25 April 2020, accepted 26 May 2020)

doi:10.1111/febs.15426

The regulation of the phosphorylation of mitogen-activated protein kinases (MAPKs) is essential for cellular processes such as proliferation, differentiation, survival, and death. Mutations within the MAPK signaling cascades are implicated in diseases such as cancer, neurodegenerative disorders, arthritis, obesity, and diabetes. MAPK phosphorylation is controlled by an intricate balance between MAPK kinases (enzymes that add phosphate groups) and MAPK phosphatases (MKPs) (enzymes that remove phosphate groups). MKPs are complex negative regulators of the MAPK pathway that control the amplitude and spatiotemporal regulation of MAPKs. MK-STYX (MAPK phosphoserine/threonine/tyrosine-binding protein) is a member of the MKP subfamily, which lacks the critical histidine and nucleophilic cysteine residues in the active site required for catalysis. MK-STYX does not influence the phosphorylation status of MAPK, but even so it adds to the complexity of signal transduction cascades as a signaling regulator. This review highlights the function of MK-STYX, providing insight into MK-STYX as a signal regulating molecule in the stress response, HDAC 6 dynamics, apoptosis, and neurite differentiation.

Introduction

Pseudoenzymes were described more than fifty years ago [1], and genomics has revealed that approximately ten percent of the proteins encoded in the human genome are pseudoenzymes [2–4]. The evolutionary conservation of these catalytically inactive homologs and their widespread existence (they can be found among the bacterial luciferases, bacterial and eukaryotic kinases, phosphatases, proteases, GTPases, synthetases, etc.) underlines the importance of understanding their functions [2,4,5]. Although use of the 'pseudo' prefix inhibited their investigation for many years, by implying a lack of function, in the past ten years, they have become appreciated and viewed as relevant and important signaling molecules—highlighting the necessity of this special issue on pseudoenzymes.

Early on, Jack Dixon's laboratory coined the term 'STYX' (phosphoserine/threonine/tyrosine-interacting protein) to designate the phosphotyrosine-binding domain of a pseudophosphatase [6]. Use of a point mutation to 'restore' catalytic activity in the STYX domain has proved to be a helpful tool to initiate the process of investigating molecules that contain STYX domains. Knockout mouse studies revealed that STYX associates with a unique RNA-binding phosphoprotein

Abbreviations
CH2, cell division cycle 25 phosphatase homology 2; DUSP, dual-specificity phosphatase; FBXW7, F-box and WD repeat domain containing 7; HDAC6, histone deacetylase 6; MAPK, mitogen-activated protein kinase; MKP, MAPK phosphatase; MK-STYX, MAPK phosphoserine/threonine/tyrosine-binding protein; MTM, myotubularin; PTP, protein tyrosine phosphatase; STYX, phosphoserine/threonine/tyrosine-interacting protein.

in spermatids termed CRHSP-24 (calcium-responsive heat-stable protein with a molecular mass of 24 kDa) that has an essential function in spermatogenesis [7]. Furthermore, cellular, siRNA, and biochemical assays revealed the role of STYX in cell fate and migration, as a signaling regulator of the ERK 1/2 MAPK signaling pathway by competing with MAP kinase phosphatase-2. STYX was also found to be a signaling regulator in ubiquitinylation that regulates the ubiquitin ligase SCF (SKP/CUL1-F-box) complex by interacting with FBXW7 (F-box and WD repeat domain containing 7) [8]. Furthermore, when misregulated, STYX plays an oncogenic role by inhibiting the function of FBXW7, resulting in metastasis and colorectal cancer [9].

Eyer's and Farhan's research teams provide an extensive and excellent review of all the pseudophosphatases, including the protein tyrosine phosphatases (PTPs) [10]. STYX domains of PTPs highlight that many pseudophosphatases have mutations within their signature active site motif (HCX_5R) that renders them inactive [4,6,11–13]. The majority of mutations lead to catalytically inactive phosphatases, but not all [14]. In addition, not all pseudophosphatases have a mutation in their active signature motif [10]; for example, the myotubularins (MTM) have mutations within their SET domain [12]. Myotubularins, which are the most prevalent pseudophosphatases, form complexes with their active homolog, resulting in enhanced phosphatase activity [15–17]. Moreover, a mutation in either the myotubularin or its active homolog leads to the Charcot–Marie–Tooth disease (neurological demyelination disorder) [11,15–17]. These mutations may render the phosphatase catalytically inactive, while maintaining the three-dimensional fold and the ability to bind phosphorylated proteins [4,12,18]. More importantly, numerous studies demonstrate that pseudophosphatases are *bona fide* signaling regulators of many cellular processes such as spermatogenesis, cell fate, migration, ubiquitylation, demyelination, oocyte-to-zygote transition, transcription, stress response, apoptosis, and neuronal differentiation [10,18–22]; affirming that the paradigm has shifted from the notion of pseudophosphatases simply serving as dominant-negative antagonists of endogenous protein phosphatases [4,12,18,23]. In addition, misregulation of pseudophosphatases has been implicated in the etiology of various diseases such as leukemia, breast cancer, Ewing sarcomas, obesity, Charcot–Marie–Tooth disorder, and neurological disorders [4,10,24,25].

This review focuses on a MK-STYX, a pseudophosphatase member of the MKP [mitogen-activated protein kinase (MAPK) phosphatase (MKP)] subfamily, and its role as a signaling regulator, beyond the role of its active homologs (MKPs), to negatively regulate MAPK. A foundation is established for why the interactors (binding partners) of MK-STYX differ from the MAPK substrates of MKPs, the active homologs [26] of MK-STYX. Moreover, MK-STYX serves as an essential regulator in signaling pathways such as apoptosis, the stress response, and neurite formation [4,13,27–29].

