Investigating Potassium Channels in Budding Yeast: A Genetic Sandbox

Timothy D. Mackie and Jeffrey L. Brodsky[1]
Department of Biological Sciences, University of Pittsburgh, Pennsylvania 15260

ABSTRACT Like all species, the model eukaryote *Saccharomyces cerevisiae*, or Bakers' yeast, concentrates potassium in the cytosol as an electrogenic osmolyte and enzyme cofactor. Yeast are capable of robust growth on a wide variety of potassium concentrations, ranging from 10 μM to 2.5 M, due to the presence of a high-affinity potassium uptake system and a battery of cation exchange transporters. Genetic perturbation of either of these systems retards yeast growth on low or high potassium, respectively. However, these potassium-sensitized yeast are a powerful genetic tool, which has been leveraged for diverse studies. Notably, the potassium-sensitive cells can be transformed with plasmids encoding potassium channels from bacteria, plants, or mammals, and subsequent changes in growth rate have been found to correlate with the activity of the introduced potassium channel. Discoveries arising from the use of this assay over the past three decades have increased our understanding of the structure–function relationships of various potassium channels, the mechanisms underlying the regulation of potassium channel function and trafficking, and the chemical basis of potassium channel modulation. In this article, we provide an overview of the major genetic tools used to study potassium channels in *S. cerevisiae*, a survey of seminal studies utilizing these tools, and a prospective for the future use of this elegant genetic approach.

KEYWORDS *S. cerevisiae*; genetics; potassium; ion channel; mutagenesis; synthetic genetic array; Trk1; Trk2

Potassium Channel Classes, Activities, and Structures

POTASSIUM channels are ubiquitous in eukaryotes and prokaryotes, and are the most diverse type of cation channel due to early evolutionary divergence (Loukin *et al.* 2005). The most salient feature of potassium channels is their ability to discriminate K^+ from smaller cations such as Na^+ (MacKinnon 2003). All potassium channels have fourfold symmetry around their central pore (MacKinnon 1991). This is typically accomplished by tetramerization of identical or homologous subunits, although K_{2P} channels are dimers in which two pore-forming domains are fused in tandem. The paradigmatic feature of all potassium channels is the eight-amino acid potassium selectivity motif, TXXTXG(Y/F)G. The alignment of the peptide backbone carbonyls of the selectivity motif mimic the hydration shell of a potassium (K^+) ion, thus favorably dehydrating K^+ ions while excluding ions with smaller van der Waals radii, such as Na^+ or Li^+ (Doyle *et al.* 1998; Roux and MacKinnon 1999).

doi: https://doi.org/10.1534/genetics.118.301026
Manuscript received April 11, 2018; accepted for publication May 15, 2018.
[1]Corresponding author: Department of Biological Sciences, University of Pittsburgh, A320 Langley Hall, Pittsburgh, PA 15260. E-mail: jbrodsky@pitt.edu

Potassium channels are broadly classified based on the number of transmembrane domains (TMD): six-TMD voltage-gated (K_v) channels, four-TMD two-pore channels (K_{2P}), and two-TMD inward-rectifying (K_{ir}) channels (Miller 2000; Enyedi and Czirják 2010).

In voltage-gated channels—also known as *Shaker*-type channels after the first such channel identified in *Drosophila melanogaster* (Tempel *et al.* 1987)—the selectivity filter is situated between TMD5 and TMD6. In addition, TMD4 is distinguished by highly conserved arginine or lysine residues at every third or fourth position along the transmembrane helix, which are essential for sensing transmembrane voltage and channel opening upon membrane depolarization (Figure 1). Calcium-gated potassium channels (K_{Ca}) are also six-TMD-containing channels and have diverged from K_v channels, though some (notably "big current" or "BK" channels) retain voltage sensitivity. As their name implies, they are activated upon binding intracellular Ca^{2+} through various mechanisms (Vergara *et al.* 1998; Ghatta *et al.* 2006).

Multicellular eukaryotes also express tandem pore domain channels, or K_{2P} channels, which appear to be the product of a gene fusion event (Figure 1). K_{2P} channels are responsible for very small "leak" currents that are voltage-insensitive and nonrectifying, yet crucial for maintaining resting membrane

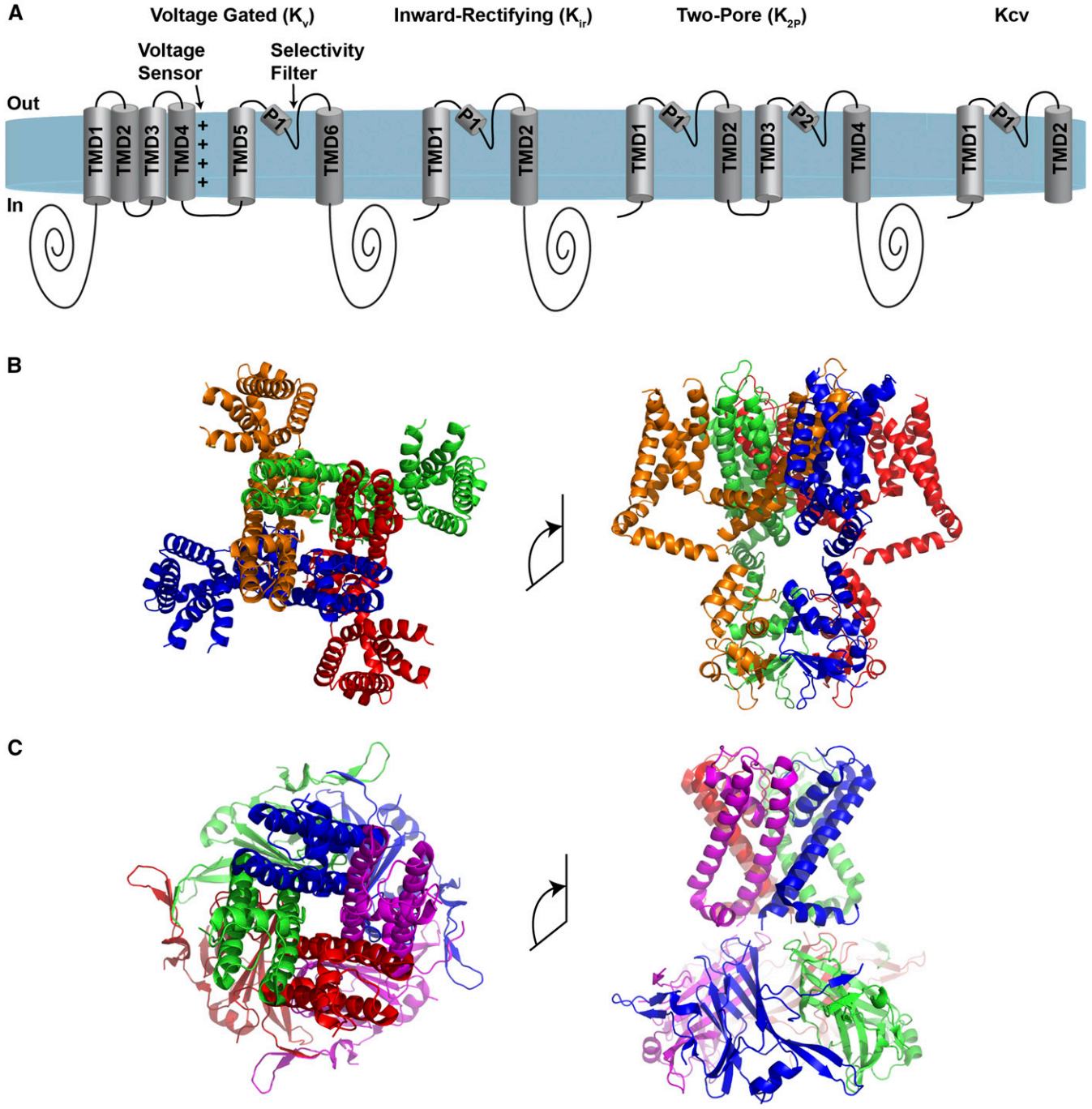

Figure 1 Topological and structural features of major potassium channel clades. (A) Cartoon representation of the transmembrane topology and key functional features of several subclasses of potassium channel monomers mentioned in this review. Note the universally conserved pore domain containing two transmembrane domains (TMDs), a pore helix, and a selectivity filter. (B) Representative structures of mammalian *Shaker* ($K_v1.2$), a six-TMD channel, and (C) bacterial KcsA, a two-TMD channel, shown looking down the pore from the extracellular side (left images) and parallel to the membrane (right images). Both structures are homotetramers with individual polypeptides rendered in different colors. The *Shaker* structure is derived from Protein Data Bank identifier (PDB ID): 2A79 (Long *et al.* 2005), and the KcsA structure is derived from PDB ID: 2WLM (Clarke *et al.* 2010). All images were rendered in PyMol (v. 1.8.6).

potential. However, K_{2P} channels can be gated by a wide variety of stimuli, such as membrane stretching or deformation, heat, and pH (Enyedi and Czirják 2010). These properties give rise to numerous specialized roles for K_{2P} channels in cells and tissues that sense physical stimuli. For example, the most highly studied K_{2P} channels—the TASK and TREK families—are involved in pain reception and are a major target of volatile anesthetics (Bayliss and Barrett 2008).

