Traffic 2009; **10:** 1181–1187

doi: 10.1111/j.1600-0854.2009.00923.x

Review

Lipins, Lipids and Nuclear Envelope Structure

Symeon Siniossoglou

Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Hills Road, Cambridge, CB2 0XY, UK
**Corresponding author: Symeon Siniossoglou, ss560@cam.ac.uk*

The lipid composition of biological membranes is crucial for many aspects of organelle function, including growth, signalling, and transport. Lipins represent a novel family of lipid phosphatases that dephosphorylate phosphatidic acid (PA) to produce diacylglycerol (DAG), and perform key functions in phospholipid and triacylglycerol biosynthesis and gene expression. In addition to its role in lipid biosynthesis, the yeast lipin Pah1p and its regulators are required for the maintenance of a spherical nuclear shape. This review summarizes recent advances in our understanding of the yeast lipin Pah1p and highlights the possible roles of phospholipid metabolism in nuclear membrane biogenesis.

Key words: DAG, ER, lipin, nuclear membrane, PA, Pah1p, phospholipids, yeast

Received 11 February 2009, revised and accepted for publication 13 April 2009, uncorrected manuscript published online 15 April 2009, published online 20 May 2009

The shape and size of many eukaryotic membrane-bound organelles is dynamic and can change during the cell cycle or development (1,2). These changes depend on the coordinated production, transport and remodelling of phospholipids, the major structural components of lipid bilayers and the activity of membrane-associated proteins. Furthermore, homeostatic mechanisms must be in place to ensure that the shape and size of organelles is appropriate for their metabolic needs. The molecular mechanisms by which cells coordinate lipid biogenesis with organelle shape, size and number remain poorly understood.

An organelle that undergoes remarkable changes during the cell cycle and development is the nucleus (3). For example, in yeast, nuclear membrane expansion allows anaphase to take place within a single nuclear compartment that partitions between the mother and the daughter cells (4). In metazoans, the nuclear membrane expands at the end of the cell cycle to accommodate chromatin decondensation (5). Moreover, a number of specialized cell types undergo 'nuclear differentiation', like the mammalian blood cells where nuclei can be highly lobed and segmented or spermatocytes and myocytes where nuclei can become very elongated (6). Maintenance of proper nuclear structure is important for cell physiology as highlighted by the identification of several diseases that are associated with changes in nuclear shape and are caused by mutations in nuclear envelope proteins (7).

During the last years there has been significant progress in characterizing protein components of the nuclear envelope and deciphering the mechanisms of nucleocytoplasmic transport. On the other hand, little is still known about the mechanisms underlying the biogenesis, remodelling of the nuclear membrane, and the role of lipid biosynthesis and signalling in these processes. Here I will review data on a novel class of lipid phosphatases, the lipins, focusing primarily on the functions of Pah1p, the *Saccharomyces cerevisae* member of this family, in lipid and nuclear membrane biogenesis. Where appropriate, insights from mammals will be discussed to highlight the similarities and the differences between the two systems.

A new player in lipid metabolism

The lipins define a novel family of Mg^{2+}-dependent phosphatidate phosphatase (PAP1) enzymes that catalyze a fundamental reaction in lipid biosynthesis, namely the dephosphorylation of PA into DAG (8). Lipins are well conserved throughout the eukaryotic kingdom and exhibit similar overall primary organization. They are relatively large proteins (close to 100 kDa) and contain a conserved amino-terminal domain (N-LIP) of unknown function, a carboxy-terminal catalytic domain (C-LIP) harbouring a DXDXT motif found in a superfamily of Mg^{2+}-dependent phosphatases with diverse specificities and a putative nuclear localization signal (NLS) (Figure 1). In addition, lipins from yeast species carry an acidic stretch at their C-terminal ends. All lipins lack transmembrane domains and therefore must first translocate onto membranes in order to dephosphorylate PA. Lipins localize to the cytoplasm but can also be found into the nucleus in adipocytes and hepatocytes (see later). Fungi, nematodes and insects express one lipin, whereas mammals express three paralogues called lipin 1, 2 and 3 (9).

In both yeast and higher eukaryotes, DAG produced by the dephosphorylation of PA is (a) acylated to produce triacylglycerols (TAG), an essential storage form of energy and fatty acids deposited in lipid droplets (10,11), and (b) used for the synthesis of phosphatidylethanolamine (PE) and phosphatidylcholine (PC), the two most abundant membrane phospholipids,

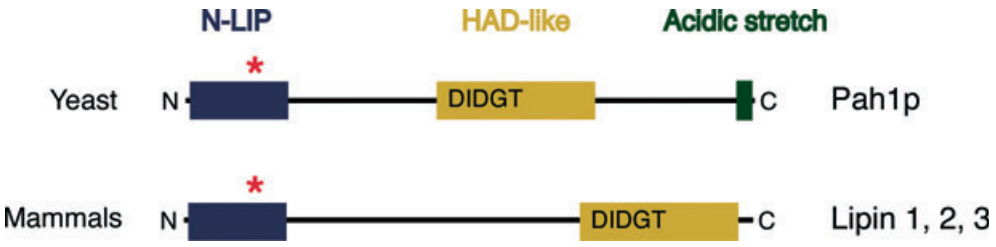

Figure 1: Domain organization of lipins. All members of the lipin family contain a conserved N-terminal domain (N-LIP) of an unknown function and a C-terminal catalytic domain that contains a HAD-like phosphatase motif (DIDGT). Within these two domains, the sequence identity between the yeast and the three mammalian orthologues is around 45%. Pah1p and lipins from other yeast species also contain a C-terminal acidic stretch. The red star indicates the position of the mutation of the conserved glycine residue (G84R, corresponding to G80 in Pah1p) identified in the *fld* mouse that causes lipodystrophy.

by the Kennedy pathway (12,13) (Figure 2). Yeast cells and hepatocytes also use PA to produce PE and PC through a second parallel route known as the cytidine diphosphate diacylglycerol (CDP-DAG) pathway (12,13) (Figure 2).

