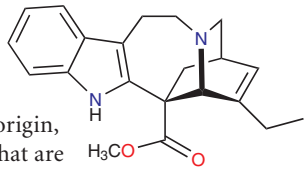
MINIMALIST PROTON CHANNEL

Voltage-gated ion channels open in response to changes in the electrical potential across a cell membrane. Prototypical voltage-gated channels contain six transmembrane segments. The first four segments form the voltage-sensing domain (VSD), which uses positively charged residues to sense voltage. The VSD then opens a pore encoded by the fifth and sixth segments of the channel. Physiological properties of voltage-gated proton channels have been widely described, including a key involvement in the oxidative burst of phagocytes. However, the protein encoding this channel has never been identified. This elusive channel has now been characterized in recent papers from Clapham and co-workers in *Nature* and from Okamura and colleagues in *Science*. Surprisingly, the channel is composed solely of a VSD that by itself functions as a proton-selective channel. Channel gating, analogously to that of known VSDs, requires voltage-sensing arginine residues in the fourth segment of the domain. Consistent with the known properties of voltage-gated proton channels, zinc can inhibit the channel. Clapham and co-workers further showed that mutation of two histidines was sufficient to prevent zinc sensitivity, suggesting that these residues are involved in chelating the metal ion. Future studies can now explore the molecular basis for VSDs functioning as proton-selective channels and the physiological role of these channels in phagocytosis and other cellular processes. (*Nature,* published online 22 March 2006, doi:10.1038/nature04700; *Science*, published online 23 March 2006, doi:10.1126/science.1122352) *JK*

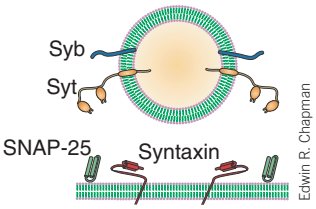
A web of genes and metabolites



Plants are an important source of medicinal compounds. For example, the Madagascar periwinkle (*Catharanthus roseus*) produces several alkaloids of mixed biosynthetic origin, such as vincristine and vinblastine, that are being used clinically for cancer treatment. Although aspects of the biosynthesis of these terpenoid indole alkaloids (TIAs) are known, the intermediates and enzymes involved in TIA biosynthesis remain incompletely characterized owing to the lack of genetic-sequence and metabolic-profiling data for many medicinal plants. Using LC-MS to detect TIA metabolites, Van Montagu, Oksman-Caldentey and colleagues showed that two plant hormones, methyl jasmonate and auxin, had differential effects on the concentrations of key TIA biosynthetic intermediates in *C. roseus*. Combining these results with transcriptional profiling for the same samples, the authors found that genes coding for enzymes or transcription factors that regulate TIA metabolism were similarly affected by hormone treatment. The profiling data were used to construct gene-gene and gene-metabolite networks that validated known aspects of TIA biosynthesis but also revealed several cytochrome P450 enzyme and transcription factor candidates that may be involved in transformations or genetic regulation of TIA metabolism. The current study suggests that selective engineering of pathway components may allow the production of new metabolites and the optimization of metabolite expression for pharmaceutical applications. (*Proc. Natl. Acad. Sci. USA* **103**, 5614–5619, 2006) *TLS*

Research Highlights written by Mirella Bucci, Joanne Kotz and Terry L. Sheppard.

Better fusion through calcium



During neuronal transmission, Ca^{2+} stimulates the exocytosis of synaptic vesicles. Complexes between SNARE proteins modulate the specificity and directionality of this process. Exocytic vesicles marked with the v-SNARE protein synaptobrevin (syb) fuse with target membranes via the t-SNAREs syntaxin and SNAP-25. The Ca^{2+}-sensing protein syntaptotagmin I (syt) binds both t-SNAREs and the anionic phospholipid PS in interactions that are thought to be critical for SNARE-complex assembly and membrane fusion. Chapman and colleagues used an *in vitro* assay that reconstitutes Ca^{2+}-regulated membrane fusion to show that syt interacts specifically with neuronal t-SNARE heterodimers in response to Ca^{2+}, a result consistent with *in vivo* observations. Ca^{2+}-bound syt (Ca^{2+}-syt) caused the aggregation of v-SNARE and t-SNARE vesicles that were mixed together and could induce full fusion of both the inner and outer membrane leaflets of vesicles containing neuronal SNAREs. The vesicle aggregation is mostly dependent on Ca^{2+}-syt interactions with PS rather than with t-SNAREs, suggesting that the PS interaction leads to aggregation whereas the SNARE interaction actually causes fusion. The authors also found that Ca^{2+}-syt acts directly to stimulate the assembly of SNAP-25 and syntaxin into functional SNARE complexes. Therefore, syt aligns SNAP-25 and syntaxin, allowing for subsequent syb binding. Separate experiments showed that the complexes are bona-fide SNARE complexes that have been stably folded together through the action of Ca^{2+}-syt. These results lead to a model where the binding of Ca^{2+}-syt-PS complexes modifies the structure of t-SNAREs so that they fold into heterodimers. (*Nat. Struct. Mol. Biol.* published online 26 March 2006 doi:10.1038/nsmb1076) *MB*

Sensing hydrogen peroxide

Hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS) play important roles in cellular signaling and in pathologies that result from oxidative stress. Detecting intracellular H_2O_2 is critical for understanding the biological roles of this molecule. Currently, dichlorofluorescein (DCF) derivatives are commonly used for detecting ROS. However, DCF is sensitive to multiple reactive nitrogen as well as oxygen species, and its intracellular localization cannot be controlled. Lukyanov and colleagues have generated a H_2O_2 sensor called 'HyPer' by fusing a fluorescent protein to the regulatory domain of OxyR, a protein that regulates transcription in response to H_2O_2 in *Escherichia coli.* OxyR is composed of a regulatory domain, which senses H_2O_2 through the oxidation of a cysteine residue, and a DNA-binding domain. HyPer generated a fluorescent signal in response to nanomolar concentrations of H_2O_2 but did not respond to other reactive oxygen or nitrogen species. This sensitivity permitted the detection of low concentrations of H_2O_2 generated during cellular stimulation with nerve growth factor. Using cytosol-localized HyPer, the authors found elevated H_2O_2 in response to Apo2L/TRAIL-induced apoptosis, which occurred downstream of caspase activation and on a timescale similar to that of the loss of mitochondrial membrane potential. Expression of Bcl-2, a regulator of apoptosis, prevented the increase in cytosolic H_2O_2. Using a mitochondrial-localized HyPer, the authors observed parallel oscillations of H_2O_2 concentration and mitochondrial transmembrane potential. These cellular studies highlight the potential of this H_2O_2-specific, genetically encoded sensor. (*Nat. Methods* 3, 281–286, 2006) *JK*