

**Figure 1** A model for the regulation of foreign DNA in bacteria. (a) Prior to horizontal gene transfer, H-NS proteins (green ovals) interact with curved regions of genomic DNA to repress gene transcription. (b) Once incorporated in the genome, foreign DNA (red), having relatively low G-C content and therefore also having regions of curvature, attracts H-NS, leading to repression of transcription. H-NS repression neutralizes the possible harmful effects of unregulated foreign gene expression on cell viability. (c) Antisilencing agents, such as competitive DNA-binding proteins (blue diamond), derepress H-NS-regulated transcription, resulting in the selective expression of genes (red arrow) that provide a fitness advantage to the cell.

with virulence regulators<sup>3</sup>. Because DNA methylation alters transcriptional repression of H-NS<sup>12</sup>, another possibility is that DNA methylation acts as an antisilencing event. Altogether, H-NS and antisilencing events may cooperate to selectively regulate expression of foreign genes to maximize bacterial fitness. Exploring the cellular events that result in selective derepression of beneficial foreign genes will be an exciting area of future work.

Considering the threat of antibiotic resistance<sup>1</sup>, it is important to discuss the ways an understanding of bacterial evolution might provide new strategies for limiting the spread of antibiotic resistance. H-NS attenuates both the conjugal transfer of DNA harboring

multidrug-resistant genes<sup>8</sup> and the expression of multidrug efflux transporters<sup>13</sup>, which suggests that antisilencing mechanisms could facilitate antibiotic resistance. Therefore, drugs targeting the antisilencers of H-NS might counteract the spread and maintenance of antibiotic resistance. Alternatively, because *hns* mutant strains of *S. typhimurium* are non-viable<sup>3</sup>, another interesting possibility is the development of new antibiotics that inhibit H-NS activity. Determining the proteins involved in bacterial fitness may reveal unanticipated drug targets and open the door to future drug development.

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## Greasing the gears of potassium channels

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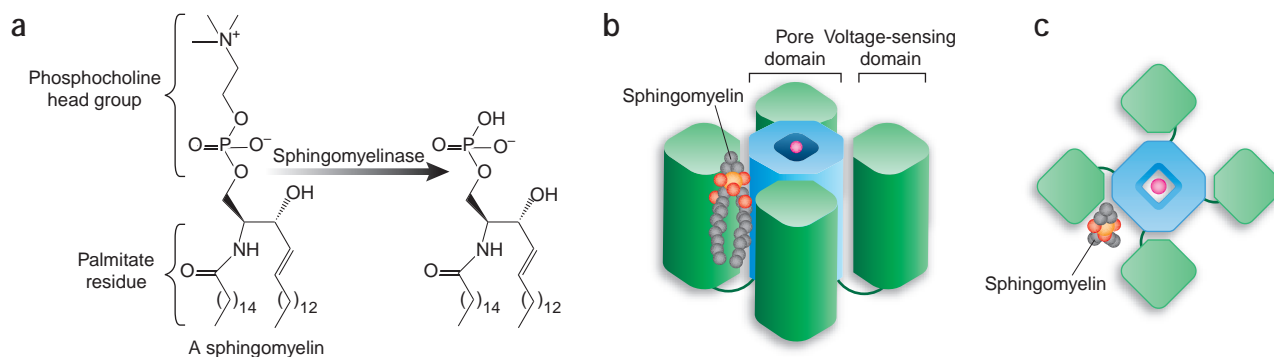
Enzymatic conversion of sphingomyelin to ceramide-1-phosphate in the external leaflet of the cellular membrane has now been shown to markedly facilitate opening of classical voltage-activated potassium channels. This discovery raises the possibility that lipids may have more prominent roles in the gating mechanism of these important ion channels than was previously appreciated.

Signaling across cell membranes has attracted the interest of scientists for generations. At some times the lipid components of the membrane have been the center of attention, whereas at

others the proteins molecules embedded in the lipid have generated the most excitement. Today there is a growing appreciation for the importance of the interface where membrane proteins and surrounding lipid molecules meet. The phosphoinositide family of lipids within the intracellular leaflet of the bilayer are a good example of lipids that interact with membrane proteins and have prominent roles in signaling mechanisms throughout biology<sup>1</sup>. There are increasing reports of relatively abundant lipids that copurify with membrane proteins, are resolved in their

crystal structures and appear to interact specifically with their protein partners<sup>2,3</sup>, as observed with the KcsA potassium channel<sup>4</sup>. The anionic phosphatidylglycerol molecules bound to KcsA seem to be required for channel function<sup>4,5</sup>, reminiscent of the cardiolipin requirement<sup>4,5</sup> of many proteins involved in cellular bioenergetics<sup>2,3</sup>. In a recent report by Ramu *et al.*<sup>6</sup>, the importance of specific lipid-protein interactions is highlighted by the discovery that two classical voltage-activated potassium (Kv) channels are exquisitely sensitive to extracellular sphingomyelinase,