MAP kinase phosphatases

Mitogen-activated protein kinase phosphatases, members of the dual-specificity phosphatase (DUSP) subfamily, dephosphorylate both threonine/serine and tyrosine residues within the TXY activation loop of MAPKs [30,31]. The dual-specificity results in the ability of these enzymes to accommodate both phosphothreonine and phosphotyrosine residues in their shallow enzymatic pockets [30]. MKPs consist of eleven mammalian members (10 catalytically active MKPs and one atypical noncatalytically active member, MK-STYX) [4,6,13,26,32]. They all possess a C-terminal catalytic phosphatase domain and an N-terminal noncatalytic domain composed of two CDC25 (cell division cycle 25)/rhodanese homology (CH2/rhodanese) domains [32–34] (Fig. 1). It is noteworthy that the rhodanese domain (rhodaneses are sulfurtransferases) is inactive in MKPs [33,34], but still has a role in modulating signaling. The C-terminal DUSP domain has conserved aspartic acid, arginine, and cysteine residues within the catalytic active site [26,30], while the N-terminal noncatalytic domain has intervening clusters of basic amino acids [26,30]. These clusters make up the kinase-interacting domain (KIM) (Fig. 1), which positions the MAPK substrate for effective catalysis [26,34]. Thus, the KIM domain serves as the modular docking site for specific MAPK isoforms, which determines the subcellular localization of the phosphatase [26,35]. MKPs are differentiated by their subcellular localization, sequence homology, and substrate specificity into three subfamilies: nuclear, cytoplasmic, and both nuclear and cytoplasmic [26,36,37]. The prototypical mammalian MKP, DUSP-1/MKP-1, has a nuclear localization signal (NLS), rendering it nuclear (Fig. 1A). DUSP-1/MKP-1 is a mitogen-activated and stress-inducible phosphatase that dephosphorylates MAPK in the nucleus [38,39]. DUSP-6/MKP-3 has a nuclear export signal (NES), which localizes it to the cytoplasm (Fig. 1A), where it regulates extracellular signal-regulated kinases (ERKs). MKPs that function in both the nucleus and cytoplasm include the JNK (c-Jun amino terminus kinase)/

p38-specific activated phosphatases [26,36,37]. These various MAPK-MKP interactions demonstrate that MKPs are essential regulators of MAPK signaling.

Intriguingly, MKPs may recognize a single class of MAPKs and inactivate them, as is the case with DUSP-6/MKP-3, which dephosphorylates the ERKs [35]. Or, MKPs can regulate multiple MAPK pathways such as nuclear DUSP-1/MKP-1. DUSP-1/MKP-1 dephosphorylates ERKs, c-Jun amino terminus kinase, and p38 [35,40]. These multiple and distinctive levels of regulation illustrate the complicated regulation of MAPKs by MKPs.

MK-STYX/DUSP-24

Considering that MK-STYX/DUSP24 has homology to DUSP-1/MKP-1 and DUSP-6/MKP-3, initial investigations addressed whether MK-STYX regulated the MAPK substrates of these active homologs. MK-STYX/DUSP24 is an inactive MKP [4,12,13,41] that is also referred to as STYXL-1 (serine/threonine/tyrosine-interacting like protein-1) [13,24]. For simplicity, it will be referred to as MK-STYX from this point. MK-STYX lacks the critical cysteine in its active site signature motif (HCX_5R), which is essential for phosphatase activity [4,12,13,41]. It has a phenylalanine and serine (FSTQGISR) in this signature motif (Fig. 1A), replacing histidine and the essential cysteine, respectively. This results in MK-STYX being catalytically inactive [4,12,13,41]. In addition, MK-STYX has a presumed kinase interaction motif (KIM), because it has a CH2/rhodanese domain [4,12,13]. KIM binds MAPK/ERK1/2 [33] (Fig. 1A). However, studies suggest that MK-STYX does not interact with or modulate MAPK/ERK1/2 activation. When HEK/293 cells were stimulated with epidermal growth factor and the phosphorylation of MAPK/ERK1/2 was assessed, MK-STYX did not change MAPK/ERK1/2 phosphorylation (unpublished data). This finding was validated by Niemi et al. [4,23,27,42], further complicating the elucidation of the role of MK-STYX.

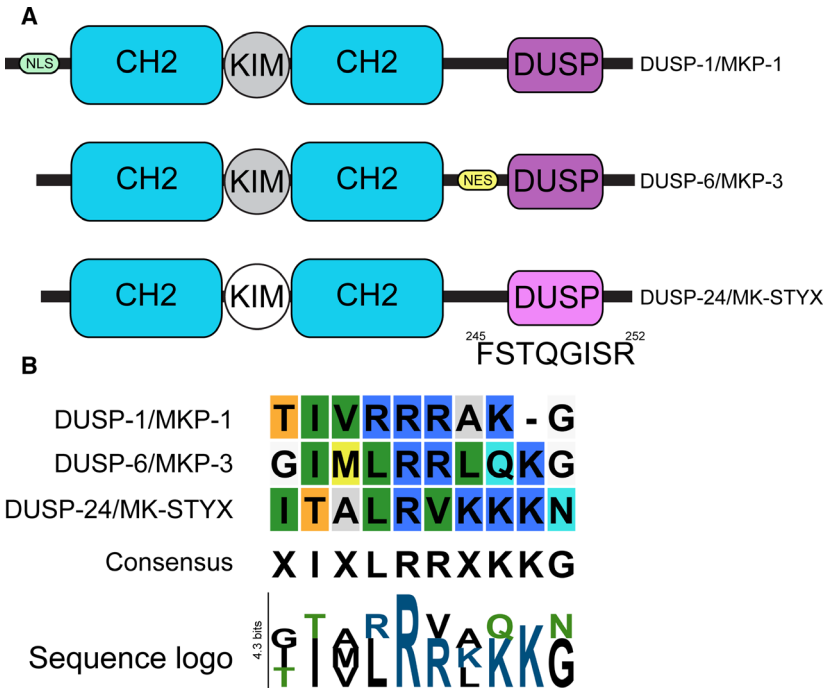

Fig. 1. Comparison of the structure of pseudophosphatase MK-STYX to that of active homologs DUSP-1/MKP-1 and DUSP-6/MKP-3. All have a C-terminal CH2/rhodanese domain interrupted by a kinase-interacting (docking) motif (KIM) and a N terminus DUSP (dual-specificity phosphatase) domain. (A) Structural depiction of DUSP-1/MKP-1(MAPK phosphatase-1) that has a nuclear localization signal to locate it to the nucleus; DUSP-6/MKP-3 that has a nuclear export signal keeping it cytosolic; DUSP-24/MK-STYX (MAPK phosphoserine/threonine/tyrosine-binding protein) has a mutated DUSP domain in the signature active site motif (instead of HCX_5R required for catalysis) and a mutated KIM domain (required for MAPK docking). (B) Alignment of DUSP-24/MK-STYX, DUSP-1/MKP-1, and DUSP-6/MKP-3 shows that DUSP-24/MK-STYX lacks essential arginines required for MAPKs to dock to MAPK phosphatases. The lower panel depicts the sequence logo to compare the consensus sequence for the KIM, which shows that MK-STYX has only one conserved amino acid (R) within the KIM. The sequence logo was built by Weblogo and is at 4.3 bits.

