A sensitive channel family replete with sense and motion

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Two new studies demonstrate that transmembrane voltage-sensing domains can act without an obvious pore-forming domain to sense membrane potential changes and make a proton-selective pore.

The modular nature of proteins is astonishing, despite the fact that there are countless examples known in soluble proteins[1]. Whether membrane proteins exhibit such modularity has been unclear. Two recent studies describe the identification and characterization of a protein family that resembles the voltage-sensor domains (VSDs) from voltage-gated ion channels but lacks a classical pore-forming domain[2,3]. We are accustomed to seeing VSDs as parts of ion-channel proteins[4]. A lone VSD is remarkable enough, but it seems that this new family of proteins (termed H_v1 (ref. 2) or voltage sensor domain only proteins, VSOPs[3]), found from tunicates to humans, can do double duty as both transmembrane voltage sensors and proton-selective pores.

For some time, scientists have been looking for the protein responsible for the voltage-sensitive proton conductance, symbolized G_{vH+}, found in phagocytes, neurons and other cell types[5]. In phagocytes, activity is linked to a phenomenon known as the 'respiratory burst', in which the electrogenic action of the transmembrane NADPH oxidase complex consumes NADPH and oxygen to generate extracellular superoxide. The electron-rich superoxide is the precursor to reactive oxygen species that have crucial roles in immune defenses that allow phagocytes to kill invaders. Without the charge compensation provided by the G_{vH+}, transport of electrons from NADPH to extracellular superoxide would rapidly depolarize the membrane.

Now, Ramsey *et al.*[2] and Sasaki *et al.*[3] have identified a family of proteins that seem to have the properties expected for G_{vH+}. Both groups used a bioinformatics approach to mine sequence databases for proteins with voltage-sensor features. Some of the work is based on the recent identification of an unusual protein from the tunicate *Ciona intestinalis*, in which there is a VSD that is part of a membrane tethered phosphoinositide phosphatase (Ci-VSP)[6]. Why is that an unusual place to start? Before Ci-VSP, VSDs were only known to be covalent parts of voltage sensitive ion-channel proteins[4]. Ion-channel VSDs are well-studied protein domains that comprise four membrane spanning regions, S1–S4 (**Fig. 1a**). S4 carries much of the VSD voltage-sensing capacity in a set of cationic side chain repeats (**Fig. 1b**). In the case of ion channels, two more transmembrane segments follow the VSD and comprise the architectural unit that makes the ion-channel pore. There are many examples of ion channels that contain a pore domain but no VSD[4]. The Ci-VSP phosphatase was the first example in which an isolated VSD was shown to sense voltage and regulate activity of something besides a channel pore. Biophysical experiments on H_v1 proteins in the two new reports now suggest that VSDs alone can not only act as voltage sensors, but also make proton selective pores.

Nature conceals many secrets that the scientific process often reveals only in portions at a time. As in the well-known parable of the blind men and the elephant, sometimes it takes a while before we are able to realize that the tail and the trunk are part of the same beast. A number of seemingly disparate and unusual observations now converge in a way that seems germane for understanding VSD properties and their relationship to the newly discovered H_v1 proton-channel proteins.

The first hint that VSDs might be able to act alone came from a provocative observation made in the course of solving the structure of the voltage-gated potassium channel K_vAP from the hyperthermophilic archaeon *Aeropyrum pernix*. In addition to a structure of the complete channel, the MacKinnon laboratory determined a high-resolution structure of the isolated K_vAP VSD (S1–S4) bound to an antibody Fab (**Fig. 2a**). Along with this crystallographic evidence demonstrating that the K_vAP VSD could fold autonomously, biochemical experiments showed that the isolated K_vAP VSD structure was likely to be native-like, because affinity columns conjugated with the K_vAP VSD were able to bind specific peptide toxins from crude spider venom that were active on the full-length channel[7]. These observations started to change the way researchers were looking at VSDs and raised the possibility that VSDs could exist as separate entities that were not completely dependent on a pore domain for folding. The recent crystal structure of the mammalian voltage-gated potassium channel $K_v1.2$ has provided further evidence for VSD modularity. In $K_v1.2$, the S1–S4 VSD appears to make very few surface contacts with the

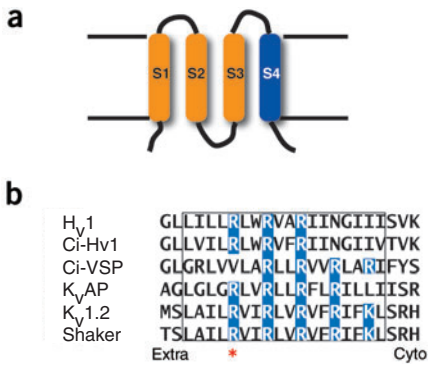

Figure 1 Voltage-sensor topology and sequences. (**a**) VSD topology. Orange, transmembrane segments S1–S3; blue, primary VSD, S4. (**b**) Comparison of S4 segments from VSD proteins. H_v1, human; Ci-H_v1, *Ciona*; Ci-VSP, Ciona voltage-sensitive phophatase; K_vAP, *A. pernix* voltage-gated potassium channel; $K_v1.2$, rat voltage-gated potassium channel; Shaker, *Drosophila* voltage-gated potassium channel. Extracellular (Extra) and intracellular (Cyto) ends of S4 are indicated. Box outlines region of highest similarity. Asterisk indicates the position of the Shaker mutations that reveal the omega pore.

