REVIEW

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Protein phosphatases of *Saccharomyces cerevisiae*

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Abstract

The phosphorylation status of a protein is highly regulated and is determined by the opposing activities of protein kinases and protein phosphatases within the cell. While much is known about the protein kinases found in *Saccharomyces cerevisiae*, the protein phosphatases are much less characterized. Of the 127 protein kinases in yeast, over 90% are in the same evolutionary lineage. In contrast, protein phosphatases are fewer in number (only 43 have been identified in yeast) and comprise multiple, distinct evolutionary lineages. Here we review the protein phosphatase families of yeast with regard to structure, catalytic mechanism, regulation, and signal transduction participation.

Keywords Protein phosphatase · Convergent evolution · *Saccharomyces cerevisiae* · Catalytic mechanism · Signal transduction

Introduction

Reversible protein phosphorylation is the most common post-translational modification in all eukaryotes. Many phosphorylation events play key regulatory roles by dictating a protein's activity, localization, or stability. Most studies of signal transduction have focused on the protein kinases that catalyze the transfer of the terminal phosphate of ATP to the hydroxyl group of serine, threonine, and tyrosine residues. While much has been learned about signal transduction by studying the protein kinases, the phosphorylation status of a protein is determined by the integration of two rates: phosphorylation and dephosphorylation. Protein phosphatases are the enzymes that hydrolyze the phosphoester bonds present in phosphorylated proteins. What may be underappreciated, by some, is the active role played by the protein phosphatases in signal transduction. Phosphatases do much more than return the system to the ground state, and in some cases, the dephosphorylation reaction is the site of regulation.

The protein phosphatases represent a case study in convergent evolution. In *S. cerevisiae*, the development of

enzymes capable of phosphoprotein dephosphorylation has been achieved by at least five distinct evolutionary lineages of proteins. This contrasts greatly with the protein kinases of yeast where the vast majority (117 of 127) are homologous and members of the same eukaryotic protein kinase superfamily (Hunter and Plowman 1997; Rubenstein and Schmidt 2007). Another difference between kinases and phosphatases is the abundance; phosphatases in general are much more abundant in the cell than kinases and have longer half-lives (Smoly et al. 2017). In addition, phosphatases are regulated more by the binding of different subunits (Abd-Rabbo and Michnick 2017), while kinases are regulated more often by phosphorylation (Rubenstein and Schmidt 2007).

Eukaryotic phosphatases were originally categorized into families based on biochemical properties such as their ability to dephosphorylate the β subunit of phosphorylase kinase and on their sensitivity to two small protein inhibitors (Cohen 1989). The advent of the DNA sequence data from numerous genomes, along with structural and catalytic information, makes clear that the classification based on biochemical properties did not always reflect evolutionary lineages. We now know that multiple families of protein phosphatases have independently evolved. The genome of *Saccharomyces cerevisiae* encodes at least 43 protein phosphatase enzymes in five distinct evolutionary lineages (Fig. 1). The three most well-known families of protein phosphatases are the PPP, PPM, and PTP families. The PPP (phosphoprotein phosphatase) superfamily of protein phosphatases includes, but is not limited to,

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PPP Family

Sub-Family

CMP2
CNA1 — PP3
PPN2

PPT1 — PP5

GLC7 — PP1

PPZ1
PPZ2 — PP1-like
PPQ1

PPG1 — PP2A-like

PPH21
PPH22 — PP2A

PPH3 — PP4
SIT4 — PP6

PPM Family

PTC1
PTC2
PTC3
PTC4 — PP2C
PTC5
PTC6
PTC7

PTP Family

CDC14
MSG5
SDP1
YVH1 — DSP
PPS1
TEP1

MIH1
YCH1 — CDC25

LTP1 — LMWP
SSU72 — SSU

OCA1
OCA2
SIW14 — PTP
PTP1
PTP2
PTP3

YMR1 — MPD

HAD Family

NEM1
PSR1
PSR2 — NIF
FCP1

RTR Family

RTR1
RTR2 — RTR

Fig. 1 Protein phosphatase families of *S. cerevisiae*. Dendrograms of the five families of protein phosphatases found in *S. cerevisiae* were constructed using the entire proteins for PPM, HAD and RTR families and the catalytic domains for the PPP and PTP families. *PPP* phosphoprotein phosphatase, *PPM* phosphoprotein phosphatase metal-dependent, *PTP* phosphotyrosine phosphatase, *DSP* dual specificity phosphatase, *HAD* haloacid dehalogenase. Phosphatases essential for viability are shown in boxes

protein-serine/threonine phosphatase type 1, type 2A, and calcineurin (type 2B). Yeast express 13 PPP phosphatases. The PPM (protein phosphatase metal-dependent) superfamily comprised of a single family of Mg^{2+}- (or Mn^{2+}-) dependent protein phosphatases. Based on biochemical properties, the PPM family was originally classified as type 2C phosphatases. However, we now know that the PPM family is a distinct evolutionary family and unrelated to the PPP phosphatases, a fact that makes the type 2C name somewhat misleading. Furthermore, the naming of the PPM family based on metal ion dependence is also unfortunate since other phosphatase families (e.g., PPP) are also dependent on metal ions in their active sites. However, it is clear that the 7 PPM family members in yeast represent a single evolutionary lineage. The third well-known family of protein phosphatases is the PTP (phosphotyrosine phosphatases) family that includes the six distinct phosphatase domains, two of which are the tyrosine phosphatases and the dual specificity phosphatases. These 17 members of the PTP family share a common catalytic mechanism distinct from the PPP and PPM phosphatases but may not all be in the same evolutionary lineage. Finally, two relatively new families of protein phosphatases are also represented in *S. cerevisiae*. These are the HAD (haloacid dehalogenase) and RTR1 families. The HAD family includes four members that catalyze dephosphorylation of proteins and includes additional enzymes that dephosphorylate a variety of small molecules and nucleic acids (Kuznetsova et al. 2015; Melcher and Entian 1992; Vance and Wilson 2001). The RTR1 family, named for its founding member, the *S. cerevisiae* phosphatase Regulator of Transcription 1, is composed of Rtr1 and its paralog, Rtr2. These phosphatases are associated with RNA polymerase II in both yeast and mammals and act as C-terminal domain phosphatases (Egloff et al. 2012; Mosley et al. 2009).

In this review, we cover the 43 protein phosphatases of *Saccharomyces cerevisiae* with a particular focus on their classification, catalytic mechanism, and participation in signal transduction pathways.

