Proteins related to animal transient receptor potential channels (TRPCs) and to voltage-gated calcium channels (VGCCs) are evident in the genomes of fungi, the group of eukaryotes closest to animals. Recent studies in budding yeast suggest that these homologs indeed function as Ca\(^{2+}\)-permeable ion channels, but their modes of regulation appear to be surprisingly different from those in animals. In yeast, the sole VGCC homolog in the plasma membrane appears to respond to the status of intracellular Ca\(^{2+}\) stores, a phenomenon frequently associated with TRPC in animal cells. The sole TRPC homolog in yeast resides in the vacuole, a lysosomelike organelle that can release Ca\(^{2+}\) in response to hyperosmotic stimuli and perhaps even voltage changes. This unusual arrangement may be typical of all fungi and possibly even the common ancestor of fungi and animals. Understanding the logic of fungal Ca\(^{2+}\) channels, therefore, may provide new insights into the organization and regulation of cellular calcium signaling networks in animals.

TRPC channels were first identified in photoreceptor cells of *Drosophila melanogaster* as critical components of the phototransduction mechanism and subsequently characterized in species ranging from nematodes to mammals (1). They typically contain a core domain of six transmembrane segments that form active ion channels after assembly into homo- or heterotetramers. Electrophysiological characterization showed that the ion selectivity and permeation rates of the channels tend to vary somewhat as a function of the specific composition of the subunits. Regulatory molecules that affect TRPC gating range from nucleotides to lipid products to proteins related to animal transient receptor potential channels (TRPCs) and to voltage-gated calcium channels (VGCCs). The specific composition of the subunits, however, exhibits little to changes in transmembrane potential and typically lack the charged amino acid residues known from VGCCs to respond to such signals (2).

The TRPC homolog in the budding yeast *Saccharomyces cerevisiae*, termed Yvc1, is localized to the limiting membrane of the vacuole, a large storage organelle that resembles animal lysosomes (3, 4). Electrophysiological studies of purified vacuole membranes revealed that Yvc1 is absolutely required for the appearance of a large-conductance inwardly rectifying cation channel that is selectively permeable to Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\), but not Cl\(^{-}\) (3, 5, 6). The vacuole contains these cations in abundance because of the action of the electrogenic H\(^{+}\) pump Vma and secondary transporters, such as the H\(^{+}\)-Na\(^{+}\) exchanger Nhx1 (7), the H\(^{+}\)-Ca\(^{2+}\) exchanger Vcx1 (8, 9), and the voltage-gated Cl\(^{-}\) channel Gef1 (10). Ca\(^{2+}\) is also sequestered in the vacuole by Pmc1 (11), a homolog of the plasma membrane Ca\(^{2+}\)-pumps of animal cells. Yvc1 channel opening was stimulated by micromolar concentrations of Ca\(^{2+}\) on the cytoplasmic face (5, 6). Additionally, Yvc1 channel opening probability peaked near -80 mV and decreased at both higher and lower potentials, suggesting that its activity may be regulated by voltage changes. From these findings, one might infer that Yvc1 functions in yeast as a cation release channel that can tap into a pool of ions, perhaps more reliable than the pool of ions available in the extracellular milieu.

How is Yvc1 regulated in vivo? Examination of the Yvc1 amino acid sequence does not reveal a voltage sensor segment or any similarities to other proteins outside of its core membrane domain. However, elegant experiments suggest that Yvc1 becomes activated in living yeast cells within seconds of a hypertonic shock (4) (Fig. 1). Cytoplasmic aequorin was used as a probe for cytosolic free Ca\(^{2+}\) to show that addition of high concentrations of salt or other osmolytes into the culture medium elevated cytosolic free Ca\(^{2+}\) in a fashion that was enhanced by Yvc1 overexpression and abolished by Yvc1 gene knockout. The Yvc1-dependent Ca\(^{2+}\) transient apparently did not require extracellular Ca\(^{2+}\), but did require the function of either Pmc1 or Vcx1. These findings suggest that Yvc1 directly (or perhaps indirectly) triggered Ca\(^{2+}\) release from the vacuole. Yvc1 might also release other cations, such as Na\(^{+}\), K\(^{+}\), or even H\(^{+}\), that would be expected to provide osmolytes to the cytoplasm and temporary relief from dehydration during hypertonic shock. The released Ca\(^{2+}\) might also help to activate calmodulin and calcineurin, which themselves promote resistance to high salt conditions (12, 13). Thus, the Yvc1 ion channel is in a position to play a pivotal role in the response to hypertonic shock. These developments also provide a powerful new experimental system for elucidating the functional domains of Yvc1 and possible regulation by upstream signaling pathways.

The observation of regulated Ca\(^{2+}\) release in yeast begs the question of whether Ca\(^{2+}\) release couples to Ca\(^{2+}\) influx in yeast. In most mammalian cell types, Ca\(^{2+}\) release from the endoplasmic reticulum upon Ca\(^{2+}\) release. However, animal TRPC channels respond little to changes in transmembrane potential and typically lack the charged amino acid residues known from VGCCs to respond to such signals (2).

The regulation of Yvc1 in vivo has garnered significant interest. Examination of the Yvc1 genetic sequence suggests that it is not regulated by upstream signals (2).