The protein basic local alignment search tool (BLAST) revealed additional mutations of MK-STYX within the KIM domains, relative to its active homologs. MK-STYX is missing critical arginine residues required for MAPK/ERK docking [35,43] (Fig. 1B). These positively charged arginine residues bind negatively charged aspartic acid residues when docking to proteins such as MAPK [44]. The KIM motif of DUSP-1/MKP-1 consists of three consecutive arginines, whereas the DUSP-6/MKP-3 consists of two repetitive arginines (Fig. 1B) [35]. MK-STYX lacks two arginines; the KIM contains one arginine, a valine, and a lysine (Fig. 1B). These mutations in the KIM of MK-STYX may explain why it does not bind MAPK/ERK and does not impact MAPK/ERK1/2 signaling (unpublished data and Niemi et al. [27]). Although the arginine replaced by lysine may not appear critical, because both residues are positively charged, it is important to note that the positive charges are not consecutive, which is a crucial aspect of the repeated arginines [35,43]. For example, the KIM of DUSP-6/MKP-3 consists of two consecutive arginines, instead of three, and maintains its ability to serve as a docking site for MAPK [35]. The sequence logos show that there is the conservation of one arginine between DUSP-1/MKP-1, DUSP-6/MKP-3, and MK-STYX—indicating that MK-STYX may not interact with MAPKs, which has been reported [27].

MK-STYX regulates stress granule formation

Pursuing MK-STYX as a regulator of MAPK/ERK1/2 revealed G3BP-1 (Ras-GTPase activating protein SH3 domain-binding protein-1), a nucleator of stress granules (SG) [45], as its first identified interactor [13]. The formation of stress granules is an immediate protective response to stressful environmental cues such as heat shock, UV irradiation, hypoxia, and oxidative stress [46]. Stress granules are cytoplasmic storage sites for abortive translational initiation complexes, which act as stations where mRNA can be routed to other sites for storage, disassembly, or degradation [47,48]. As part of the cellular response to stress, the SG cycle involves assembly, coalescence, and disassembly. We discovered that MK-STYX binds G3BP-1 and inhibits G3BP-1-induced stress granules and those (Fig. 2A) induced by sodium arsenite [13]. Furthermore, this interaction is independent of the phosphorylation status of G3BP-1 at Ser 149 [29], where dephosphorylation at this site was thought to be critical for stress granule formation [45]. Recently, Pana et al. reported that phosphorylation at Ser 149 does not influence stress granule formation [49], which is consistent with our observations. Point mutations, where phenylalanine and serine in MK-STYX are restored to a histidine and the critical cysteine within the active site signature motif of MK-STYX, generate an active phosphatase [4,13] that is referred to as MK-STYX$_{(\text{active mutant})}$ [13,29]. We noticed that MK-STYX$_{(\text{active mutant})}$ causes G3BP-1-induced stress granule formation (Fig. 2B) [13,29], eliciting the opposite effect of wild-type MK-STYX.

Stress granules represent a complex assemblage of translational initiation factors such as eIF3 and eIF4, proteins involved in translational control, the microtubule array, and chaperone proteins [50–53]. Phosphorylation of eIF2α (eukaryotic initiation factor 2α) by several stress-activated kinases triggers the formation of stress granules by reducing the frequency of the initiation of translation [47]. Heat shock also induces stress granules and activates many heat shock proteins (Hsps). For example, Hsp70 expression increases as SG disappear, leading to translational recovery [53]. Therefore, there are many avenues that MK-STYX may use to decrease stress granules, but the role of MK-STYX in the stress response pathway is not directly through the eIF2α signaling or Hsp70 (unpublished data). There are many other recent reports and exciting signaling networks that MK-STYX may exert its function to decrease stress granules, which are ongoing and promising studies such as regulation of histone deacetylase isoform 6.

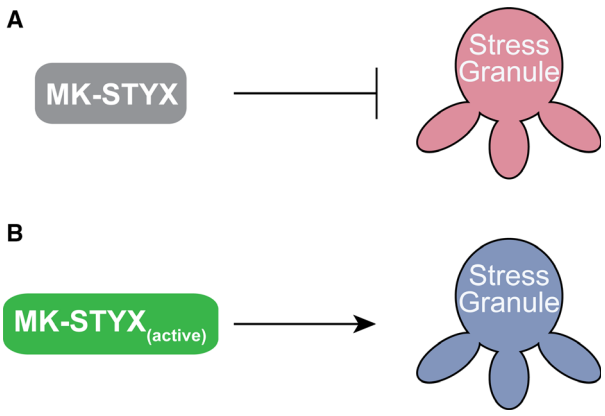

Fig. 2. (A) Model of pseudophosphatase MK-STYX effects on stress granules. (A) MK-STYX inhibits stress granules (stalled mRNA as a protective response to stress) [46]. (B) The signature active site motif of MK-STYX was mutated from FSX$_5$R to HCX$_5$R to restore phosphatase activity [4,13]. This mutant referred to as MK-STYX$_{(\text{active mutant})}$ induces stress granules.

MK-STYX promotes dephosphorylation of histone deacetylase 6

Histone deacetylase isoform 6 (HDAC6) has also been implicated in the stress granule life cycle [54]. Members of the HDAC superfamily remove acetyl groups from histones, repressing gene expression [55,56]. HDACs are primarily localized in the nucleus [57]; however, HDAC6 is cytoplasmic [58] and is involved in the stress response [59–61]. HDAC6 contains two catalytic domains for deacetylation, which are separated by the dynein-binding domain [57,62,63], a SE14 domain, and a C terminus cysteine- and histidine-rich ZnF-UBP (zinc-finger ubiquitin-binding protein) domain [59]. In particular, the ZnF-UBP domain plays a critical role in the function of HDAC6 in the cellular stress response pathway [64,65]. HDAC6 binds G3BP-1 to regulate stress granule formation [66]. The finding that both HDAC6 and MK-STYX interact with G3BP-1 led us to investigate whether MK-STYX has an impact on the dynamics of HDAC6. We found that HDAC6 localizes in both the cytosol and nucleus in the presence of MK-STYX, instead of solely in the cytosol (Fig. 3A) [67]. In addition, HDAC6 phosphorylation at Ser 22 decreases in the presence of MK-STYX (Fig. 3B) [67], illustrating that MK-STYX influences HDAC6 dynamics in both its subcellular localization and post-translational modification [67]. Thus, a strong link for the role of MK-STYX in HDAC6 signaling has been established, and MK-STYX as a regulator in the stress response pathway has been further supported, but how MK-STYX inhibits stress granules remains elusive.

