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Review article

Structure and regulation of MAPK phosphatases

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Abstract

MAP kinases (MAPKs), which control mitogenic signal transduction in all eukaryotic organisms, are inactivated by dual specificity MAPK phosphatases (DS-MKPs). Recent studies reveal that substrate specificity and enzymatic activity of MKPs are tightly controlled not only by the conserved C-terminal phosphatase domain but also by an N-terminal (NT) kinase-binding domain. Notably, MKPs that consist of a kinase-binding domain and a phosphatase domain exhibit little phosphatase activity in the absence of their physiological substrates. MKP binding to a specific MAPK results in enzymatic activation of the phosphatase in a substrate-induced activation mechanism. This direct coupling of inactivation of an MAPK to activation of an MKP provides a tightly controlled regulation that enables these two key enzymes to keep each other in check, thus guaranteeing the fidelity of signal transduction. This review discusses the recent understanding of structure and regulation of the large family of dual specificity MKPs, which can be divided into four subgroups according to their functional domains and mechanism of substrate recognition and enzymatic regulation. Moreover, detailed comparison of the structural basis between this unique substrate-induced activation mechanism and the common auto-inhibition mechanism is provided.

Keywords: MAPK phosphatases; Dual specificity MAPK phosphatases; N-terminal

1. Introduction

Protein phosphorylation presents a mechanism by which the activity of numerous proteins in the form of enzymes, receptors, transporters, docking and scaffolding proteins is switched on or off across a wide array of biological processes from cell growth and differentiation through apoptosis to disease [1–3]. Studies on *Saccharomyces cerevisiae* suggest that the human genome may encode as many as 2000 protein kinases to phosphorylate amino acid residues serine, threonine and tyrosine within these proteins in various tissues, cell lines and organelles [4]. Once phosphorylated, proteins must be unphosphorylated in order to attain a perfect harmony within the cellular machinery. This task of undoing the phosphorylation or removing the phosphate group from the proteins falls to an equally impressive array of enzymes termed protein phosphatases [5]. There are many distinct families of phosphatases designed to meet the demand for structurally and functionally distinct protein targets. One such family of phosphatases has come to be known as mitogen-activated protein (MAP) kinase phosphatases (MKPs) [6] due to the virtue of its ability to target a group of proteins called MAP kinases (MAPKs) [7].

MAPKs transduce extracellular signals from hormones, growth factors, cytokines and environmental stresses that elicit a diverse array of physiological functions including cell proliferation, differentiation, development and apoptosis [8–13] (Fig. 1). The biological significance of MAPKs is further underscored by their involvement in important cellular functions throughout the animal kingdom [7,14–17]. The three major subfamilies of MAP kinases include: the extracellular signal-regulated kinases (ERK-1/2), c-Jun N-terminal kinases (JNK-1/2/3) and p38 proteins (p38-α/β/γ/δ) [18–21]—which all consist of a homodimer of molecular mass of about 80 kDa and contain the signature sequence –TXY–, where T and Y are threonine and tyrosine, and X is glutamate, proline or glycine, respectively, in ERK, JNK or p38 [22–24]. Phosphorylation of both the threonine and tyrosine within this signature sequence is essential for MAP kinase activity and is achieved through

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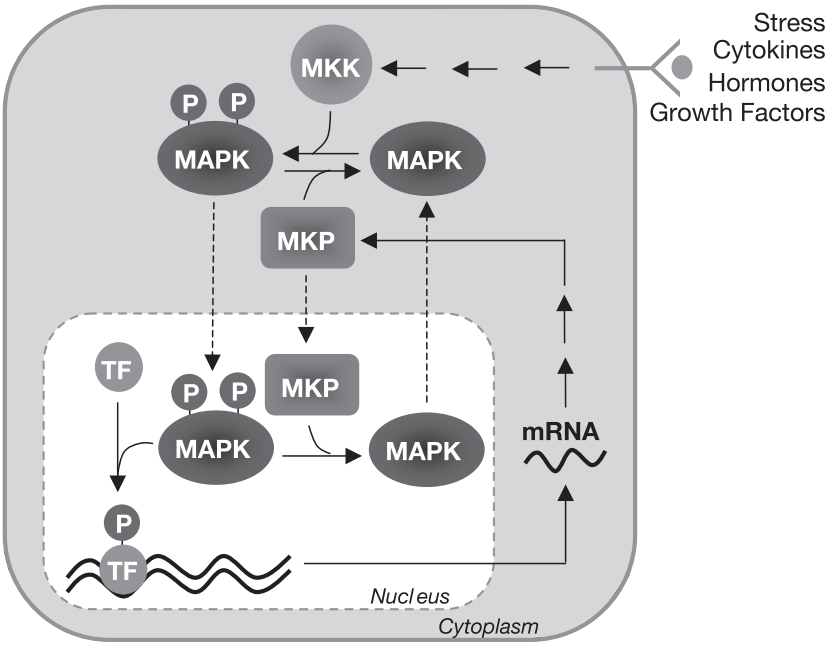

Fig. 1. MAPK signaling and down-regulation by MKPs. Stimuli such as stress, cytokines, hormones and growth factors activates MKKs. MKKs in turn phosphorylate tyrosine and threonine residues within the motif –pTXpY– located in the activation loop of MAPKs—such dual phosphorylation results in dimerization and subsequent activation of MAPKs. MAPKs may then interact with and phosphorylate cytoplasmic proteins, or alternatively translocate to the nucleus, where MAPKs may interact with specific transcription factors (TFs) leading to gene transcriptional activation of specific proteins including MKPs. MKPs in turn provide a negative feedback regulatory mechanism by inactivating MAPKs via dual dephosphorylation of –pTXpY– in the cytoplasm and the nucleus.

