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Roles of phosphatidate phosphatase enzymes in lipid metabolism

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Abstract

Phosphatidate phosphatase (PAP) enzymes catalyze the dephosphorylation of phosphatidate, yielding diacylglycerol and inorganic phosphate. In eukaryotic cells, PAP activity has a central role in the synthesis of phospholipids and triacylglycerol through its product diacylglycerol, and it also generates and/or degrades lipid-signaling molecules that are related to phosphatidate. There are two types of PAP enzyme, Mg^{2+} dependent (PAP1) and Mg^{2+} independent (PAP2), but only genes encoding PAP2 enzymes had been identified until recently, when a gene (*PAH1*) encoding a PAP1 enzyme was found in *Saccharomyces cerevisiae*. This discovery has revealed a molecular function of the mammalian protein lipin, a deficiency of which causes lipodystrophy in mice. With molecular information now available for both types of PAP, the specific roles of these enzymes in lipid metabolism are being clarified.

Phosphatidate phosphatase

Phosphatidate phosphatase (PAP, 3-*sn*-phosphatidate phosphohydrolase, EC 3.1.3.4) catalyzes the dephosphorylation of phosphatidate (PtdOH), yielding diacylglycerol (DAG) and inorganic phosphate [1] (Figure 1). PAP enzymes have roles in both the synthesis of lipids and the generation or degradation of lipid-signaling molecules in eukaryotic cells. They are classified as either Mg^{2+}-dependent (referred to as PAP1 enzymes) or Mg^{2+}-independent (PAP2 or lipid phosphate phosphatase [LPP] enzymes) with respect to their cofactor requirement for catalytic activity [2–7]. In both yeast and mammalian systems, PAP2 enzymes are known to be involved in lipid signaling [3,6,8,9]; by contrast, the physiological roles of PAP1 enzymes have remained unclear owing to a lack of molecular information. The recent identification of a gene encoding a PAP1 enzyme, however, has shown that this enzyme has roles in *de novo* lipid synthesis [10], thereby revealing that the two types of PAP are responsible for different physiological functions.

Here, we focus primarily on the PAP enzymes of the budding yeast *Saccharomyces cerevisiae*; for the most part, however, the roles of PAP enzymes in yeast parallel those in mammalian cells (see Refs [6,8,9] for reviews on mammalian PAP enzymes).

Roles of PAP enzymes in lipid synthesis and lipid signaling

In both yeast and higher eukaryotic cells, the PAP reaction is the committed step in the synthesis of the storage lipid triacylglycerol (TAG), which is formed from PtdOH through the intermediate DAG [11] (Figure 2). The reaction product DAG is also used in the synthesis of the membrane phospholipids phosphatidylcholine (PtdCho) and phosphatidylethanolamine [6,12,13] (Figure 2). The substrate PtdOH is used for the synthesis of all membrane phospholipids (and the derivative inositol-containing sphingolipids) through the intermediate CDP-DAG [12,13] [Figure 2; note that in mammalian cells CDP-DAG is used for the synthesis

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of only phosphatidylinositol (PtdIns), phosphatidylglycerol and cardiolipin]. Thus, regulation of PAP activity might govern whether cells make storage lipids and phospholipids through DAG or phospholipids through CDP-DAG. In addition, PAP is involved in the transcriptional regulation of phospholipid synthesis [14] (see later).

The PAP enzymes are also involved in lipid signaling in mammalian cells as part of the phospholipase D and PAP pathway, where they generate DAG for the activation of protein kinase C [15–17]. In addition, PAP activity can attenuate the bioactive functions of PtdOH, which include promoting cell growth and proliferation, vesicular trafficking, secretion and endocytosis [5,8,17–20].