For a long time it has been known that a soluble PAP1 activity translocates onto the cytosolic side of intracellular membranes, but the identity of the enzyme(s) was unknown (14–16). The *Saccharomyces cerevisae* lipin Pah1p/Smp2p was the first PAP1 enzyme to be cloned and characterized at the molecular level (8). Deletion of *PAH1* led to the accumulation of PA but also the concomitant reduction of DAG and TAG levels and changes in PE and PC amounts (8). Further studies demonstrated

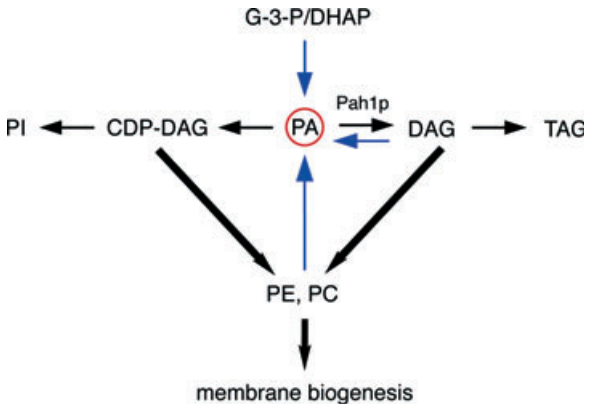

Figure 2: Simplified overview of phospholipid biosynthetic pathways in yeast and the role of Pah1p. PA is a key precursor that is used for the synthesis of the major phospholipids phosphatidylethanolamine (PE) and phosphatidylcholine (PC) through cytidine diphosphate diacylglycerol (CDP-DAG) or, in the presence of choline and ethanolamine, through DAG (Kennedy pathway). Therefore, regulation of the conversion of PA to DAG, catalyzed by Pah1p, will affect both pathways. CDP-DAG and DAG give rise to PI (phosphatidylinositol) and TAG (triacylglycerols), respectively. Pathways that generate PA are indicated in blue: glycerol 3-phosphate (G-3-P) or dihydroxyacetone phosphate (DHAP) are converted through a series of acylations (not depicted here) into PA. PA can also be generated by phosphorylation of DAG by Dgk1p and by hydrolysis of PC by the phospholipase D Spo14p.

that the mammalian lipin 1, 2 and 3 are also PAP1 enzymes (8,17,18) and that mammalian lipins can rescue the yeast *pah1*Δ mutant (19). Consistent with the role of lipins in TAG production, lipin 1 was originally identified as the gene mutated in the *f*atty *l*iver *d*ystrophy (*fld*) mouse (9). This mouse displays features of generalized lipodystrophy, characterized by significant reduction in the adipose tissue mass and in the cellular lipid droplet content (9). The mutated residue in the *fld* mouse is a conserved glycine in the N-LIP domain required for the enzymatic activity of both Pah1p and lipin 1 (17,18,20). Together these data established that Pah1p and lipin 1 have essential roles in lipid droplet and phospholipid metabolism. In addition to their biosynthetic roles, PA and DAG are also important in signal transduction cascades. Regulation of the ''signalling'' pools of PA and DAG is thought to involve a second type of PA phosphatase activity that does not require Mg^{2+} as a cofactor and is known as PAP2 or LPP (14,21). In contrast to PAP1, these transmembrane enzymes are relatively non-selective for their substrate as, in addition to PA, they act on several other phosphorylated lipids.

Regulation of Pah1p function

PAH1/SMP2 was independently identified as a dosage suppressor of the *spo7*Δ and *nem1*Δ deletions (22). Spo7p and Nem1p genetically interact with components of the nuclear pore and form a transmembrane complex that localizes to the nuclear/ER membrane and dephosphorylates Pah1p (22,23). Nem1p, a member of the CPD phosphatase family (24), is the catalytic subunit while Spo7p is the regulatory subunit that binds to the catalytic domain of Nem1p and is required for the phosphatase activity of the holoenzyme (22). Interestingly, the fission yeast lipin Ned1 displays genetic and two-hybrid interactions with a nucleoporin and factors involved in nucleocytoplasmic transport, providing another link between lipin and nuclear envelope function (25).

Pah1p is phosphorylated *in vivo* on at least 12 sites, including seven Ser/Thr-Pro (S/T-P) motifs (26). Mobility shift and dephosphorylation assays indicate that

some of these phospho-S/T-P residues are targeted by the Nem1p–Spo7p complex (26). A septuple S/T-P Pah1p phosphorylation null mutant (Pah1p-7P) displayed higher specific activity when compared to the wild-type enzyme (26). Thus, phosphorylation of Pah1p inhibits its PAP1 activity and consistent with this *nem1*Δ and *spo7*Δ deletion strains accumulate phosphorylated Pah1p and display the same phenotypes as those described for the *pah1*Δ knockout (22,23) (see later).