the action of MAP kinase kinases (MKKs). Upon activation, MAPKs mediate key cellular events in the cytoplasm including phosphorylation of membrane-associated and cytoplasmic proteins such as kinases, cytoskeletal elements, phospholipase A_2 and stathmin [25]. They may translocate to the nucleus to phosphorylate specific transcription factors such as c-Jun, c-Fos, Elk-1 and c-Myc [11,18,21,26,27]. Activation of transcription factors can result in immediate gene transcription of important cellular proteins and cytokines as well as MKPs. The expression of MKPs provides a negative feedback mechanism for MAP kinase activity. The role of MAP kinases in orchestrating many important cellular functions thus cannot be overemphasized but how their activity is down-regulated by MKPs remains largely elusive.

MKPs are a family of protein phosphatases that inactivate MAPKs through dephosphorylation of threonine and/or tyrosine residues within the signature sequence –pTXpY– located in the activation loop of MAP kinases, where pT and pY are phosphothreonine and phosphotyrosine, respectively. MKPs are divided into three major categories depending on their preference for dephosphorylating tyrosine, serine/threonine or both the tyrosine and threonine (dual specificity). The tyrosine-specific MKPs (TS-MKPs) include PTP-SL [28], STEP [28] and HePTP [29], while serine/threonine-specific MKPs (SS-MKPs) are PP2A [30] and PP2C [30]. The TS-MKPs and SS-MKPs have been covered in detail in a number of excellent recent reviews on MKPs [6,31]. The focus of this current review is on the recent advances and breakthroughs made in understanding the mechanism, function and regulation of dual specificity MKPs (DS-MKPs).

2. Catalytic properties of DS-MKPs

DS-MKPs preferentially act upon MAPKs containing dual phosphorylation of threonine and tyrosine residues within the consensus motif –pTXpY–. The DS-MKP MKP1 was the first MAPK phosphatase discovered over a decade ago in 1991 [32]. To date, 13 of nearly 30 dual specificity phosphatases (DSPs) identified are DS-MKPs (Table 1). All DS-MKPs contain a highly conserved signature motif HCXXXXXR that is characteristic of all protein phosphatases but not Ser/Thr phosphatases [33]. The cysteine and arginine residues within this signature sequence located in the active site loop, and an highly conserved aspartate residue located in the general acid loop are positioned close to each other in the active site of protein phosphatases and their role in the dephosphorylation of MAPKs by DS-MKPs is essential (Fig. 2).

Dephosphorylation begins with nucleophilic attack by thiolate anion of cysteine within the DS-MKP signature sequence –HCXXXXXR– on the phosphorus atom of phosphotyrosine within MAPKs, while at the same time an aspartate in the general acid loop donates a proton to the phenolic oxygen atom of phosphotyrosine. This results in the formation of a transient MKP-phosphate intermediate

Table 1
Subgrouping of DS-MKP family

Subgroup	MAPK specificity	Expasy	Species	Alternative names	Residues
Type I					
VHR	ERK≫JNK ~ p38	P51452	Human	–	185
		Q9D7X3	Mouse	TDSP11	185
DSP2	p38 ~ JNK≫ERK	Q99N11	Mouse	LMW-DSP2	184
MKP6	ERK ~ JNK≫p38	O95147	Human	–	198
		Q9JLY7	Mouse	–	198
Type II					
MKP1	p38 ~ JNK≫ERK	P28562	Human	VH1, CL100, PTPN10	367
		P28563	Mouse	ERP, 3CH134, PTPN10	367
		Q64623	Rat	CL100	367
PAC1	ERK≫p38 ~ JNK	Q05923	Human	–	314
		Q05922	Mouse	–	314
MKP2	ERK ~ JNK ~ p38	Q13115	Human	VH2, TYP1	394
		Q62767	Rat	–	395
		Q9PW71	Chicken	–	375
VH3	–	Q16690	Human	B23	384
		O54838	Rat	CPG21	384
MKP3	ERK≫JNK ~ p38	Q16828	Human	PYST1	381
		Q9DBB1	Mouse	–	381
		Q64346	Rat	VH6	381
PYST2	–	Q16829	Human	B59	320
		Q91Z46	Mouse	–	329
		Q63340	Rat	MKPX	–
MKP4	ERK ~ JNK ~ p38	Q99956	Human	PYST3	384
Type III					
MKP5	p38 ~ JNK>ERK	Q9Y6W6	Human	–	482
		Q9ESS0	Mouse	–	483
Type IV					
VH5	JNK ~ p38≫ERK	Q13202	Human	–	625
		O09112	Mouse	M3/6, NTTP1	663
MKP7	JNK ~ p38≫ERK	Q9BY84	Human	KIAA1700	665
		Q920R2	Mouse	3830417M17RIK	660

and the release of dephosphorylated MAPK [34]. The aspartate in the general acid loop then accepts a proton from a water molecule and the resulting hydroxyl anion attacks the phosphorus atom of the cysteinyl-phosphate intermediate leading to release of inorganic phosphate and regeneration of a thiolate anion at the active site cysteine in the free enzyme. A number of other residues in the signature sequence –HCXXXXXR– also play a critical role in the dephosphorylation reaction. For example, the arginine residue coordinates with the phosphate group of phosphotyrosine or phosphothreonine of an MAPK substrate. The histidine residue within the signature is believed to decrease the pK_a of the active site cysteine to enable it to exist in a thiolate form necessary for its initial nucleophilic attack of phosphotyrosine or phosphothreonine under physiological conditions. The pK_a value of a typical cysteine within a protein is about 8.5 [35], whereas the pK_a of the active site cysteine in the DS-MKP VHR has been shown to be about 5.5 [36]. Dephosphorylation of phosphothreonine within MAPKs is believed to proceed through a similar mechanism [33].