PAP1

PAP1 enzymes have been purified and characterized from the membrane and cytosolic fractions of yeast [3]. As mentioned earlier, a gene (*PAH1*, formerly known as *SMP2*) has now been identified to encode a PAP1 enzyme in yeast [10]. The *PAH1*-encoded PAP1 enzyme is found in the cytosolic and membrane fractions of the cell, and its association with the membrane is peripheral in nature [10]. As expected from the multiple forms of PAP1 that have been purified from yeast [3], *pah1*Δ mutants still contain PAP1 activity [10], indicating the presence of an additional gene or genes encoding PAP1 that are yet to be identified and characterized.

The analysis of mutants lacking the *PAH1*-encoded PAP1 has provided evidence that this enzyme generates the DAG used for lipid synthesis [10]. Cells containing the *pah1*Δ mutation accumulate PtdOH and have reduced amounts of DAG and its acylated derivative TAG [10]. Phospholipid synthesis predominates over the synthesis of TAG in exponentially growing yeast, whereas TAG synthesis predominates over the synthesis of phospholipids in the stationary phase of growth [10,21,22]. The effects of the *pah1*Δ mutation on TAG content are most evident in the stationary phase [10]. For example, stationary phase cells devoid of the *PAH1* gene show a reduction of >90% in TAG content [10]. Likewise, the *pah1*Δ mutation shows the most marked effects on phospholipid composition (e.g. the consequent reduction in PtdCho content) in the exponential phase of growth [10]. The importance of the *PAH1*-encoded PAP1 enzyme to cell physiology is further emphasized because of its role in the transcriptional regulation of phospholipid synthesis [14]. In addition, *pah1*Δ mutants show a temperature sensitivity phenotype [10,14]. At present, however, it is unclear whether the molecular basis of this phenotype is directly related to a change in lipid metabolism.

PAP2

Nearly all PAP2 activity in yeast is encoded by the *DPP1* [23] and *LPP1* [24] genes, with the former being the principal contributor of this activity [24]. The *DPP1*- and *LPP1*-encoded enzymes are integral membrane proteins with six transmembrane spanning regions and are localized, respectively, to the vacuole [25,26] and Golgi [27] compartments of the cell. Unlike the *PAH1*-encoded PAP1 enzyme, which is specific for PtdOH, the *DPP1*- and *LPP1*-encoded PAP2 enzymes have broad substrate specificity. In addition to PtdOH, these enzymes use various lipid phosphate substrates including diacylglycerol pyrophosphate (DGPP), lysoPtdOH, sphingoid base phosphates and isoprenoid phosphates [23,24,28–30]. The *DPP1*-encoded enzyme shows a preference for DGPP as a substrate [28], whereas the *LPP1*-encoded enzyme has similar substrate specificity for both PtdOH and DGPP [29]. For the *DPP1*-encoded enzyme, only PtdOH and DGPP have been shown to be substrates *in vivo* [24].

Research on plants indicates that DGPP might function as a signaling molecule under conditions of stress [4,31]. Accumulation of DGPP is transient and coincides with an increase in PtdOH [4,31]. By analogy, the *DPP1*-encoded and *LPP1*-encoded PAP2 enzymes might function during stress conditions to regulate specific cellular pools of PtdOH and DGPP [4].

Consistent with this hypothesis, the yeast *DPP1* gene is induced under the stress condition of zinc deprivation [25], and its encoded PAP2 activity regulates the cellular quantities of PtdOH and DGPP in the vacuole membrane [26]. The impact of this regulation on vacuolar membrane structure and/or function remains unclear. Like the yeast PAP2 enzymes, the mammalian counterparts have broad substrate specificity [6,7,32] and are responsible for attenuating the bioactive functions of lipid phosphate molecules such as lysoPtdOH [5,6,8,33,34].