MK-STYX regulates apoptosis

Studies by Niemi et al. on MK-STYX have been a great contribution to elucidating the molecular mechanism of this pseudophosphatase [4,27,28]. They established that MK-STYX localizes to the mitochondria, and is a regulator of apoptotic signaling [27]. MK-STYX is required to decrease chemosensitivity and induce stress-activated mitochondrial-dependent apoptosis [27]. Moreover, the interaction of MK-STYX with the mitochondrial phosphatase, PTPM1 (PTP localized to the mitochondrion 1) is required for MK-STYX to function as a regulator of apoptosis [28]. In the absence of MK-STYX, PTPM1 inhibits apoptosis [27,28]. In the presence of MK-STYX, the pseudophosphatase negatively regulates PTPM1 by binding it and inhibiting its catalytic activity, thereby promoting apoptosis (Fig. 4) [4,28]. An MK-STYX:PTPM1 complex is essential for proper function, similar to the MTM pseudophosphatases:phosphatase enzyme complexes. To date, MK-STYX has not been reported to bind any of its active MKP homologs to enhance their activity. Instead, MK-STYX binds to members of a different class of phosphatases and decreases their activity. This further validates that MK-STYX has very different interactors, as well as signaling roles compared with its active homologs.

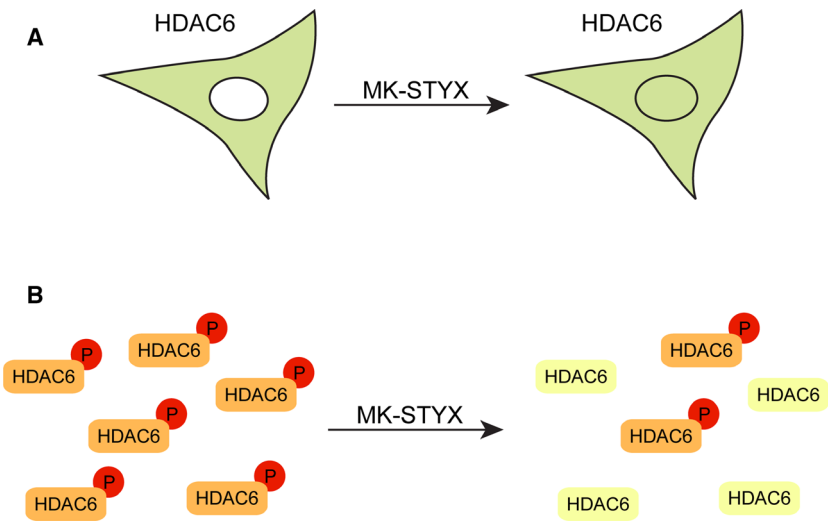

Fig. 3. Model of the effects of MK-STYX on HDAC6 dynamics. (A) Histone deacetylase 6 (HDAC6) is cytoplasmic [58]; however, it partially localizes to the nucleus in the presence of MK-STYX. (B) HDAC6 phosphorylation at Ser 22, which has a role in the deacetylase activity of HDAC6 [80], is decreased in the presence of MK-STYX.

Fig. 4. Model of the effects of MK-STYX as regulator of apoptosis. Phosphatase activity of PTPM1 (mitochondrial phosphatase) prevents apoptosis; therefore, MK-STYX regulates apoptosis by binding PTPM1 and inhibiting its PTP activity. Thus, when a complex of MK-STYX-PTPM1 forms, apoptosis is promoted by inhibiting the inhibitor.

MK-STYX regulates neurite formation

Because G3BP-1 and stress granules have been linked to neurological disorders [15,23,68,69], we also focused on the role of MK-STYX in neurite formation. We discovered that MK-STYX dramatically increases the number of primary neurites in rat pheochromocytoma (PC-12) cells [42], as well as secondary neurites [4,23,70]. MK-STYX decreases RhoA activation, which increases when MK-STYX is down-regulated. MK-STYX-overexpressing cells produce more actin growth cones than control cells [4,70]. MK-STYX also affects the actin-binding protein cofilin, a downstream player of RhoA. MK-STYX decreases the phosphorylation of cofilin in unstimulated cells, but increases its phosphorylation in NGF-stimulated cells, whereas knocking down MK-STYX caused opposite effects [4,42]. Furthermore, MK-STYX sustains actin expression in the absence or presence of NGF [70]. The effects of MK-STYX on RhoA activation, cofilin, and actin expression implicate it as a regulator of neurite formation through the RhoA signaling pathway (Fig. 5) [4,23,42,70]. Furthermore, MK-STYX also affects hippocampal primary neurons [70]. MK-STYX-expressing neurons produce more than the normal number of primary neurites [4,70].

These dynamic morphological changes in neurites caused by MK-STYX indicate that MK-STYX has an effect on various cytoskeletal proteins and/or networks responsible for the morphology of cells [71]. Intriguingly, proteomic analysis reveals that MK-STYX differentially interacts with various cytoskeletal proteins (Fig. 6) [72]. Affinity purification mass spectrometry data revealed six cytoskeletal proteins, MYO1B (unconventional myosin-1B), ACTA 1 (actin, alpha skeletal muscle), MYO1C (unconventional myosin-1C), VIM (vimentin), SPTBN1 (spectrin beta chain, nonerythrocytic 1), and SPTAN1 (spectrin alpha chain, nonerythrocytic 1) as high-confidence candidates that interact with MK-STYX (false discovery rate (FDR) ≤ 1 indicates that interaction is real). Cytoskeleton proteins specifically interact with MK-STYX, but not prototypical STYX (Fig. 6). Four of these proteins, MYO1B, SPTBN1, VIM, and SPTAN1, have regulatory roles in neuronal development such as neurite outgrowths, projections, vesicle transport, and axon guidance [73–75]. Moreover, there have been a number of correlations pointing toward a key role of MK-STYX in neuronal development. A missense mutation of *MK-STYX* is highly expressed in intellectual disability and epilepsy [24], but may not directly contribute to disease phenotype [76]. Taken together, this highlights the emergence of MK-STYX as an important signaling molecule in neurodegenerative diseases.