3. Structural and functional diversity of DS-MKPs

DS-MKPs are structurally and functionally distinct—they may be composed of a single catalytic domain or contain additional domains flanking the catalytic domain for higher regulatory control. For this reason, we group members of the DS-MKP family into four categories (types I–IV) on the basis of their structural and functional characteristics (Fig. 3 and Table 1).

3.1. Type I DS-MKPs

Type I DS-MKPs are approximately 200 amino acid residues in length and contain only a DSP domain. So far only three members have been identified. These include the VHR [37], DSP2 [38] and MKP6 [39]. They share between 25% and 35% sequence identity and display unique MAPK specificity. While VHR is highly specific for ERK [40], DSP2 and MKP6 respectively show marked preference for p38/JNK [38] and ERK/JNK [39].

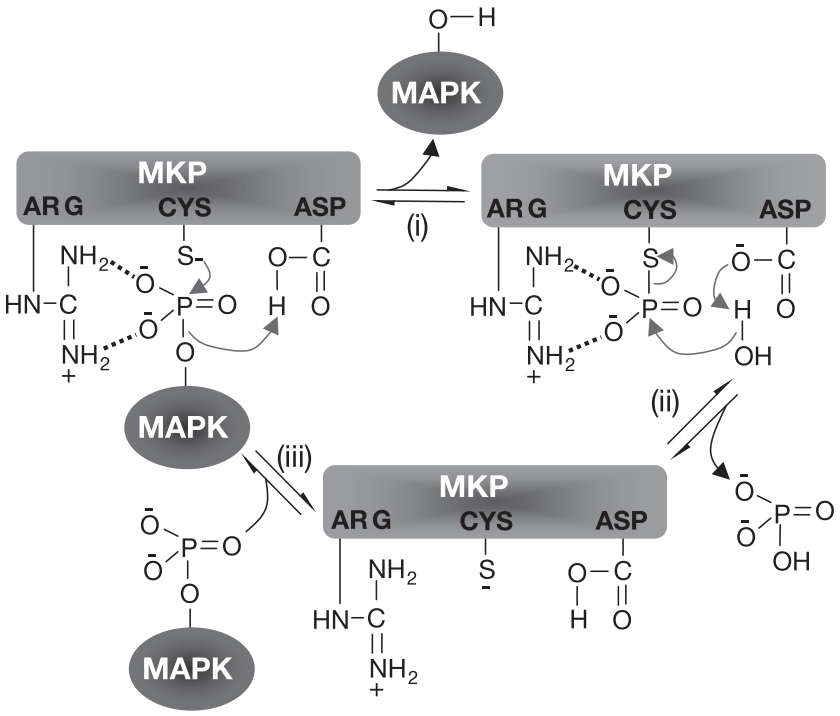

Fig. 2. Catalytic mechanism of dephosphorylation of MAPKs by MKPs. (i) Nucleophilic attack of the thiolate anion of the active site Cys of an MKP on the phosphate of pY of an MAPK results in the formation of a transient phospho-enzyme intermediate with concomitant release of MAPK-Y aided by the donation of a proton from the active site Asp acting as a general acid. (ii) The active site Asp, acting as a general base, accepts a proton from a water molecule and the resulting hydroxyl group attacks the phosphate atom within the phospho-enzyme intermediate to eliminate phosphate and regenerate a thiolate anion at the active site Cys of the MKP. (iii) The regenerated thiolate anion of the MKP binds phosphorylated MAPK and the catalytic cycle is repeated.

3.2. Type II DS-MKPs

Type II DS-MKPs are usually between 300 and 400 amino acid residues in length and contain an N-terminal (NT) MAP kinase-binding (MKB) domain in addition to the DSP domain. Members identified so far include MKP1 [41–44], PAC1 [43,45,46], MKP2 [43,47–50], VH3 [51,52], MKP3 [50,53–56], PYST2 [50,53,56,57] and MKP4 [58,59]. These display different specificities towards their MAPK substrates. For example, MKP3 and PAC1 selectively dephosphorylate ERK1/2; MKP1 dephosphorylates p38/JNK, MKP2 and MKP4 exhibit broad phosphatase activity to dephosphorylate all three known classes of MAPKs. Little is known about the specificity of VH3 and PYST2 towards MAPKs—although both efficiently dephosphorylate the ERK MAP kinase.

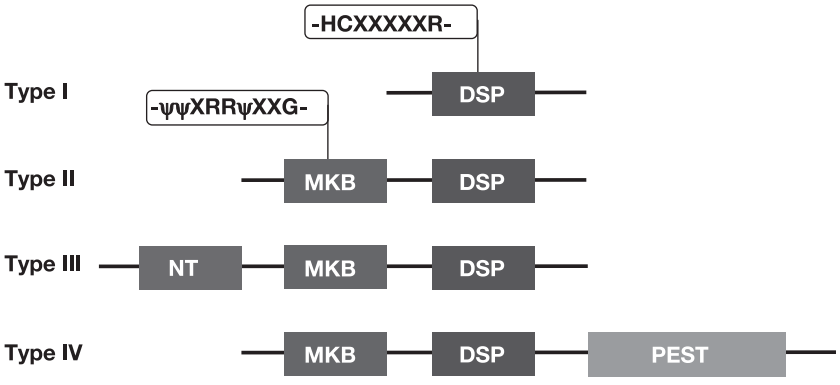

Fig. 3. Subgrouping of the DS-MKP family. Type I DS-MKPs contain only a DSP domain characterized by the phosphatase signature sequence HCXXXXXR. Type II DS-MKPs contain a MKB domain N-terminal to the DSP domain. In Type III DS-MKPs, the MKB domain is flanked by an NT domain and a C-terminal DSP domain. In Type IV DS-MKPs, the DSP domain is flanked by an N-terminal MKB domain and a C-terminal region rich in PEST-like sequences (PEST).