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central pore-forming domain[8] (**Fig. 2b**). Taking these structural data together with the functional evidence that the Ci-VSP VSD can actually act in the absence of a channel-pore domain[6], as well as the new data on H_v1 proteins[2,3], it now seems that VSDs are modular protein domains in the fullest sense.

The other line of research that connects VSD properties in ion channels with those in H_v1 proteins is the work showing that potassium-channel VSDs can be coaxed into acting like pores. The Bezanilla[9] and Isacoff[10] groups have shown that mutation of the most extracellular arginine in the VSD of the voltage-gated potassium channel Shaker can open a conduction pathway that is active when the sensors are in the hyperpolarized state. The S4 mutation R362H opens a proton-selective hole. Other substitutions at this position allow cations as large as guanidinium ions through. This hole, termed the 'omega pore'[10], is not the same hole used for passage of permeant ions and seems to have a fundamental role in facilitating the motion of the VSD relative to the pore-forming domain (**Fig. 2c**). In particular, it is proposed that the omega pore is the usual route for movement of the S4 arginine side chains as they detect and respond to changes in transmembrane voltage[10].

The two new papers on H_v1 provide a different lens with which to view VSDs. H_v1 VSDs alone not only act in the expected way as voltage sensors but can also form a proton-conducting channel. Thus, neither of the VSD properties that have been worked out in channels seems to require association with a pore domain. The fact that H_v1 proteins are present from a member of the earliest branch of chordates, *Ciona*, through humans suggests that the original observation that an archaeal VSD can fold on its own is not a quirk of a hyperthermophilic protein. Rather, it may be a deeply rooted property of this functional unit that has been exploited by nature to many ends. We are used to seeing modular domain organization in soluble proteins[1]. The fact that membrane proteins are modular too should not be surprising. Nevertheless, the convergent work on VSDs now provides a concrete example of how membrane-embedded modules can be mixed and matched with other components to make proteins with new properties (**Fig. 2**). Undoubtedly, as structural studies of membrane proteins advance, we are likely to see more examples of this sort of modularity.

The two new papers leave many fundamental questions unanswered that will ignite vigorous research efforts. Do H_v1 proton channels function as monomers or multimeric complexes? Do H_v1 proteins act alone? Mutations in H_v1 S4 show

