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CELL BIOLOGY

# Lipid Signaling and Homeostasis: PA- Is Better than PA-H, But What About Those PIPs?

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**Although cellular membranes are composed of hundreds of distinct lipid species, the lipid composition is maintained within a narrow range. The regulatory circuit responsible for this homeostasis in yeast depends on a membrane-bound transcriptional repressor that translocates to the nucleus in response to the abundance of its lipid ligand on the membrane. Feedback control in this system is provided because the lipid ligand is also an end product of the activity of the transcription factor. This basic design is also evident in higher eukaryotes such as *Drosophila* and mammals, but with important differences in the lipid being sensed, the composition of the sensors, and the fine-tuning of the response. New work indicates that regulation of intracellular pH levels in yeast by glucose availability may fine-tune the binding of the repressor to its lipid ligand, providing a mechanism that connects phospholipid metabolism to nutrient sensing. The importance of pH effects in this pathway raises the possibility that additional lipid-signaling pathways may be regulated by the protonation state of the lipid or its effector.**

## Introduction

Hundreds of distinct lipid species make up cell membranes, but the underlying purpose of this complexity is currently unknown. In contrast, the homeostatic control mechanisms that are responsible for maintaining this complexity within a narrow range under widely varying growth conditions are beginning to become clear. For the yeast *Saccharomyces cerevisiae*, the phospholipid phosphatidic acid (PA) has a central role in the transcriptional regulation of genes involved in phospholipid synthesis (*1*). This is noteworthy on two counts: It takes PA away from its usual signaling role downstream of phospholipase D or diacylglycerol kinase activation (*2*), and, given that PA is a precursor for most phospholipids, it makes the yeast regulatory circuit analogous to other eukaryotes for which lipid homeostasis depends on one of the lipid products as a sensor (Fig. 1). Young *et al*. reported that PA acts as a sensor in conjunction to changes in intracellular pH brought about by extracellular glucose concentrations, potentially tying phospholipid biosynthesis with nutrient availability (*3*).

The PA effector in yeast is the transcriptional repressor Opi1, which is bound to the endoplasmic reticulum (ER) through interactions with the protein Scs2 and PA (*1*). The model that emerged from previous experiments proposed that Opi1 localization depends on extracellular inositol concentrations, which determine intracellular PA availability. In the absence of extracellular inositol, which results in plentiful amounts of PA on the ER, Opi1 is kept out of the nucleus, and genes involved in inositol synthesis are transcribed. Upon addition of inositol, PA is consumed to generate phosphatidylinositol (PI) (Fig. 1), and Opi1 translocates from the ER to the nucleus, where it represses the genes involved in inositol synthesis (Fig. 2). Given that PI and phosphatidylcholine (PC) synthesis are coordinately regulated in yeast, the above-mentioned model may also be relevant for sensing phospholipid biosynthetic activity in general under varying growth conditions (*4*).

However, recognition of PA by Opi1 is not a simple story. In an unbiased genetic screen for deletion mutants unable to grow in the absence of inositol, Young *et al*. identified various genes involved in the regulation of intracellular pH, including the subunits of the vacuolar adenosine triphosphatase complex (V-ATPase), the ER chaperones involved in its assembly, and the plasma membrane $H^+$-ATPase that is regulated by the V-ATPase (*3*). With the use of yeast genetics, lipid biochemistry, and subcellular imaging, Young *et al*. then showed that cytosolic acidification is coupled to phospholipid metabolism because it derepresses Opi1. The reason for this is that PA is a better ligand for Opi1 when it is deprotonated (that is, PA- is better than PA-H). A potentially relevant physiological stimulus is glucose, given that (i) components of glucose signaling were identified in the genetic screen by Young *et al*. and (ii) the intracellular pH of yeast cells starved of glucose drops rapidly, enabling Opi1 to translocate to the nucleus where it represses its target genes. This model explains how nutrients can affect phospholipid metabolism and provides