Inward-rectifying channels have a simpler structure of two TMDs flanking the selectivity filter (Hibino *et al.* 2010)

(Figure 1). They are presumably the basal clade of potassium channels with widely distributed homologs, even in prokaryotic genomes (Loukin *et al.* 2005). Their bias for inward over outward currents (in apparent violation of the Nernst equation) occurs via transient block by intercellular divalent cations, primarily Mg^{2+} and polyamines (Lopatin *et al.* 1994). These divalent anions interact at multiple sites in the extended cytoplasmic pore of K_{ir} channels (Nichols and Lopatin 1997). However, in mammalian physiological settings, K_{ir} channels rarely encounter sufficient voltage to allow potassium influx and are usually efflux channels. The "classical" mammalian K_{ir} channels ($K_{ir}2.x$) are constitutively active—*i.e.*, they have a high open probability (P_o) and are regulated primarily by the amount of protein at the cell surface—while other classes have a low P_o until activated by G-proteins (*e.g.*, $K_{ir}3.x$) or intracellular nucleotides (*e.g.*, K_{ATP} or $K_{ir}6.x$) (Hibino *et al.* 2010).

Based on their activities, regulation, and distribution, potassium channels mediate a broad range of biological processes. For example, in humans they are involved in nerve conduction (Mathie and Veale 2009), cardiac contraction (Sanguinetti and Tristani-Firouzi 2006), hearing (Wangemann 2006), maintenance of blood pressure (Wang *et al.* 2010), immune responses (Cahalan *et al.* 2001), and numerous other processes beyond the scope of this review. Moreover, polymorphisms in human potassium channels have been linked to dozens of genetic disorders affecting the nervous system, heart, kidneys, endocrine glands, and immune cells [see Kim (2014) for an extensive review of channelopathies]. Unfortunately, investigating potassium channel activity, modulation, and biogenesis is often labor-intensive, especially in mammalian cells. The development of a rapid, genetically tractable model in which these studies can be conducted has significantly aided our understanding of potassium channel physiology.

Potassium Channels and Potassium Homeostasis in the Yeast *Saccharomyces cerevisiae*

The budding yeast *Saccharomyces cerevisiae* presents several properties that make it an attractive system in which to probe the function of potassium channels. First, genetic transformations are famously facile in *S. cerevisiae* as compared to other commonly studied organisms. Second, strain libraries have been developed in which every gene in the yeast genome has been deleted, can be conditionally deactivated, or is present at reduced copy. Third, these unicellular eukaryotes can be propagated on either solid or in liquid growth medium, so yeast numbers are eminently scalable and the organism is amenable to high-throughput manipulations. Fourth, laboratory strains can be transitioned between the haploid and diploid state, allowing for genetic recombination and analyses of recessive and dominant phenotypes. Fifth, the resting membrane potential in *S. cerevisiae* is believed to be high relative to mammalian cells (see below), thus driving inward cation currents. Finally, despite nearly a billion years of evolutionary separation from humans, the vast majority of subcellular processes that animate yeast are conserved. Therefore, insights from yeast research almost always translate into multicellular eukaryotes.

Plasma membrane alkali metal transport in *S. cerevisiae* is adapted to efficiently import potassium and exclude sodium from the cytosol [for a detailed review, see Ariño *et al.* (2010)] (Figure 2). In brief, the electromotive force for potassium influx is generated by the electrogenic plasma membrane proton ATPase Pma1. Transmembrane potential in yeasts is believed to be quite high due to the activity of this proton transporter, but is notoriously difficult to measure directly with electrophysiological methods due to the high rigidity of the yeast cell wall (Vacata *et al.* 1981; Volkov 2015). Pma1 is the most abundant plasma membrane protein in yeast and consumes ~20% of cellular ATP.

The Pma1-dependent transmembrane potential provides the electromotive force for potassium uptake through two high-efficiency channels, Trk1 and Trk2 (Figure 2). Trk1 was identified as the primary avenue for potassium influx (Gaber *et al.* 1988; Ko and Gaber 1991) and its structure is quite distinct from mammalian potassium channels. Most prominently, the pore-forming unit is a single polypeptide consisting of four two-TMD units, which together form a four-fold symmetric pore. Under most growth conditions, Trk1 is responsible for nearly all potassium influx, but it is not essential. Yeast lacking *TRK1* (*trk1*Δ) have slightly increased potassium requirements that nevertheless fall within the bounds of ordinary laboratory growth medium. These yeast survive due to the presence of Trk2, which is a Trk1 paralog with a lower affinity for potassium and far lower expression in wild-type yeast. In contrast, doubly mutated *trk1*Δ *trk2*Δ yeast have a drastically increased requirement for potassium supplementation in growth medium, usually ~100 mM. That these yeast are able to survive at all speaks to the presence of a very low-affinity potassium uptake system, termed "nonspecific cation channel 1" (Nsc1) (Bihler *et al.* 1998). The gene encoding this putative channel is as yet unidentified, and in fact may not even exist because potassium may leak into *trk1*Δ *trk2*Δ cells through various nutrient transporters. Indeed, mutations in hexose or amino acid transporters are a common cause of phenotypic reversion in *trk1*Δ *trk2*Δ yeast (Ko *et al.* 1993; Wright *et al.* 1997; Liang *et al.* 1998). This can be prevented by propagating *trk1*Δ *trk2*Δ cells on high-potassium medium up until the time that key experiments are conducted (see below). Nevertheless, *trk1*Δ *trk2*Δ cells are hyperpolarized and are sensitive to low pH (Ko and Gaber 1991), to alkali metals other than potassium (Bertl *et al.* 2003), and to a variety of toxic cations such as hygromycin B (Madrid *et al.* 1998).

In addition to the Trk channels, *S. cerevisiae* express a voltage-gated outward-rectifying potassium channel, Tok1. Like K_v channels in animals, Tok1 opens upon membrane depolarization. Tok1 is neither essential nor does deletion of *TOK1* make *trk1*Δ *trk2*Δ yeast more sensitive to growth on low-potassium medium; however, compared to *trk1*Δ *trk2*Δ yeast, the triple-mutant strain exhibits increased

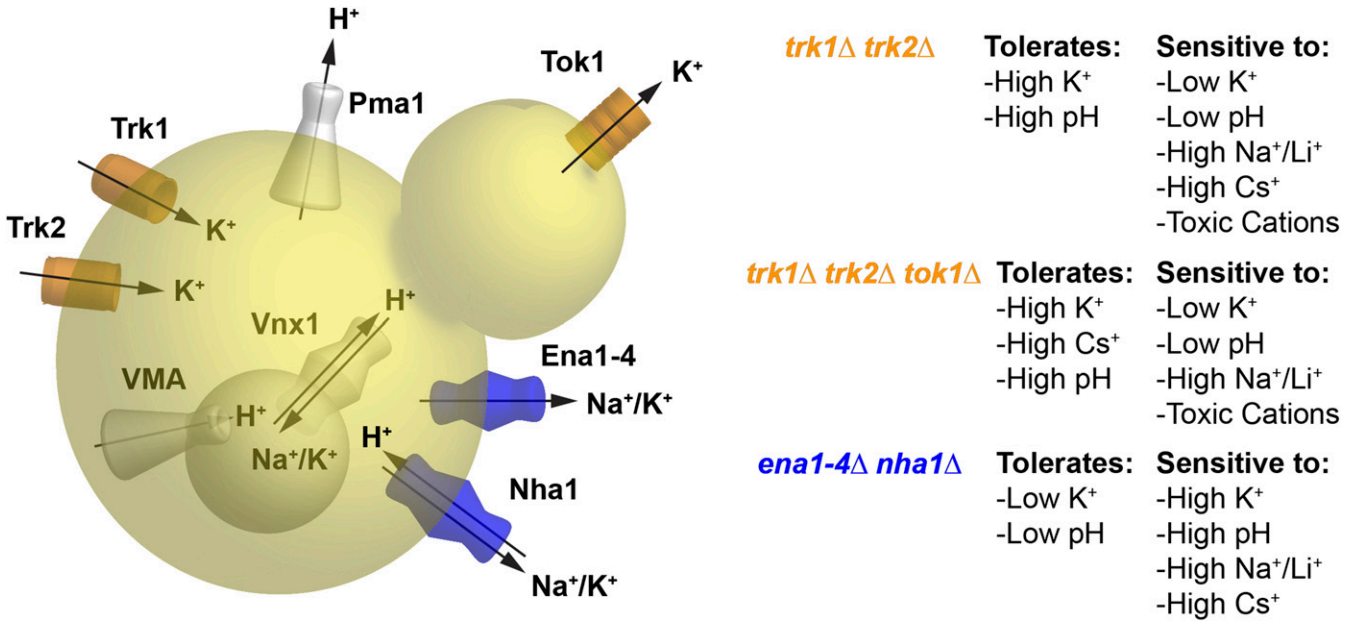

Figure 2 Schematic of plasma membrane proton and alkali metal channels and transporters in *S. cerevisiae*. Native yeast channels and transporters mentioned in this text are shown here with representative ion currents. This figure has been adapted and simplified from previously published work (Ariño *et al.* 2010). Select phenotypes of the potassium channel and transporter deletion strains mentioned in the text are shown to the right (Bañuelos *et al.* 1998; Bertl *et al.* 2003) Channels deleted in the *trk1*Δ *trk2*Δ and *trk1*Δ *trk2*Δ *tok1*Δ strains are shown in orange, and transporters deleted in the *ena1-4*Δ *nhx1*Δ strain are shown in blue.

tolerance to high cesium, which competes for potassium (Bertl *et al.* 2003). In addition, whereas mammals lack Trk1 or Trk2 homologs, Tok1 is homologous to the vertebrate two-pore K_{2P} channels (Wolfe and Pearce 2006). Therefore, select studies have utilized the *trk1*Δ *trk2*Δ *tok1*Δ potassium-sensitive strain (see below).