Conclusion and perspective

Converting a phosphotyrosine-binding domain into an active DUSP [6] prompted excitement for these catalytically inactive enzymes but did not revolutionize their investigation. Almost a quarter of a century later, pseudophosphatases and pseudoenzymes, in general, are receiving well-deserved attention and categorization of these molecules as integral regulatory components of signaling pathways. Through genomic analysis, it became apparent that pseudoenzymes are widespread [2,5,10,14,77], and it thus became important to understand more of their actions [1,4,10]. This review detailed substantial and compelling evidence that one such protein, MK-STYX, is a critical signaling regulator of signaling pathways such as apoptosis, stress response, RhoA, and neuronal differentiation [4,13,27–29,42,70].

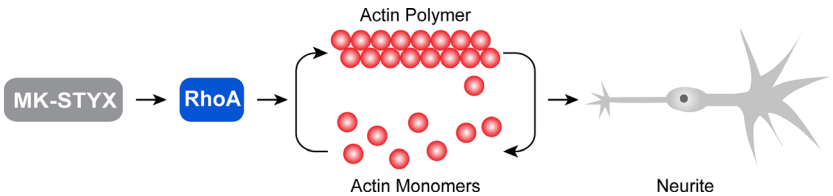

Fig. 5. Model of the effects of MK-STYX on neurite formation. The working model suggests that MK-STYX induces neurites by regulating the RhoA signaling pathway, which changes actin dynamics to induce neurite formation.

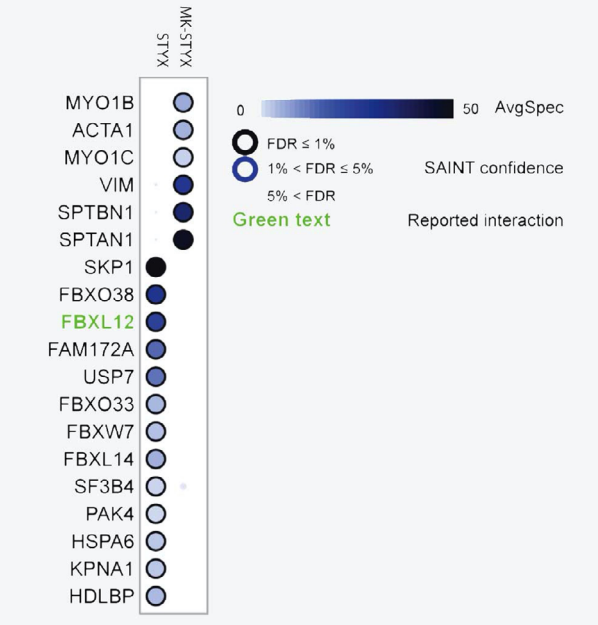

Fig. 6. MK-STYX interacts with cytoskeletal proteins. Prototypical pseudophosphatase STYX (phosphoserine/threonine/tyrosine-interacting protein) or MK-STYX was cloned in Flp-In T-REx (with a FLAG epitope) and transfected in the Flp-In T-REx HEK293 system to create an isogenic cell pool of the recombinant protein under the control of a tetracycline-inducible promoter [72]. FLAG affinity purification mass spectrometry data represented as a dot plot. Interactions between archetypical STYX and MK-STYX are very distinct; STYX interacts with SKP1 and several F-box proteins, which has also been reported by another laboratory [8], while MK-STYX was found to interact with cytoskeletal components (Adapted from St-Denis *et al.*) [72].

Characterizing the relationship of pseudophosphatases to their interactors is important to understanding their role as regulators. This point is elegantly shown by *Caenorhabditis elegans* EGG 4/5, which provided the initial evidence that pseudophosphatases are signal regulators [4,21,22]. These studies emphasize the importance of continuing to develop new tools and techniques to investigate pseudoenzymes. Proteomics and biochemical approaches were instrumental in understanding the molecular mechanism of MK-STYX as a regulator of apoptosis [4,27,28]. We have made significant contributions to the biological function of MK-STYX and have developed an important phenotypic model (neurite extensions) [4,13,29,42,67]. Our recent report of the impact of MK-STYX on HDAC6 dynamics [67] demonstrates that it has many functions as a signaling molecule. HDAC6 is an intensively investigated target for neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases, further validating the importance of continuing to pursue the role of MK-STYX in neuronal development.

These catalytically inactive molecules must be studied with comparable rigor to their active homologs. Tools such as those of structural biology, biochemistry, and *in vivo* models must be used to elucidate the molecular mechanisms and biological functions of these proteins [4,77]. For example, crystal structures of pseudokinases revealed insight into their role as allosteric regulators, hubs for assembling protein complexes, and switches [3]. Obtaining the crystal structure of MK-STYX and its active mutant will provide important insight into its molecular mechanism, just as structures revealed the mechanism of PTPs [11]. It will allow the implementation of structure–function studies to decipher structural changes that MK-STYX confers on its interactors. These studies will help visualize how the active site pocket and KIM have been disrupted by mutations [2], which will help identify unique signaling networks that may lead to complex pathologies. One elegant structure–function study demonstrated that structure features of the KIM/docking motifs were critical for MAPKs (c-Jun N-terminal kinase, p38, and extracellular signal-regulated kinase) to differentiate between specific partners [78]. Ultimately, it will be imperative for laboratories investigating pseudophosphatases to invest in knockout models to have an overall understanding of the function of these molecules, which may catapult interest in designing inhibitors against the noncatalytic phosphatases. The field has passed the crossroads, and the time is now to elucidate the molecular mechanisms of all pseudophosphatases and establish their roles in pathologies. The development of substrate-trapping mutants (inactive PTPs that maintain the ability to bind substrates [79]) illustrated that PTPs display exquisite substrate specificity, allowing them to function as highly selective regulators of signal transduction [11]. This indicates the importance of the 'naturally occurring' substrate-trapping mutants [11,18], pseudophosphatases, as critical selective regulators of signaling cascades, which is highlighted in this special edition.