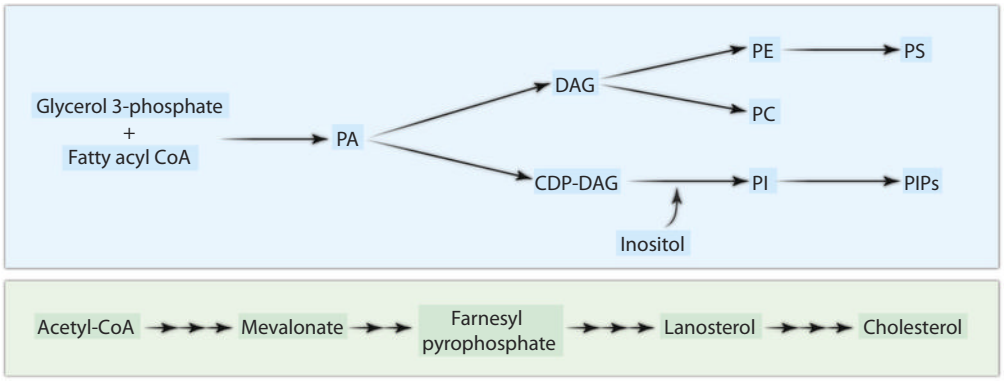


**Fig. 1.** Pathways of phospholipid and cholesterol synthesis. Simplified schemes for the synthesis of most major phospholipids from glycerol 3-phosphate and fatty acyl CoA, with PA as an intermediate (**top**), and for the synthesis of cholesterol from acetyl CoA (**bottom**). DAG, diacylglycerol; CDP, cytidine 5′-diphosphate; PS, phosphatidylserine; PIPs, phosphatidylinositol phosphates.

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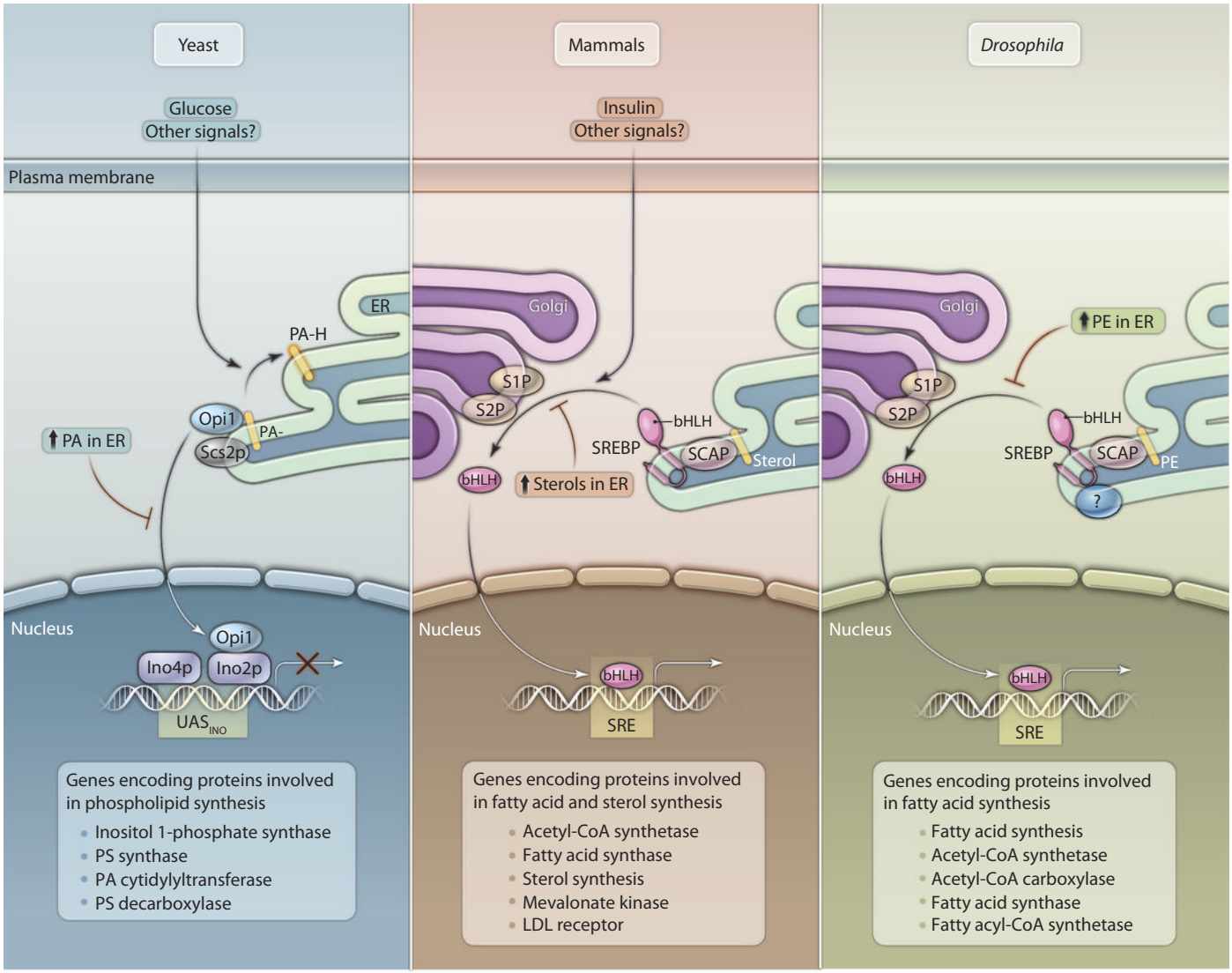