The regulation of Pah1p and lipins by phosphorylation appears to be complex and is probably mediated by multiple kinases. Pah1p is phosphorylated in mitotic cells in a Cdk1/Cdc28p-dependent manner (22). In addition, recent high-throughput studies have identified another Cdk, Pho85p and the Dbf2p–Mob1p complex, a component of the mitotic exit network, as putative Pah1p kinases (27–29). Similar to budding yeast, Ned1 (25) and the mammalian lipins 1 and 2 (19) are mitotically phosphorylated and in the latter case phosphorylation decreases the PAP1 activity of the enzymes. This in turn could contribute to the inhibition of phospholipid biosynthesis that takes place in mammalian cells at the G2/M phase (30). In addition, lipin 1 is phosphorylated in response to insulin treatment in rat adipocytes in an mTOR-dependent manner (31), whereas in mouse adipocytes, phosphorylation of lipin 1 on multiple sites correlates with a decrease of its microsomal-bound pool (18). Consequently, phosphorylation of lipins could also control their subcellular localization. Thus, it is possible that distinct kinases/phosphatases might be involved in the recruitment and the activation of Pah1p/lipins to different organelles to control PA and DAG levels in response to cellular needs.

Considering the multitude of functions of PA and DAG in biosynthetic and signalling pathways, this complexity is not surprising. In yeast, phospholipids used for biogenesis of membranes can be synthesized by two pathways (13) (Figure 2). The dephosphorylation of PA by Pah1p is a branching point that may govern whether phospholipids will be synthesized via the Kennedy pathway, or via the CDP-DAG pathway (Figure 2). Moreover, as Pah1p also produces DAG necessary for the synthesis of TAG, regulation of its activity could be important for TAG homeostasis during the cell cycle and the availability of TAG-derived fatty acids that are used for membrane biogenesis (32). DAG has many other roles such as in secretion and Golgi function (33), but currently it is not known whether these DAG pools are affected by Pah1p.

The role of Pah1p in the regulation of phospholipid biosynthesis

Besides these metabolic roles, Pah1p has a key signalling function in the transcriptional regulation of genes encoding phospholipid biosynthetic enzymes. In yeast, PA levels co-ordinately control the transcription of many genes encoding for enzymes of the CDP-DAG and the Kennedy pathways (34). This regulation depends on three factors: (a) a sequence in the promoters of these genes known as UAS_{INO} (35–37), (b) the heterodimeric Ino2p–Ino4p complex that binds to UAS_{INO} and activates transcription of the downstream genes (38,39) and (c) the PA-binding protein Opi1p that represses transcription of these genes (40,41). When the levels of PA are high, Opi1p is excluded from the nucleus by binding to PA on the ER membrane and Ino2p-Ino4p-driven transcription is derepressed. When the PA levels are low, Opi1p is released from the membranes and translocates into the nucleus to repress transcription (41). Conditions such as inositol availability, growth phase and nutrient depletion can affect PA levels and, as a result, influence UAS_{INO}-dependent transcription (34).

Mutations in the Nem1p–Spo7p complex that result in hyperphosphorylation of Pah1p (22), or mutations in Pah1p itself (20,22), result in transcriptional derepression of UAS_{INO}-containing genes. On the other hand, overexpression of the more active Pah1p-7P phosphorylation null mutant causes inositol auxotrophy (26). Inositol auxotrophy is caused by the constitutive repression of the inositol 1-phosphate synthase (*INO1*) gene, the most highly regulated UAS_{INO}-containing gene (34). Notably, the inositol auxotrophy of the Pah1p-7P cells can be rescued by the deletion of the Opi1p repressor (26). The fact that the transcription of UAS_{INO}-containing genes responds to changes in the catalytic activity of Pah1p indicates that Pah1p-derived PA regulates transcription through the Opi1p signalling pathway. However, when compared with the single *opi1*Δ mutant, the *pah1*Δ*opi1*Δ double mutant exhibits a synergistic effect on the transcriptional derepression of two UAS_{INO}-containing genes, *INO1* and *OPI3* (26). This suggests that Pah1p regulates the transcription of these genes in an Opi1p-independent mechanism as well. The nuclear envelope plays an important role in the transcriptional regulation of many genes (42), including *INO1* (43). As the activity of Pah1p can dramatically affect nuclear envelope structure (see below), additional effects on the expression of UAS_{INO}-containing genes cannot be ruled out.

Chromatin immunoprecipitation studies have shown that a pool of a Pah1p-protein A fusion associates with the promoters of three UAS_{INO}-containing genes (22). This has led to the suggestion that Pah1p might interact with the transcription machinery to regulate gene expression. Interestingly, in addition to its cytosolic localization, mammalian lipin 1 can also localize to the nucleus in hepatocytes in an NLS-dependent manner (44) and in adipocytes (9). Nuclear lipin 1 can act as a transcriptional co-activator of fatty acid oxidation and adipogenic genes, through direct interactions with nuclear receptors (45,46). This has led to the suggestion that lipins can regulate lipid metabolism at multiple levels (47), but currently there is no evidence that Pah1p has similar functions in yeast. Nevertheless, a pool of dephosphorylated Pah1p may also enter the nucleus in order to regulate

PA/DAG homeostasis at the inner nuclear membrane. Nem1p and Spo7p contain putative NLS's (48), although direct evidence that they can localize to the inner nuclear membrane is lacking. Moreover Dullard, the mammalian orthologue of Nem1p that dephosphorylates lipin 1, localizes to the inner nuclear membrane (49,50). The determination of the subcellular localization of the endogenous Pah1p will be crucial to address further some of these questions.