3.3. Type III DS-MKPs

MKP5 is the only known member of this subgroup of DS-MKPs [60]. Its structural features include the presence of an N-terminal domain of unknown function in addition to the MKB and DSP domains that are characteristic of Type II DS-MKPs. MKP5 shows preference for dephosphorylating JNK and p38 MAPKs but not ERK [60]. We hypothesize that the novel NT domain is possibly a protein interaction domain that may be involved in interaction with other cellular proteins and thereby providing a mechanism for cross-talk between MAPK and other signaling pathways involved in regulating cellular functions. The NT domain thus suggests another element of complexity in the biological function of DS-MKPs.

3.4. Type IV DS-MKPs

Type IV DS-MKPs are between 600 and 700 amino acid residues in length and, like Type II and Type III DS-MKPs, contain both the MKB and DSP domains. Their unique feature however is that they contain a sequence of about 300 residues C-terminus to the DSP domain that is rich in prolines, glutamates, serines and threonines (PEST). The function of this region remains to be elucidated but, in analogy with other proteins containing PEST-like sequences, it has been suggested that it may be involved in rapid degradation of Type IV DS-MKPs through uniquitin-mediated proteolysis [61] and thus may provide an important regulatory mechanism. Members include VH5 [62] and MKP7 [63]—both of which are specific for JNK and p38 but not ERK.

4. The DSP domain is the business end of DS-MKPs

The DSP domain among different DS-MKPs exhibits between 25% and 75% sequence identity—arguing strongly in support of structural and functional differences among the various members of this MKP subfamily. The DSP domain is characterized by the phosphatase signature sequence –HCXXXXXR– and a conserved aspartate residue that acts as the general acid/base in the catalysis reaction [33]. The DSP domain is thus the business part of DS-MKPs. In order to understand the molecular details of how the DSP domain dephosphorylates its substrates, knowledge of structural information is of paramount importance.

To date, atomic structures of DSP domain from VHR [64,65], MKP3 [66] and PAC1 [67] have been solved. The structures of the DSP domain, constructed on Rossmann fold with a central β-sheet sandwiched between helices on each side, are remarkably similar with some expected differences (Fig. 4). Thus, while one face of the central β-sheet in MKP3 and PAC1 contains only α3, the same face in VHR additionally contains helices α2 and α4—while α4 is substituted by the strand β4, α2 is totally absent in MKP3 and PAC1. Conversely, strand β4 is missing in the structure of VHR. Furthermore, the second face of the central β-sheet in MKP3 and PAC1 contains helices α5, α6, α7 and α8, whereas the same face in VHR contains an additional helix termed α1. Notably, subtle differences are also observed in the DSP domain structures of MKP3 and PAC1, which share nearly 50% sequence identity within this domain. Specifically, while α3 is one turn shorter in MKP3, this trend is reversed in the case of α7. In addition, the relative orientation of α6 and α8 helices are different between MKP3 and PAC1. It has been suggested that such differences between the DSP domain of MKP3 and PAC1 may be due to differences in the buffer conditions used in the two separate studies [66,67].

4.1. Active site configuration

The active site cleft of DS-MKPs within the DSP domain is shallow, with a depth of only about 5.5 Å, and thus enabling the active site to accommodate both pY and pT. This is in contrast to the much deeper 10 Å active site cleft observed in the classical PTP1B phosphatase, which is only capable of hydrolyzing substrates exclusively containing pY [68]. The active site residues cysteine and arginine within the signature sequence –HCXXXXXR– are located in the loop β6–α6, while the general acid/base aspartate is found within the loop β5–α5 (Fig. 4). As expected, these loops lie in close proximity to each other and thereby leading to the construction of enzymatic active site from residues that are distant in sequence but close in space.

The structures described above reveal the key insights into the molecular details of the mechanism of action of DS-MKPs. Particularly, while the active-site C124 and R130 in the signature sequence –HCXXXXXR–, and D92, the general acid/base aspartate, assume a catalytically active conformation within VHR, the corresponding residues in MKP3 and PAC1 are somewhat perturbed. Thus, the loop β5–α5 containing the general acid/base aspartate, D262 in MKP3 and D226 in PAC1, is flipped away from the active site signature sequence –HCXXXXXR–, containing the other catalytically important residues, C293 and R299 in MKP3 and C257 and R263 in PAC1, by about 10–20 Å—the resulting orientation of the loop places the aspartate much further away from cysteine and arginine and thus making it unavailable to act as a general acid/base in the dephosphorylation reaction. In addition, the active site arginines, R299 in MKP3 and R263 in PAC1, are not optimally positioned to coordinate with the phosphate moiety of pY within the –pTXpY– motif of MAPK substrates. The active site within the DSP domain of MKP3 and PAC1, unlike that of VHR, thus clearly exists in a disordered state. It is argued that in order for the DSP domain of MKP3 and PAC1 to efficiently bind and dephosphorylate pY or pT, it must undergo a conformational change upon interaction with ERK2 resulting in the closure of the loop β5–α5 over the active site [66,67].