While potassium influx in yeast involves gradient-dependent, passive transport through the Trk1 and Trk2 channels, potassium efflux under conditions of excess cytosolic potassium requires active transport. Yeast have two primary systems to expel excess alkali metals into the extracellular environment: the ENA (*exitus natru*) uniporters and the Nha1 Na^+/H^+ antiporter (Figure 2). Although they primarily transport sodium, these systems are also able to export excess potassium (Bañuelos *et al.* 1998). ENA uniporters are encoded as tandem repeats at a single locus on chromosome IV with high (~97%) conservation. The number of *ENA* isoforms varies among laboratory strains, with W3031-A having four copies and S288C having five. This may partially explain the observation that these two common laboratory workhorses differ in their tolerance to sodium and other cations (Petrezselyova *et al.* 2010). Deletion of the *ENA* cluster renders yeast sensitive to both sodium and lithium. ENA expression is typically low but is strongly induced upon exposure to high sodium or lithium via the action of the osmoresponsive transcription factor Hog1 (Märquez and Serrano 1996). In contrast, Nha1 is constitutively expressed at low levels, and seems to play a housekeeping role in regulating intracellular potassium under low pH and in expelling the small quantities of Na^+ that enter through other transporters, such as the Na^+/PO_4^{3-} symporter Pho89 (Persson *et al.* 1998). Both Nha1 and Ena1 promote growth in high-K^+ medium, and a strain lacking both the *ENA* cluster (*ena1-4*Δ) and *NHA1* fail to propagate on high concentrations of potassium or any other alkali metal (Bañuelos *et al.* 1998; Kinclová *et al.* 2001).

Yeast are also capable of sequestering alkali metals in organelles, particularly the vacuole. Vnx1 is a vacuole-localized antiporter that can exchange vacuolar protons for cytosolic sodium or potassium (Cagnac *et al.* 2007). Vnx1 likely plays an important role in detoxifying the cytosol upon sodium stress as *vnx1*Δ yeast are sensitive to high sodium (Cagnac *et al.* 2007) and the genomes of halotolerant fungi such as *Hortaea werneckii* contain multiple paralogs of *VNX1* (Plemenitaš *et al.* 2016). While concentrating potassium in the vacuole can contribute to cellular turgor and help to balance the negative charge of vacuolar polyphosphate (Klionsky *et al.* 1990), it is unclear whether the vacuole can function as a "strategic reserve" upon acute potassium starvation.

The Awesome Power of the Potassium Growth Phenotype: Discoveries with *trk1*Δ *trk2*Δ Yeast

In this next section, we survey the many uses of *trk1*Δ *trk2*Δ yeast and then discuss several studies that have significantly aided our understanding of potassium channel function, regulation, biogenesis, and pharmacology. For the sake of brevity, we limit discussion to seminal studies and the most recent findings. For further information, we point the reader to the studies outlined in Table 1, which provides a more

Table 1 Select studies utilizing expression of an exogenous channel or transporter in *trk1*Δ *trk2*Δ, *trk1*Δ *trk2*Δ *tok1*, or *ena1-4*Δ *nha1*Δ yeast

Channel	Primary method	Full citation
K_{2P}2.1	Drug screen	Bagriantsev *et al.* (2013)
K_{ir}2.1	Drug screen	Zaks-Makhina *et al.* (2004)
K_{ir}2.1	Drug screen	Zaks-Makhina *et al.* (2009)
K_{ir}3.2 (GIRK2)	Drug screen	Kawada *et al.* (2016)
Akt1	Expression	Sentenac *et al.* (1992)
AKT2	Expression	Cao *et al.* (1995)
HIV-1 Vpu	Expression	Herrero *et al.* (2013)
Hkt1	Expression	Schachtman and Schroeder (1994)
Kat1	Expression	Anderson *et al.* (1992)
Kat1	Expression	Schachtman *et al.* (1992)
K_{ir}2.1	Expression	Hasenbrink *et al.* (2005)
K_{ir}2.1 (IRK1)	Expression	Tang *et al.* (1995)
K_{ir}6.1/6.2	Expression	Graves and Tinker (2000)
K_v11.1 (hERG)	Expression	Schwarzer *et al.* (2008)
ORK1	Expression	Goldstein *et al.* (1996)
RhAG (not a K channel)	Expression	André *et al.* (2000)
SKD1,2 (Transporters)	Expression	Périer *et al.* (1994)
TcCat	Expression	Jimenez and Docampo (2012)
AKT1	Random mutagenesis	Ros *et al.* (1999)
Hak5	Random mutagenesis	Alemán *et al.* (2014)
HvHAK	Random mutagenesis	Mangano *et al.* (2008)
Kat1	Random mutagenesis	Nakamura and Gaber (2009)
Kat1	Random mutagenesis	Nakamura *et al.* (1997)
Kat1	Random mutagenesis	Lai *et al.* (2005)
KcsA	Random mutagenesis	Paynter *et al.* (2008)
K_{ir}3.1	Random mutagenesis	Sadja *et al.* (2001)
K_{ir}3.2	Random mutagenesis	Bichet *et al.* (2004)
K_{ir}1.1 (ROMK)	Random mutagenesis	Paynter *et al.* (2010)
K_{ir}2.1	Random mutagenesis	Cho *et al.* (2000)
K_{ir}2.1	Random mutagenesis	Minor *et al.* (1999)
K_{ir}3.2 (GIRK2)	Random mutagenesis	Yi *et al.* (2001)
Kcv	Random mutagenesis	Chatelain *et al.* (2009)
K_{2P}2.1 (TREK-1)	Random mutagenesis	Bagriantsev *et al.* (2011)
K_{ir}2.1	Random mutagenesis	Chatelain *et al.* (2005)
TRVP4	Random mutagenesis	Loukin *et al.* (2010)
Kcv	Synthetic biology	Cosentino *et al.* (2015)
K_{ir}1.1 (ROMK)	Synthetic genetic array	Mackie *et al.* (2018)
K_{ir}2.1	Synthetic genetic array	Kolb *et al.* (2014)
K_{ir}2.1	Trafficking assays	Grishin *et al.* (2006)
K_{ir}2.1	Trafficking assays	Bernstein *et al.* (2013)
K_{2P}3.1 (TASK-1)		
K_{2P}9.1 (TASK-3)		
K_{ir}3.2 (GIRK2)	Trafficking screening	Bagriantsev *et al.* (2014)
K_{ir}6.1	Trafficking assays	Zerangue *et al.* (1999)
Kcv, Kesv	Trafficking assays	Balss *et al.* (2008)
K_{ir}1.1 (ROMK)	Trafficking assays	O'Donnell *et al.* (2017)
K_{ir}2.1	Trafficking screening	Shikano *et al.* (2005)

comprehensive list of discoveries in which exogenous potassium channels expressed in *trk1*Δ *trk2*Δ yeast played a key role.

The identification of uncharacterized potassium channels from cDNA libraries

The Gaber laboratory first noted the potential of exogenous potassium channels to substitute for the endogenous potassium uptake system in yeast (Ko and Gaber 1991; Nakamura and Gaber 1998). Numerous studies over the past two decades have exploited this phenotypic assay to identify novel classes of potassium channels from various organisms. By transforming cDNA libraries into *trk1*Δ *trk2*Δ yeast and identifying clones that enable growth on low-potassium media, researchers have identified new potassium channels rapidly and with minimal bias. This technique offers several advantages over traditional screens for new channels. In this case, cRNA libraries are injected into *Xenopus laevis* oocytes and then ion current is measured. This labor-intensive protocol led to the identification of many potassium channels [see for example Ho *et al.* (1993)]. In contrast, the yeast selection scheme is cheaper, there is no need for electrophysiology "rigs," and the isolation of positive clones does not require sequential subdivision of clonal oocyte pools and the isolation and amplification of unique clones because transformed yeast only express single clones. This technique has provided a particular boon for identifying potassium channels as well as transporters from the plant kingdom.

Potassium is a key macronutrient for plants that is absorbed from the soil through the root system and concentrated in cells to maintain cellular turgor. Because plants lack contractile tissues, potassium fluxes underpin various tropisms that allow them to respond to environmental stimuli (Dreyer and Uozumi 2011). The first plant potassium channel, KAT1, was identified by coopting the *trk1*Δ *trk2*Δ growth phenotype on low potassium (Anderson *et al.* 1992; Schachtman *et al.* 1992). KAT1 is a *Shaker*-type voltage-gated channel with inward-rectifying properties, is highly expressed in guard cells, and its activation in response to hyperpolarization causes the stoma to swell and open. Dozens of subsequent studies have since identified previously unknown potassium channels and transporters in both model and crop plants, including AKT1, a strongly inward-rectifying potassium uptake channel in root hairs (Sentenac *et al.* 1992; Hirsch *et al.* 1998), as well as HKT, which was the first plant high-affinity potassium transporter identified (Schachtman and Schroeder 1994) (see Table 1). Potassium transporters are functionally and morphologically distinct from potassium channels and, as such, fall outside the scope of this review, but they have also been extensively studied in yeast [see Rodríguez-Navarro and Rubio (2006) for review].