Differentiation of PAP enzymes by catalytic motifs

Sensitivity to the thioreactive compound *N*-ethylmaleimide (NEM) has been used to differentiate PAP1 (NEM-sensitive) from PAP2 (NEM-insensitive) activity in mammalian cells [2,11]. It has been found that the yeast *DPP1*-encoded PAP2 activity is insensitive to NEM [28], whereas *LPP1*-encoded PAP2 activity is sensitive to NEM [29]. Likewise, the *PAH1*-encoded PAP1 activity is insensitive to NEM, whereas the PAP1 activity remaining in *pah1*Δ mutants is sensitive to NEM [10]. Thus, yeast PAP1 and PAP2 enzymes cannot be differentiated on the basis of their NEM sensitivity. Sequence information indicates that NEM-sensitive enzymes have more cysteine residues than do NEM-insensitive ones. For example, Lpp1p contains ten cysteine residues, whereas Dpp1p and Pah1p contain only three. One reagent that might be useful in differentiating PAP enzymes in yeast is bromoenol lactone, which seems to inhibit PAP1 activity selectively in mammalian cells [35]. Its specificity for yeast PAP enzymes, however, has yet to be determined.

The requirement of Mg^{2+} ions as a cofactor for PAP enzymes is correlated with the catalytic motifs that govern the phosphatase reactions of these enzymes. For example, the *PAH1*-encoded PAP1 enzyme has a DxDxT catalytic motif within a haloacid dehalogenase (HAD)-like domain (Figure 3a). This motif is found in a superfamily of Mg^{2+}-dependent phosphatase enzymes, and its first aspartate residue is responsible for binding the phosphate moiety in the phosphatase reaction [36,37]. By contrast, the *DPP1*-and *LPP1*-encoded PAP2 enzymes contain a three-domain lipid phosphatase motif that is localized to the hydrophilic surface of the membrane [23,24,26,38,39] (Figure 3b). This catalytic motif, which comprises the consensus sequences KxxxxxxRP (domain 1), PSGH (domain 2) and SRxxxxxHxxxD (domain 3), is shared by a superfamily of lipid phosphatases that do not require Mg^{2+} ions for activity [38,40,41]. The conserved arginine residue in domain 1 and the conserved histidine residues in domains 2 and 3 are essential for the catalytic activity of PAP2 enzymes [38,39,42].

Another difference between the PAP1 and PAP2 enzymes is the nature of their membrane association. As predicted by their primary sequences, the *DPP1*- and *LPP1*-encoded PAP2 enzymes are integral membrane proteins with six transmembrane spanning regions [23,24], whereas the *PAH1*-encoded enzyme is a cytosolic and peripheral membrane protein [10].

Regulators of PAP1 and PAP2 activity

Defined studies on the biochemical regulation of the yeast PAP1 and PAP2 enzymes have been facilitated by using enzymes that have been purified to near homogeneity [28,43,44]. On the one hand, PAP1 activity is stimulated by phospholipids synthesized through CDP-DAG, including CDP-DAG itself, PtdIns and cardiolipin [45]. The activation constants for these phospholipids are similar to the concentrations found in yeast cells [45], indicating that stimulation of PAP1 activity by these lipids might be physiologically relevant. For example, such regulation would attenuate phospholipid synthesis through CDP-DAG and favor lipid synthesis through DAG. On the other hand, PAP1 activity is inhibited by sphingoid bases (e.g. phytosphingosine, and sphinganine) [46] and by nucleotides (e.g. ATP and CTP) [47]. Inhibition by these molecules would favor phospholipid synthesis through CDP-DAG. The regulation of PAP1 activity by sphingoid bases and ATP correlates with observed changes in

the synthesis of phospholipids and TAG when the concentration of these molecules varies *in vivo* [21,22,47–49].

In contrast to the PAP1 enzyme, the DGPP phosphatase activity of the *DPP1*-encoded PAP2 enzyme is inhibited by CDP-DAG [50]. This PAP2 activity is also inhibited by Zn^{2+} ions in a mechanism that involves the formation of DGPP–Zn^{2+} complexes [25]. As indicated earlier, expression of the *DPP1* gene and its encoded enzyme product is induced by zinc deprivation [25]. The inhibition of DGPP phosphatase activity by Zn^{2+} ions might counterbalance the induction of the enzyme by zinc deprivation.