This special edition on pseudoenzymes is timely; it emphasizes that pseudoenzymes are critical regulators of signal transduction. We are excited about the continued development of new techniques. Initially, comparing pseudophosphatases to their active homologs was thought to be the best option to understand their function. Such a comparison is meritorious; however, it must be combined with the perspective that each pseudophosphatase may perform its own role as a regulator in signaling pathways. It is imperative to study each and every pseudoenzyme in detail [77]. With the

interest of new investigators, and collaborative efforts, it is likely that the molecular mechanisms these catalytically inactive phosphatases exert to achieve these cellular processes will be discovered in the near future [4]. Collectively accomplishing this goal will be a pivotal moment for the field and science. Moreover, elucidating the mechanisms of actions of these catalytically inactive phosphatases may provide insight into how the allosteric interactions of the active PTPs regulate biological function.

Acknowledgements

Gratitude is extended to Justinn E. Barr, who performed the pilot BLAST analysis of MK-STYX and DUSP-1/MKP-1. We thank Dr William R. Eckberg for critical reading of the manuscript. We thank Dr Lizabeth A. Allison for her thoughtful discussion of figures and manuscript, and Vincent Roggero for his thoughtfulness and assistance in transforming sketches for this manuscript into figures. This work was supported by the National Science Foundation Grant MCB1909316 to SDH.

Conflict of interest

The authors declare no conflict of interest.

References

1 Murphy JM, Farhan H & Eyers PA (2017) Bio-Zombie: the rise of pseudoenzymes in biology. *Biochem Soc Trans* **45**, 537–544.
2 Todd AE, Orengo CA & Thornton JM (2002) Sequence and structural differences between enzyme and nonenzyme homologs. *Structure* **10**, 1435–1451.
3 Murphy JM, Mace PD & Eyers PA (2017) Live and let die: insights into pseudoenzyme mechanisms from structure. *Curr Opin Struct Biol* **47**, 95–104.
4 Hinton SD (2019) The role of pseudophosphatases as signaling regulators. *Biochim Biophys Acta* **1866**, 167–174.
5 Abudukelimu A, Mondeel T, Barberis M & Westerhoff HV (2017) Learning to read and write in evolution: from static pseudoenzymes and pseudosignalers to dynamic gear shifters. *Biochem Soc Trans* **45**, 635–652.
6 Wishart MJ, Denu JM, Williams JA & Dixon JE (1995) A single mutation converts a novel phosphotyrosine binding domain into a dual-specificity phosphatase. *J Biol Chem* **270**, 26782–26785.
7 Wishart MJ & Dixon JE (2002) The archetype STYX/dead-phosphatase complexes with a spermatid mRNA-binding protein and is essential for normal sperm production. *Proc Natl Acad Sci USA* **99**, 2112–2117.
8 Reiterer V, Figueras-Puig C, Le Guerroue F, Confalonieri S, Vecchi M, Jalapothu D, Kanse SM, Deshaies RJ, Di Fiore PP, Behrends C *et al.* (2017) The pseudophosphatase STYX targets the F-box of FBXW7 and inhibits SCFFBXW7 function. *EMBO J* **36**, 260–273.
9 He D, Ma Z, Fang C, Ding J, Yang W, Chen P, Huang L, Wang C, Yu Y, Yang L *et al.* (2019) Pseudophosphatase STYX promotes tumor growth and metastasis by inhibiting FBXW7 function in colorectal cancer. *Cancer Lett* **454**, 53–65.
10 Reiterer V, Eyers PA & Farhan H (2014) Day of the dead: pseudokinases and pseudophosphatases in physiology and disease. *Trends Cell Biol* **24**, 489–505.
11 Tonks NK (2013) Protein tyrosine phosphatases–from housekeeping enzymes to master regulators of signal transduction. *FEBS J* **280**, 346–378.
12 Wishart MJ & Dixon JE (1998) Gathering STYX: phosphatase-like form predicts functions for unique protein-interaction domains. *Trends Biochem Sci* **23**, 301–306.
13 Hinton SD, Myers MP, Roggero VR, Allison LA & Tonks NK (2010) The pseudophosphatase MK-STYX interacts with G3BP and decreases stress granule formation. *Biochem J* **427**, 349–357.
14 Chen MJ, Dixon JE & Manning G (2017) Genomics and evolution of protein phosphatases. *Sci Signal* **10**, 1–17.
15 Gal J, Kuang L, Barnett KR, Zhu BZ, Shissler SC, Korotkov KV, Hayward LJ, Kasarskis EJ & Zhu H (2016) ALS mutant SOD1 interacts with G3BP1 and affects stress granule dynamics. *Acta Neuropathol* **132**, 563–576.
16 Hnia K, Vaccari I, Bolino A & Laporte J (2012) Myotubularin phosphoinositide phosphatases: cellular functions and disease pathophysiology. *Trends Mol Med* **18**, 317–327.
17 Robinson FL & Dixon JE (2006) Myotubularin phosphatases: policing 3-phosphoinositides. *Trends Cell Biol* **16**, 403–412.
18 Tonks NK (2009) Pseudophosphatases: grab and hold on. *Cell* **139**, 464–465.
19 Bulgari D, Jha A, Deitcher DL & Levitan ES (2018) Myopic (HD-PTP, PTPN23) selectively regulates synaptic neuropeptide release. *Proc Natl Acad Sci USA* **115**, 1617–1622.
20 Gahloth D, Heaven G, Jowitt TA, Mould AP, Bella J, Baldock C, Woodman P & Tabernero L (2017) The open architecture of HD-PTP phosphatase provides new insights into the mechanism of regulation of ESCRT function. *Sci Rep* **7**, 9151.
21 Parry JM, Velarde NV, Lefkovith AJ, Zegarek MH, Hang JS, Ohm J, Klancer R, Maruyama R, Druzhinina MK, Grant BD *et al.* (2009) EGG-4 and EGG-5 link events of the oocyte-to-embryo transition with meiotic progression in *C. elegans*. *Curr Biol* **19**, 1752–1757.