One limitation of this system bears mention. Full-length K_v channels from animals are unable to rescue the growth of *trk1*Δ *trk2*Δ yeast on low potassium. This phenomenon arises due to the fact that mammalian K_v channels fail to open in the hyperpolarizing potential across the yeast membrane; in contrast, plant Kv channels open under these conditions, which is why KAT1 and other related channels could be isolated in yeast (Table 1). However, the K_v channel rat *ether-à-go-go*-related gene-1 (rEAG1) was able to restore growth in *trk1*Δ *trk2*Δ cells if the N-terminal 190 amino acids (*i.e.*, the entire cytosolic N-terminal domain) were truncated (Schwarzer *et al.* 2008). This result, while potentially opening many doors for studying mammalian K_v channels in yeast, is

puzzling as the N-terminal PAS domain of hERG, the human homolog of rEAG1, is necessary for channel activity in native systems (Gianulis and Trudeau 2011). Fortunately, many mammalian K_{ir} channels function in *trk1*Δ *trk2*Δ and *ena1-4*Δ *nha1*Δ yeast [*e.g.*, Graves and Tinker (2000), Yi *et al.* (2001), and Paynter *et al.* (2010), and also see Table 1]. The first of these—and to date the most extensively studied—is the cardiac inward-rectifier $K_{ir}2.1$, which was originally called *IRK1* (Tang *et al.* 1995) (see below).

New potassium channels can currently be predicted by *in silico* recognition of core features based on DNA sequence analysis. Nonetheless, *trk1*Δ *trk2*Δ yeast remain a valuable tool to validate the function of putative potassium channels detected in the ever-expanding collection of sequenced genomes. This approach is particularly useful for exotic potassium channels that lack the "canonical" potassium selectivity motif. Many of these noncanonical channels have been found in the genomes of viruses or intracellular parasites, such as *Trypanosoma cruzi* (Jimenez and Docampo 2012).

Viroporins are small transmembrane proteins encoded in viral genomes that insert into the host cell plasma membrane, increase cell permeability, and play important roles in virion maturation and budding (Nieva *et al.* 2012). Because they are exposed to the extracellular environment, viroporins are also attractive therapeutic targets. Expression of viroporins from various viral taxa in *trk1*Δ *trk2*Δ yeast established that these proteins function as potassium channels. Notable viroporins studied in this system include *Paramecium bursaria Chorella* virus-1 Kcv (Chatelain *et al.* 2009), human immunodeficiency virus-1 protein U (Vpu) (Herrero *et al.* 2013), and porcine epidemic diarrhea virus ORF3 (Wang *et al.* 2012). Vpu lacks a canonical potassium selectivity motif, has a pentameric (rather than a tetrameric) pore structure, and only weakly selects potassium over other small monovalent cations, such as sodium and ammonium (Kukol and Arkin 1999). In contrast, a potassium selectivity filter was predicted in Kcv based on its presence in a viral genome (Plugge *et al.* 2000), even though Kcv is the smallest canonical potassium channel identified (each subunit is only 94 amino acids) and completely lacks a cytosolic C-terminal domain (Figure 1A) (Gazzarrini *et al.* 2003). While Kcv forms a functional ion channel at the host plasma membrane, Kesv, a similar viroporin from a related virus, is sorted to the mitochondria. Expression in *trk1*Δ *trk2*Δ yeast showed that Kesv mitochondrial targeting is dependent on the length of TMD2 and that a slight extension of TMD2 retargets the channel to the secretory pathway (Balss *et al.* 2008). Therefore, yeast expression studies have elucidated some unexpected features of viral potassium channels.

Structure–function relationships via mutagenesis and genetic selection

Random mutagenesis has long been used as a rapid and nonbiased method to identify critical residues, motifs, and domains in ion channels. The approach may target specific regions, in which degenerate primer sets scramble a small segment of the coding sequence, or the entire coding sequence or a sizable portion thereof through error-prone PCR. Gene shuffling can also be used to efficiently mutagenize large genes and to perform *in vitro* backcrosses to reduce the number of mutations per clone, or to ensure the presence of a particular mutation in a screen for second-site suppressors (Stemmer 1994; Bichet *et al.* 2004). These studies are easily undertaken in yeast as transformed yeast cells only uptake single clones from which mutations can be quickly identified. In addition, homologous recombination can be used to join multiple linear DNA fragments, one of which can contain a mutagenized insert (created, for example, by PCR) and one that contains the expression plasmid via direct transformation into recipient yeast strains (Weir and Keeney 2014).

Targeted mutagenesis studies of KAT1 focused on the selectivity filter. The conserved "YG" site in the canonical selectivity filter TXXTXG**YG** was mutated to 400 possible combinations using a degenerate primer set (Nakamura *et al.* 1997). While some conservative mutations of the tyrosine produced nominally functional channels with impaired selectivity (*i.e.*, growth on low potassium in the absence of sodium), only the most conservative YG→FG mutation resulted in a fully selective channel that allowed growth of *trk1*Δ *trk2*Δ yeast on low-potassium media in the presence of high sodium. When the residues flanking the selectivity motif were instead mutated, multiple mutations increased Na^+ exclusion, as evidenced as well by increased growth on low potassium as well as high sodium. Curiously, the mutations in the flanking region, which led to increased Na^+ exclusion, also allowed the transport of large toxic cations such as Cs^+ and triethylammonium that normally block the channel. These data suggest that the entire pore region has evolved under dual selective pressure to exclude cations both larger and smaller than K^+ (Nakamura and Gaber 2009).

While residues conferring selectivity are confined to a small but highly conserved region in potassium channels, residues that regulate gating are often more broadly distributed. Jan and colleagues performed mutagenesis to determine the importance of the voltage-sensing TMD4 helix in relation to the pore forming domain in TMD5 in KAT1. Mutations in TMD5 that conferred a mild loss-of-function phenotype were isolated and then random mutagenesis of TMD4 uncovered second-site suppressors, thus providing evidence of the close proximity of TMD4 and TMD5, particularly when the channel is in the open state (Lai *et al.* 2005). Similar studies from the same laboratory focused on identifying the gating mechanisms of mammalian K_{ir} channels. Specifically, TMD1 and TMD2 in $K_{ir}2.1$ were randomly mutagenized and, based on the relative effects of the mutations on channel function in *trk1Δ trk2Δ* yeast, the authors predicted the lipid-facing, pore-facing, and helix-packing surfaces of the TMDs (Minor *et al.* 1999). These predictions would later be validated by an analysis of the crystallographic structure of a bacterial inward-rectifying channel, KirBac1.1, in the closed state (Kuo *et al.* 2003).

Of the mammalian K_{ir} channels, $K_{ir}2.1$ is the most amenable to studies in *trk1*Δ *trk2*Δ yeast as it is highly expressed,

traffics efficiently to the plasma membrane, and its P_o is high (Minor *et al.* 1999). A random mutagenesis screen in *trk1*Δ *trk2*Δ yeast for $K_{ir}2.1$ channels resistant to barium block indicated that the dipole moment of the pore helix does not play a major role in stabilizing cations in the pore, as barium-resistant mutants with positively-charged substitutions at the C-terminus of the pore helix did not exhibit reduced single-channel potassium current (Chatelain *et al.* 2005).

Unlike $K_{ir}2.1$ and other classical K_{ir} channels, the G-protein-activated inward-rectifying potassium channel-2 (GIRK2, also known as $K_{ir}3.2$) has a much lower P_o (~0.01) unless activated by $G_{\beta\gamma}$ binding. A random mutagenesis screen of the entire GIRK2 coding sequence identified five residues in the pore domain that are necessary for gating, and substitution at any of these positions increased the P_o independently of G-protein stimulation. The authors also identified a specific valine in TMD2 that plays a critical role during channel opening (Yi *et al.* 2001). A similar analysis performed contemporaneously by another group also found pore domain mutations necessary for GIRK1 ($K_{ir}3.1$) channel gating in the absence of G-proteins (Sadja *et al.* 2001). A follow up to the initial mutagenesis of $K_{ir}3.2$ utilized gene shuffling to find second-site suppressors of the constitutively active but selectivity-deficient S177W mutation. This study identified several mutations in the TMDs that were able to functionally restore potassium selectivity (Bichet *et al.* 2004).

K_{2P} channels can be gated by a much wider variety of physical and chemical stimuli than either K_{ir} or K_v channels. For example, the highly-studied human TREK-1 ($K_{2P}2.1$) channel is gated by both intra- and extracellular low pH, heat, mechanical membrane deformation, and anesthetics (Enyedi and Czirják 2010). Random mutagenesis of TREK-1 and expression in *trk1*Δ *trk2*Δ yeast revealed that TREK-1's response to these disparate stimuli relies on a common gating mechanism. Specifically, a well-conserved extracellular region of TMD4 containing multiple aromatic residues was shown to be necessary for channel gating in TREK-1 and other K_{2P} channels (Bagriantsev *et al.* 2011).