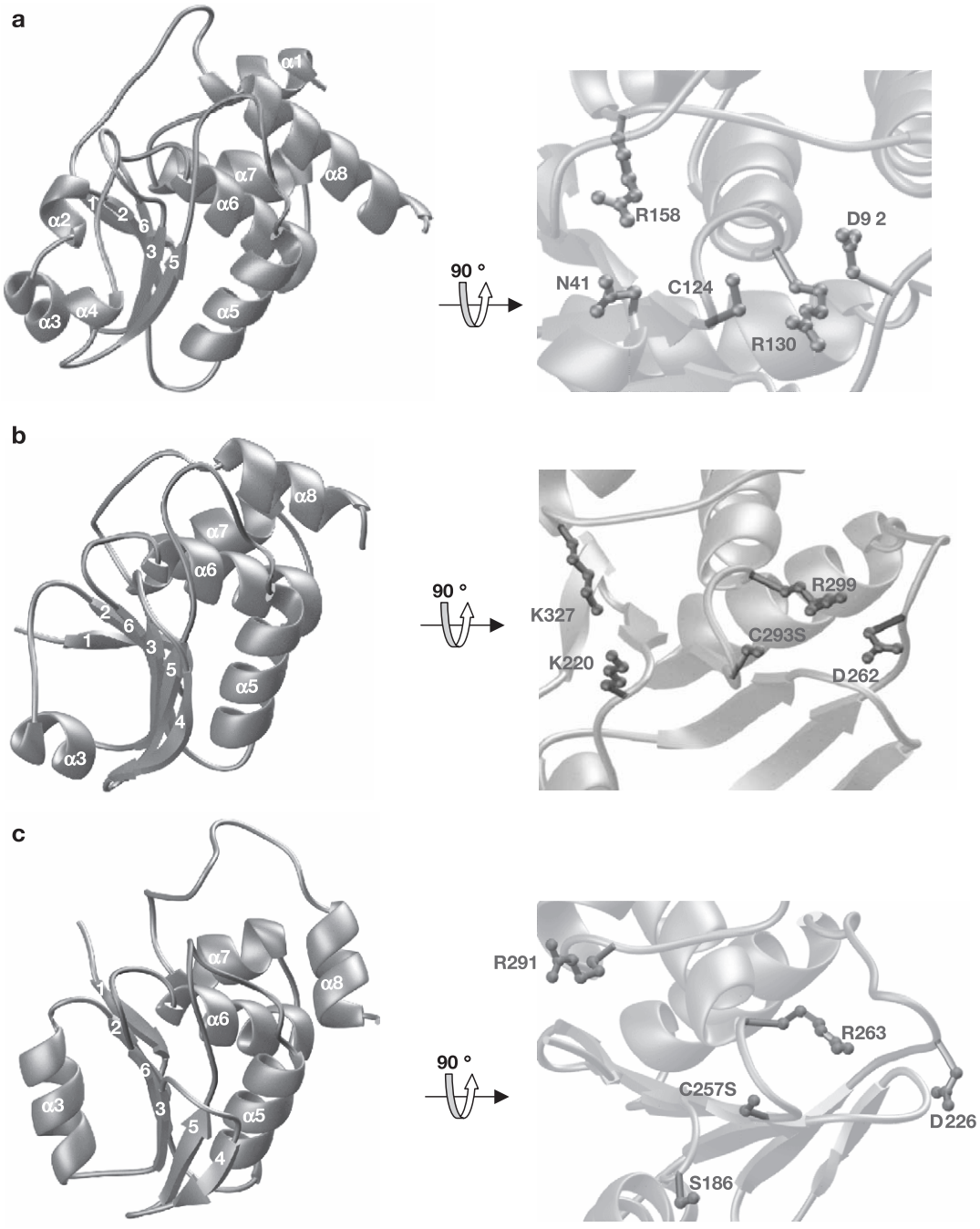

Fig. 4. Structures of the DSP domains of VHR (a), MKP3 (b) and PAC1 (c). The left panel shows the global view of the ribbon representation of the domain. The active site and general acid loops are colored red. The secondary structural elements are colored purple and the loops gray. Strands are numbered 1–6, while helices are denoted by α1–α8. The right panel shows a close-up view of the domain's two phospho-amino acid binding sites. The side chains of key residues involved in binding and catalysis of pT and pY within the MAPK signature –pTXpY- motif are shown in green.

4.2. Substrate binding

How does the MAPK substrate containing the –pTXpY– motif bind to the active site within the DSP domain of DS-MKPs? Schumacher et al. [65] recently solved a structure of VHR in complex with the peptide DDE(Nle)pTGpYVATR containing the MAPK consensus motif –pTXpY–. The structure is virtually identical to that of the unliganded state solved previously and the two structures superimpose upon each other with a backbone RMSD of 0.53 Å [64]. The conformation and positioning of key residues in both the active site loop and the general acid loop are remarkably identical in both structures, indicating that the free VHR exists in a catalytically active conformation, and substrate binding would not cause any major structural change of this enzyme. This is in contrast to MKP3 and

PAC1, which, due to their disordered active site residues, would require some sort of conformational change upon substrate binding to generate a productive and catalytically efficient active site.

In the above structure of VHR complexed to a peptide, two distinct binding sites for pT and pY are observed. The pY binds to the active site loop β6–α6 containing the signature sequence –HCXXXXXR–, while pT is loosely tethered into a nearby site clustered with basic residues. The cysteine and arginine residues within the signature sequence of VHR are C124 and R130, while D92 is the aspartate in the general acid loop β5–α5. These residues are positioned optimally for an efficient dephosphorylation of bound pY. The pT binding site is constituted by residues N41 located in the loop β2–α2 and R158 in the loop α7–α8. While the side chain of N41 appears to stabilize pT via hydrogen bonding to the phosphate moiety, the role of R158 is largely to create a positive surface.