**Fig. 2.** Simplified diagrams of the three lipid sensing circuits in yeast (**left**), mammals (**middle**), and *Drosophila* (**right**). In all cases, a transcription factor enters the nucleus in response to lipid sensing on the ER and regulates genes that control phospholipid, fatty acid, or cholesterol synthesis as indicated. For yeast, the transcriptional repressor is Opi1, which binds to the ER through deprotonated PA and Scs2p. A signal generated from glucose sensing results in protonation of PA and translocation of Opi1 to the nucleus where it represses genes containing a inositol-sensitive upstream activating sequence ($UAS_{INO}$) response element. For mammals, cholesterol depletion in the ER causes the SREBP-SCAP complex to be transported to the Golgi, where two proteases generate the transcriptional activator bHLH from the N terminus of SREBP. This domain enters the nucleus and activates genes containing SREBP response elements (SRE). The abundance of SREBP and, perhaps, its activity are controlled by insulin availability upstream of mTOR signaling. For *Drosophila*, SREBP senses PE instead of cholesterol on the ER, SCAP is not essential for the response (and Insig is absent), and upstream signals have not been identified. However, the basic design of the circuit and the proteins involved are analogous to that of the mammals. LDL, low-density lipoprotein.

additional points of control linking growth state with lipid homeostasis (*3*).

The way that the yeast findings fit with lipid homeostatic mechanisms in other eukaryotes, such as flies and mammals, is notable both in terms of similarities of the overall design and of differences characteristic of each organism. In addition, the work by Young *et al*. suggests the possibility that pH changes could affect other pathways of lipid signaling by modifying either the signal itself or its effector.

## Lipid Homeostasis in Yeast, *Drosophila*, and Mammals

At a basic level, the design of the sensing circuits in the three organisms is remarkably similar. Lipid sensing occurs on the ER, and the end result is the nuclear translocation of a transcription factor with a broad set of target genes involved in lipid biosynthesis (Fig. 2). In mammalian cells, the circuit senses sterol concentrations on the ER through a multipass transmembrane protein termed SCAP [sterol regulatory element–binding protein (SREBP) cleavage-activating protein] (*5*, *6*). SCAP associates with SREBP, a two-pass transmembrane protein that contains, at its cytosolic N terminus, a basic helix-loop-helix (bHLH) transcription factor, which is the active domain that ultimately enters the nucleus (*7*). High ER cholesterol concentrations maintain the SCAP-SREBP complex at the ER through binding of SCAP to another ER protein, Insig (not

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shown in Fig. 2), in a cholesterol-sensitive fashion. Cholesterol depletion causes SCAP-SREBP to move to the Golgi, where SREBP is cleaved by two resident proteases S1P and S2P, with S2P effecting the final cleavage within the transmembrane domain of the SREBP fragment to generate the active transcription factor (*5*, *6*).

There are at least two additional control elements in this system. HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, the rate controlling enzyme in cholesterol biosynthesis that is resident on ER membranes, is degraded in response to high ER sterol concentrations, thus further slowing cholesterol synthesis (*8*, *9*). In addition, the abundance (and perhaps activity) of SREBP is controlled by the kinase mammalian target of rapamycin (mTOR), which is downstream of insulin and phosphatidylinositol 3-phosphate (PI3P) kinase signaling (*10*, *11*). This complex regulatory circuit must accomplish some challenging tasks: (i) to maintain cholesterol concentrations within a narrow range under steady-state conditions; (ii) to accommodate synthesis rates varying by more than two orders of magnitude, depending on external cholesterol concentrations and growth conditions; and (iii) to balance cholesterol concentrations with those of other phospholipids such that their relative amounts are kept within a narrow range (*6*).