Protein phosphatase families

PPP family

Members of the PPP family account for the great majority of serine/threonine phosphatase activity in eukaryotic cells
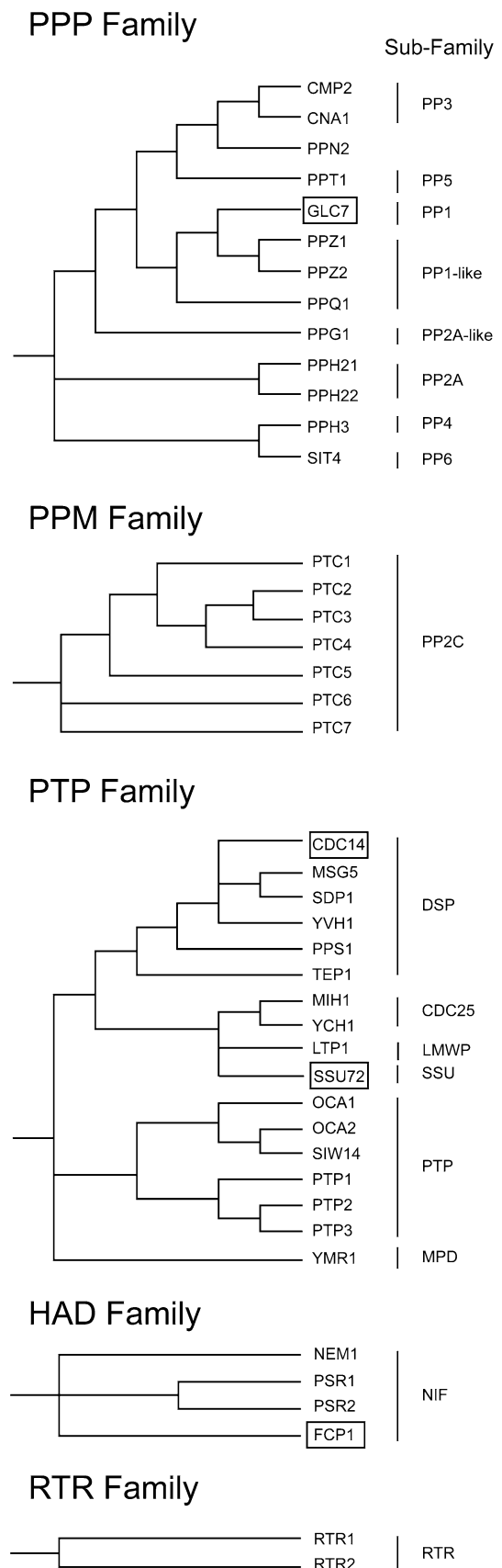
(Castermans et al. 2012). The *S. cerevisiae* genome encodes 13 phosphatases in the PPP family and dozens of regulatory subunits. Members of the PPP family have been classified into multiple sub-families (Fig. 1) based primarily on their interactions with distinct regulatory subunits (Brautigan and Shenolikar 2018). Members of the PPP family have regions of amino acid primary sequence that are highly conserved (Fig. 2a). Outside of the catalytic domain, the N- and C-terminal domains of these proteins vary in size and show little similarity (Fig. 2b). Crystal structures of several members of this family have been solved and show a central β sandwich that coordinates two metal ions (Goldberg et al. 1995; Hurley et al. 2007; Ye et al. 2013).

Catalytic mechanism

The catalytic mechanism of the PPP phosphatases utilizes a metal-activated water to directly hydrolyze the phosphoester bond (Egloff et al. 1995; Goldberg et al. 1995). The identity of the coordinated metal ion varies between different PPP phosphatases, and in most cases, has not been determined with certainty. Calcineurin binds one atom of Fe^{2+} and one of Zn^{2+} at its active site (King and Huang 1984), while PP1 is thought to bind Mn^{2+} and possibly Fe^{2+} (Goldberg et al. 1995). The structure of a PPP phosphatase and the mechanism of catalysis are depicted in Fig. 2c, d.
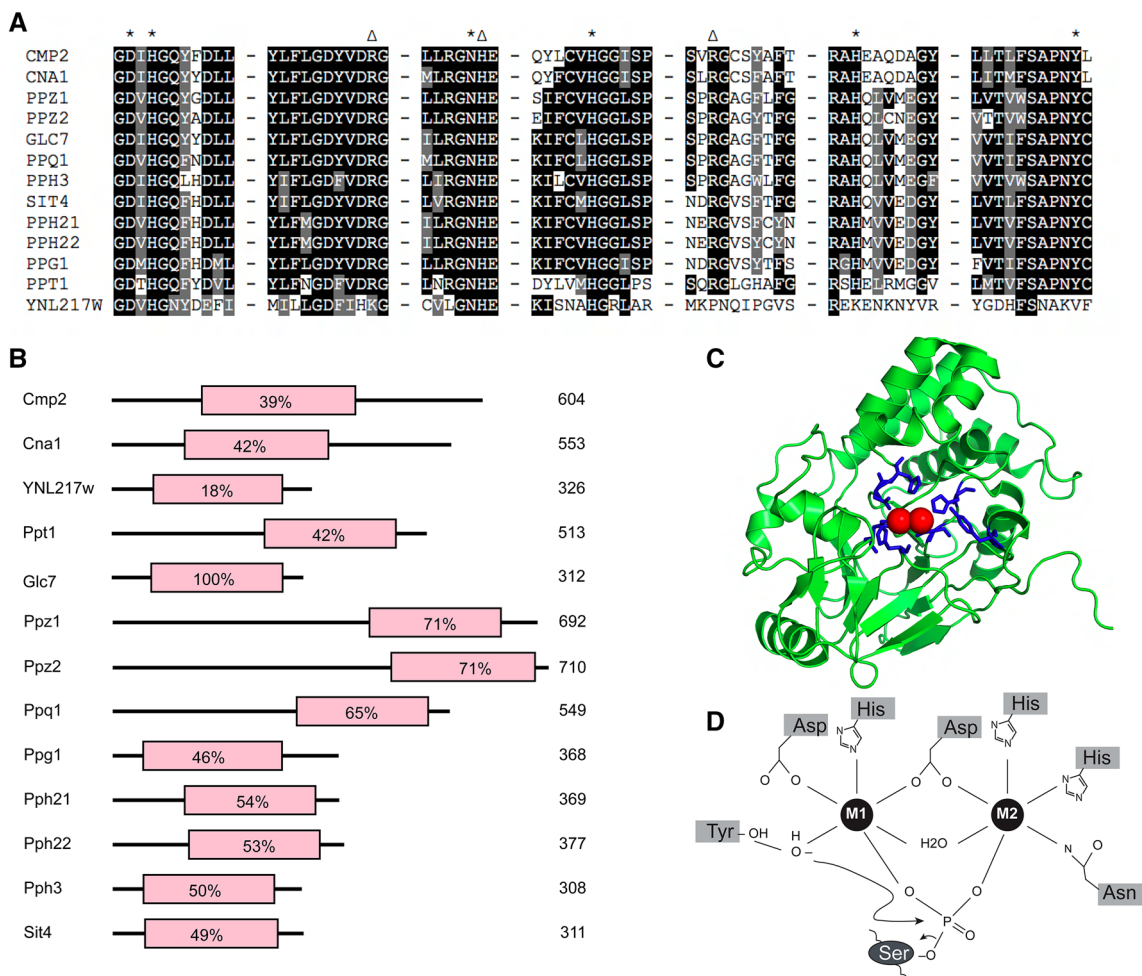

Fig. 2 PPP phosphatases of *S. cerevisiae*. **a** Multiple sequence alignment of the conserved catalytic domains of the PPP phosphatases. Each catalytic domain is approximately 200 residues in length. Breaks (denoted with a hyphen) have been introduced in the alignment to highlight the most highly conserved sequences. Residues predicted to make contact with the two metal ions (*) and the phosphoryl group on the substrate (Δ) are identified based on their conservation with rabbit PP1 (Goldberg et al. 1995). **b** Schematic representation of the PP1 phosphatases showing the total size of the protein, the position of the catalytic domain and the percentage identity when aligned with the catalytic domain of Glc7. **c** Structure of a PPP catalytic domain is portrayed showing the metal ions at the active site (red spheres) and their coordinating residues (blue sticks). Structural model is based on human PP1 using PDB file 1S70 (Terrak et al. 2004). **d** Proposed catalytic mechanism for the PPP family showing the coordination or the metal ions and activated water molecule that acts as the nucleophile-attacking phosphoserine

Regulation

A hallmark of the PPP phosphatases is the formation of multimeric complexes composed of a single catalytic subunit and one or more regulatory subunits that are believed to control catalytic activity (King and Huang 1984). The specificity of the PPP phosphatases is generated, to a large degree, through the association with regulatory subunits. Thus, while yeast may only express 13 catalytic subunits in the PPP family, there are likely to be hundreds of distinct holoenzymes that differ in the identity and number of regulatory subunits.