Linking lipin function to nuclear membrane biogenesis in yeast

The nucleus is delimited by the nuclear envelope, which consists of two closely opposed lipid bilayers, the outer and inner nuclear membranes (5). The outer membrane is continuous with the endoplasmic reticulum and functions in secretion and lipid biosynthesis while the inner membrane faces the nucleoplasm and functions as a platform for the binding of chromatin. The outer and inner nuclear membranes are connected at the highly curved domains present around the nuclear pore complexes (NPCs).

In yeast, nuclear envelope structure can undergo dramatic structural reorganization, during its so-called 'closed mitosis' (4), mating (51) and ascospore formation (52). Moreover, changes in the protein composition of the nuclear membrane can affect its structure. For example, mutations or overexpression of nuclear pore (53) or inner nuclear envelope proteins (54) disrupts nuclear envelope organization. Furthermore, mutations of certain components involved in ER to Golgi transport can cause nuclear envelope defects (55,56). Finally, mutations in the acetyl-CoA carboxylase *MTR7/ACC1*, a key enzyme involved in fatty acid biosynthesis result in the separation of the outer and inner nuclear membranes (57).

Deletion of *NEM1*, *SPO7* or *PAH1* has a unique effect on nuclear structure: instead of the round nuclei typical of wild-type yeast, these mutants display irregularly shaped nuclei with long stacks of membranes that contain nuclear pores and appear to be in contact with the nuclear envelope (22,23) (Figure 3). Similar defects were described in mutants of the fission yeast Ned1, which is also impaired in chromosome maintenance (25). In wild-type cells, the nucleolus adopts a crescent-like morphology and it is found in close association with part of the nuclear membrane (4). In *spo7* and *pah1* mutants the expansion of the nuclear membrane takes place in the area of the nuclear envelope that is associated with the nucleolus while the remaining nuclear membrane that is associated with chromatin appears to resist expansion (58). As a result, the organization of the bulk DNA does not appear severely affected in *nem1*, *spo7* and *pah1* mutants (22,23,58). However, it is still possible that the lipid composition of the nuclear/ER membrane might impact on the function of chromatin or on its nuclear envelope anchoring given the multiple synthetic lethal interactions of *pah1*Δ with enzymes involved in DNA metabolism such as histone modifications, transcription or DNA repair (59,60). Notably, an analogous situation where the nucleolus dissociates from bulk chromatin while the nucleus changes shape, has been described in cells exposed to mating pheromone, but whether this depends on the Pah1p pathway is not known (61).

Three observations suggest that changes in PA homeostasis are responsible for the nuclear membrane expansion. Firstly, the membrane expansion can be caused by a catalytically dead Pah1p (20). Secondly, mutations in upstream biosynthetic steps that lower PA levels decrease the nuclear membrane expansion in *pah1*Δ cells (62). Thirdly, overexpression of Dgk1p, a novel nuclear/ER membrane DAG kinase that generates PA, can phenocopy the effects of *pah1*Δ (62,63). Thus a distinct nuclear membrane subdomain expands as a result of changes in PA metabolism but how direct is the PA involvement in this process is not known. Given the role of PA in the transcriptional induction of phospholipid biosynthesis, one possibility could be that increased membrane lipid biogenesis accounted for by the CDP-DAG pathway,

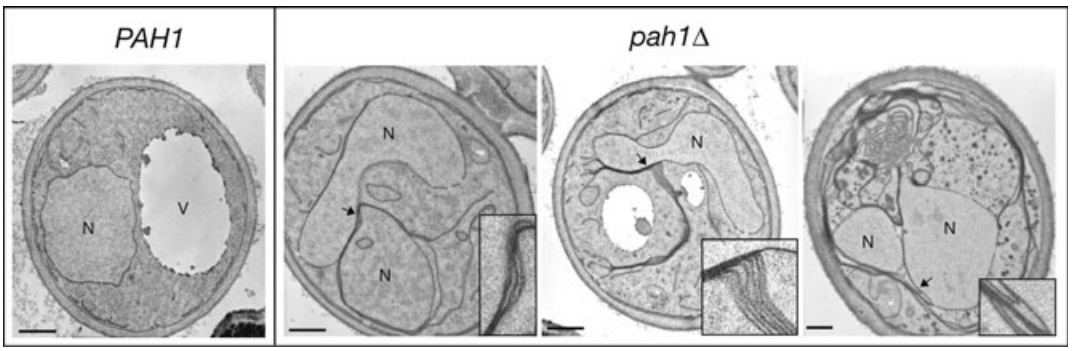

Figure 3: Nuclear structure in *pah1*Δ cells. Thin section electron microscopy of wild-type (*PAH1*) or *pah1*Δ cells stained with potassium permanganate. Inset panels show enlargements of areas of the nuclear envelope in *pah1*Δ cells (highlighted by arrows) that display membrane proliferation. N, nucleus; V, vacuole. Bars, 0.5 μm. Reprinted by permission from Macmillan Publishers Ltd: EMBO J. (22) © (2005).