22 Cheng KC, Klancer R, Singson A & Seydoux G (2009) Regulation of MBK-2/DYRK by CDK-1 and the pseudophosphatases EGG-4 and EGG-5 during the oocyte-to-embryo transition. *Cell* **139**, 560–572.
23 Dahal A & Hinton SD (2017) Antagonistic roles for STYX pseudophosphatases in neurite outgrowth. *Biochem Soc Trans* **45**, 381–387.
24 Isrie M, Zamani Esteki M, Peeters H, Voet T, Van Houdt J, Van Paesschen W & Van Esch H (2015) Homozygous missense mutation in STYXL1 associated with moderate intellectual disability, epilepsy and behavioural complexities. *Eur J Med Genet* **58**, 205–210.
25 Siligan C, Ban J, Bachmaier R, Spahn L, Kreppel M, Schaefer KL, Poremba C, Aryee DN & Kovar H (2005) EWS-FLI1 target genes recovered from Ewing's sarcoma chromatin. *Oncogene* **24**, 2512–2524.
26 Caunt CJ & Keyse SM (2013) Dual-specificity MAP kinase phosphatases (MKPs): shaping the outcome of MAP kinase signalling. *FEBS J* **280**, 489–504.
27 Niemi NM, Lanning NJ, Klomp JA, Tait SW, Xu Y, Dykema KJ, Murphy LO, Gaither LA, Xu HE, Furge KA *et al*. (2011) MK-STYX, a catalytically inactive phosphatase regulating mitochondrially dependent apoptosis. *Mol Cell Biol* **31**, 1357–1368.
28 Niemi NM, Sacoman JL, Westrate LM, Gaither LA, Lanning NJ, Martin KR & MacKeigan JP (2014) The pseudophosphatase MK-STYX physically and genetically interacts with the mitochondrial phosphatase PTPMT1. *PLoS One* **9**, e93896.
29 Barr JE, Munyikwa MR, Frazier EA & Hinton SD (2013) The pseudophosphatase MK-STYX inhibits stress granule assembly independently of Ser149 phosphorylation of G3BP-1. *FEBS J* **280**, 273–284.
30 Huang CY & Tan TH (2012) DUSPs, to MAP kinases and beyond. *Cell Biosci* **2**, 24.
31 Guan KL, Broyles SS & Dixon JE (1991) A Tyr/Ser protein phosphatase encoded by vaccinia virus. *Nature* **350**, 359–362.
32 Dickinson RJ & Keyse SM (2006) Diverse physiological functions for dual-specificity MAP kinase phosphatases. *J Cell Sci* **119**, 4607–4615.
33 Keyse SM & Ginsburg M (1993) Amino acid sequence similarity between CL100, a dual-specificity MAP kinase phosphatase and cdc25. *Trends Biochem Sci* **18**, 377–378.
34 Bordo D & Bork P (2002) The rhodanese/Cdc25 phosphatase superfamily. Sequence-structure-function relations. *EMBO Rep* **3**, 741–746.
35 Owens DM & Keyse SM (2007) Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* **26**, 3203–3213.
36 Camps M, Nichols A & Arkinstall S (2000) Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB J* **14**, 6–16.
37 Theodosiou A & Ashworth A (2002) MAP kinase phosphatases. *Genome Biol* **3**, REVIEWS3009.
38 Sun H, Charles CH, Lau LF & Tonks NK (1993) MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. *Cell* **75**, 487–493.
39 Tonks NK (2013) Special issue: Protein phosphatases: from molecules to networks: introduction. *FEBS J* **280**, 323.
40 Slack DN, Seternes OM, Gabrielsen M & Keyse SM (2001) Distinct binding determinants for ERK2/p38alpha and JNK map kinases mediate catalytic activation and substrate selectivity of map kinase phosphatase-1. *J Biol Chem* **276**, 16491–16500.
41 Tonks NK (2006) Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* **7**, 833–846.
42 Flowers BM, Rusnak LE, Wong KE, Banks DA, Munyikwa MR, McFarland AG & Hinton SD (2014) The pseudophosphatase MK-STYX induces neurite-like outgrowths in PC12 cells. *PLoS One* **9**, e114535.
43 Peti W & Page R (2013) Molecular basis of MAP kinase regulation. *Prot Sci* **22**, 1698–1710.
44 Tanoue T, Adachi M, Moriguchi T & Nishida E (2000) A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat Cell Biol* **2**, 110–116.
45 Tourriere H, Chebli K, Zekri L, Courselaud B, Blanchard JM, Bertrand E & Tazi J (2003) The RasGAP-associated endoribonuclease G3BP assembles stress granules. *J Cell Biol* **160**, 823–831.
46 Anderson P & Kedersha N (2002) Visibly stressed: the role of eIF2, TIA-1, and stress granules in protein translation. *Cell Stress Chaperones* **7**, 213–221.
47 Kedersha NL, Gupta M, Li W, Miller I & Anderson P (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. *J Cell Biol* **147**, 1431–1442.
48 Kedersha N & Anderson P (2002) Stress granules: sites of mRNA triage that regulate mRNA stability and translatability. *Biochem Soc Trans* **30**, 963–969.
49 Panas MD, Kedersha N, Schulte T, Branca RM, Ivanov P & Anderson P (2019) Phosphorylation of G3BP1-S149 does not influence stress granule assembly. *J Cell Biol* **218**, 2425–2432.
50 Ivanov PA, Chudinova EM & Nadezhdina ES (2003) Disruption of microtubules inhibits cytoplasmic ribonucleoprotein stress granule formation. *Exp Cell Res* **290**, 227–233.
51 Kedersha N, Stoecklin G, Ayodele M, Yacono P, Lykke-Andersen J, Fritzler MJ, Scheuner D, Kaufman RJ, Golan DE & Anderson P (2005) Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J Cell Biol* **169**, 871–884.