PP1 phosphatases The type 1 protein phosphatase, PP1, is an extreme case when it comes to the formation of multiple distinct holoenzymes. In mammalian cells, over 180 PP1 regulatory subunits have been identified (Heroes et al. 2012). In yeast, no fewer than 17 regulatory subunits for Glc7, the sole yeast PP1 enzyme, have been identified (Cannon 2010) with the probability that many more await discovery. Bioinformatics approaches to identify PP1 interacting proteins (PIPs) have proven to be challenging since these proteins have limited sequence similarity. In general, PIPs tend to be proteins with large regions that are inherently disordered. This property allows them to wrap around the PP1 catalytic subunit forming multiple low affinity contacts that, when added together, provide both specificity and strength of interaction (Heroes et al. 2012).

Short sequence motifs present on the PIPs interact with distinct docking sites on the surface of PP1. The best characterized of these short sequence motifs is the RVxF motif (Hendrickx et al. 2009). Most PIPs contain this motif, and it is required for their stable association with the PP1 enzyme. The conserved valine and phenylalanine side chains from the RVxF sequence fit into a hydrophobic channel on the surface of the PP1 enzyme opposite from the active site. The hydrophobic channel is created by two C-terminal β sheets that are conserved in all PPP phosphatases (Heroes et al. 2012). In yeast, the PIPs whose interactions with PP1 are known to require a RVxF motif are shown in Fig. 3a. Sequence analyses of the RVxF motifs used by the yeast PIPs reveal that the consensus is actually [KR]xVRF (Fig. 3b). Mutations in this motif eliminate or greatly reduce interaction with PP1 (Bharucha et al. 2008a, b; Chang et al. 2002; Dombek et al. 1999; Knaus et al. 2005; Kozubowski et al. 2003; Wu and Tatchell 2001).

While the RVxF motif is required for PP1 binding, additional determinants are likely important. Mammalian PIPs use additional short sequence motifs to stabilize the interaction with PP1 (Heroes et al. 2012). However, these additional PP1 interaction motifs are not recognizable in yeast PIPs. For instance, the myosin phosphatase N-terminal element (MyPhoNE) with the consensus sequence RxxQ[VIL]

[KR]x[YW] is found seven times in the yeast proteome but not in any of the known yeast PIPs. Despite the challenges in identifying PP1 interaction motifs through computational means, the likelihood remains that additional interactions over the surface of the PP1 enzyme are important for specific associations, since point mutations on the surface of yeast PP1 at sites distinct from the hydrophobic groove cause pleiotropic phenotypes and interfere with association of distinct PIPs (Baker et al. 1997; Connor et al. 2000).

The regulation of yeast PP1 activity, localization, and substrate specificity is conferred by association with distinct PIPs. In mammalian cells, the PIPs form contacts near the active site of PP1 in a manner that can either restrict access to specific substrates (Ragusa et al. 2010) or block the active site entirely (Hurley et al. 2007). Similar mechanisms are likely to be utilized in yeast. For instance, the PP1-like phosphatase, Ppz1, has two inhibitory subunits, Vhs3 and Hal3 (Ruiz et al. 2004). Hal3 binds to the C-terminal catalytic domain and negatively regulates the phosphatase (de Nadal et al. 1998). Despite having a KLHVLF motif, Hal3 interacts with Ppz1 at two alternative points. These interactions are necessary for negative regulation of Ppz1 (Munoz et al. 2004). What is clear for the yeast enzyme is that distinct PIPs control the localization of the PP1 enzyme (Knaus et al. 2005; Kozubowski et al. 2003; Pedelini et al. 2007; Peggie et al. 2002; Pinsky et al. 2006; Tachikawa et al. 2001). Further studies will be needed to determine whether the control of the localization of the Glc7 protein is sufficient to dictate substrate selection or whether additional mechanisms for substrate selection are at work.

PP2A phosphatases Yeast encodes 5 phosphatases that can be considered to be PP2A or PP2A-like phosphatases (Fig. 1). The canonical PP2A phosphatase is a heterotrimer composed of an A-subunit, a B-subunit, and a single catalytic C-subunit (Fig. 4a). Yeast encodes two PP2A catalytic subunits (Pph21 and Pph22), a single A-subunit (Tpd3) and two alternative B subunits (Cdc55 and Rts1) (Jiang 2006). In addition, PP2A-like phosphatases also form other heterotrimeric complexes. They can bind Tap42, a distinct regulatory subunit unrelated to the A and B subunits. Binding of Tap42 is required for proper function and substrate targeting of Sit4 and Pph21, suggesting that Tap42 possibly binds and regulates all PP2A phosphatases (Cherkasova and Hinnebusch 2003; Wang et al. 2003).