Studies on potassium channel trafficking in the yeast model

The whole-cell potassium conductance, which determines the potassium current for a given driving force, is the product of the single-channel conductance, the open probability, and the total number of channels at the cell surface. Therefore, cells must tightly control the plasma membrane residence of ion channels by regulating synthesis, intramembrane trafficking, and degradation. Like all classes of transmembrane proteins, potassium channels interface with the intramembrane trafficking machinery through specific amino acid motifs (Griffith 2001). Most of these trafficking signals are short linear motifs, similar to canonical signals shared by a wide variety of membrane proteins, but they can also be found at the interface between two subunits (Ma *et al.* 2011; Li *et al.* 2016). Endoplasmic reticulum (ER)-retention motifs are short linear motifs found on the cytosolic face of transmembrane proteins and promote retrograde transport from the Golgi apparatus to the ER, thereby preventing cargo proteins from reaching the cell surface. One such sequence is the di-arginine "RXR" motif that interacts directly with the coatamer (COPI) (Zuzarte *et al.* 2009; Okamoto and Shikano 2011) and becomes occluded as the channel folds and assembles (Zerangue *et al.* 1999). RXR was first characterized in $K_{ir}6.2$ and is present in many mammalian K_{ir} channels. Channels that lack this motif, such as $K_{ir}2.1$, are robustly trafficked to the plasma membrane in both yeast and mammalian cells (Grishin *et al.* 2006). To identify novel ER exit/forward trafficking signals that may override RXR, Shikano and colleagues fused a random peptide library to $K_{ir}2.1$-RXR and screened for increased channel activity in *trk1*Δ *trk2*Δ yeast. As a result of this analysis, the authors identified SWTY as a 14-3-3 protein interactor (Shikano *et al.* 2005). 14-3-3 proteins bind to phosphoserine/threonine motifs to regulate myriad processes (Muslin *et al.* 1996; Kumar 2017). In both yeast and multicellular eukaryotes, the -2 threonine of the SWTY motif must be phosphorylated to bind 14-3-3 proteins, and this phenomenon was demonstrated using both model substrates as well as naturally occurring proteins with SWTY-like sequences (Shikano *et al.* 2005). In a similar study, novel modulators of GIRK2 were identified by fusing either a peptide library that contained random 15 amino acid motifs flanked by a well-folded domain from *Staphylococcus aureus* protein Z, or a randomly mutagenized version of protein Z itself to either the N- or C-terminus of GIRK2. This multipronged approach made for a more comprehensive screen by preventing bias against poorly folding peptides that are nonetheless strong positive regulators of GIRK2. As a result of this study, the authors identified a peptide that augments GIRK2 current by increasing plasma membrane residence (Bagriantsev *et al.* 2014).

In contrast to the identification of embedded sequences that regulate potassium channel trafficking, the yeast screen has also been used to find extragenic regulators of trafficking. This was accomplished through the use of synthetic genetic array (SGA) strategies (Tong and Boone 2006) in which a genome-wide analysis can be performed to identify genes that, when deleted, alter the growth of potassium channel-expressing *trk1*Δ *trk2*Δ yeast on low-potassium media (Figure 3). Based on the strength of the phenotype, the candidates can also be ranked.

The first instance in which an SGA was performed in yeast expressing a potassium channel used a targeted library of deletion strains lacking components associated with the early secretory pathway. The library was used to query yeast expressing a constitutively active GIRK variant ($K_{ir}3.2_{S177W}$) that increases Na^+ influx (Yi *et al.* 2001). As a result of this phenotype, yeast growth was slowed on high-sodium media. Therefore, isolated strains with increased growth on high sodium were hypothesized to lack a factor that promotes $K_{ir}3.2_{S117W}$ biosynthesis and forward trafficking. The genes identified in this analysis were primarily involved in sphingolipid biosynthesis, which may facilitate channel opening at the plasma

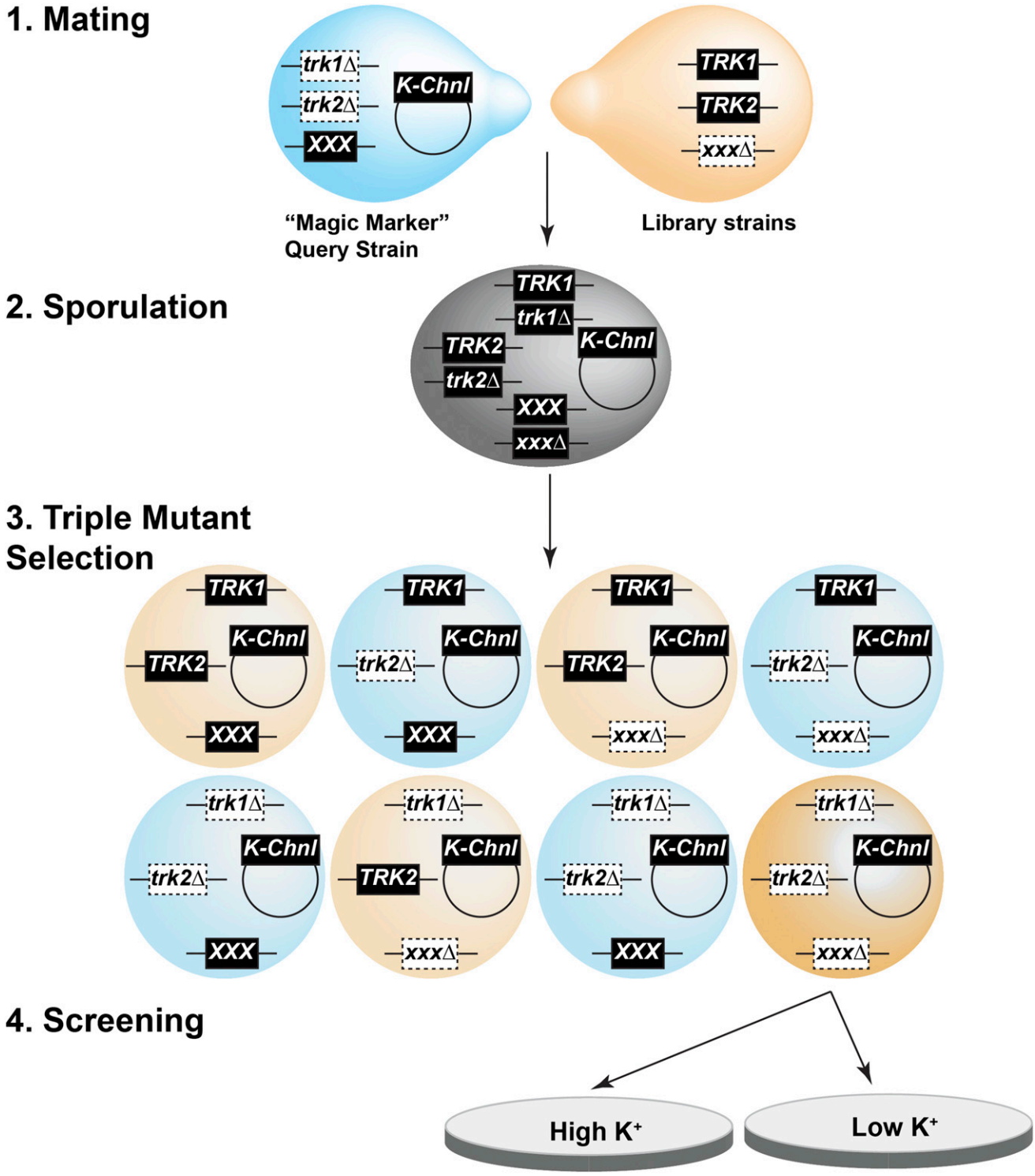

Figure 3 Visual representation of Synthetic Gene Array (SGA) technology to screen for potassium channel regulators. (1) The "magic marker" yeast strain, which contains the *can1*Δ marker for dominant selection against diploid cells and *HIS3* under the control of the *STE2* promoter to select for *MAT***a** haploids, is additionally deleted for *trk1*Δ and *trk2*Δ. This strain is then transformed with a potassium channel ("*K-ChnI*") and mated to the yeast deletion library ("*xxx*Δ"). (2) Yeast colonies are transferred to selective media to propagate diploids, then onto low-nitrogen media to induce sporulation. (3) Sporulated yeast are grown on a series of selective media to isolate *MAT***a** haploid progeny harboring *trk1*Δ *trk2*Δ and the library hypomorph. All growth media in steps 1–3 should be supplemented with 100 mM K^+ to prevent intergenic suppression of potassium sensitivity. (4). Triple-mutant ("*trk1*Δ *trk2*Δ *xxx*Δ") yeast are transferred to high- and low-potassium media and growth is assessed. Adapted from Kolb *et al.* (2014) and the materials and methods section in Mackie *et al.* (2018).

membrane (Epshtein *et al.* 2009), and COPII vesicle cargo packing of GPI-anchored proteins, which may indirectly impact ER protein export. These data indicate a preeminent role of these pathways in K_{ir} channel biogenesis and function (Haass *et al.* 2007).

Subsequent studies from our group have applied an SGA to identify and rank regulators of potassium channels with the entire nonessential gene library, which contains > 5000 gene knockout strains. For this analysis, *TRK1* and *TRK2* were deleted in the "magic-marker" SGA query strain (Tong and Boone 2006), which resulted in slow growth on low-potassium media. The strain was then transformed with a plasmid encoding $K_{ir}2.1$ and mated to each strain in the deletion collection. Yeast displaying increased growth on low-potassium media were hypothesized to lack negative regulators of $K_{ir}2.1$. The result of this screen led to the isolation of yeast lacking genes encoding factors involved in vacuolar protein sorting (VPS) and several subunits of the endosomal complexes required for trafficking (ESCRT) (Kolb *et al.* 2014). These data suggested that $K_{ir}2.1$, while primarily being targeted for destruction by the ER-associated degradation (ERAD) pathway (see below), is also routed to the vacuole for degradation; therefore, slowed vacuole-dependent degradation liberated additional $K_{ir}2.1$ channels to the plasma membrane, which facilitated growth on low-potassium media. Consistent with this hypothesis, inhibition of the ESCRT pathway via knockdown strategies in human cells increased the steady-state pool of $K_{ir}2.1$.