The circuit in *Drosophila* is similar to the one described above, but with a few differences (*12*). Because this organism does not synthesize cholesterol, the molecule being sensed is phosphatidylethanolamine (PE), an abundant phospholipid that predominates over PC (which is the predominant species in mammalian membranes) (*13*) in *Drosophila* membranes. Despite this difference, the protein components involved in PE feedback control in flies are similar to mammalian cells and include SREBP, SCAP, and the two proteases (S1P and S2P). [Two additional differences are the absence of Insig and the fact that SCAP function is not essential in *Drosophila* and may be supplemented by additional proteins (*14*).] Thus, in mammals and flies, the structure of the circuit is similar, but a different lipid species is sensed.

Yeast do not contain proteins related to the SREBP pathway, and its equivalent regulator, Opi1, lacks counterparts in higher eukaryotes. Therefore, it is not surprising that the conservation in the design of the circuits was not apparent until the yeast components were functionally characterized (*4*); the similarities between the circuits are quite pronounced. In a further simplification to *Drosophila*, the sensed lipid in yeast (PA) is the earliest precursor in phospholipid biosynthesis (*15*). In addition, there is apparently no ER-to-Golgi transport step for the processing of the transcription factor to its active form. However, the basic design of a membrane-bound protein translocating to the nucleus in response to the abundance of its ligand on the membrane is maintained. The key characteristic, that one of the lipid end products of the transcriptional activity is the ligand of the transcription factor, is also maintained. I would also argue that the findings of Young *et al*. are congruent with the observed regulation in higher eukaryotes. These circuits must be able to sense extracellular conditions to coordinate lipid synthesis with nutrient-dependent growth rates. In mammals and, presumably, in *Drosophila*, the circuit is connected to insulin availability (*10*). In yeast, according to the genetic and biochemical data of Young *et al*., the circuit is connected to glucose availability, which is probably the most critical nutrient for these cells (*3*).

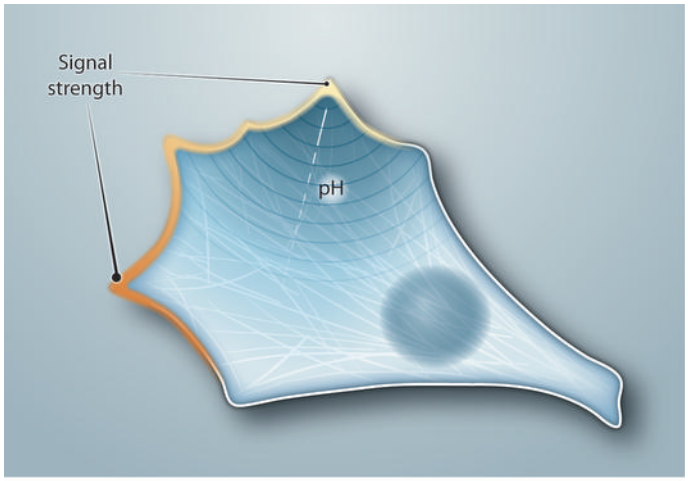


**Fig. 3.** A pH gradient can alter the strength of a lipid signal. The orange band represents a region of a membrane where a lipid signal has been generated. If this signal is affected by pH (either by the protonation state of the lipid or of its effector), then local pH changes would affect how the signal interacts with its effectors. In the simple color scheme shown here, this is reflected by changes in the intensity of the orange color. The magnitude of the signal in response to pH changes is arbitrary.

## Alterations in Cytosolic pH May Regulate Lipid Signals

Cells expend considerable resources to keep intracellular pH within a narrow range. Under resting conditions, the pH in the cytosol is maintained at ~7.2, even when the external pH varies by several pH units. Nevertheless, although overall pH in the cytosol is fairly constant, the use of sensitive pH indicators has revealed examples of ligands and conditions that induce pH alterations, either throughout the cytosol or within more localized regions (*16*–*18*). The work by Young *et al*. shows that PA can function as a pH biosensor, implying the possibility that a pH gradient may alter the strength of a lipid signal (Fig. 3).