drives the growth of the nuclear membrane. Inactivation of the PA signals downstream of Pah1p by either deleting the transcriptional activator Ino2p or overexpressing the repressor Opi1p, can restore normal nuclear shape in *nem1*Δ *spo7*Δ or *pah1*Δ deletion mutants (22). This observation suggests that *de novo* phospholipid synthesis is necessary for nuclear expansion in these cells. On the other hand, transcriptional derepression of phospholipid biosynthesis cannot, solely, account for the membrane expansion because the *opi1*Δ cells appear to have round nuclei (26). Therefore, it is likely that besides increased lipid synthesis, additional factors are involved. It must be noted that *pah1* mutants produce membranes with a significantly altered phospholipid composition (8,20) that could, by either changing the physical properties of lipid bilayers or the binding of effector proteins, promote membrane expansion. Previous studies have shown that nuclear envelope targeting of amphipathic proteins that could promote membrane curvature, induce nuclear membrane proliferation by an unknown mechanism. These include Nup53 in yeast (64), CTP:phosphocholine cytidylyltransferase-α (CCTα) in mammals (65) or Kugelkern in *Drosophila* (66). Changes in certain lipids at the nuclear membrane of *pah1*Δ cells might play an analogous role: PA and the PC:PE ratio can affect the physical properties and curvature of membranes and return of their levels to normal correlates with re-establishment of wild-type nuclear shape in *dgk1*Δ *pah1*Δ cells (62).

Given that the nuclear-ER membranes form one continuous compartment, changes in the structural organization of the ER could also impact on the shape of the nucleus. Recent evidence in mammals suggests that nuclear envelope dynamics depend on the restructuring of ER membrane domains mediated by reticulons, conserved ER proteins that are necessary to maintain proper ER tubular structure (67–69). In yeast, the ER adopts a more sheet-like appearance in *spo7*Δ cells (58); on the other hand the overexpression of Dgk1p does not disrupt tubular ER morphology (62). In *C. elegans*, two very recent studies reported that downregulation of Lpin-1, the single lipin orthologue in that organism, disrupts ER organization and causes the appearance of membrane sheets (70,71). In addition, it also causes defects in nuclear lamina disassembly, nuclear envelope breakdown (NEBD), chromosome segregation and nuclear structure (70,71). The defects in NEBD can be significantly rescued by lamin co-depletion (70). Whether lipin regulates directly the nuclear envelope dynamics or whether the nuclear envelope defects are a consequence of the disruption of the ER network remains to be determined.

How the nuclear membrane expands in yeast during anaphase to allow sister chromatid separation within a single nuclear compartment is not known. By controlling the amount and the type of phospholipids synthesized at the nuclear/ER membrane, Pah1p activity may play an important role in this process. Because chromatin binding to the inner nuclear envelope may restrict gross remodelling of nuclear structure, the nucleolus-associated membrane might define a more plastic area of the envelope where lipids and membrane can be added or reorganized, as previously suggested for the *spo7* mutants (58). Moreover, two membrane fission events of the nuclear envelope, take place in proximity of the nucleolar-associated membrane. The first is the resolution of the mother–daughter nuclei at the end of anaphase (4), and the second is the pinching off of part of the nucleus into the vacuole during the piecemeal microautophagy of the nucleus (72). Whether these events, that require extensive membrane bending and remodelling, are also influenced by Pah1p-dependent changes on lipid metabolism is currently not known.

Lipins in mammalian cells may also regulate organelle membrane biogenesis. Lipin 1 is highly upregulated during the increased phospholipid biosynthesis that drives ER and Golgi expansion when B-lymphocytes differentiate into antibody-secreting cells (73), or during ER membrane expansion resulting from the overexpression of the transcription factor XBP1 (74). Lipin 1 may be involved in these processes through its function in the biosynthetic production of DAG that is needed for membrane formation. Therefore, both Pah1p and lipins could have roles in the biogenesis of membrane-bound organelles, although the specific pathways involved in yeast and higher eukaryotes may differ.

Concluding remarks

Since Pah1p has been only very recently identified and our understanding of its function is still limited, there are several questions that need to be addressed. Pah1p is a soluble enzyme that must respond to changes in membrane composition. Understanding the temporal and spatial regulation of the membrane recruitment of Pah1p is essential in order to define its functions in lipid and organelle biogenesis. Moreover, apart from its role in the biosynthetic production of phospholipids, it is also possible that Pah1p can regulate more local PA/DAG pools. This is particularly relevant to a number of mammalian cell types that co-express all three lipin paralogues, raising the possibility that different lipins could control phospholipid homeostasis of different membranes within the same cell.

One unexpected finding was that mammalian lipin 1 has a distinct intranuclear pool with functions in gene expression. How the two roles, that of the lipid biosynthetic enzyme and transcriptional regulator, are related is not well understood. It will be important to address the possible intranuclear and inner nuclear membrane functions of Pah1p. The role of Pah1p in nuclear structure highlights the importance of phospholipid metabolism in nuclear membrane biogenesis, an area of research where little is still known. How changes in PA or DAG are translated into an expanded subdomain of the nuclear envelope and do these lipids, or other metabolically linked ones,

have a role during cell cycle or developmentally induced changes of nuclear shape? Is there a role for PA and DAG in the structural organization of ER subdomains? It will also be important to differentiate between PA- and DAG-dependent mechanisms and examine the role of their protein effectors in the function of Pah1p. Finally, advances into the biology of Pah1p may also provide valuable insights into the function of lipins and the pathways that regulate fat metabolism in mammals.