Additionally, the PP2A phosphatases undergo forms of post-translational modifications that affect the regulation of these proteins. The C terminus of the catalytic subunit ends with a conserved YFL motif undergoes reversible methylation that affects the phosphatase's ability to associate with the A and B subunits (Fig. 4a) (Wei et al. 2001). Interestingly, this YFL motif is present at the C terminus of only five proteins in the yeast proteome. These five proteins are all
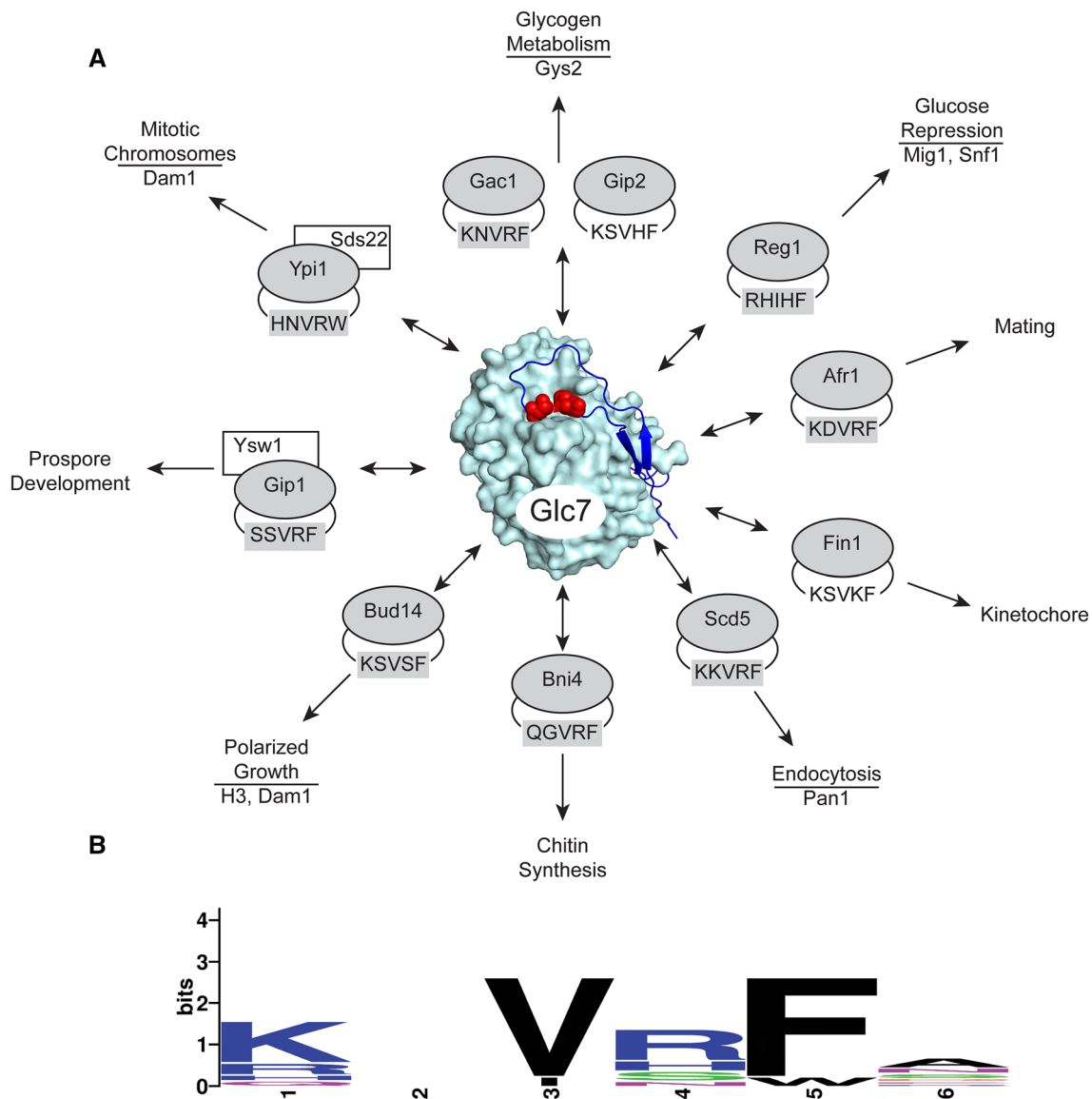
Fig. 3 Regulatory subunits compete for binding to a common site on PP1 phosphatase Glc7. **a** Many of the PP1 regulatory subunits associate with Glc7 through interactions between a hydrophobic groove present on the back surface of PP1 (shown in cyan) and a PP1 binding motif known as the RVxF motif. The hydrophobic residues in this motif (Val and Phe) are shown as red spheres. The regulatory subunits direct the phosphatase to different subcellular localizations, biological processes and substrates as shown. The sequence of the RVxF motif for each subunit is shown below. Those present in a shaded box have experimental evidence showing their importance for Glc7 association (Bharucha et al. 2008a, b; Chang et al. 2002; Dombek et al. 1999; Knaus et al. 2005; Kozubowski et al. 2003; Nakamura et al. 2017; Wu and Tatchell 2001). Unshaded sequences indicate potential PP1 interaction motifs that have yet to be verified. **b** Logo representation of the confirmed PP1 interaction motifs in *S. cerevisiae* indicating that the yeast consensus is [KR]xVRF

PP2A and PP2A-like phosphatases (Fig. 4b). The carboxy-methylation of the C-terminal leucine in Pph21 and Pph22 is required for activity and association with the B subunits (Castermans et al. 2012). This methylation is performed by the methyltransferase Ppm1 (Wei et al. 2001).

PP2A phosphatases have also been found to bind to additional proteins that influence their regulation. Members of the PP2A family require the peptidyl-prolyl cis/trans-isomerase activity of Rrd1 and Rrd2 proteins (formerly known as Ypa1 and Ypa2) for proper folding and activity (Chao et al. 2006; Jordens et al. 2006). The action of Rrd1 and Rrd2 promotes binding with other proteins and subunits and is a critical activator of PP2A and PP2A-like phosphatases (Van Hoof et al. 2005). Sit4 interacts with a number of proteins called SIT4 Associating Proteins (SAPs) that differ in functions. These interactions are required for proper function; however, their detail mechanisms are not yet known (Luke et al. 1996).
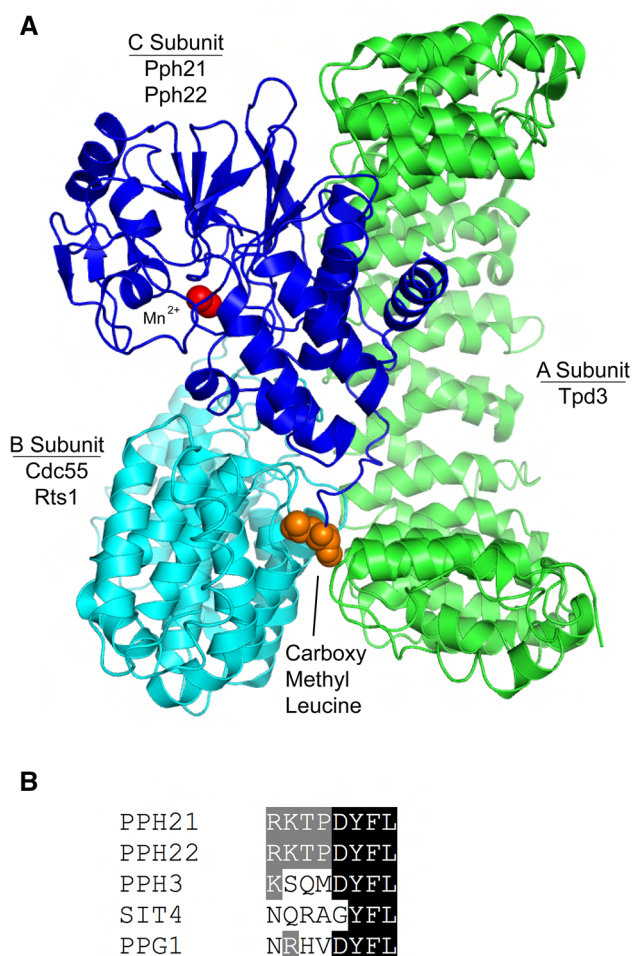
Fig. 4 C-terminal modification of the yeast protein phosphatase 2A. **a** Structural model of the yeast PP2A showing the location of the C-terminal carboxymethyl leucine (orange spheres) sandwiched between the A and B subunits of the PP2A heterotrimer. The manganese ions at the active site of the catalytic C subunit are shown (red spheres). **b** Alignment of the C termini of the PP2A and PP2A-like phosphatases

PP2B/calcineurin phosphatases The PP2B phosphatases, also known as the calcineurin phosphatases, are heterodimers. These phosphatases have a catalytic A-subunit (Cna1 or Cna2) and a regulatory subunit (Cnb1) (Roy et al. 2007). Full function of calcineurin requires both Ca^{2+} binding as well as the recruitment of calmodulin (Connolly et al. 2018). Calcium binding to the 4 EF hand domains in the regulatory subunit induces conformational changes that are important for activation of the phosphatase (Connolly et al. 2018). The calcineurin active site contains two metal ions: one molecule of Zn^{2+} and one of Fe^{2+} (Wang et al. 1996). Metal binding induces a conformational change and is required for activity of the phosphatase (Ren et al. 2009). Calcineurin binds to a number of substrates using short peptide sequences as recognition motifs. For instance, calcineurin binding to the transcription factor Crz1 requires the PxIxIT motif (Gold-man et al. 2014; Roy et al. 2007) while binding to Rcn1 requires the LxVP motif (Grigoriu et al. 2013). Additionally, the immunosuppressive drugs FK506 in a complex with Fpr1 and cyclosporin in a complex with Cpr1 act as an inhibitors of calcineurin (Heitman et al. 1991).