Role of PAP1 in regulation of phospholipid biosynthesis

Yeast Pah1p has been identified as a protein that couples phospholipid synthesis to growth of the nuclear/endoplasmic reticulum (ER) membrane [14]. Mutants defective in the *PAH1* gene show massive expansion of the nuclear/ER membrane and, at the same time, abnormally high expression of key phospholipid biosynthetic genes (i.e. *INO1*, involved in PtdIns synthesis, and *OPI3*, involved in PtdCho synthesis) [14]. The expression of these genes, which contain upstream activating sequence inositol-responsive (UAS_{INO}) elements in their promoters, is controlled at the level of transcription [12]. Expression of *INO1* and *OPI3* is maximized by interaction of the Ino2p–Ino4p complex with the UAS_{INO} elements, whereas it is repressed by interaction of Opi1p with Ino2p [12,51]. The repressive effect of Opi1p on the expression of the UAS_{INO}-containing genes is most marked when cells are supplemented with inositol [12].

PtdOH, which tethers Opi1p at the nuclear/ER membrane together with Scs2p (a vesicle-associated protein homolog), has a crucial role in the Opi1p-mediated repression of *INO1* [52]. A reduction in PtdOH concentration, brought about by inositol supplementation, promotes translocation of Opi1p from the nuclear/ER membrane into the nucleus, where it interacts with Ino2p to repress the expression of *INO1* [52] (Figure 4). The same mechanism of regulation should apply to other UAS_{INO}-containing genes (Figure 2), including *OPI3* [52]. The reduction in PtdOH concentration can be attributed in part to an increase in PtdIns synthesis [52], which draws on the pool of PtdOH through CDP-DAG [12] (Figure 2). The *PAH1*-encoded PAP1 enzyme also controls the cellular quantities of PtdOH [10], and PAP1 activity is increased in inositol-supplemented cells [48]. Pah1p is phosphorylated by cyclin-dependent Cdc28p (also known as Cdk1p) kinase [14,53], and its dephosphorylation (which stimulates PAP1 activity [54]) by a Nem1p–Spo7p phosphatase complex is required both for regulated expression of *INO1* and *OPI3* and for normal growth of the nuclear/ER membrane [14]. Thus, the PtdOH-mediated control of Opi1p repressor function is governed by the regulation of *PAH1*-encoded PAP1 activity.

The lipin connection

Lipin 1 has been identified as a protein that regulates fat metabolism in mammalian cells [55–60]. In mice, lipin 1 deficiency prevents normal development of adipose tissue and results in lipodystrophy and insulin resistance, whereas an excess of lipin 1 promotes obesity and insulin sensitivity [55,56]. Despite the importance of lipin 1, the mechanism by which it affects lipodystrophy and obesity has been unclear owing to a lack of information on its molecular function. Lipin 1 shares sequence homology with the *PAH1*-encoded PAP1 protein (Pah1p) in evolutionarily conserved N-terminal and C-terminal regions of the protein [55]. In fact, the conserved C-terminal region of these proteins consists of a HAD-like domain with a DxDxT catalytic motif [10] (Figure 3). Indeed, the recent biochemical characterization of recombinant human lipin 1 has indicated that it is a PAP1 enzyme [10]. PAP1 is the penultimate enzyme in the pathway to synthesize TAG (Figure 2); thus, a similar molecular function of lipin 1 provides

a mechanistic basis for why this protein has such a great effect on fat metabolism in mammalian cells.

Lipin 1 is phosphorylated in response to insulin treatment in rat adipocytes, and its phosphorylation is mediated by the mammalian target of rapamycin pathway [61]. The effects of this phosphorylation on the PAP1 activity of lipin 1 are, however, unknown.