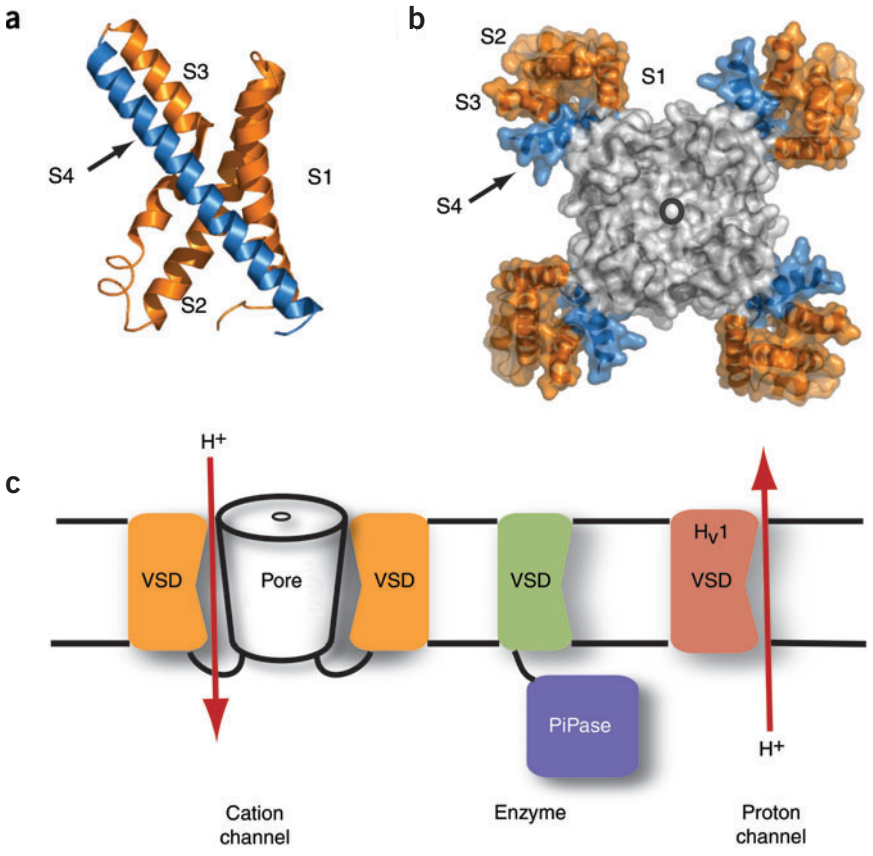


Figure 2 Structures of channel VSDs. (**a**) Ribbon diagram of the isolated KvAP VSD (PDB entry 1ORS). S1–S4 are indicated and colored as in **Figure 1**. Fab is not shown. (**b**) Surface representation of $K_v1.2$ (PDB entry 2A79) viewed from the extracellular side of the membrane. Pore-forming domain is colored white. Black circle indicates ion-conduction pore. (**c**) Modular nature of VSD membrane proteins. Left, schematic of a voltage-sensitive cation channel. Pore and VSDs are indicated. Two of the four VSDs are not shown. Putative path of omega-pore protons is shown. Center, schematic of the voltage-sensitive phosphoinositide phosphatase. PiPase denotes phosphatase domain. Right, H_v1. Potential proton path is indicated.

that it is indeed part of the voltage-sensing mechanism[2,3]; however, this result cannot formally exclude the possibility that H_v1 pore formation requires some other protein component that is supplied endogenously by the test cells. Is there some other partner subunit? Where exactly is the pore and what is the mechanism by which proton passage across the membrane is facilitated? In contrast to the potassium-channel omega pore, which it is active when the membrane is hyperpolarized, H_v1 currents are active when membranes are depolarized. Although the standard textbook view of the membrane is that it contains a monolithic slab of low dielectric through which is difficult for charged species to pass, experimental[11] and computational studies[12–14] suggest that moving charges across the bilayer may not be as difficult as it might appear at first glance. Recent work suggests that proteins may exploit the varied functionality of lipid headgroups[12] and lipid flexibility to distort the membrane so that the distance from one side to the other is not that far and the energetic cost not insurmountable. Moreover, effects of water networks[13] and bilayer deformations[14] may also be important. Do H_v1 channels have a catalytic machinery to exploit these mechanisms? Do the omega-pore function of channel VSDs and the proton-pore function of H_v1s use the same type of mechanism? If some VSDs can really be isolated actors, can we harness their potential to endow voltage-sensing properties to proteins that are not normally voltage sensitive?

Finally, one must keep in mind that protein activity can go awry as a result of mutations. Defects in other components of the respiratory-burst pathway cause immune deficits[15]. Are there H_v1 channel defects that are also associated with human diseases? There is no question that this new family of proteins will stir

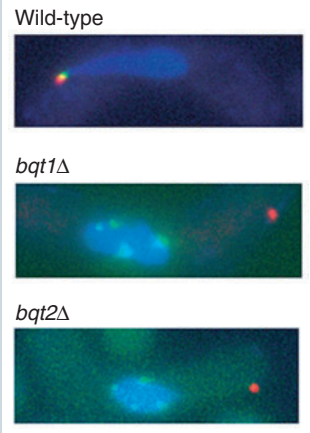
excitement about all of these questions and open a new era in the study of voltage-gated channels. Stay tuned to this new family channel.

1. Petsko, G.A. & Ringe, D. *Protein Structure and Function* (New Science Press Ltd. London, 2004).
2. Ramsey, I.S., Moran, M.M., Chong, J.A. & Clapham, D.E. *Nature*, advance online publication 22 March 2006 (doi:10.1038/nature04700).
3. Sasaki, M., Takagi, M. & Okamura, Y. *Science*, published online 23 March 2006 (doi:10.1126/science.1122352).
4. Hille, B. *Ion Channels of Excitable Membranes* (Sinauer Associates, Inc., Sunderland, Massachusetts, USA, 2001).
5. Decoursey, T.E. *Physiol. Rev.* **83**, 475–579 (2003).
6. Murata, Y., Iwasaki, H., Sasaki, M., Inaba, K. & Okamura, Y. *Nature* **435**, 1239–1243 (2005).
7. Jiang, Y. *et al. Nature* **423**, 33–41 (2003).
8. Long, S.B., Campbell, E.B. & Mackinnon, R. *Science* **309**, 903–908 (2005).
9. Starace, D.M. & Bezanilla, F. *Nature* **427**, 548–553 (2004).
10. Tombola, F., Pathak, M.M. & Isacoff, E.Y. *Neuron* **45**, 379–388 (2005).
11. Hessa, T., White, S.H. & von Heijne, G. *Science* **307**, 1427 (2005).
12. Freites, J.A., Tobias, D.J., von Heijne, G. & White, S.H. *Proc. Natl. Acad. Sci. USA* **102**, 15059–15064 (2005).
13. Tepper, H.L. & Voth, G.A. *Biophys. J.* **88**, 3095–3108 (2005).
14. Wilson, M.A. & Pohorille, A. *J. Am. Chem. Soc.* **118**, 6580–6587 (1996).
15. Babior, B.M. *Curr. Opin. Immunol.* **16**, 42–47 (2004).