The structures of the DSP domain of MKP3 and PAC1 in complex with an ERK2 peptide containing the consensus motif –pTXpY– are eagerly awaited. Preliminary studies however reveal that the DSP domain of PAC1 shows very weak binding to both ERK2 peptides and the full-length ERK2, implying that it is unable to recognize its substrates in the absence of MKB domain. Nonetheless, a few important inferences can be made regarding the possible mode of substrate binding to the DSP domain in an analogy with the crystal structure of VHR in complex with the peptide DDE(Nle)pTGpYVATR [65]. It appears that the DSP domains of both MKP3 and PAC1 also contain a second phospo-binding site for accommodating pT in addition to pY that binds at the enzyme active site. The residues in MKP3 that correspond to N41 and R158 in VHR for binding pT are respectively K220 (in the loop β2–α3) and K327 (in the loop α7–α8) (Fig. 4). In the crystal structure of the DSP domain of MKP3, these residues are located close to the active site of the enzyme in an analogous position to the pT binding site in VHR. Together with K324 and K326, these residues constitute a basic patch that would accommodate pT while pY is bound at the active site.

The residues in PAC1 that correspond to N41 and R158 in VHR and, K220 and K327 in MKP3 for binding pT are respectively S186 (in the loop β2–α3) and R291 (in the loop α7–α8) (Fig. 4). In the structure of the DSP domain of PAC1, these residues are also positioned close to the active site of the enzyme in an analogous position to the pT binding sites in VHR and MKP3. Recent studies based on NMR titration of the DSP domain with *p*-nitrophenylphosphate (pNPP) have indeed pointed to the presence of a second phospho-binding site comprised of a highly basic patch constituted by residues K288, R290 and R291 [67]. Thus, R291 is likely directly involved in stabilizing pT either by creating a positive surface or possibly via hydrogen bonding but the role of S186, which could also stabilize pT through hydrogen bonding, appears less certain. Unlike the analogous residues N41 in VHR and K220 in MKP3, which are both positioned optimally to interact with pT, the loop β2–α3 containing S186 is flipped away from the loop α7–α8 containing R291 by about 20 Å in PAC1—implying that S186 is not optimally positioned in the DSP domain of PAC1 to interact with pT. The possibility that the pT binding site in PAC1 is also disordered however cannot be excluded. Thus, like the ordering of the active site residues, the pT binding site may also be constructed in the presence of the substrate.

4.3. Mechanism of dephosphorylation

The above studies suggest that two distinct phospho-binding sites exist in the DSP domain of DS-MKPs for the binding of pY and pT within the MAPK motif –pTXpY–. Thus, while pY binds to the active site constituted by the phosphatase signature sequence –HCXXXXXR– within the loop β6–α6, pT is tethered into a nearby site clustered with largely basic residues located within the loops β2–α2/α3 and α7–α8. The structural features discussed above thus suggest strongly that the dephosphorylation of pY precedes

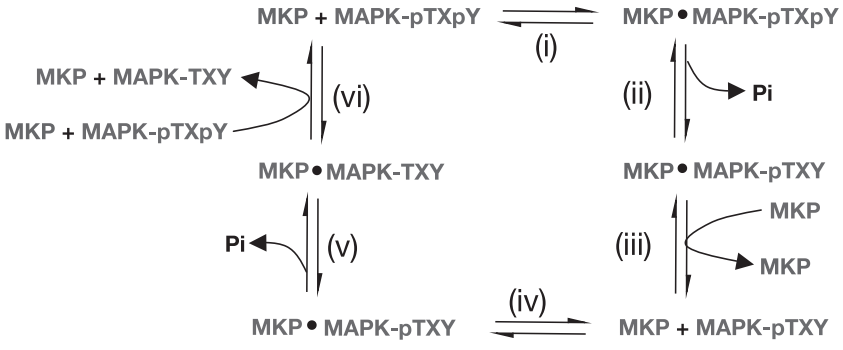

Fig. 5. A generalized ordered and distributive mechanism for the dual dephosphorylation of MAPKs by MKPs. (i) An MKP molecule is recruited to the dually phosphorylated MAPK-pTXpY. (ii) MKP dephosphorylates pY and generates the intermediate MAPK-pTXY. (iii) After dephosphorylation of pY, MKP dissociates from the monophosphorylated MAPK-pTXY substrate. (iv) A second MKP molecule then binds to the monophosphorylated MAPK-pTXY. (v) The second MKP molecule dephosphorylates pT generating the non-phosphorylated MAPK-TXY. (vi) After dephosphorylation of pT, the second MKP molecule dissociates from MAPK-TXY and the enzymatic cycle is repeated.

that of pT. Recent kinetic analysis by Zhao and Zhang [34] indeed indicated that pY is dephosphorylated first followed by that of pT. These investigators went further to demonstrate that the hydrolysis of pY within the dually phosphorylated ERK2/pTXpY by MKP3 generates not only monophosphorylated ERK2/pTXY but also leads to dissociation of MKP3 from ERK2/pTXY intermediate (Fig. 5). A second MKP3 then binds ERK2/pTXY intermediate to produce fully dephosphorylated ERK2/TXY. The structural and kinetic data thus together show that dephosphorylation of MAPKs by MKPs occurs via an ordered and distributive mechanism—in contrast to random and processive mechanism. It is worth pointing out here that VHR is unique among the DS-MKP subfamily in that it shows a marked preference for dephosphorylating pY over pT [40]. In order for pT to be dephosphorylated, it must be able to bind to the active site containing residues C124, R130 and D92. The crystal structure reveals that pT may not be able to efficiently bind to the active site of VHR as the presence of residues E126 and Y128, at positions X_1 and X_2 within the signature sequence $-HCXX_1XX_2XR-$, at the entrance of this active site may cause steric clash with pT [65]. Notably, all other members of DS-MKP, which are able to dephosphorylate both pY and pT, respectively contain the smaller Ala and Ile/Val at positions X_1 and X_2 in the signature sequence.