More recently, we published on a related screen for regulators of the renal outer medullary potassium channel ROMK (also known as $K_{ir}1.1$). ROMK was the first K_{ir} gene identified in mammals (Ho *et al.* 1993) and is primarily expressed in the distal nephron, where it serves as a master regulator of extracellular potassium in response to changes in dietary intake. ROMK also plays an important role in sodium balance and the urinary concentrating mechanism (Welling and Ho 2009). ROMK is inhibited when the intracellular pH drops below 6.0 (Paynter *et al.* 2010), which necessitated the mutation of a pH-titratable lysine to a methionine (K80M) for functional studies in *trk1*Δ *trk2*Δ yeast. Unlike $K_{ir}2.1$, ROMK primarily resides in the ER at steady-state until Serine 44 becomes phosphorylated (Yoo *et al.* 2005), so a phosphomimic was next introduced at position 44, converting a serine to an aspartic acid (O'Donnell *et al.* 2017). An SGA screen with a ROMK-expressing query strain revealed that many of the same VPS genes responsible for removing $K_{ir}2.1$ from the plasma membrane similarly regulated ROMK, but an added contribution of the class C core vacuole/endosome tethering (CORVET) complex was evident (Mackie *et al.* 2018). CORVET is a heterohexameric complex that acts upstream of ESCRT by tethering postendocytic vesicles to form early endosomes (Balderhaar *et al.* 2013). As neither $K_{ir}2.1$ nor ROMK is native to yeast, it is likely that endosomal sorting represents a conserved mechanism to regulate K_{ir} channels. As for $K_{ir}2.1$, surface expression of ROMK increased when ESCRT subunits were silenced in cultured mammalian cells (Kolb *et al.* 2014; Mackie *et al.* 2018).

As mentioned in *Potassium Channel Classes, Activities, and Structures*, mutations in potassium channels give rise to a range of human diseases, and ROMK is no exception. Over 50 loss-of-function mutations in ROMK have been linked to the autosomal recessive salt-wasting disease Bartter Syndrome Type II (Welling and Ho 2009). We hypothesized that many of these mutants compromise ROMK folding or tetramerization, which would trap the channels in the ER and target them for ERAD. Yeast and multicellular eukaryotes utilize ERAD to recognize and degrade misfolded proteins in both the ER lumen and the ER membrane (Vembar and Brodsky 2008). As the ER lumen lacks a major degradative protease, the AAA+ ATPase Cdc48/p97 retrotranslocates substrates to the cytosol for degradation in the proteasome (Ye *et al.* 2001). Mutations in proteins that enter the secretory pathway, including several classes of ion channels, have been linked to many genetic diseases and lead to protein misfolding and ERAD targeting (Guerriero and Brodsky 2012). Indeed, we found that select Bartter Syndrome mutations in the C-terminus of ROMK cause a loss-of-function phenotype in *trk1*Δ *trk2*Δ yeast by increasing the ERAD of these mutants, thus establishing Bartter Syndrome Type II as yet another ERAD-linked channelopathy (O'Donnell *et al.* 2017).

Pharmacological studies of potassium channels in yeast

As a large and functionally multifaceted class of proteins, potassium channels represent a wellspring of potential targets for new additions to the pharmacopoeia (Kaczorowski *et al.* 2008; Wulff *et al.* 2009). Drugs that regulate potassium channels have proved efficacious in clinical settings. For example, rosiglitazone, which stimulates insulin release from pancreatic β-cells through inhibition of K_{ATP} ($K_{ir}6.1$), has been used to treat diabetes and hypertension (Yu *et al.* 2012), while retigabine, a potentiator of $K_v7.2/K_v7.3$ channels, has shown promise as an antiepileptic (Ihara *et al.* 2016). Conversely, the human *ether-à-go-go*-related gene (hERG or $K_v11.1$) is a common nemesis of drug trials as it is inhibited by many structurally and functionally unrelated compounds (Priest *et al.* 2008). Therefore, there is an unmet need for drugs that target potassium channels that have been heretofore refractory to medicinal chemistry or that exhibit fewer side effects. To these ends, potassium channel pharmacology lends itself naturally to the *trk1*Δ *trk2*Δ system as molecules can be added directly to growth media to interact with the plasma membrane pool of potassium channels. As many of these compounds interact with the extracellular face of the channel pore, their efficacy is unlikely to be affected by *S. cerevisiae*'s well-known arsenal of multidrug resistance ABC transporters.

In pioneering work, a library of > 10,000 synthetic compounds was screened for their ability to inhibit $K_{ir}2.1$-mediated rescue of *trk1*Δ *trk2*Δ yeast, as determined by the optical density of cultures in low-potassium medium. From a list of preliminary hits, compounds were excluded that inhibited other potassium channels, such as GIRK2 or KAT1. Ultimately, a synthetic compound was found that blocked the ion-conducting pore in $K_{ir}2.1$ and protected against potassium efflux-mediated caspase activation and apoptosis in primary

cultured neurons (Zaks-Makhina *et al.* 2004). A follow-up study using a natural products library identified gambogic acid as a potent and long-lasting inhibitor of $K_{ir}2.1$ that alters the interaction of the channel with the membrane microenvironment (Zaks-Makhina *et al.* 2009). These screening techniques were then modified to find regulators of the mechano- and thermosensitive K_{2P} channel TREK-1 ($K_{2P}2.1$). K_{2P} channels are of broad interest in medicine due to their roles in pain, protection from ischemia, migraine, and depression. By screening a library of $>$ 100,000 compounds in *trk1*Δ *trk2*Δ yeast that expressed TREK-1 and were grown in a variety of K^+ concentrations, one compound was identified that activates TREK-1 and its close relatives, but not more distally related K_{2P} channels or other classes of potassium channels (Bagriantsev *et al.* 2013).

In another successful application of this technology, the *KCNJ6* gene was used. *KCNJ6*, which encodes GIRK2, is located on chromosome 21 in humans, and its duplication in trisomy-21 (Down syndrome) has been linked to cognitive impairment (Rachidi and Lopes 2007). In a screen of $>$ 2000 compounds in GIRK-expressing *trk1*Δ *trk2*Δ *tok1*Δ yeast, proflavine and several derivatives were identified as voltage-dependent blockers of GIRK2 (Kawada *et al.* 2016). It should be noted that the use of *trk1*Δ *trk2*Δ *tok1*Δ rather than *trk1*Δ *trk2*Δ allowed the authors to employ Cs^+ as a positive control for GIRK2 channel blockage. In each of the other drug screens, a more pleiotropic positive control for cell death was used, such as SDS. In contrast, Cs^+ specifically targets the exogenously expressed potassium channels in *trk1*Δ *trk2*Δ *tok1*Δ yeast.

Synthetic Biology and the Future of Potassium Channel Research in Yeast

Potassium channels and other ion channels are critical to maintain homeostasis and allow for information transduction and stimuli responses on much shorter timescales than any other biological process (Isacoff *et al.* 2013). Due to their ion-conducting properties and selectivity, potassium channels have attracted significant attention among synthetic biologists, who have endeavored to engineer novel gating mechanisms in potassium channels. Screens for neofunctionalized channels in yeast allow synthetic biologists to benefit from many of the same high-throughput molecular evolution techniques described thus far. For example, voltage sensitivity can be engineered into the Kcv channel upon fusion with a K_v-like voltage-sensing domain from a phosphatase (Arrigoni *et al.* 2013). More recently, a blue light-activatable potassium channel was created by fusing the light-sensitive LOV2 module from an *Avena sativa* phototropin to the N-terminus of Kcv. Error-prone PCR mutagenesis was then used to identify clones that rescue growth of *trk1*Δ *trk2*Δ yeast on low potassium when activated by blue light. The researchers then expressed this new potassium channel, termed blue light-induced potassium channel-1 (BLINK1), in *Zebrafish* embryos and found that their escape response reflex in response to touch was fully and reversibly inhibited upon exposure to blue light (Cosentino *et al.* 2015). This study represents the first instance of an optogenetically controlled potassium channel and will enable the creation of transgenic animals expressing photo-activatable potassium channels in specific neurons.

In the future, we envision continued use of the yeast screen for therapeutics, as many channel modulators lack desired specificity. Drugs are also needed for other potassium channels, particularly those whose function increases the severity of various cancers (Huang and Jan 2014). Moreover, we envision screens for modulators of mutated potassium channels for which treatments are currently lacking. For example, the expression of Bartter Syndrome mutant forms of ROMK, which are trapped in the early secretory pathway and degraded by ERAD (see above), might respond to small-molecule protein folding correctors. In fact, corrector molecules have proved to be efficacious treatments for other disease-causing mutations in ion channels targeted for ERAD, such as CFTR (Van Goor *et al.* 2011). A small molecule screen might uncover pharmacological chaperones that promote the folding of mutant ROMK and allow it to escape ERAD and other forms of protein quality control.

In sum, recent studies from multiple laboratories, spanning a wide breadth of disciplines, have demonstrated the continuing utility of a technique originally developed a quarter century ago. From the earliest identification of novel potassium channels before the genomic era to frontiers in molecular medicine and biotechnology, yeast screens have contributed significantly to our understanding of potassium channel biology. To date, a scan of the potassium channel literature demonstrates that most investigations have focused on small group of channels—KAT1, $K_2P_{2.1}$, Kcv, $K_{ir}2.1$, and $K_{ir}3.x$—which are either isolated from well-studied model organisms or are intimately linked to human biology. However, this list barely scratches the surface of this diverse and ubiquitous class of biomolecules. As potassium channel science continues to advance, yeast-based assays will undoubtedly continue to play an important part.

Acknowledgments

We thank Paul Welling, Allyson O'Donnell, and Arohan Subramanya for their assistance in preparing the document. Studies on potassium channel physiology in the Brodsky laboratory are supported by National Institutes of Health grants GM-075061 and DK-79307. The authors declare no conflicts of interest.