Acknowledgments

I am grateful to Nickolas Ktistakis, Fulvio Reggiori and Helena Santos-Rosa for the critical reading of the manuscript and to Mátyás Gorjánácz, Iain Mattaj and Orna Cohen-Fix for communicating their papers prior publication. Work in my laboratory is funded by the Medical Research Council.

References

1. Nunnari J, Walter P. Regulation of organelle biogenesis. Cell 1996;84:389–394.
2. Warren G, Wickner W. Organelle inheritance. Cell 1996;84:395–400.
3. Burke B, Ellenberg J. Remodelling the walls of the nucleus. Nat Rev Mol Cell Biol 2002;3:487–497.
4. Wente SR, Gasser SM, Caplan AJ. The nucleus and nucleocytoplasmic transport in *Saccharomyces cerevisiae*. In: Pringle JR, Broach JR, Jones EW, editors. The molecular and cellular biology of the yeast *Saccharomyces*. Volume 3: Cell cycle and cell biology. New York: CHSL Press; 1997, 471–546.
5. Hetzer MW, Walther TC, Mattaj IW. Pushing the envelope: structure, function and dynamics of the nuclear periphery. Annu Rev Cell Dev Biol 2005;21:347–380.
6. Leitch AR. Higher levels of organization in the interphase nucleus of cycling and differentiated cells. Microbiol Mol Biol Rev 2000; 64:138–152.
7. Mounkes L, Kozlov S, Burke B, Stewart CL. The laminopathies: nuclear structure meets disease. Curr Opin Genet Dev 2003;13: 223–230.
8. Han GS, Wu WI, Carman GM. The *Saccharomyces cerevisiae* Lipin homolog is a Mg2+-dependent phosphatidate phosphatase enzyme. J Biol Chem 2006;281:9210–9218.
9. Peterfy M, Phan J, Xu P, Reue K. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. Nat Genet 2001;27:121–124.
10. Athenstaedt K, Daum G. The life cycle of neutral lipids: synthesis, storage and degradation. Cell Mol Life Sci 2006;63:1355–1369.
11. Coleman RA, Lee DP Enzymes of triacylglycerol synthesis and their regulation. Prog Lipid Res 2004;43:134–176.
12. Lykidis A, Jackowski S. Regulation of mammalian cell membrane biosynthesis. Prog Nucleic Acid Res Mol Biol 2001;65:361–393.
13. Carman GM, Henry SA. Phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae* and interrelationship with other metabolic processes. Prog Lipid Res 1999;38:361–399.
14. Brindley DN. Lipid phosphate phosphatases and related proteins: signaling functions in development, cell division, and cancer. J Cell Biochem 2004;92:900–912.
15. Nanjundan M, Possmayer F. Pulmonary phosphatidic acid phosphatase and lipid phosphate phosphohydrolase. Am J Physiol Lung Cell Mol Physiol 2003;284:L1–23.
16. Carman GM. Phosphatidate phosphatases and diacylglycerol pyrophosphate phosphatases in *Saccharomyces cerevisiae* and *Escherichia coli*. Biochim Biophys Acta 1997;1348:45–55.
17. Donkor J, Sariahmetoglu M, Dewald J, Brindley DN, Reue K. Three mammalian lipins act as phosphatidate phosphatases with distinct tissue expression patterns. J Biol Chem 2007;282:3450–3457.
18. Harris TE, Huffman TA, Chi A, Shabanowitz J, Hunt DF, Kumar A, Lawrence JC Jr. Insulin controls subcellular localization and multisite phosphorylation of the phosphatidic acid phosphatase, lipin 1. J Biol Chem 2007;282:277–286.
19. Grimsey N, Han GS, O'Hara L, Rochford JJ, Carman GM, Siniossoglou S. Temporal and spatial regulation of the phosphatidate phosphatases lipin 1 and 2. J Biol Chem 2008;283:29166–29174.
20. Han GS, Siniossoglou S, Carman GM. The cellular functions of the yeast lipin homolog PAH1p are dependent on its phosphatidate phosphatase activity. J Biol Chem 2007;282:37026–37035.
21. Oshiro J, Han GS, Carman GM. Diacylglycerol pyrophosphate phosphatase in *Saccharomyces cerevisiae*. Biochim Biophys Acta 2003; 1635:1–9.
22. Santos-Rosa H, Leung J, Grimsey N, Peak-Chew S, Siniossoglou S. The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. EMBO J 2005;24:1931–1941.
23. Siniossoglou S, Santos-Rosa H, Rappsilber J, Mann M, Hurt E. A novel complex of membrane proteins required for formation of a spherical nucleus. EMBO J 1998;17:6449–6464.
24. Kobor MS, Archambault J, Lester W, Holstege FC, Gileadi O, Jansma DB, Jennings EG, Kouyoumdjian F, Davidson AR, Young RA, Greenblatt J. An unusual eukaryotic protein phosphatase required for transcription by RNA polymerase II and CTD dephosphorylation in *S. cerevisiae*. Mol Cell 1999;4:55–62.
25. Tange Y, Hirata A, Niwa O. An evolutionarily conserved fission yeast protein, Ned1, implicated in normal nuclear morphology and chromosome stability, interacts with Dis3, Pim1/RCC1 and an essential nucleoporin. J Cell Sci 2002;115:4375–4385.
26. O'Hara L, Han GS, Peak-Chew S, Grimsey N, Carman GM Siniossoglou S. Control of phospholipid synthesis by phosphorylation of the yeast lipin Pah1p/Smp2p Mg2+-dependent phosphatidate phosphatase. J Biol Chem 2006;281:34537–34548.
27. Dephoure N, Howson RW, Blethrow JD, Shokat KM, O'Shea EK. Combining chemical genetics and proteomics to identify protein kinase substrates. Proc Natl Acad Sci U S A 2005;102: 17940–17945.
28. Ptacek J, Devgan G, Michaud G, Zhu H, Zhu X, Fasolo J, Guo H, Jona G, Breitkreutz A, Sopko R, McCartney RR, Schmidt MC, Rachidi N, Lee SJ, Mah AS et al. Global analysis of protein phosphorylation in yeast. Nature 2005;438:679–684.
29. Mah AS, Elia AE, Devgan G, Ptacek J, Schutkowski M, Snyder M, Yaffe MB, Deshaies RJ. Substrate specificity analysis of protein kinase complex Dbf2-Mob1 by peptide library and proteome array screening. BMC Biochem 2005;6:22.
30. Jackowski S. Coordination of membrane phospholipid synthesis with the cell cycle. J Biol Chem 1994;269:3858–38567.
31. Huffman TA, Mothe-Satney I, Lawrence JC Jr. Insulin-stimulated phosphorylation of lipin mediated by the mammalian target of rapamycin. Proc Natl Acad Sci U S A 2002;99:1047–1052.
32. Kurat CF, Wolinski H, Petschnigg J, Kaluarachchi S, Andrews B, Natter K, Kohlwein SD. Cdk1/Cdc28-dependent activation of the major triacylglycerol lipase Tgl4 in yeast links lipolysis to cell-cycle progression. Mol Cell 2009;33:53–63.
33. Bankaitis VA, Morris AJ. Lipids and the exocytotic machinery of eukaryotic cells. Curr Opin Cell Biol 2003;15:389–395.
34. Carman GM, Henry SA. Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. J Biol Chem 2007;282:37293–37297.
35. Kodaki T, Nikawa J, Hosaka K, Yamashita S. Functional analysis of the regulatory region of the yeast phosphatidylserine synthase gene, PSS. J Bacteriol 1991;173:7992–7995.