Additional examples of pH-mediated regulation of lipid binding have been discovered. The FYVE domain, a lipid-binding module that recognizes PI3P, shows a stronger preference for its target lipid when the cytosolic pH is acidic. The molecular basis of this phenomenon is a pair of histidine residues in the active site of the FYVE domain, which, when protonated, enhances lipid binding, thus making the FYVE domain a low-pH biosensor (*19*). Similar findings have been reported for the binding of the epsin ENTH domain and the AP180 ANTH domain to phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$]. For both domains, lipid binding is enhanced by acidic pH, and the protonation state of a histidine residue within the active site of the domain partially underlies this effect (*20*). Another example in which a local pH field may regulate lipid binding concerns the function of cofilin during membrane protrusion. The actin-severing activity of cofilin generates free barbed ends that become sites of actin assembly at the leading edge of motile cells. Cofilin binds $PI(4,5)P_2$ at the plasma membrane, which is enhanced by acidic pH and involves the protonation state of a histidine residue (*21*). Given that cell migration may depend, in part, on a cytosolic pH gradient (*22*), the data suggest that, under resting conditions, cofilin binds to $PI(4,5)P_2$ at the plasma

membrane, whereas under activating conditions and increased pH, cofilin may bind to actin to generate free barbed ends for motility. In this view, binding to $PI(4,5)P_2$ competes with binding to actin, with local pH contributing to the balance point of the interaction (*21*).

In addition, several other lipid-binding proteins show pH dependency, which is explained by protonation state of key residues on the protein (*16*, *23*). The paper by Young *et al*. invokes a second mechanism by which pH may influence lipid binding. In the case of Opi1, pH alters the electrostatics of the lipid itself, which is recognized by the effector. A second protein that appears to behave similarly is Spo20, which is involved in sporulation downstream of the yeast phospholipase D (an enzyme that generates PA from PC) and binds to PA (*3*). The biophysical basis for this mechanism appears to lie in the unusual ionization properties of PA (*24*). Because the phosphate group of PA is attached to the backbone as a monoester, the second $pK_a$ (where $K_a$ is the acid dissociation constant) of PA falls within a physiological pH range between 5 and 8, and it is sensitive to the surrounding membrane composition. In practical terms, this means that PA can be differentially protonated, depending on the pH and its membrane context (*24*, *25*). Given that most proteins recognize PA through electrostatic interactions using stretches of positively charged residues (*2*, *24*), the protonation state of PA in response to pH changes could, in principle, help to switch it on or off as a signal in other cases. Could this be a broader principle in lipid signaling, given that other phosphoinositides contain phosphate groups with similar ionization properties (*26*)? It is worth considering, but in my opinion, it may be too simple an idea. The cytosol contains milimolar amounts of divalent cations, likely to affect the ionization properties of these lipids and perhaps mask any pH-dependent effects. On the other hand, a mechanism involving pH-dependent alterations, on both the effectors and the signal, that synergize to change binding affinities may be more likely. In any event, the work by Young *et al*. provides a framework to address some of these questions in the future.