Literature Cited

Alemán, F., F. Caballero, R. Ródenas, R. M. Rivero, V. Martínez *et al.*, 2014 The F130S point mutation in the Arabidopsis high-affinity K(+) transporter AtHAK5 increases K(+) over Na(+) and Cs(+) selectivity and confers Na(+) and Cs(+) tolerance to yeast under heterologous expression. Front. Plant Sci. 5: 430. https://doi.org/10.3389/fpls.2014.00430

Anderson, J. A., S. S. Huprikar, L. V. Kochian, W. J. Lucas, and R. F. Gaber, 1992 Functional expression of a probable Arabidopsis thaliana potassium channel in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 89: 3736–3740.

André, B., B. Chérif-Zahar, A.-M. Marini, G. Matassi, V. Raynal *et al.*, 2000 The human Rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast. Nat. Genet. 26: 341–344.

Ariño, J., J. Ramos, and H. Sychrová, 2010 Alkali metal cation transport and homeostasis in yeasts. Microbiol. Mol. Biol. Rev. 74: 95–120. https://doi.org/10.1128/MMBR.00042-09

Arrigoni, C., I. Schroeder, G. Romani, J. L. Van Etten, G. Thiel *et al.*, 2013 The voltage-sensing domain of a phosphatase gates the pore of a potassium channel. J. Gen. Physiol. 141: 389–395. https://doi.org/10.1085/jgp.201210940

Bagriantsev, S. N., R. Peyronnet, K. A. Clark, E. Honoré, and D. L. Minor, 2011 Multiple modalities converge on a common gate to control K2P channel function. EMBO J. 30: 3594–3606. https://doi.org/10.1038/emboj.2011.230

Bagriantsev, S. N., K.-H. Ang, A. Gallardo-Godoy, K. A. Clark, M. R. Arkin *et al.*, 2013 A high-throughput functional screen identifies small molecule regulators of temperature- and Mechano-sensitive K_{2P} channels. ACS Chem. Biol. 8: 1841–1851. https://doi.org/10.1021/cb400289x

Bagriantsev, S. N., F. C. Chatelain, K. A. Clark, N. Alagem, E. Reuveny *et al.*, 2014 Tethered protein display identifies a novel Kir3.2 (GIRK2) regulator from protein scaffold libraries. ACS Chem. Neurosci. 5: 812–822. https://doi.org/10.1021/cn5000698

Balderhaar, H. J., J. Lachmann, E. Yavavli, C. Bröcker, A. Lürick *et al.*, 2013 The CORVET complex promotes tethering and fusion of Rab5/Vps21-positive membranes. Proc. Natl. Acad. Sci. USA 110: 3823–3828. https://doi.org/10.1073/pnas.1221785110

Balss, J., P. Papatheodorou, M. Mehmel, D. Baumeister, B. Hertel *et al.*, 2008 Transmembrane domain length of viral K+ channels is a signal for mitochondria targeting. Proc. Natl. Acad. Sci. USA 105: 12313–12318. https://doi.org/10.1073/pnas.0805709105

Bañuelos, M. A., H. Sychrova, C. Bleykasten-Grosshans, J.-L. Souciet, and S. Potier, 1998 The Nhal antiporter of Saccharomyces cerevisiae mediates sodium and potassium efflux. Microbiology 144: 2749–2758.

Bayliss, D. A., and P. Q. Barrett, 2008 Emerging roles for two-pore-domain potassium channels and their potential therapeutic impact. Trends Pharmacol. Sci. 29: 566–575. https://doi.org/10.1016/j.tips.2008.07.013

Bernstein, J. D., Y. Okamoto, M. Kim, and S. Shikano, 2013 Potential use of potassium efflux-deficient yeast for studying trafficking signals and potassium channel functions. FEBS Open Bio 3: 196–203. https://doi.org/10.1016/j.fob.2013.04.002

Bertl, A., J. Ramos, J. Ludwig, H. Lichtenberg-Fraté, J. Reid *et al.*, 2003 Characterization of potassium transport in wild-type and isogenic yeast strains carrying all combinations of trk1, trk2 and tok1 null mutations. Mol. Microbiol. 47: 767–780.

Bichet, D., Y.-F. Lin, C. A. Ibarra, C. S. Huang, B. A. Yi *et al.*, 2004 Evolving potassium channels by means of yeast selection reveals structural elements important for selectivity. Proc. Natl. Acad. Sci. USA 101: 4441–4446.

Bihler, H., C. L. Slayman, and A. Bertl, 1998 NSC1: a novel high-current inward rectifier for cations in the plasma membrane of Saccharomyces cerevisiae. FEBS Lett. 432: 59–64.

Cagnac, O., M. Leterrier, M. Yeager, and E. Blumwald, 2007 Identification and characterization of Vnx1p, a novel type of vacuolar monovalent cation/H+ antiporter of Saccharomyces cerevisiae. J. Biol. Chem. 282: 24284–24293.

Cahalan, M. D., H. Wulff, and K. G. Chandy, 2001 Molecular properties and physiological roles of ion channels in the immune system. J. Clin. Immunol. 21: 235–252.

Cao, Y., J. M. Ward, W. B. Kelly, A. M. Ichida, R. F. Gaber *et al.*, 1995 Multiple genes, tissue specificity, and expression-dependent modulationcontribute to the functional diversity of potassium channels in Arabidopsis thaliana. Plant Physiol. 109: 1093–1106.

Chatelain, F. C., N. Alagem, Q. Xu, R. Pancaroglu, E. Reuveny *et al.*, 2005 The pore helix dipole has a minor role in inward rectifier channel function. Neuron 47: 833–843.

Chatelain, F. C., S. Gazzarrini, Y. Fujiwara, C. Arrigoni, C. Domigan *et al.*, 2009 Selection of inhibitor-resistant viral potassium channels identifies a selectivity filter site that affects barium and amantadine block. *PLoS One* 4: e7496. https://doi.org/10.1371/journal.pone.0007496

Cho, H. C., R. G. Tsushima, T. T. Nguyen, H. R. Guy, and P. H. Backx, 2000 Two critical cysteine residues implicated in disulfide bond formation and proper folding of Kir2.1. Biochemistry 39: 4649–4657.

Clarke, O. B., A. T. Caputo, A. P. Hill, J. I. Vandenberg, B. J. Smith *et al.*, 2010 Domain reorientation and rotation of an intracellular assembly regulate conduction in Kir potassium channels. Cell 141: 1018–1029.

Cosentino, C., L. Alberio, S. Gazzarrini, M. Aquila, E. Romano *et al.*, 2015 Optogenetics. Engineering of a light-gated potassium channel. Science 348: 707–710.

Doyle, D. A., J. Morais Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis *et al.*, 1998 The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science 280: 69–77.

Dreyer, I., and N. Uozumi, 2011 Potassium channels in plant cells. FEBS J. 278: 4293–4303. https://doi.org/10.1111/j.1742-4658.2011.08371.x

Enyedi, P., and G. Czirják, 2010 Molecular background of leak K^+ currents: two-pore domain potassium channels. Physiol. Rev. 90: 559–605. https://doi.org/10.1152/physrev.00029.2009

Epshtein, Y., A. P. Chopra, A. Rosenhouse-Dantsker, G. B. Kowalsky, D. E. Logothetis *et al.*, 2009 Identification of a C-terminus domain critical for the sensitivity of Kir2.1 to cholesterol. Proc. Natl. Acad. Sci. USA 106: 8055–8060. https://doi.org/10.1073/pnas.0809847106

Gaber, R. F., C. A. Styles, and G. R. Fink, 1988 TRK1 encodes a plasma membrane protein required for high-affinity potassium transport in Saccharomyces cerevisiae. Mol. Cell. Biol. 8: 2848–2859.

Gazzarrini, S., M. Severino, M. Lombardi, M. Morandi, D. DiFrancesco *et al.*, 2003 The viral potassium channel Kcv: structural and functional features. FEBS Lett. 552: 12–16.

Ghatta, S., D. Nimmagadda, X. Xu, and S. T. O'Rourke, 2006 Large-conductance, calcium-activated potassium channels: structural and functional implications. Pharmacol. Ther. 110: 103–116.

Gianulis, E. C., and M. C. Trudeau, 2011 Rescue of aberrant gating by a genetically encoded PAS (Per-Arnt-Sim) domain in several long QT syndrome mutant human ether-á-go-go-related gene potassium channels. J. Biol. Chem. 286: 22160–22169. https://doi.org/10.1074/jbc.M110.205948

Goldstein, S. A., L. A. Price, D. N. Rosenthal, and M. H. Pausch, 1996 ORK1, a potassium-selective leak channel with two pore domains cloned from Drosophila melanogaster by expression in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 93: 13256–13261.

Graves, F. M., and A. Tinker, 2000 Functional expression of the pore forming subunit of the ATP-sensitive potassium channel in saccharomyces cerevisiae. Biochem. Biophys. Res. Commun. 272: 403–409.

Griffith, L. C., 2001 Potassium channels: the importance of transport signals. Curr. Biol. 11: R226–R228.

Grishin, A., H. Li, E. S. Levitan, and E. Zaks-Makhina, 2006 Identification of gamma-aminobutyric acid receptor-interacting factor 1 (TRAK2) as a trafficking factor for the K+ channel Kir2.1. J. Biol. Chem. 281: 30104–30111.

Guerriero, C. J., and J. L. Brodsky, 2012 The delicate balance between secreted protein folding and endoplasmic reticulum-associated degradation in human physiology. Physiol. Rev. 92: 537–576. https://doi.org/10.1152/physrev.00027.2011

Haass, F. A., M. Jonikas, P. Walter, J. S. Weissman, Y.-N. Jan *et al.*, 2007 Identification of yeast proteins necessary for cell-surface function of a potassium channel. Proc. Natl. Acad. Sci. USA 104: 18079–18084.