Perspectives

Over the past few years much attention has been paid to the PAP2 enzyme because of its lipid-signaling role in mammalian cells. Studies on PAP2 enzymes have been facilitated by molecular genetic approaches and the identification of their genes [6,8,9]. By contrast, progress in understanding the roles of the PAP1 enzyme has lagged behind owing to a lack of molecular information on this enzyme. The recent discovery of PAP1 genes in yeast [10] and mammalian cells [10,55], however, has provided the molecular tools with which to explore in greater depth the roles of PAP1 in lipid metabolism and cell physiology.

Several questions need to be addressed concerning regulation of the yeast *PAH1*-encoded PAP1 enzyme. What signals control expression of *PAH1* during the cell cycle, and in cells grown in the absence and presence of nutrients such as inositol? What is the significance of Pah1p localization? What causes Pah1p to associate with the nuclear/ ER membrane, where it catalyzes the dephosphorylation of PtdOH? Which genes encode the NEM-sensitive PAP1 enzymes expressed in the *pah1*Δ mutant? Lastly, what are the specific contributions of the different PAP1 enzymes to TAG synthesis, to phospholipid synthesis through DAG and CDP-DAG, and to the transcriptional regulation of phospholipid synthesis?

The DxDxT catalytic motif is present in two other mammalian lipin proteins [55] (Figure 3a). The molecular functions of these lipin proteins have not been characterized, but it is reasonable to predict that they are also PAP1 enzymes on the basis of their sequence similarity to lipin 1. The sequence identity of lipin 2 and lipin 3 to lipin 1 is 49% and 46%, respectively. Moreover, the phosphatidate phosphatase catalytic motif is conserved in all three lipin proteins. The different forms of the mammalian lipin proteins might be responsible for specific PAP1 functions in different tissues. Moreover, the PAP1 activity of the mammalian lipin proteins might represent an important pharmaceutical target for controlling body fat in humans. For example, a drug that inhibits PAP1 activity might be used to treat obesity, whereas a drug that activates PAP1 activity might be used to treat lipodystrophy. With these molecular reagents available and knowledge of their molecular functions, a greater understanding of the physiological functions of PAP1 enzymes in yeast and in mammalian cells should be forthcoming.

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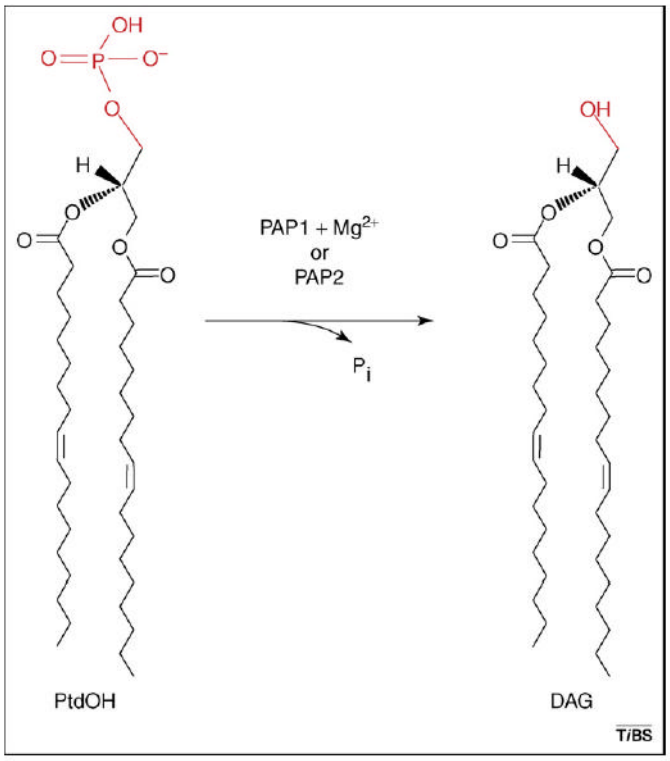

Figure 1.
PAP catalyzes the dephosphorylation of PtdOH to yield DAG. The structures of the substrate and product are shown with fatty acyl groups containing 18 carbon atoms and one double bond.