### References


1. C. J. R. Loewen, M. L. Gaspar, S. A. Jesch, C. Delon, N. T. Ktistakis, S. A. Henry, T. P. Levine, Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. *Science* **304**, 1644–1647 (2004).
2. C. L. Stace, N. T. Ktistakis, Phosphatidic acid- and phosphatidylserine-binding proteins. *Biochim. Biophys. Acta* **1761**, 913–926 (2006).
3. B. P. Young, J. J. H. Shin, R. Orij, J. T. Chao, S. C. Li, X. L. Guan, A. Khong, E. Jan, M. R. Wenk, W. A. Prinz, G. J. Smits, C. J. R. Loewen, Phosphatidic acid is a pH biosensor that links membrane biogenesis to metabolism. *Science* **329**, 1085–1088 (2010).
4. G. M. Carman, S. A. Henry, Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **282**, 37293–37297 (2007).
5. J. L. Goldstein, R. A. DeBose-Boyd, M. S. Brown, Protein sensors for membrane sterols. *Cell* **124**, 35–46 (2006).
6. M. S. Brown, J. L. Goldstein, Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J. Lipid Res.* **50**, (Suppl), S15–S27 (2009).
7. C. Yokoyama, X. Wang, M. R. Briggs, A. Admon, J. Wu, X. Hua, J. L. Goldstein, M. S. Brown, SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell* **75**, 187–197 (1993).
8. G. Gil, J. R. Faust, D. J. Chin, J. L. Goldstein, M. S. Brown, Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. *Cell* **41**, 249–258 (1985).
9. M. Nakanishi, J. L. Goldstein, M. S. Brown, Multivalent control of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Mevalonate-derived product inhibits translation of mRNA and accelerates degradation of enzyme. *J. Biol. Chem.* **263**, 8929–8937 (1988).
10. S. Li, M. S. Brown, J. L. Goldstein, Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3441–3446 (2010).
11. M. Laplante, D. M. Sabatini, mTORC1 activates SREBP-1c and uncouples lipogenesis from gluconeogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3281–3282 (2010).
12. R. B. Rawson, The SREBP pathway—insights from Insigs and insects. *Nat. Rev. Mol. Cell Biol.* **4**, 631–640 (2003).
13. I. Y. Dobrosotskaya, A. C. Seegmiller, M. S. Brown, J. L. Goldstein, R. B. Rawson, Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila*. *Science* **296**, 879–883 (2002).
14. K. A. Matthews, C. Ozdemir, R. B. Rawson, Activation of sterol regulatory element binding proteins in the absence of Scap in *Drosophila melanogaster*. *Genetics* **185**, 189–198 (2010).
15. K. Athenstaedt, G. Daum, Phosphatidic acid, a key intermediate in lipid metabolism. *Eur. J. Biochem.* **266**, 1–16 (1999).
16. J. R. Casey, S. Grinstein, J. Orlowski, Sensors and regulators of intracellular pH. *Nat. Rev. Mol. Cell Biol.* **11**, 50–61 (2010).
17. J. Han, K. Burgess, Fluorescent indicators for intracellular pH. *Chem. Rev.* **110**, 2709–2728 (2010).
18. A. Roos, W. F. Boron, Intracellular pH. *Physiol. Rev.* **61**, 296–434 (1981).
19. S. A. Lee, R. Eyeson, M. L. Cheever, J. Geng, V. V. Verkhusha, C. Burd, M. Overduin, T. G. Kutateladze, Targeting of the FYVE domain to endosomal membranes is regulated by a histidine switch. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13052–13057 (2005).
20. R. A. Hom, M. Vora, M. Regner, O. M. Subach, W. Cho, V. V. Verkhusha, R. V. Stahelin, T. G. Kutateladze, pH-dependent binding of the Epsin ENTH domain and the AP180 ANTH domain to PI(4,5)P2-containing bilayers. *J. Mol. Biol.* **373**, 412–423 (2007).
21. C. Frantz, G. Barreiro, L. Dominguez, X. Chen, R. Eddy, J. Condeelis, M. J. Kelly, M. P. Jacobson, D. L. Barber, Cofilin is a pH sensor for actin free barbed end formation: role of phosphoinositide binding. *J. Cell Biol.* **183**, 865–879 (2008).
22. S. P. Denker, D. L. Barber, Cell migration requires both ion translocation and cytoskeletal anchoring by the Na-H exchanger NHE1. *J. Cell Biol.* **159**, 1087–1096 (2002).
23. J. Srivastava, D. L. Barber, M. P. Jacobson, Intracellular pH sensors: design principles and functional significance. *Physiology (Bethesda)* **22**, 30–39 (2007).
24. E. E. Kooijman, K. N. Burger, Biophysics and function of phosphatidic acid: a molecular perspective. *Biochim. Biophys. Acta* **1791**, 881–888 (2009).
25. A. Bhushan, M. G. McNamee, Correlation of phospholipid structure with functional effects on the nicotinic acetylcholine receptor. A modulatory role for phosphatidic acid. *Biophys. J.* **64**, 716–723 (1993).
26. E. E. Kooijman, K. E. King, M. Gangoda, A. Gericke, Ionization properties of phosphatidylinositol polyphosphates in mixed model membranes. *Biochemistry* **48**, 9360–9371 (2009).


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