36. Lopes JM, Hirsch JP, Chorgo PA, Schulze KL, Henry SA. Analysis of sequences in the INO1 promoter that are involved in its regulation by phospholipid precursors. Nucleic Acids Res 1991;19:1687–1693.
37. Schüller HJ, Hahn A, Tröster F, Schütz A, Schweizer E. Coordinate genetic control of yeast fatty acid synthase genes FAS1 and FAS2 by an upstream activation site common to genes involved in membrane lipid biosynthesis. EMBO J 1992;11:107–114.
38. Nikoloff DM, McGraw P, Henry SA. The INO2 gene of *Saccharomyces cerevisiae* encodes a helix–loop–helix protein that is required for activation of phospholipid synthesis. Nucleic Acids Res 1992;20:3253.
39. Hoshizaki DK, Hill JE, Henry SA. The *Saccharomyces cerevisiae* INO4 gene encodes a small, highly basic protein required for derepression of phospholipid biosynthetic enzymes. J Biol Chem 1990; 265:4736–4745.
40. White MJ, Hirsch JP, Henry SA. The OPI1 gene of *Saccharomyces cerevisiae*, a negative regulator of phospholipid biosynthesis, encodes a protein containing polyglutamine tracts and a leucine zipper. J Biol Chem 1991;266:863–872.
41. Loewen CJ, Gaspar ML, Jesch SA, Delon C, Ktistakis NT, Henry SA, Levine TP. Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. Science 2004;304:1644–1647.
42. Akhtar A, Gasser SM. The nuclear envelope and transcriptional control. Nat Rev Genet 2007;8:507–517.
43. Brickner JH, Walter P. Gene recruitment of the activated INO1 locus to the nuclear membrane. PLoS Biol 2004;2:e342.
44. Khalil MB, Sundaram M, Zhang HY, Links PH, Raven JF, Manmontri B, Sariahmetoglu M, Tran K, Reue K, Brindley DN, Yao Z. The level and compartmentalization of phosphatidate phosphatase-1 (lipin-1) control the assembly and secretion of hepatic VLDL. J Lipid Res 2009;50:47–58.
45. Finck BN, Gropler MC, Chen Z, Leone TC, Croce MA, Harris TE, Lawrence JC Jr, Kelly DP. Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. Cell Metab 2006;4:199–210.
46. Koh Y-K, Lee M-Y, Kim J-W, Kim M, Moon J-S, Lee Y-J, Ahn Y-H, Kim K-S. Lipin1 is a key factor for the maturation and maintenance of adipocytes in the regulatory network with CCAAT/Enhancer-binding protein α and peroxisome proliferator-activated receptor γ2. J Biol Chem 2008;283:34896–34906.
47. Reue K, Brindley DN. Multiple roles for lipins/phosphatidate phosphatase enzymes in lipid metabolism. J Lipid Res 2008;49:2493–2503.
48. Lusk CP, Blobel G, King MC. Highway to the inner nuclear membrane: rules for the road. Nat Rev Mol Cell Biol 2007;8:414–420.
49. Schirmer EC, Florens L, Guan T, Yates JR III, Gerace L. Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science 2003;301:1380–1382.
50. Kim Y, Gentry MS, Harris TE, Wiley SE, Lawrence JC Jr, Dixon JE. A conserved phosphatase cascade that regulates nuclear membrane biogenesis. Proc Natl Acad Sci U S A 2007;104:6596–6601.
51. Rose MD. Nuclear fusion in the yeast *Saccharomyces cerevisiae*. Annu Rev Cell Dev Biol 1996;12:663–695.
52. Neiman AM. Ascospore formation in the yeast *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 2005;69:565–584.
53. Fabre E, Hurt E. Yeast genetics to dissect the nuclear pore complex and nucleocytoplasmic trafficking. Annu Rev Genet 1997; 31:277–313.
54. Hattier T, Andrulis ED, Tartakoff AM. Immobility, inheritance and plasticity of shape of the yeast nucleus. BMC Cell Biol 2007;8:47.
55. Kimata Y, Lim CR, Kiriyama T, Nara A, Hirata A, Kohno K. Mutation of the yeast epsilon-COP gene ANU2 causes abnormal nuclear morphology and defects in intracellular vesicular transport. Cell Struct Funct 1999;24:197–208.