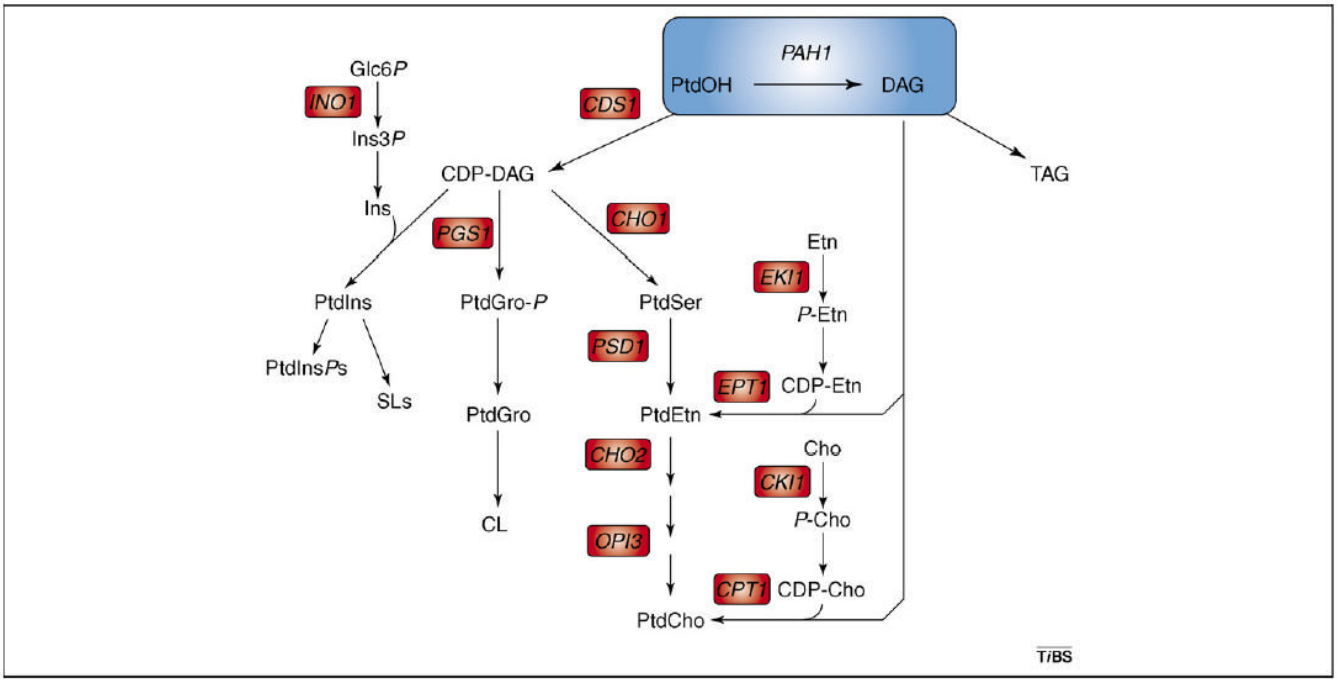

Figure 2.

PAP1 is important in the synthesis of lipids in yeast. The product DAG is used for the synthesis of PtdEtn and PtdCho and for the synthesis of TAG. The substrate PtdOH is used for the synthesis all phospholipids and PtdIns-derived sphingolipids through the intermediate CDP-DAG. The UAS_{INO}-containing genes that are subject to inositol-mediated regulation are indicated in red. Complete details of the lipid biosynthetic pathways in yeast can be found elsewhere [12,13]. PtdOH, phosphatidate; DAG, diacylglycerol; TAG, triacylglycerol; Glc6*P*, glucose-6-phosphate; Ins3*P*, inositol-3-phosphate; Ins, inositol; PtdIns, phosphatidylinositol; PtdIns*P*s, phosphatidylinositol phosphates; PtdGro-*P*, phosphatidylglycerophosphate; PtdGro, phosphatidylglycerol; PtdSer, phosphatidylserine; PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; Etn, ethanolamine; *P*-Etn, phosphoethanolamine; Cho, choline, *P*-Cho, phosphocholine.

