Keeping sphingolipid levels n*ORM*al

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Lipids are essential for life as the principal components of biomembranes. In addition, many lipids act as signaling molecules both in and outside the cell. For these structural and regulatory functions it is crucial that the abundance of different lipids is controlled in a coordinated fashion to maintain the right balance between them. Aberrations in this equilibrium could result in altered membrane fluidity, increased membrane permeability, or depletion of signaling precursors. Particularly in the plasma membrane, a delicate balance between major lipid classes, namely glycerophospholipids, sterols, and sphingolipids, is maintained. Whereas important paradigms of sterol regulation have emerged principally from the work of the Brown and Goldstein laboratory, little is yet known about the regulation of sphingolipid levels. Two reports from the laboratories of Jonathan Weissman and Amy Chang published in *Nature* and PNAS, respectively, have started to uncover a fascinating regulatory mechanism for sphingolipid synthesis by the evolutionarily conserved family of endoplasmic reticulum (ER)-resident Orm proteins (1, 2).

Starting from the observation a few years ago that deletion of *ORM* genes reduces fitness of yeast grown on media containing agents that induce ER stress (3), Han et al. found the unfolded protein response (UPR) that monitors the ER and homeostatically regulates its functions activated in *ORM* mutants. A functional link to sphingolipid metabolism is provided by a comprehensive genetic analysis generated in the Weissman laboratory, showing that overexpression of Orm proteins has a genetic interaction profile similar to the one of the hypomorphic alleles of *LCB1* and *LCB2*, encoding subunits of serine–palmitoyl–transferase (SPT) that catalyze the committing step of sphingolipid synthesis in the ER. Conversely, deletion of the *ORM* genes resulted in a phenotypic signature opposite to that of *lcb1* and *lcb2*. Interestingly, both groups found that this relationship is reflected in a physical complex of Orm proteins with SPT (1, 2).

These results prompted the analysis of sphingolipid synthesis intermediates, which revealed highly elevated levels of long chain sphingoid bases such as phytosphingosine in the *orm1*Δ*orm2*Δ mutants (1, 2). Breslow et al. (1) also found that Orm protein overexpression results in the opposite, namely reduction of long chain sphingoid base levels. Because long chain sphingoid bases are the product of SPT, the model emerging from these studies posits that Orm proteins negatively regulate sphingolipid synthesis at the committing step (Fig. 1). In this scenario, disruption of this negative regulation would relieve SPT from inhibition and lead to increased production of long chain sphingoid bases. This in turn would produce the pleiotropic phenotypes on ER stress, ER-to-Golgi trafficking, and inositol–phospholipid metabolism observed by Han et al. (2). In agreement with this model, most phenotypes of *orm* mutants are suppressed by nonlethal concentrations of SPT inhibitors that lower levels of long chain sphingoid bases (2). Interestingly, the regulation by Orm proteins might function as a homeostatic feedback loop. In an elegant experiment, Breslow et al. show that wild-type cells maintain normal long chain sphingoid base levels in the presence of increasing concentrations of SPT inhibitors up to a certain point where the system breaks. *ORM* mutants on the other hand respond by linearly decreasing long chain sphingoid base levels over the whole range of inhibitor concentrations (1). This is exactly the behavior expected from a homeostatic system, such as a climate control: In its presence, and as long as it is not overwhelmed by an external perturbation, it regulates a parameter, such as temperature or sphingolipid levels, in a narrow range. In its absence, the parameter that is controlled goes to an extreme, but responds directly to disturbances.

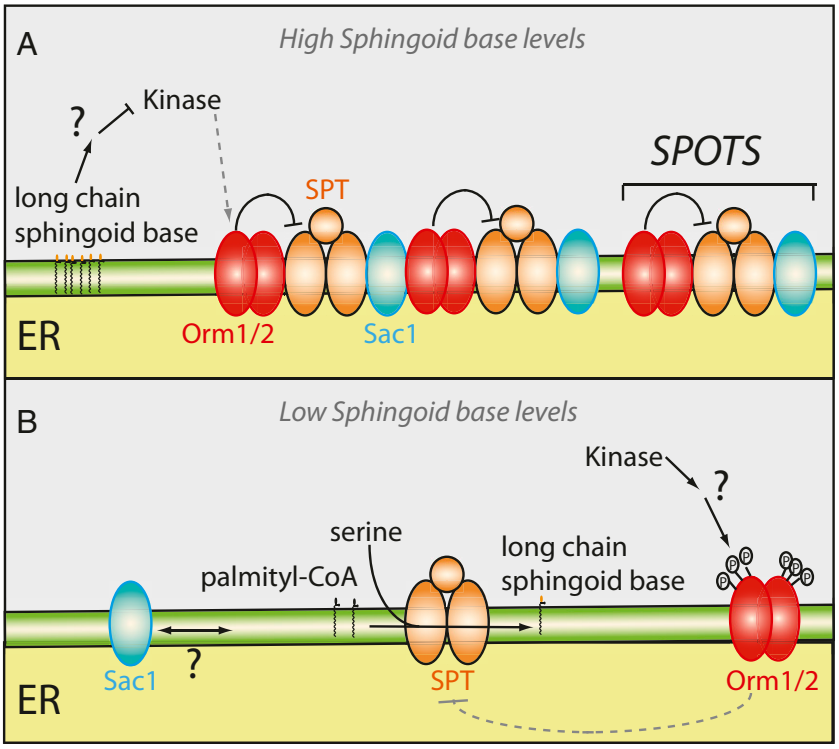

Fig. 1. Model of the homeostatic regulation of SPT activity by Orm proteins. See text for details.