Signal transduction

Members of the PPP family are involved in a vast number of cellular processes. Glc7, the most well-studied and only essential PPP phosphatase in yeast, is involved in a myriad of cellular processes. An extraordinary amount of Glc7 regulation is mediated by its interacting proteins. Regulatory subunits may compete for interaction with a pool of available Glc7, as increasing the expression of particular subunits diminishes Glc7 activity in other pathways. Indeed, the expression levels of some (but not all) of the Glc7-regulatory subunits vary with certain cellular conditions (Yiu et al. 2008). For instance, expression of the Gip1 regulatory subunit increases in meiosis (Ramaswamy et al. 1998; Tu et al. 1996), when it is required for proper septin organization and localization of Glc7 to the prospore membranes (Tachikawa et al. 2001).

Numerous environmental stresses generate a calcium signal that activates calcineurin signaling (Cyert 2003). Calcineurin acts directly on the transcription factor Crz1. Dephosphorylation of Crz1 promotes translocation to the nucleus (Stathopoulos-Gerontides et al. 1999). The strength of interaction between calcineurin and Crz1 dictates the magnitude of the transcriptional response (Roy et al. 2007). Overall, as many as 160 genes may be transcriptionally regulated by calcineurin signaling (Yoshimoto et al. 2002).

PPM family

The PPM family of *S. cerevisiae* contains a single subfamily, the PP2C phosphatases. Members of this family lack sequence or structural similarity to the PPP phosphatases (Figs. 2, 5) but share a similar catalytic mechanism with two metal ions at the active site (Das et al. 1996). However, the overall folds of the PPP and PPM proteins are completely unrelated (Figs. 2c, 5b), as are the residues that coordinate the metal ions (Figs. 2d, 5c). In contrast with the PPP phosphatases, PP2C phosphatases tend to function as monomers and do not utilize accessory subunits for regulation (Cohen 1989). While Ptc2 and Ptc3 are closely related (60% sequence identity), other members of the family show a much lower degree of sequence identity (15–30%).

Catalytic mechanism

Both PPP and PPM phosphatases use metal ions to activate a water molecule that acts to directly hydrolyze the
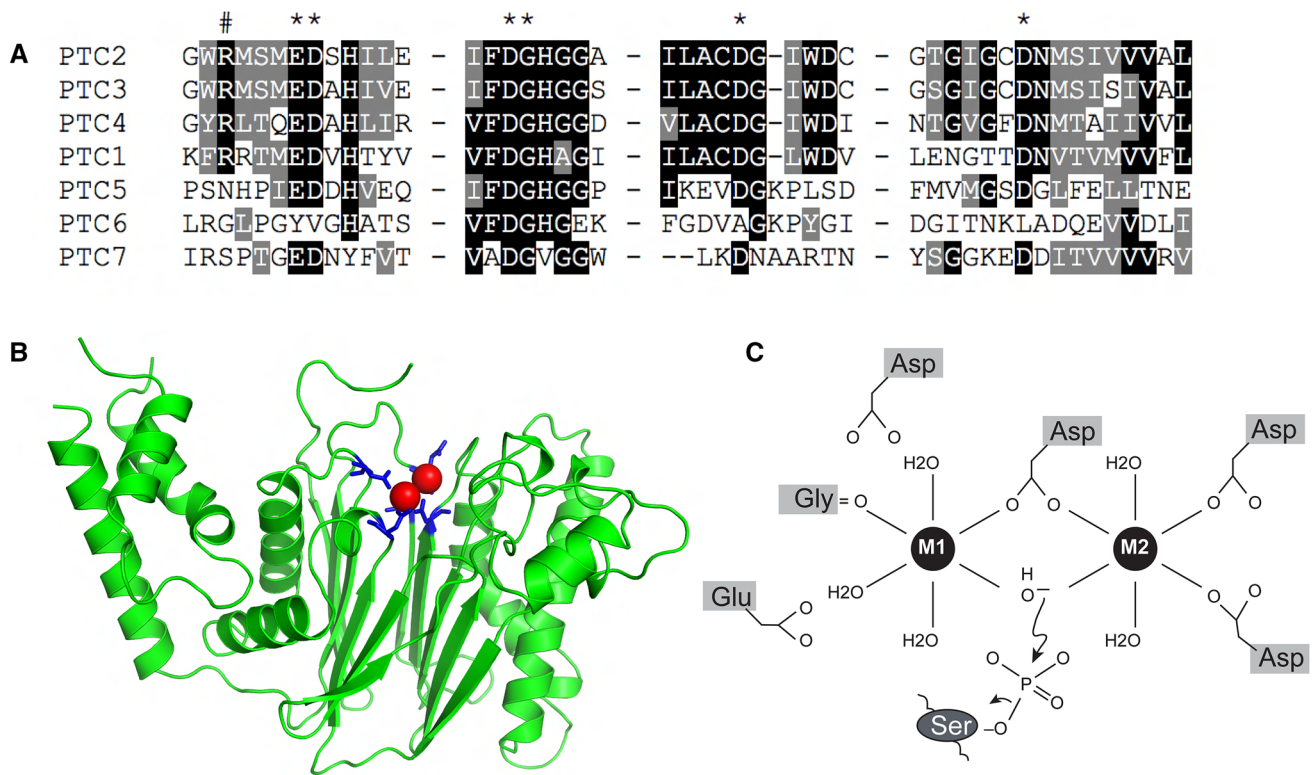
Fig. 5 PPM phosphatase family. **a** Multiple sequence of the most highly conserved segments of the yeast PPM phosphatases. Gaps are indicated with hyphens. Homology to human PP2Ca protein (ref) was used to predict residues that coordinate the metal ions at the active site (asterisk) and interact with the phosphate group of the substrate (hash). **b** Structure of a PPM phosphatase in cartoon representation showing the pair of metal ions (red spheres). Residues that coordinate the metal ions are shown in blue. The phosphatase shown is human PP2C using PDB file 1A6Q (Das et al. 1996). **c** Proposed catalytic mechanism for PP2C showing the activated water molecule that acts as the nucleophile

phosphoester bond (Das et al. 1996). The PPM phosphatases use four aspartate residues to coordinate two Mn^{2+} ions that then activate a water molecule (Cohen 1989). This water molecule utilizes hydrogen bonds to coordinate the three phosphate oxygens of the phosphate group. Catalysis then occurs in a two-step manner. First, the metal ion-activated water acts as a nucleophile to attack the phosphorous. Then, a second water molecule protonates the leaving group and completes the reaction. In this, it is important to note that the metal ion coordinating residues are invariant (Fig. 5a), supporting this model of catalysis (Das et al. 1996). Figure 5b, c illustrates the structure of a PPM phosphatase as well as the phosphatase reaction.