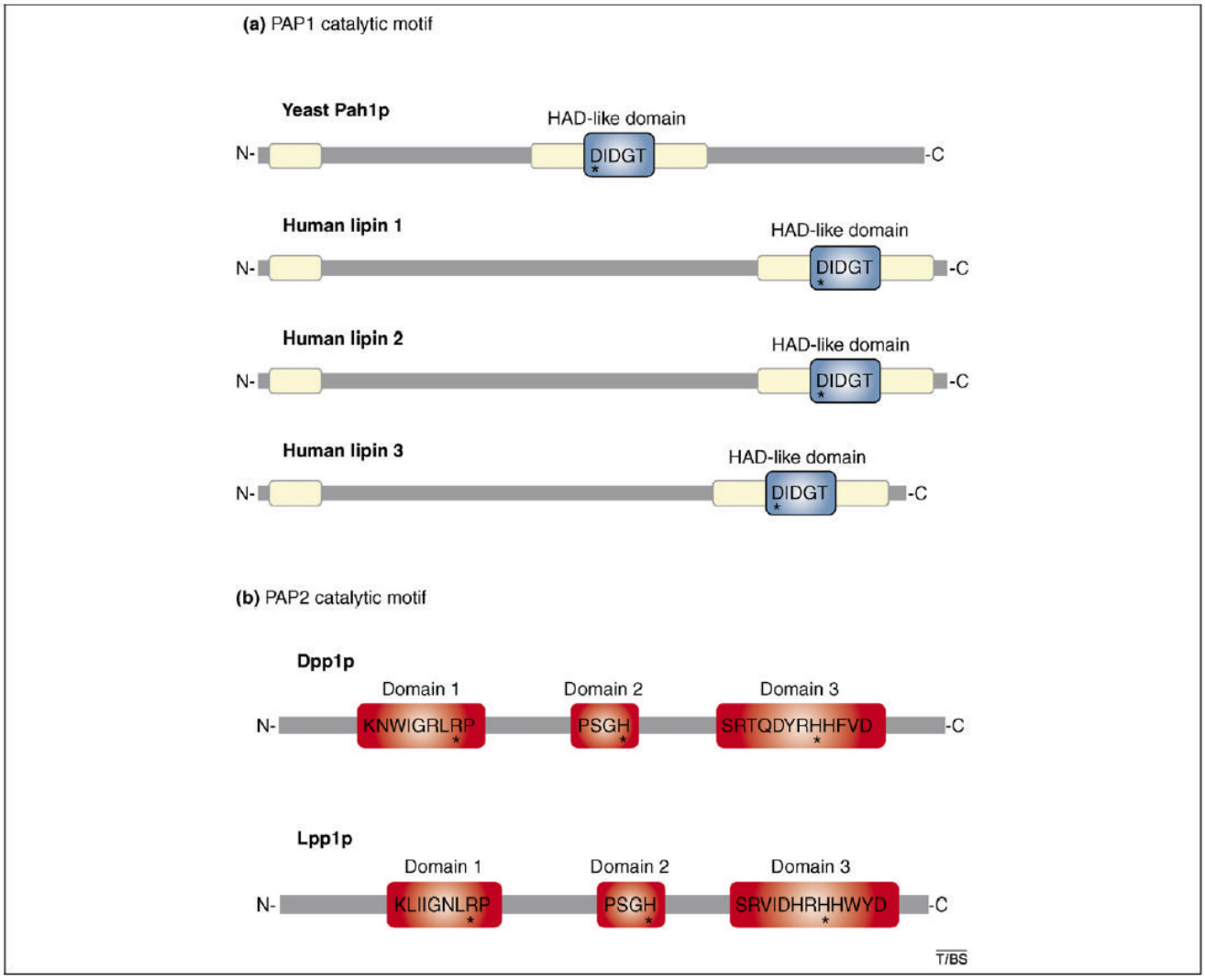

Figure 3.