5. The MKB domain in DS-MKPs acts as the docking site for MAPKs

It has been reported that the catalytic activity of the DSP domain of MKP3 increases by as much as 6 orders of magnitude upon binding to its substrate ERK2 and that this binding is achieved through the interaction of the MKB domain [34,55,69]. What is the basis of this catalytic activation? One might be driven to speculate that, in the absence of MAPK substrate, the MKB domain perhaps somehow blocks the active site of the DSP domain and inactivates the enzyme via the auto-inhibition mechanism as observed in the protein tyrosine phosphatase SHP2 [70]. The fact that an isolated DSP domain alone is virtually inactive towards its MAPK substrates upon truncation of the MKB domain [55,69] argues that the catalytic activation of the full-length DS-MKPs is achieved through a substrate-induced activation mechanism rather than the auto-inhibition mechanism. In an effort to understand how substrate binding to MKB induces activation of the DSP domain and the basis of specificity of this interaction with MAPKs, we recently solved the atomic structure of the MKB domain of MKP3 [71].

As in the case of DSP domains of VHR, MKP3 and PAC1, the solution structure of the MKB domain of MKP3 is also constructed on Rossmann fold with a central five-stranded parallel β-sheet with two α-helices on one face of the sheet and three α-helices on the other face (Fig. 6). In addition, a small anti-parallel β-sheet is found at right angle to the main central β-sheet. The MKB domain among various DS-MKPs shares between 25% and 40% sequence identity and it is characterized by the motif $-\psi\psi XRR\psi XXG-$, where ψ is a hydrophobic residue and X is any residue. Mutagenesis and structural studies indicate that this motif represents the binding site for ERK2 MAPK [71–73]. This motif is located in the loop β3–α3 that also contains β4—which is stabilized by β7 at the C terminus of the MKB domain that directly leads to the DSP domain. Substitution of L63, R64 and R65, corresponding to XRR in the motif $-\psi\psi XRR\psi XXG-$, leads to complete abolishment of MKB domain binding to ERK2 [71,73].

The importance for leucine within the motif $-\psi\psi XRR\psi XXG-$ suggests that electrostatic interactions as well as hydrophobic contacts are critical for interaction with MAPKs. Interestingly, a majority of absolutely conserved residues within the MKB domain of various DS-MKPs are localized on the side of the domain that contains the MAPK docking site [71]. This implies a similarity in binding of other MKB domain to MAPKs. However, local conformation of the loop β3–α3 that contains the MAPK docking site $-\psi\psi XRR\psi XXG-$ may be different due to the high degree of sequence variation within this region of MKB

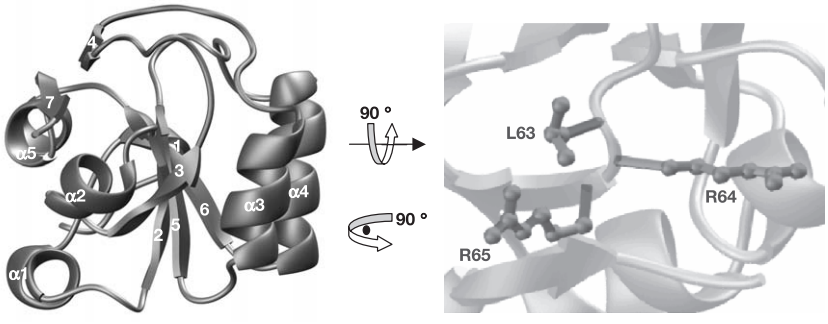

Fig. 6. Structure of the MKB domain of MKP3. The left panel shows the global view of the ribbon representation of the domain. Strands are numbered 1–6, while helices are denoted by α1–α8. The secondary structural elements are colored blue and the loops gray. The loop colored red and containing the strand β4 contains the signature motif—ψψXRRψXXG—that is important for interaction with MAPKs. The right panel shows a close-up view of the domain's docking site for MAPKs, including the side chains of the residues located within the motif—ψψXRRψXXG—that are critical for binding MAPKs.

domains. In addition to the highly conserved G69, which corresponds to the G in −ψψXRRψXXG−, residues such as P59, G60 and P72 are non-conserved. The presence of these non-conserved residues in the immediate vicinity of the motif −ψψXRRψXXG− may dictate the precise local conformation of the loop β3−α3 and hence could hold the clue to the specificity of MKB domains to specific MAPKs.