How could such a feedback loop be achieved mechanistically? Initial insights from Breslow et al. (1) suggest that formation of SPT-containing complexes and a signal transduction cascade is involved. They found that Orm proteins associate together with SPT in higher-order assemblies and that the interactions involved are regulated by sphingolipids. Specifically, assembly is decreased after incubation with SPT inhibitors. In addition, Orm proteins are phosphorylated and the level of phosphorylation is altered in response to blocking sphingolipid synthesis. Importantly, mutation of phosphorylation sites in Orm proteins results in a reduction

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of long chain sphingoid base levels and decreases the association of SPT in large assemblies. In addition, Breslow and colleagues found increased levels of not only long chain sphingolipids but also complex sphingolipids such as ceramide or $M(IP)_2C$ that make up the bulk of sphingolipids in yeast. On the basis of these data, they suggest that this feedback loop participates in the regulation of overall sphingolipid levels. However, an important discrepancy between the two papers remains unresolved. In contrast to the data outlined above, Han et al. (2) actually saw a decrease of ceramides in *orm* mutants and argue that the flux through the sphingolipid synthesis pathway is not increased. In this scenario, the regulation of SPT by Orm proteins would have a more local effect in the sphingolipid synthesis metabolic network, and other regulatory mechanisms could partially compensate for its absence.

How and where are sphingolipids perceived and how is the information on their relative abundance relayed to Orm proteins? At the moment these are open questions. Previously, two kinase modules were implicated in sphingolipid regulation. Conditional alleles of the Target of Rapamycin kinase complex 2 (TORC2) have a defect in ceramide synthesis (4). In addition, Pkh kinases are regulated by sphingolipids (5–7). These two signaling pathways converge on the Ypk kinase level of the signaling network (8–10). Both TORC2 and Pkh kinases localize to the plasma membrane, where the bulk of sphingolipids is located (6, 8, 11). For Pkh kinases, an intriguing mechanism of regulation by sphingolipids based on localization of an inhibitory plasma membrane protein, Nce102, was recently suggested (6). In this model, Pkh kinases are localized to only one distinct domain of the plasma membrane, where they are inhibited by colocalizing Nce102. Upon lowering complex sphingolipid levels, Nce102 moves out of this domain and relieves the repression of Pkh kinases. This might explain how changes in the levels of complex sphingolipids might be detected by the cell. In addition, long chain sphingoid bases have been reported to directly activate kinases of this network, possibly leading to integration of information on several sphingolipid metabolism intermediates (5, 12).

Activation of the Pkh/Ypk pathway by an increase of complex sphingolipids was recently shown in a PNAS paper by Roelants et al. to down-regulate another downstream kinase (Fpr1) that in turn phosphorylates and regulates a phospholipid flippase (7). This or a similar pathway would be an attractive candidate to phosphorylate Orm proteins, thereby activating sphingolipid synthesis.

To balance the relative levels of different lipid classes, sphingolipid synthesis regulation is coordinated with the abundance of other lipids. For example, the Fpr1-dependent regulation of bilayer asymmetry of some glycerophospholipids [phosphatidylethanolamine (PE) and phosphatidylserine (PS)] by the flippases might coordinate levels of these lipids in the outer plasma membrane leaflet with the amounts of sphingolipids, thought to be mainly present there. Numerous reports have also shown coordination between the regulation of sterols and sphingolipids, but the molecular mechanism of how this is achieved is still unclear (e.g., ref. 13).

Interestingly, the complex containing SPT and the Orm proteins also contained Sac1, a phosphoinositide phosphatase, as a member of the newly defined SPT, Orm1/2, Tsc3, and Sac1 (SPOTS) complex (1, 14). Cross-regulation of sphingolipid and phosphoinositide levels was already known. In part it is thought to be mediated by plasma membrane signaling proteins binding phosphoinositides (e.g., ref. 14). However, Sac1 was also implicated in sphingolipid regulation and proposed to exert its effect by generating substrates for complex sphingolipid synthesis (15). The new data now suggest that Sac1 might play a much more direct function in sphingolipid regulation and its coordination with phosphoinositide levels.

Is the regulation of sphingolipid levels restricted to simple eukaryotes? As such organisms have to deal with large temperature shifts that affect membrane fluidity, which is also a function of sphingolipid concentration, it is possibly more pronounced in them. However, salient features of the Torc2/Pkh/Ypk-kinase network are conserved through evolution. Similarly, the Orm proteins and other major subunits of the SPOTS complex are evolutionarily conserved and associate in human cells (1). This raises the intriguing possibility that sphingolipid levels are also regulated homeostatically by Orm proteins in higher eukaryotic cells. Particularly interesting in this context is that a human Orm protein (*ORMDL3*) was recently identified as a risk factor for childhood asthma (16). Some of the disease-associated polymorphisms were shown to increase its expression level and thereby are predicted to inhibit sphingolipid synthesis. In addition, single-nucleotide polymorphisms close to *ORMDL3* are associated with a variety of other diseases ranging from Crohn's disease to type I diabetes (17, 18). The discovery of how cells keep their sphingolipid levels normal might therefore not only lead to unique insights into this fascinating topic, but also offer unique therapeutic avenues for treatment of these pathologies.

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