**Keywords:** Calcium, Yeast, TRPC, VGCC, Vacuole

**References:**
free Ca$^{2+}$ concentrations, both of which depend on the functions of Cch1. Cells overexpressing Pmc1 or Vcx1 also exhibit high rates of Ca$^{2+}$ influx through Cch1, because of competition with Pmr1 for substrate. A second plasma membrane protein, known as Mid1, is also required for Ca$^{2+}$ influx under these conditions. Mid1 binds Cch1 (15) and is required for Cch1 activity under many conditions in vivo (16-19), suggesting that Mid1 functions as a regulatory subunit of Cch1 analogous to the α2-δ subunits of animal VGCCs (2). These findings are consistent with the existence of a CCE-like mechanism in yeast that couples depletion of secretory Ca$^{2+}$ stores to stimulation of a four-domain VGCC composed of Mid1 and Cch1.

It is too soon to know whether the CCE-like phenomenon in yeast is mechanistically related to CCE in animal cells. The CCE mechanism in animal cells has not been elucidated, and CCE channels have not been conclusively identified. The evidence that VGCCs in animal cells respond to depletion of Ca$^{2+}$ stores is very sparse, but it is difficult to rule out this possibility completely. The closest relatives of Cch1 in animals have been identified in the genomes of nematode, fruit fly, and mammals, but are not yet characterized functionally (20). An unidentified small molecule was proposed as a component of the CCE mechanism in vertebrates, and a functionally similar molecule may accumulate in yeast mutants that lack Pmr1 (21). Another small molecule (glucose 1-phosphate or a related metabolite) stimulated the Ca$^{2+}$ influx activity of Cch1 (22), although its potential role in the response to depletion of secretory Ca$^{2+}$ has not been evaluated. The regulatory mechanism linking intracellular Ca$^{2+}$ stores with Cch1 remains obscure and therefore difficult to compare with the equally obscure process in animal cells. One must now consider the possibility that Yvc1 becomes activated in response to Ca$^{2+}$ depletion in the organelles of the secretory pathway, much like certain TRPCs in animals. These concepts are now experimentally tractable in the yeast system.

Studies of Cch1 regulation have revealed another unexpected twist: Ca$^{2+}$ influx activity of Cch1 can be stimulated by agents that perturb protein, carbohydrate, or lipid biosynthesis in the endoplasmic reticulum (23). Drugs that block N-glycosylation or disulfide bonding of secretory proteins in the endoplasmic reticulum stimulate Cch1 activity, and similar effects were observed in mutants lacking the normal functions of many different molecular chaperones and quality control factors in the endoplasmic reticulum. Cch1 was also strongly activated by drugs that block ergosterol biosynthesis in endoplasmic reticulum (23) and by mutants unable to degrade sphingosine 1-phosphate in the endoplasmic reticulum (24). Thus, diverse insults to the endoplasmic reticulum can increase Cch1 activity. This phenomenon appears to be conserved broadly in fungi, and several species of pathogenic fungi appear to require the Ca$^{2+}$ influx and downstream signaling pathways for resistance to commonly prescribed antifungal drugs (25, 26). Cch1 and downstream effectors, therefore, represent excellent targets for the development of new antifungal drugs.

In yeast, Cch1 responds to stress within the secretory pathway, and perhaps it is this stress response, rather than a CCE-like mechanism, that explains the Ca$^{2+}$ phenotypes of mutants lacking Pmr1 (15) or Spf1, another possible ion pump in the endoplasmic reticulum (27). Similar stress responses may be coupled to Ca$^{2+}$ influx in mammalian cells. For example, disrupting presenilin-1 function in the endoplasmic reticulum of neurons also affects several aspects of Ca$^{2+}$ influx and signaling (28, 29), which might contribute to familial Alzheimer disease. Endoplasmic reticulum dysfunction may contribute to a number of pathological states through effects on Ca$^{2+}$ influx and signaling (30, 31). The possibility that animal and fungal cells use a similar mechanism to trigger Ca$^{2+}$ influx in response to secretory stress warrants further examination.

The genome sequences of about 20 diverse fungal species have been initiated or completed. Orthologs of Cch1 and Yvc1 are already evident in most, if not all, of these fungal species. The incomplete genome of the slime mold Dictyostelium dis-
coideum seems to contain a two-domain VGCC homolog (or possibly two one-domain homologs). Moving to even deeper branches in the tree of life, the plant Arabidopsis thaliana contains a two-domain VGCC homolog (32) similar to the two-domain proteins found in mammals (33), but not fruit fly or nematode. A one-domain VGCC homolog from the bacterium Bacillus halodurans was recently identified and characterized (34). Clearly, the origins of the VGCC and TRPC families are extremely ancient, possibly predating the divergence of prokaryotes and eukaryotes. Mammals have retained a greater diversity of VGCC and TRPC, whereas the more streamlined genomes of fruit fly, nematode, and especially fungi appear to have dispensed with certain subtypes. It is quite likely that ancestral modes of VGCC and TRPC regulation persist today in these species. If Dobzhansky was correct in his statement that “nothing in biology makes sense except in the light of evolution” (35), then our understanding of Ca2+ channel regulation and function in mammalian cells stands to benefit tremendously from studies of the fungal systems.

References