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**Figure 1** Conversion of sphingomyelin to ceramide-1-phosphate and interaction of sphingomyelin with Kv channels. **(a)** Removal of the choline portion of the lipid headgroup by sphingomyelinase. **(b,c)** Side view (within the membrane) **(b)** and extracellular view **(c)** of an illustration of a tetrameric Kv channel, showing the hypothetical positioning of a sphingomyelin molecule at the interface between voltage-sensing and pore domains. Each voltage-sensing domain is comprised of the S1–S4 helices from a single subunit, and the central pore domain is comprised of the S5–S6 segment from four subunits. The pink sphere is a potassium ion bound within the selectivity filter, marking the location of the ion-permeation pathway.

an enzyme that cleaves the choline headgroup from sphingomyelins in the outer leaflet of the membrane.

The discovery by Ramu *et al.* has a rather unusual and interesting origin, having little to do with the interaction of lipids and ion channels. The authors were screening venom from poisonous creatures in search of small protein toxins that might bind to the Kv2.1 voltage-activated potassium channel. Although this type of work has been going on for a very long time, predating the discovery of  $\alpha$ -bungarotoxin for acetylcholine receptor channels over 30 years ago<sup>7</sup>, each venom contains hundreds of distinct protein toxins and it is clear that we have only begun to discover the interesting tools they harbor. The Ramu *et al.* team was chasing down an intriguing activity in the venom of the brown recluse spider, *Loxosceles reclusae*. Spider venom, like that of other venomous creatures, is loaded with small protein toxins, most of which act as ion-channel inhibitors. This venom caught the researchers' attention, however, because adding a component of the venom to the extracellular solution caused the Kv2.1 channel to open at negative membrane voltages where the channel would normally be closed. When they examined the voltage dependence of channel opening over a wide range of voltages, it became apparent that the channel remains fully voltage activated, but that a venom component shifts activation of the channel to more negative voltages by about 30 mV.

At this point one might imagine that Ramu *et al.* had found an 'opener' of the Kv2.1 channel, perhaps akin to the tarantula toxins that bind to the voltage sensors in these channels<sup>8</sup>. The voltage-sensor toxins described thus far work by stabilizing closed states of Kv channels, but it is reasonable to imagine that some varieties might stabilize the open state, as has

been found for some sodium channel toxins<sup>9</sup>. Instead, the team determined that the active fraction contains sphingomyelinase D (SMase D), a previously described lipase that removes the choline portion of the polar head of sphingomyelin, leaving behind the anionic ceramide-1-phosphate (Fig. 1a). That SMase D is the Kv channel activating activity in *Loxosceles* venom was confirmed by demonstrating similar activity with recombinant SMase D and by showing that the activity was dependent on  $Mg^{2+}$ , a known cofactor of SMase D. Mutation of the two histidine residues that are crucial for lipase activity also ablates the Kv channel-activating effects of SMase D, suggesting that the effects of SMase D require lipase activity. Similar activity is observed against the Shaker Kv channel, and in that case Ramu *et al.* were able to also show that SMase D has a pronounced effect on movement of the voltage sensors. Although the mechanism by which SMase D alters the gating of Kv channels remains to be further explored, this discovery clearly shows that altering sphingomyelin has a dramatic effect on the gating behavior of these important ion channels. The authors favor the possibility that sphingomyelin interacts in a relatively specific fashion with some Kv channels, in part because SMase D shifts the voltage-activation relation of Kv channels to variable extents, ranging from about 30 mV for Kv2.1 and 10 mV for the Shaker Kv channel, to undetectable alterations for EAG Kv channels and Slo Kv channels.

The discovery by Ramu *et al.* has two important implications that are likely to energize the study of lipid-protein interactions within the ion channel community. The first is that seemingly conventional voltage-activated ion channels can be robustly activated by something other than a change in membrane voltage. Voltage-activated channels are expressed in an array of non-excitable eukaryotic cells and in

prokaryotes, where changes in the membrane lipids (instead of the voltage) might actually be the primary stimulus that opens and closes the ion-permeation pathway. The second is that lipids may interact intimately with the gating machinery in voltage-activated ion channels, perhaps even interacting with the crucial S4 arginine residues in the apparent space between voltage-sensing and pore domains reported in the X-ray structures of the KvAP and Kv1.2 potassium channels<sup>10,11</sup> (Fig. 1b,c). If lipid-channel interactions are a fundamental part of the gating mechanism in voltage-activated channels, it will be important to understand why some channels are insensitive to SMase D. Perhaps there are structural barriers that prevent access of the enzyme to the bound sphingomyelin, or possibly lipids other than sphingomyelin have a similar role. It is also possible that the SMaseD-insensitive channels hold on to their sphingomyelin tightly and thus are less likely to see the modified version of the lipid in the surrounding membrane. At this point the possibilities abound. There is clearly much to learn about the greasy molecules that may be present in the gears of this important family of membrane proteins.

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