6. Substrate-induced activation of DS-MKPs by MAPKs

On the basis of high sequence similarity (25−40%) in the MKB domain among various DS-MKPs, it is reasonable to assume that the catalytic activation of the DSP domain via the interaction of the MKB domain with MAPK substrate may be a general feature of all members of this subgroup of DS-MKP family. The MKB and DSP domains in DS-MKPs are connected by a variable length of sequence termed the ''linker region''. Although the motif −ψψXRRψXXG− appears to be the primary docking site for ERK2 and other MAPKs, several lines of evidence suggest that both the linker region (LR) and the DSP domain present additional binding sites for ERK2 and that the binding affinity to ERK2 increases in the order MKP3>(MKB+LR)>MKB [66,71,74]. Although ERK2 is a powerful activator of MKP3, ERK2 mutant D319N is completely disabled in its ability to either bind or activate MKP3 [75,76]. This suggests that D319 and residues flanking it may represent a potential site in ERK2 for binding the MKB domain of MKP3. Indeed, this region in the C lobe of ERK2 appears to be characterized by an highly conserved motif −D(P/T)X(D/E)− in all MAPKs and has been also suggested to be a docking site for MKKs and other activators and regulators of MAPKs [73,77].

Using ERK2 chimeras, further regions in the C lobe of ERK2 that interact with the MKB domain of MKP3 have also been identified [72]. A recent study, based on kinetic and mutagenesis analysis, shows that the binding of MKP3 to ERK2 not only requires the region containing the motif −D(P/T)X(D/E)− on the back of the molecule but also residues lining the ERK2 substrate binding site located at the front side between the two lobes [78]. Thus, although the motifs −ψψXRRψXXG− and −D(P/T)X(D/E)− appear primary contact points in MKPs and MAPKs, additional interactions are likely to define both the specificity and high affinity binding between these partners. The recent MKP3 study has demonstrated that the side of the MKB domain containing the motif −ψψXRRψXXG−, which acts as the docking site for MAPK substrates, also interacts with the DSP domain [71]. In other words, the DSP domain is physically associated with the MKB domain in the absence of substrate−implying that this interaction, which mutually stabilizes the two domains [67], must be disrupted upon the binding of ERK2 substrate. Such a disruption could in turn trigger a sequence of events leading to activation of the DSP domain itself.

In light of the above findings, we postulate a substrate-induced activation mechanism for the activation of DS-MKPs by MAPKs (Fig. 7). In this model, binding of MAPK to the MKB domain of MAPK phosphatases alters its interactions with the DSP domain such that this interaction allosterically triggers the active site residues cysteine and arginine within the signature sequence −HCXXXXXR− and an aspartate in the general acid loop to reconfigure to a conformation optimal for the dephosphorylation of MAP kinases. Such a model could account for the requirement of the MKB domain in the activation of the DSP domain of MKPs by MAPK substrates.

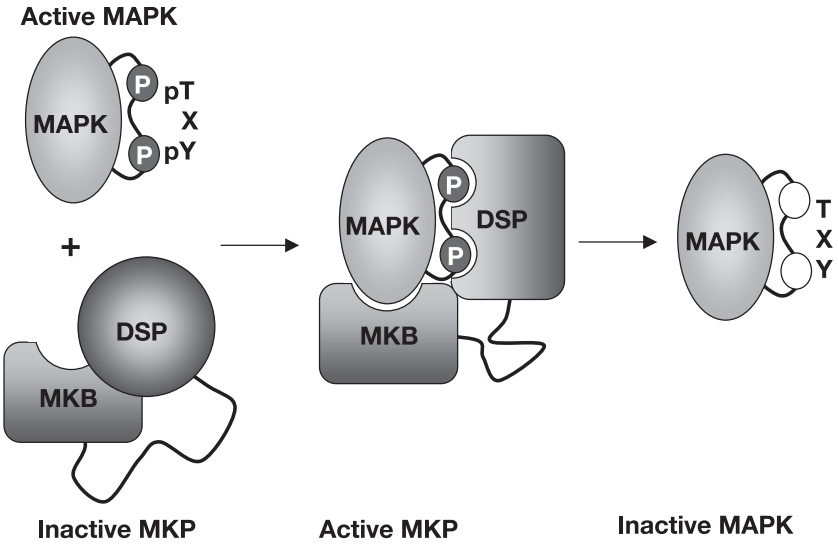

Fig. 7. A model for the substrate-induced catalytic activation of MKPs by MAPKs. In the absence of its substrate, the DSP domain of MKP exists in an inactive state. Binding of the MKB domain of MKP to dual-phosphorylated activated MAPK alters interactions between the DSP and the MKB domains of MKP. This conformational effect, along with the interaction of the DSP domain to MAPK, allosterically triggers the active site residues, cysteine and arginine within the signature sequence −HCXXXXXR− and an aspartate in the general acid loop, to recon figure to a conformation optimal for dephosphorylation of MAPK.

7. Conclusions and future perspectives

The last decade has witnessed the emergence of a new family of protein phosphatases that have come to be known as dual specificity MAPK phosphatases due to the virtue of their unique ability to down-regulate MAP kinases via dephosphorylation of both the phosphotyrosine and phosphothreonine within the consensus motif –pTXpY– of MAPK substrates. Although many important milestones have been reached, we are still a long way from having conquered the detailed structural basis of enzyme-substrate interactions and the mechanism of dephosphorylation of MAPKs by DS-MKPs. Specifically, we need to understand how the active site in the DSP domain becomes re-organized in the presence of the substrate and how the recognition of MAPKs by the MKB domain leads to allosteric activation of the enzyme. In order to answer these questions, we need the three-dimensional structures of a full-length DS-MKP, both alone and in complex with its MAPK substrate. The presence of additional domains of unknown function in Type III and Type IV DS-MKPs also needs a close scrutinization. In the type III subgroup, the role of an additional N-terminal domain could be to provide a cross-talk between MAPK and other signaling pathways. We thus need to search for new binding partners and understand their molecular basis of interaction. In the type IV subgroup, the additional C-terminal domain is believed to play a key role in determining the half-life of the protein. How exactly this is played out in the cell needs some light.

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