Regulation

Unlike the PPP phosphatases, the PPM family phosphatases are not stably associated with regulatory subunits that specify localization and substrate targeting. Instead, PP2C phosphatases can be inactivated through competitive inhibition at the Mg^{2+}/Mn^{2+} binding site by ions such

as Ca^{2+}, Zn^{2+}, and Ni^{2+} (Das et al. 1996). Additionally, there is evidence that PP2C phosphatases may use transient interactions with adaptor proteins to help target the phosphatases to the appropriate substrates. This was seen with Ptc1 and the SH3 domain-containing adapter protein, Nbp2. Nbp2 stabilizes the interaction between Ptc1 and its substrate, Pbs2, a kinase in the HOG pathway (Ota and Mapes 2007).

Interestingly, alternative splicing may play a role in the regulation of PPM phosphatases. In particular, the Ptc7 mRNA can be alternatively spliced generating different proteins that display distinct localization patterns (Juneau et al. 2009). The Ptc7 protein derived from spliced mRNA has variable expression dependent on carbon source and can localize to the mitochondria, while the unspliced form contains several transmembrane domains and localizes to the nuclear envelope. The unspliced Ptc7 helps the yeast cell cope with Latrunculin A toxicity. Both isoforms carry the characteristic and conserved PP2C motifs and house phosphatase activity (Juneau et al. 2009).

Signal transduction

The phosphatases of the PPM family are involved in a number of cellular functions. These phosphatases are functionally redundant, particularly in regard to cold response, high temperature stress, as well as with Li^{2+}, Na^{2+}, and caffeine sensitivity (Sharmin et al. 2015). Ptc1 is required for the transition between the G2 phase and M phase of the cell cycle by activating the cell wall integrity (CWI) pathway through dephosphorylation of Mkk1, a MAPK kinase (Tatjer et al. 2016). Ptc1 is required for cortical ER inheritance to help facilitate ER tubule spreading across the bud cortex (Li et al. 2013). Ptc6 is involved in the CWI pathway by negatively regulating the MAP kinase, Slt2 (Sharmin et al. 2015). Ptc7 dephosphorylates the hydrolase Coq7 (Cat5) activating coenzyme Q6 biosynthesis (Martin-Montalvo et al. 2013). While this is just a snapshot of the processes PP2C phosphatases are involved in, it is clear that PP2C phosphatases have overlapping functions and are involved in a variety of cellular processes.

PTP family

The PTP family of protein phosphatases is a diverse family with very limited sequence identity between members. Outside of the catalytic core, the members of the PTP family show very little sequence similarity and may even represent multiple evolutionary lineages (Fig. 6a). PTP phosphatases have an expanded substrate specificity to include proteins with phosphorylated serine, threonine, and tyrosine residues. While yeast lacks tyrosine-specific protein kinases, it encodes several dual specificity kinases that are capable of phosphorylating tyrosine residues. Indeed, although small in number, the phosphorylation of tyrosine residues in yeast is critical to important signal transduction pathways, including cell cycle checkpoint regulation (Sia et al. 1996; Zhan et al. 1997) and stress response signaling (Hahn and Thiele 2002; Mattison et al. 1999). Additionally, members of the PTP family are highly important in facilitating numerous intracellular signaling pathways (Barford et al. 1998). The wide variety of substrates and roles within the cell may explain the diversity of phosphatase domains within the PTP protein phosphatase family.

Catalytic mechanism

The PTP family members share a common catalytic mechanism with a conserved sequence motif represented by C-x5-R (Fig. 6b). This motif includes the invariant cysteine residue that serves as the nucleophile in the dephosphorylation reaction (Fig. 6c) and the arginine residue that acts to stabilize the covalent phosphoryl-enzyme intermediate (Zhang et al. 1994). The presence of phosphopeptide substrates induces a conformational change, aligning the catalytic residues with the substrate and allowing for catalysis to occur (Barford et al. 1998).

Regulation

Members of the PTP family differ in the regulatory domains attached to either side of the catalytic subunit. Regulation of catalysis, substrate specificity, and localization is influenced by the presence of these domains (Barford et al. 1998). PTP phosphatases may be regulated by other means, such as through sequestration or by binding with an inhibitor protein, as seen with the essential phosphatase, Cdc14. Prior to returning to G1 in the cell cycle, Cdc14 interacts with its inhibitor, Net1, which sequesters Cdc14 in the nucleolus (Shou et al. 1999; Visintin et al. 1999). Release of Cdc14 is required for cell cycle progression. Additionally, Cdc14 must dimerize for proper function, since disruption of the binding interface interferes with Cdc14 function (Kobayashi and Matsuura 2017). Cdc14 is also regulated by various signaling cascades, including the FEAR and the MEN signaling cascades, which influence proper localization of Cdc14 (Stegmeier and Amon 2004).

Signal transduction

The PTP family is composed of a diverse group of protein phosphatases that share a conserved catalytic motif. Due to the limited sequence identity and wide substrate specificity, the phosphatases within the PTP family are involved in a wide variety of functions and roles within the cell. In fact, there are as many as 455 potential substrates for Cdc14 (Kao et al. 2014). Cdc14 is involved in a number of functions, including chromosome segregation (Matos-Perdomo and Machin 2018; Ramos et al. 2017), autophagy induction (Kondo et al. 2018), recombinational DNA repair (Villoria et al. 2017), cytokinesis (Kuilman et al. 2015; Miller et al. 2015), and spindle stability and duplication (Fox et al. 2017; Villoria et al. 2017). Cdc14 is activated in times of starvation and has been connected to TORC1 inactivation (Villoria et al. 2017). The Mih1 phosphatase (Mitotic Inducer Homolog 1) is a dual specificity protein phosphatase that contains a Rhodanese-like domain (Fig. 6a). Mih1 is the yeast ortholog of mammalian and *S. pombe* Cdc25. These important phosphatases activate cyclin-dependent protein kinases that control cell cycle progression such as Cdc28 in *S. cerevisiae* (Sia et al. 1996) and cdc2/cyclinB in humans (Strausfeld et al. 1991).