56. Ryan KJ, Wente SR. Isolation and characterization of new *Saccharomyces cerevisiae* mutants perturbed in nuclear pore complex assembly. BMC Genet 2002;3:17.
57. Schneiter R, Hitomi M, Ivessa AS, Fasch EV, Kohlwein SD, Tartakoff AM. A yeast acetyl coenzyme A carboxylase mutant links very-long-chain fatty acid synthesis to the structure and function of the nuclear membrane-pore complex. Mol Cell Biol 1996;16:7161–7172.
58. Campbell JL, Lorenz A, Witkin KL, Hays T, Loidl J, Cohen-Fix O. Yeast nuclear envelope subdomains with distinct abilities to resist membrane expansion. Mol Biol Cell 2006;17:1768–1778.
59. Pan X, Ye P, Yuan DS, Wang X, Bader JS, Boeke JD. A DNA integrity network in the yeast *Saccharomyces cerevisiae*. Cell 2006; 124:1069–1081.
60. Lin YY, Qi Y, Lu JY, Pan X, Yuan DS, Zhao Y, Bader JS, Boeke JD. A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. Genes Dev 2008 ;22:2062–2074.
61. Stone EM, Heun P, Laroche T, Pillus L, Gasser SM. MAP kinase signaling induces nuclear reorganization in budding yeast. Curr Biol 2000;10:373–382.
62. Han GS, O'Hara L, Carman GM, Siniossoglou S. An unconventional diacylglycerol kinase that regulates phospholipid synthesis and nuclear membrane growth. J Biol Chem 2008;283:20433–20442.
63. Han GS, O'Hara L, Siniossoglou S, Carman GM. Characterization of the yeast DGK1-encoded CTP-dependent diacylglycerol kinase. J Biol Chem 2008;283:20443–20453.
64. Marelli M, Lusk CP, Chan H, Aitchison JD, Wozniak RW. A link between the synthesis of nucleoporins and the biogenesis of the nuclear envelope. J Cell Biol 2001;153:709–724.
65. Gehrig K, Cornell RB, Ridgway ND. Expansion of the nucleoplasmic reticulum requires the coordinated activity of lamins and CTP:phosphocholine cytidylyltransferase alpha. Mol Biol Cell 2008; 19:237–247.
66. Brandt A, Papagiannouli F, Wagner N, Wilsch-Bräuninger M, Braun M, Furlong EE, Loserth S, Wenzl C, Pilot F, Vogt N, Lecuit T, Krohne G, Grosshans J. Developmental control of nuclear size and shape by Kugelkern and Kurzkern. Curr Biol 2006;16:543–552.
67. Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA. A class of membrane proteins shaping the tubular endoplasmic reticulum. Cell 2006;124:573–586.
68. Audhya A, Desai A, Oegema K. A role for Rab5 in structuring the endoplasmic reticulum. J Cell Biol 2007;178:43–56.
69. Anderson DJ, Hetzer MW. Reshaping of the endoplasmic reticulum limits the rate for nuclear envelope formation. J Cell Biol 2008; 182:911–924.
70. Gorjánácz M, Mattaj IW. Lpin-1 is required for efficient nuclear envelope breakdown in *Caenorhabditis elegans*. J Cell Sci 2009;in press.
71. Golden A, Liu J, Cohen-Fix O. Inactivation of the *C. elegans* homolog of lipin leads to endoplasmic reticulum disorganization and defects in nuclear envelope breakdown and reassembly. J Cell Sci 2009; in press.
72. Kvam E, Goldfarb DS. Nucleus–vacuole junctions and piecemeal microautophagy of the nucleus in *S. cerevisiae*. Autophagy 2007; 3:85–92.
73. Fagone P, Sriburi R, Ward-Chapman C, Frank M, Wang J, Gunter C, Brewer JW, Jackowski S. Phospholipid biosynthesis program underlying membrane expansion during B-lymphocyte differentiation. J Biol Chem 2007;282:7591–7605.
74. Sriburi R, Bommiasamy H, Buldak GL, Robbins GR, Frank M, Jackowski S, Brewer JW. Coordinate regulation of phospholipid biosynthesis and secretory pathway gene expression in XBP-1(S)-induced endoplasmic reticulum biogenesis. J Biol Chem 2007;282:7024–7034.