Gathering bouquets

Chromosome segregation involves pairing and recombination of homologous chromosomes. During the meiotic cell cycle, a single round of replication is followed by two consecutive nuclear divisions. Both events require major chromosomal movements, and missegregation during meiosis can result in developmental defects. During meiotic prophase, telomeres, which are usually spread along the nuclear envelope during the mitotic cycle, cluster together at the nuclear envelope. This telomere grouping results in a 'bouquet' arrangement of chromosomes that is characteristic in many organisms, suggesting a conserved role for telomeres in meiosis.

Schizosaccharomyces pombe is an amenable system for studying bouquet formation. During normal mitosis in *S. pombe*, the centromeres are associated with the spindle pole body (SPB). However, during meiosis, the chromosomes instead become tethered to the SPB through the telomeres. This telomere-SPB association has been shown to facilitate homologous chromosome pairing and recombination and is required for formation of the meiotic bouquet.



There are several proteins in both the telomeres and SPB known to be important in bouquet formation. Two of them, Rap1 (human RAP1) and Taz1 (human TRF1 and TRF2), are found constitutively associated with telomeres in *S. pombe*. Taz1 binds directly to the telomeric repeats and is required for Rap1 telomere binding. Although these proteins are non-essential for mitotic growth, *rap1* or *taz1* deletion strains have defects in meiotic telomere clustering. Sad1 is an SPB component crucial for mitotic growth, whereas Kms1 is an SPB component important in meiosis. Cells lacking Kms1 show defects in telomeric clustering: rather than forming a single spot at the SPB, Sad1 forms several distinct foci at the nuclear envelope, to which the telomeres localize.

Despite what is known about the importance of SPB and telomere components during meiosis, there is little information about what brings these complexes together in preparation for chromosome segregation. Now, Hiraoka and colleagues (*Cell* **125**, 59–69, 2006) have identified two novel proteins that are required to physically link the telomeres to the SPB during bouquet formation. Because the mating pheromone response induces meiotic telomere clustering in *S. pombe*, the authors looked for genes whose expression was induced in response to mating-pheromone signaling and systematically disrupted each of these genes. One strain showed loss of telomere clustering, and they named that gene *bqt1* (for bouquet formation). Disruption of a second gene, *bqt2*, identified by a two-hybrid screen using Bqt1 as bait, resulted in loss of bouquet formation during meiotic prophase. Although the authors found no obvious homologs for Bqt1 or Bqt2, Bqt1 shares weak sequence similarity with Dam1, a fungal centromere protein found associated with the kinetochore during mitosis.

Characterization of GFP-tagged Bqt1 and Bqt2 showed that both proteins colocalized with Taz1 and Sad1 at the telomere-SPB cluster during meiosis. Deletion of *bqt2* resulted in dispersed telomeres (green), with Bqt1 colocalized with Sad1 (red) at the SPB. In the absence of Bqt1, Bqt2 had diffuse nuclear localization and the telomeres were not localized at the SPB. Additional experiments defined interactions between telomere and SPB components, showing that Bqt1 binds directly to Sad1, whereas Bqt2 can only bind Sad1 when Bqt1 is present. Both Bqt1 and Bqt2 are required to interact with Rap1. Artificial expression of Bqt1 and Bqt2 in mitotic cells showed that both can recruit Sad1 to the telomeres, confirming that these two proteins are sufficient to bridge SPB and telomere components. Time-lapse imaging of meiotic cells shows that Sad1, initially localized at the SPB, transiently disperses to Rap1-bound telomeres and then returns to the SPB.

The authors propose a model for bouquet formation in which Bqt1 and Bqt2 recruit Sad1 to Rap1 at the telomeres along the nuclear envelope. Sad1 then brings the complex back to the SPB, where efficient chromosome pairing and recombination can occur. The authors suggest that Kms1, which has been shown to interact with the cytoskeletal motor protein dynein, may be responsible for bringing Sad1, and the attached telomeres, back to the SPB. This role would explain the Sad1 foci observed in Kms1-deficient cells. These studies provide new insight into how the cell performs the dramatic chromosomal movements associated with meiosis.

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