Ssu72, the other essential PTP phosphatase, is highly involved with transcription and interacts with RPB2, a subunit of RNA polymerase II (Pappas and Hampsey 2000). Ssu72 dephosphorylate residues of the carboxy-terminal domain (CTD) of RNA polymerase II (Ganem et al. 2006;
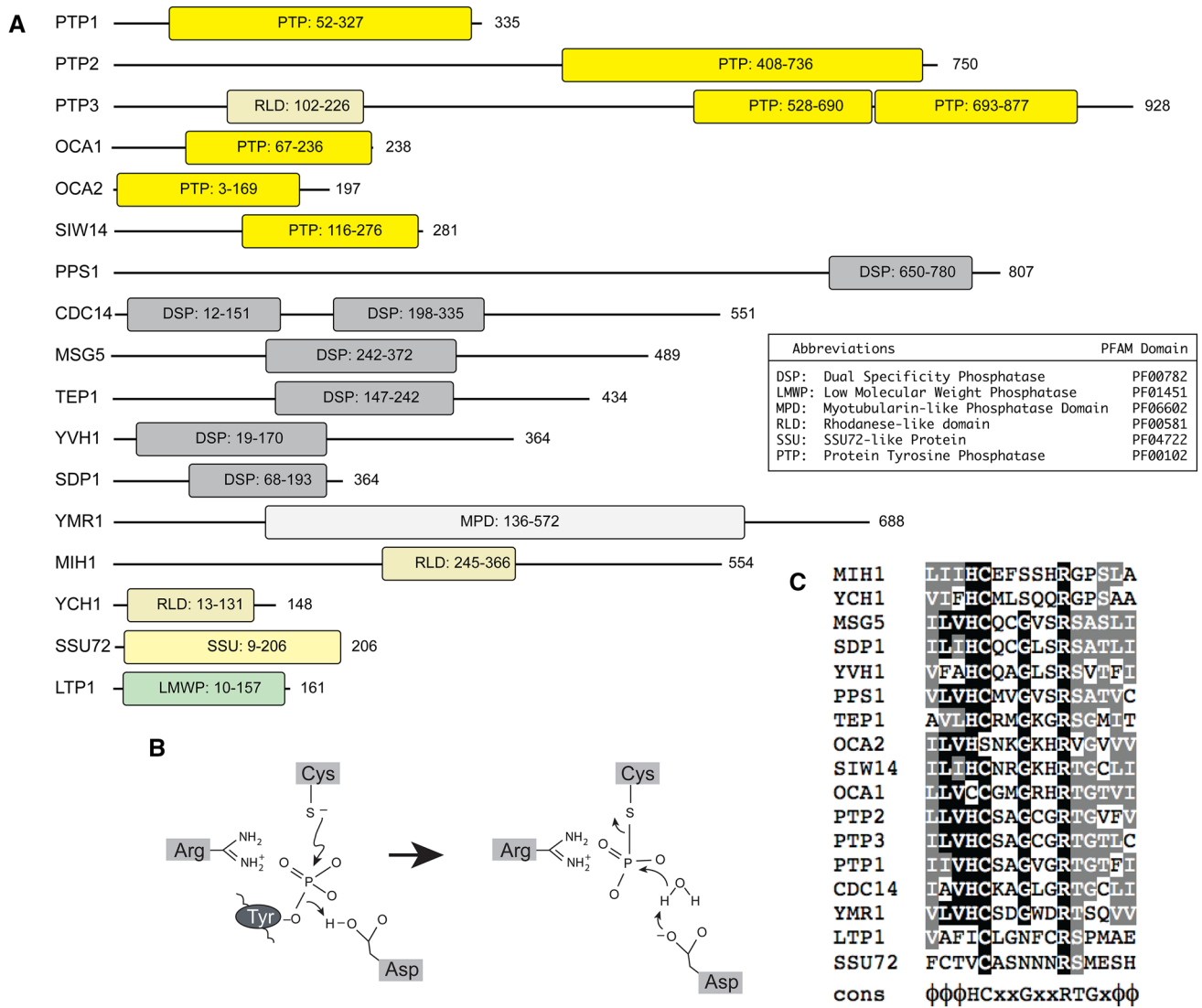
Fig. 6 Protein tyrosine phosphatase family. **a** Schematic representation of the yeast PTP family showing the protein size and the location of recognizable PFAM domains. **b** Proposed catalytic mechanism of the PTP family showing the conserved cysteine residue acting as the nucleophile and the conserve arginine acting to stabilize the phosphoryl intermediate. **c** Multiple sequence alignment of the catalytic core of the PTP family members and the conserved C-x5-R motif. Hydrophobic residues are represented in the consensus as ø

Krishnamurthy et al. 2004) with a preference for Ser7 (Zhang et al. 2012) and indirectly influence the dephosphorylation of Ser2 during initiation (Rosado-Lugo and Hampsey 2014). Interestingly, Ssu72 has roles both in the initiation–elongation transition phase (Dichtl et al. 2002; Pappas and Hampsey 2000; Rosado-Lugo and Hampsey 2014) as well as the termination phase of transcription (Ansari and Hampsey 2005; Dichtl et al. 2002; Ganem et al. 2003; He et al. 2003; Krishnamurthy et al. 2004; Reyes-Reyes and Hampsey 2007; Zhang et al. 2012). Ssu72 is required for the formation of gene loops during transcription (Ansari and Hampsey 2005; Rosado-Lugo and Hampsey 2014; Tan-Wong et al. 2012). Additionally, Ssu72 interacts with the cleavage and polyadenylation machinery (Ansari and Hampsey 2005; Dichtl et al. 2002; He et al. 2003; Steinmetz and Brow 2003). With this in mind, it is clear that Ssu72 plays an important role in the regulation of the RNA polymerase II and transcription.

HAD family

The HAD family of protein phosphatases was discovered when the identity of a phosphatase that acted on the RNA polymerase II carboxy-terminal domain (CTD) was discovered (Chambers and Dahmus 1994; Chambers and Kane 1996). The HAD family is not related to other protein phosphatase families by sequence, but is instead related to the haloacid dehalogenase superfamily (Burroughs et al.

2006). The members of this family are characterized by the DxDx(T/V) signature motif (Fig. 7a) located in the catalytic center (Kamenski et al. 2004). Yeast encodes at least four protein phosphatases in the HAD family and additional HAD enzymes that dephosphorylate small molecules (Melcher and Entian 1992) and nucleic acids (Deshpande and Wilson 2004). However, the signature motif is relatively common in the yeast proteome, and the absence of sequence similarity outside of this motif makes it difficult to determine how many other yeast proteins are members of the HAD family of enzymes.

Catalytic mechanism

Members of the HAD family bind a magnesium ion at the active site, and phosphatase activity is dependent on the presence of this magnesium ion (Kuznetsova et al. 2015). Additionally, these phosphatases use the first aspartate from the DxDx(T/V) motif (Fig. 7a) as the initial nucleophile (Fig. 7b). The covalent phosphoryl-enzyme intermediate is then hydrolyzed by water to release a free phosphate and regenerate the active site (Ghosh et al. 2008). Structural analyses of Fcp1 from *Schizosaccharomyces pombe*, a member of the HAD family, suggest that this mechanism is conserved in fission yeast (Ghosh et al. 2008).

Regulation

To date, minimal research has been conducted into understanding how members of the HAD family are regulated in *S. cerevisiae*. With that in mind, there is evidence that Psr1 and Psr2, members of the HAD protein phosphatase family, utilize adaptor proteins to assist with proper localization and substrate targeting (Kaida et al. 2002). Fcp1, an essential phosphatase in the HAD family, is an RNA polymerase II C-terminal domain (CTD) modifier that works to recycle RNA polymerase II. Following dissociation of the transcription machinery, Fcp1 interacts with free RNAPII, demonstrating some substrate specificity (Kong et al. 2005). However, the diversity of the members of this family, combined with low specificity substrates, may provide evidence of convergent evolution (Kuznetsova et al. 2015).

Signal transduction

HAD phosphatases have been implicated in a number of cellular processes. As previously noted, the Fcp1 phosphatase plays a key role in transcription by dephosphorylating the CTD of RNA polymerase II (Kong et al. 2005). Additionally, Psr1 and Psr2 function in mediating responses to sodium stress by inducing transcription of Ena1, the major sodium extrusion pump (Siniossoglou et al. 2000).