52 Kedersha N & Anderson P (2007) Mammalian stress granules and processing bodies. *Methods Enzymol* **431**, 61–81.
53 Thomas MG, Loschi M, Desbats MA & Boccaccio GL (2010) RNA granules: the good, the bad and the ugly. *Cell Signal* **23**, 324–334.
54 Youn JY, Dunham WH, Hong SJ, Knight JDR, Bashkurov M, Chen GI, Bagci H, Rathod B, MacLeod G, Eng SWM *et al.* (2018) High-density proximity mapping reveals the subcellular organization of mRNA-associated granules and bodies. *Mol Cell* **69**, 517–532, e11.
55 Simoes-Pires C, Zwick V, Nurisso A, Schenker E, Carrupt PA & Cuendet M (2013) HDAC6 as a target for neurodegenerative diseases: what makes it different from the other HDACs? *Mol Neurodegener* **8**, 7.
56 Zeb A, Park C, Rampogu S, Son M, Lee G & Lee KW (2018) Structure-based drug designing recommends HDAC6 inhibitors to attenuate microtubule-associated tau-pathogenesis. *ACS Chem Neurosci* **10**, 1326–1335.
57 de Ruijter AJ, van Gennip AH, Caron HN, Kemp S & van Kuilenburg AB (2003) Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* **370**, 737–749.
58 Verdel A & Khochbin S (1999) Identification of a new family of higher eukaryotic histone deacetylases. Coordinate expression of differentiation-dependent chromatin modifiers. *J Biol Chem* **274**, 2440–2445.
59 Boyault C, Sadoul K, Pabion M & Khochbin S (2007) HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. *Oncogene* **26**, 5468–5476.
60 Li L & Yang XJ (2015) Tubulin acetylation: responsible enzymes, biological functions and human diseases. *Cell Mol Life Sci* **72**, 4237–4255.
61 Li T, Zhang C, Hassan S, Liu X, Song F, Chen K, Zhang W & Yang J (2018) Histone deacetylase 6 in cancer. *J Hematol Oncol* **11**, 111.
62 Verdin E, Dequiedt F & Kasler HG (2003) Class II histone deacetylases: versatile regulators. *Trends Genet* **19**, 286–293.
63 Grozinger CM, Hassig CA & Schreiber SL (1999) Three proteins define a class of human histone deacetylases related to yeast Hda1p. *Proc Natl Acad Sci USA* **96**, 4868–4873.
64 Li ZY, Zhang C, Zhang Y, Chen L, Chen BD, Li QZ, Zhang XJ & Li WP (2017) A novel HDAC6 inhibitor tubastatin A: controls HDAC6-p97/VCP-mediated ubiquitination-autophagy turnover and reverses temozolomide-induced ER stress-tolerance in GBM cells. *Cancer Lett* **391**, 89–99.
65 Ryu HW, Won HR, Lee DH & Kwon SH (2017) HDAC6 regulates sensitivity to cell death in response to stress and post-stress recovery. *Cell Stress Chaperones* **22**, 253–261.
66 Kwon S, Zhang Y & Matthias P (2007) The deacetylase HDAC6 is a novel critical component of stress granules involved in the stress response. *Genes Dev* **21**, 3381–3394.
67 Cao Y, Banks DA, Mattei AM, Riddick AT, Reed KM, Zhang AM, Pickering ES & Hinton SD (2019) Pseudophosphatase MK-STYX alters histone deacetylase 6 cytoplasmic localization, decreases its phosphorylation, and increases detyrosination of tubulin. *Int J Mol Sci* **20**, 1455.
68 Martin S, Zekri L, Metz A, Maurice T, Chebli K, Vignes M & Tazi J (2013) Deficiency of G3BP1, the stress granules assembly factor, results in abnormal synaptic plasticity and calcium homeostasis in neurons. *J Neurochem* **125**, 175–184.
69 Vanderweyde T, Yu H, Varnum M, Liu-Yesucevitz L, Citro A, Ikezu T, Duff K & Wolozin B (2012) Contrasting pathology of the stress granule proteins TIA-1 and G3BP in tauopathies. *J Neurosci* **32**, 8270–8283.
70 Banks DA, Dahal A, McFarland AG, Flowers BM, Stephens CA, Swack B, Gugssa A, Anderson WA & Hinton SD (2017) MK-STYX alters the morphology of primary neurons, and outgrowths in MK-STYX overexpressing PC-12 cells develop a neuronal phenotype. *Front Mol Biosci* **4**, 76.
71 Fuchs E & Karakesisoglou I (2001) Bridging cytoskeletal intersections. *Genes Dev* **15**, 1–14.
72 St-Denis N, Gupta GD, Lin ZY, Gonzalez-Badillo B, Veri AO, Knight JD, Rajendran D, Couzens AL, Currie KW, Tkach JM *et al.* (2016) Phenotypic and interaction profiling of the human phosphatases identifies diverse mitotic regulators. *Cell Rep* **17**, 2488–2501.
73 Wang Y, Ji T, Nelson AD, Glanowska K, Murphy GG, Jenkins PM & Parent JM (2018) Critical roles of alphaII spectrin in brain development and epileptic encephalopathy. *J Clin Invest* **128**, 760–773.
74 Ju Lee H, Bartsch D, Xiao C, Guerrero S, Ahuja G, Schindler C, Moresco JJ, Yates JR III, Gebauer F, Bazzi H *et al.* (2017) A post-transcriptional program coordinated by CSDE1 prevents intrinsic neural differentiation of human embryonic stem cells. *Nat Commun* **8**, 1456.
75 Iuliano O, Yoshimura A, Prosperi MT, Martin R, Knolker HJ & Coudrier E (2018) Myosin 1b promotes axon formation by regulating actin wave propagation and growth cone dynamics. *J Cell Biol* **217**, 2033–2046.
76 Hengel H, Schelling Y, Keimer R, Deigendesch W, Bauer P & Schols L (2019) A previously identified missense mutation in STYXL1 is likely benign. *Eur J Med Genet* **62**, 103582.
77 Eyers PA & Murphy JM (2016) The evolving world of pseudoenzymes: proteins, prejudice and zombies. *BMC Biol* **14**, 98.

78 Garai A, Zeke A, Gogl G, Toro I, Fordos F, Blankenburg H, Barkai T, Varga J, Alexa A, Emig D *et al*. (2012) Specificity of linear motifs that bind to a common mitogen-activated protein kinase docking groove. *Sci Signal* **5**, ra74.

79 Flint AJ, Tiganis T, Barford D & Tonks NK (1997) Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. *Proc Natl Acad Sci USA* **94**, 1680–1685.

80 Du J, Zhang L, Zhuang S, Qin GJ & Zhao TC (2015) HDAC4 degradation mediates HDAC inhibition-induced protective effects against hypoxia/reoxygenation injury. *J Cell Physiol* **230**, 1321–1331.