PAP1 and PAP2 type enzymes have different catalytic motifs. **(a)** The reaction catalyzed by the PAP1 type enzyme uses a DxDxT motif present in a HAD-like domain. The evolutionarily conserved N-terminal region and HAD-like domain in yeast Pah1p and the human lipin proteins are indicated in yellow. Asterisk indicates the conserved aspartate residue responsible for phosphate binding in the phosphatase reaction. **(b)** The reaction catalyzed by the PAP2 type enzyme uses a three-domain lipid phosphatase motif. Asterisk indicates the amino acids that are conserved in each domain and required for phosphatase activity.

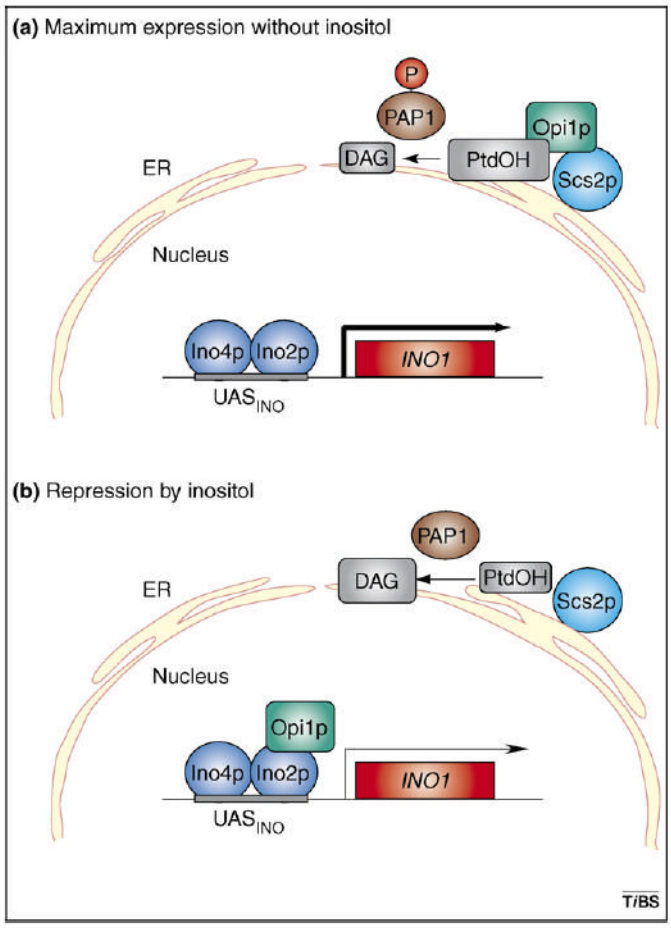

Figure 4.

Model of the role of PAP1 in the transcriptional regulation of UAS_{INO}-containing genes by inositol. **(a)** UAS_{INO}-containing genes (e.g. *INO1*) are maximally expressed (thick arrow) when wild-type yeast is grown in the absence of inositol. Expression of *INO1* is dependent on interaction of the Ino2p–Ino4p complex with the UAS_{INO} element in the gene promoter. Under this growth condition, the repressor Opi1p is associated with the nuclear/ER membrane through interactions with PtdOH and Scs2p (a vesicle-associated protein homolog). The PAP1 enzyme is phosphorylated and has reduced catalytic activity. **(b)** When inositol is added to the growth medium, transcription of *INO1* is attenuated (thin arrow) by the interaction of Opi1p with Ino2p. Dissociation of Opi1p from the nuclear/ER membrane and its translocation into the nucleus are caused by a decrease in PtdOH concentration, which is mediated by the stimulation of PAP1 activity after dephosphorylation of the enzyme.