RTR family

Great interest in transcriptional regulation has led to the identification of the multiple kinases and phosphatases acting on the C-terminal domain (CTD) of RNA polymerase II (Egloff and Murphy 2008). Ssu72 and Fcp1, members of the PTP and HAD families of protein phosphatases, are
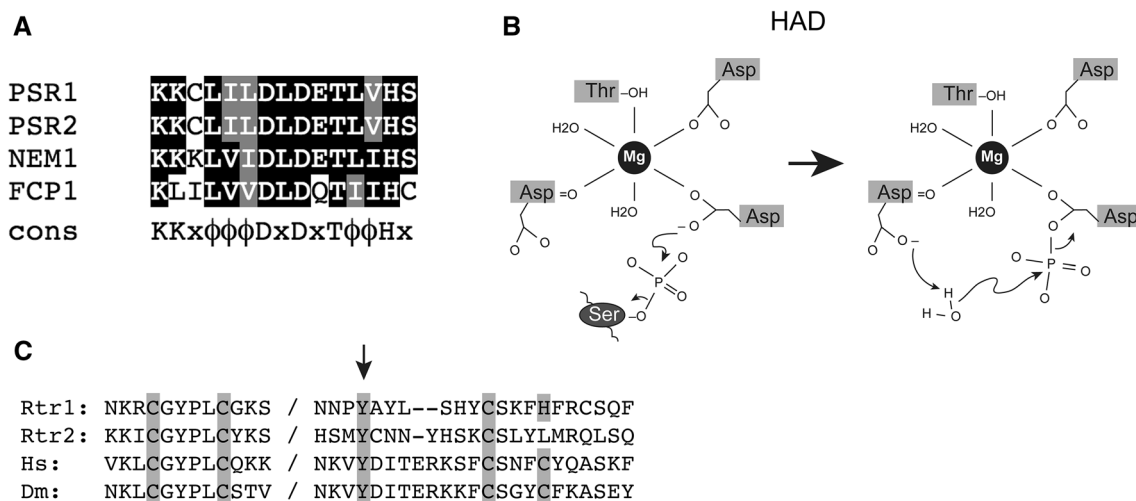

Fig. 7 HAD and RTR phosphatases. **a** Conserved catalytic core of the HAD protein phosphatases in yeast showing the DxDxT motif. Hydrophobic residues are represented in the consensus as ø. The catalytic aspartate is the first aspartate in the DxDxT motif. **b** Proposed catalytic mechanism for the HAD protein phosphatases showing the residues that coordinate the magnesium ion and the catalytic aspartate residue that acts as the nucleophile. **c** Conserved sequences in the RTR phosphatases from yeast (Rtr1 and Rtr2) as well as human (Hs) and Drosophila (Dm) showing the conserved cysteine and histidine residues that coordinate a zinc ion. A proposed catalytic mechanism for this family envisions a conserved tyrosine residue (arrow) acting as the nucleophile (Irani et al. 2016)

known CTD phosphatases. A third CTD phosphatase, Rtr1, was identified as an RNA polymerase binding protein (Gibney et al. 2008), capable of dephosphorylating the serine-5 phosphorylation on the CTD in vitro (Mosley et al. 2009). Cells lacking the RTR1 gene displayed increased serine-5 phosphorylation *in vivo* (Hunter et al. 2016). Homologs of Rtr1 are found throughout eukaryotes, including mammalian species (Fig. 7c).

Catalytic mechanism

Previous studies in *Kluyveromyces lactis* suggested that Rtr1 did not house an active site or any catalytic activity in the purified protein (Xiang et al. 2012). However, recent structural studies in *S. cerevisiae* have indicated otherwise. The zinc finger domain and a pair of helices may form a substrate binding pocket. Mutagenesis of residues within this site dramatically reduced catalytic activity, suggesting that this is the location of the phosphoryl transfer reaction (Irani et al. 2016). The exact mechanism of catalysis has yet to be fully elucidated; however, Tyr105 is required for catalytic function. As this residue is located at the base of the newly discovered phosphate binding pocket, it may be the nucleophile in catalysis (Irani et al. 2016). Difficulty ensuring stability and proper folding of Rtr1 in vitro (Irani et al. 2016) may explain previous reports of Rtr1 lacking catalytic activity (Xiang et al. 2012).

The Rtr1 family is a distinct phosphatase lineage with few similarities to other protein phosphatase families. For example, Rtr1 is dissimilar to the HAD family, as it does not require the presence of a magnesium ion, nor does it have a di-metal active site which is characteristic of the PPP family. Neither does the Rtr1 family have a conserved cysteine residue available to act as a nucleophile. The presence of catalytic activity in recombinant Rtr1 rules out the possibility that this protein acts as a scaffold in yeast to recruit a phosphatase to the CTD (Irani et al. 2016). Thus, Rtr1 represents a novel and atypical protein phosphatase family.

Regulation

Due to the recent discovery of the Rtr1 protein phosphatase family, there remains much research to be done to fully understand how members of this phosphatase family are regulated. In terms of regulating binding to RNA polymerase II, the phosphorylation status of the CTD mediates the interaction with Rtr1. Rtr1 preferentially binds to the CTD when serine-2, serine-5, and serine-7 are phosphorylated during elongation. Additionally, Rtr1 substrate specificity and phosphorylation status is dependent on CTDK-1 (Smith-Kinnaman et al. 2014). In terms of mRNA stability, Rtr1 has the ability to destabilize its own mRNA,

providing a level of regulation post-transcriptionally. This process is dependent on the proteins Dhh1 and Rex2/Rex3 (Hodko et al. 2016).

Signal transduction

The interactome of Rtr1 has not yet been extensively studied; however, Rtr1 plays a role in transcription from the initiation stage to elongation and termination by dephosphorylating Ser5 of the CTD (Gibney et al. 2008, Hsu et al. 2014; Hunter et al. 2016; Irani et al. 2016; Mosley et al. 2009; Smith-Kinnaman et al. 2014) and possibly Tyr1 as well (Hsu et al. 2014). Additionally, Rtr1 is involved in the regulation of various co-transcriptional processes and affects methylation of lysine 36 of histone H4, thereby establishing a role in chromosome maintenance and integrity (Hunter et al. 2016).

Conclusion

Protein kinases and protein phosphatases act in opposition to dictate the phosphorylation status of proteins in eukaryotic cells. Though tied together in function, protein kinases and phosphatases show distinct differences in the number of enzymes and the evolutionary lineages that gave rise to them. Kinases are numerous in yeast and represent fewer lineages. Of the 127 protein kinases in yeast, 117 are members of the same serine/threonine protein kinase superfamily (Rubenstein and Schmidt 2007). The remaining ten atypical protein kinases are related to bacterial kinases or inositol kinases. Thus, almost all of the protein kinases ($> 92\%$) share the same catalytic domain structure and mechanism. In contrast, phosphatases are fewer with only 43 in yeast and they comprise multiple independent evolutionary lineages, each with distinct structures and mechanisms. Some families utilize metal ions to activate a water molecule to serve as the nucleophile (PPP, PPM). Others use nucleophilic amino acid side chains such as cysteine (PTP) or aspartate (HAD) to first generate a phosphoryl-enzyme intermediate prior to hydrolysis. The PTP family shares a common catalytic mechanism but may itself constitute multiple evolutionary lineages. While few in number, protein phosphatases are abundant proteins that generate enzymatic diversity through interactions with regulatory subunits. The complex interplay of the protein kinases and phosphatases provides the rich diversity for numerous signal transduction pathways that control cellular responses to a vast array of external and internal stimuli.

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