Hasenbrink, G., S. Schwarzer, L. Kolacna, J. Ludwig, H. Sychrova *et al.*, 2005 Analysis of the mKir2.1 channel activity in potassium influx defective Saccharomyces cerevisiae strains determined as changes in growth characteristics. FEBS Lett. 579: 1723–1731.

Herrero, L., N. Monroy, and M. E. González, 2013 HIV-1 Vpu protein mediates the transport of potassium in *Saccharomyces cerevisiae*. Biochemistry 52: 171–177. https://doi.org/10.1021/bi3011175

Hibino, H., A. Inanobe, K. Furutani, S. Murakami, I. Findlay *et al.*, 2010 Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol. Rev. 90: 291–366. https://doi.org/10.1152/physrev.00021.2009

Hirsch, R. E., B. D. Lewis, E. P. Spalding, and M. R. Sussman, 1998 A role for the AKT1 potassium channel in plant nutrition. Science 280: 918–921.

Ho, K., C. G. Nichols, W. J. Lederer, J. Lytton, P. M. Vassilev *et al.*, 1993 Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. Nature 362: 31–38.

Huang, X., and L. Y. Jan, 2014 Targeting potassium channels in cancer. J. Cell Biol. 206: 151–162.

Ihara, Y., Y. Tomonoh, M. Deshimaru, B. Zhang, T. Uchida *et al.*, 2016 Retigabine, a Kv7.2/Kv7.3-channel opener, attenuates drug-induced seizures in knock-in mice harboring Kcnq2 mutations. PLoS One 11: e0150095. https://doi.org/10.1371/journal.pone.0150095

Isacoff, E. Y., L. Y. Jan, and D. L. Minor, 2013 Conduits of life's spark: a perspective on ion channel research since the birth of neuron. Neuron 80: 658–674.

Jimenez, V., and R. Docampo, 2012 Molecular and electrophysiological characterization of a novel cation channel of Trypanosoma cruzi. PLoS Pathog. 8: e1002750. https://doi.org/10.1371/journal.ppat.1002750

Kaczorowski, G. J., O. B. McManus, B. T. Priest, and M. L. Garcia, 2008 Ion channels as drug targets: the next GPCRs. J. Gen. Physiol. 131: 399–405. https://doi.org/10.1085/jgp.200709946

Kawada, H., A. Inanobe, and Y. Kurachi, 2016 Isolation of proflavine as a blocker of G protein-gated inward rectifier potassium channels by a cell growth-based screening system. Neuropharmacology 109: 18–28. https://doi.org/10.1016/j.neuropharm.2016.05.016

Kim, J.-B., 2014 Channelopathies. Korean J. Pediatr. 57: 1–18.

Kinclová, O., J. Ramos, S. Potier, and H. Sychrová, 2001 Functional study of the Saccharomyces cerevisiae Nha1p C-terminus. Mol. Microbiol. 40: 656–668.

Klionsky, D. J., P. K. Herman, and S. D. Emr, 1990 The fungal vacuole: composition, function, and biogenesis. Microbiol. Rev. 54: 266–292.

Ko, C. H., and R. F. Gaber, 1991 TRK1 and TRK2 encode structurally related K+ transporters in Saccharomyces cerevisiae. Mol. Cell. Biol. 11: 4266–4273.

Ko, C. H., H. Liang, and R. F. Gaber, 1993 Roles of multiple glucose transporters in Saccharomyces cerevisiae. Mol. Cell. Biol. 13: 638–648.

Kolb, A. R., P. G. Needham, C. Rothenberg, C. J. Guerriero, P. A. Welling *et al.*, 2014 ESCRT regulates surface expression of the Kir2.1 potassium channel. Mol. Biol. Cell 25: 276–289. https://doi.org/10.1091/mbc.E13-07-0394

Kukol, A., and I. T. Arkin, 1999 vpu transmembrane peptide structure obtained by site-specific fourier transform infrared dichroism and global molecular dynamics searching. Biophys. J. 77: 1594–1601.

Kumar, R., 2017 An account of fungal 14–3-3 proteins. Eur. J. Cell Biol. 96: 206–217. https://doi.org/10.1016/j.ejcb.2017.02.006

Kuo, A., J. M. Gulbis, J. F. Antcliff, T. Rahman, E. D. Lowe *et al.*, 2003 Crystal structure of the potassium channel KirBac1.1 in the closed state. Science 300: 1922–1926.

Lai, H. C., M. Grabe, Y. N. Jan, and L. Y. Jan, 2005 The S4 voltage sensor packs against the pore domain in the KAT1 voltage-gated potassium channel. Neuron 47: 395–406.

Li, X., B. Ortega, B. Kim, and P. A. Welling, 2016 A common signal patch drives AP-1 protein-dependent Golgi export of inwardly rectifying potassium channels. J. Biol. Chem. 291: 14963–14972. https://doi.org/10.1074/jbc.M116.729822

Liang, H., C. H. Ko, T. Herman, and R. F. Gaber, 1998 Trinucleotide insertions, deletions, and point mutations in glucose transporters confer K+ uptake in Saccharomyces cerevisiae. Mol. Cell. Biol. 18: 926–935.

Long, S. B., E. B. Campbell, and R. Mackinnon, 2005 Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. Science 309: 897–903.

Lopatin, A. N., E. N. Makhina, and C. G. Nichols, 1994 Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. Nature 372: 366–369.

Loukin, S., Z. Su, X. Zhou, and C. Kung, 2010 Forward genetic analysis reveals multiple gating mechanisms of TRPV4. J. Biol. Chem. 285: 19884–19890.

Loukin, S. H., M. M.-C. Kuo, X.-L. Zhou, W. J. Haynes, C. Kung *et al.*, 2005 Microbial K+ channels. J. Gen. Physiol. 125: 521–527.

Ma, D., T. K. Taneja, B. M. Hagen, B.-Y. Kim, B. Ortega *et al.*, 2011 Golgi export of the Kir2.1 channel is driven by a trafficking signal located within its tertiary structure. Cell 145: 1102–1115. https://doi.org/10.1016/j.cell.2011.06.007

Mackie, T. D., B.-Y. Kim, A. R. Subramanya, D. J. Bain, A. F. O'Donnell *et al.*, 2018 The endosomal trafficking factors CORVET and ESCRT suppress plasma membrane residence of the renal outer medullary potassium channel (ROMK). J. Biol. Chem. 293: 3201–3217. https://doi.org/10.1074/jbc.M117.819086

MacKinnon, R., 1991 Determination of the subunit stoichiometry of a voltage-activated potassium channel. Nature 350: 232–235.

MacKinnon, R., 2003 Potassium channels and the atomic basis of selective ion conduction (Nobel Lecture). Angew. Chem. Int. Ed. Engl. 43: 4265–4277.

Madrid, R., M. J. Gómez, J. Ramos, and A. Rodríguez-Navarro, 1998 Ectopic potassium uptake in trk1 trk2 mutants of Saccharomyces cerevisiae correlates with a highly hyperpolarized membrane potential. J. Biol. Chem. 273: 14838–14844.

Mangano, S., S. Silberstein, and G. E. Santa-María, 2008 Point mutations in the barley HvHAK1 potassium transporter lead to improved K + -nutrition and enhanced resistance to salt stress. FEBS Lett. 582: 3922–3928.

Märquez, J., and R. Serrano, 1996 Multiple transduction pathways regulate the sodium-extrusion gene PMR2/ENA1 during salt stress in yeast. FEBS Lett. 382: 89–92.

Mathie, A., and E. L. Veale, 2009 Neuronal potassium channels., pp. 2792–2797 in *Encyclopedia of Neuroscience.* Springer, Berlin, Heidelberg.

Miller, C., 2000 An overview of the potassium channel family. Genome Biol. 1: REVIEWS0004.

Minor, D. L., S. J. Masseling, Y. N. Jan, and L. Y. Jan, 1999 Transmembrane structure of an inwardly rectifying potassium channel. Cell 96: 879–891.

Muslin, A. J., J. W. Tanner, P. M. Allen, and A. S. Shaw, 1996 Interaction of 14–3-3 with signaling proteins is mediated by the recognition of phosphoserine. Cell 84: 889–897.

Nakamura, R. L., and R. F. Gaber, 1998 Studying ion channels using yeast genetics. *Methods Enzymol.* 293: 89–104.

Nakamura, R. L., and R. F. Gaber, 2009 Ion selectivity of the Kat1 K^+ channel pore. Mol. Membr. Biol. 26: 293–308. https://doi.org/10.1080/09687680903188332

Nakamura, R. L., J. A. Anderson, and R. F. Gaber, 1997 Determination of key structural requirements of a K+ channel pore. J. Biol. Chem. 272: 1011–1018.
Nichols, C. G., and A. N. Lopatin, 1997 Inward rectifier potassium channels. Annu. Rev. Physiol. 59: 171–191.
Nieva, J. L., V. Madan, and L. Carrasco, 2012 Viroporins: structure and biological functions. Nat. Rev. Microbiol. 10: 563–574. https://doi.org/10.1038/nrmicro2820
O'Donnell, B. M., T. D. Mackie, A. R. Subramanya, and J. L. Brodsky, 2017 Endoplasmic reticulum-associated degradation of the renal potassium channel, ROMK, leads to type II Bartter syndrome. J. Biol. Chem. 292: 12813–12827. https://doi.org/10.1074/jbc.M117.786376
Okamoto, Y., and S. Shikano, 2011 Phosphorylation-dependent C-terminal binding of 14–3-3 proteins promotes cell surface expression of HIV co-receptor GPR15. J. Biol. Chem. 286: 7171–7181. https://doi.org/10.1074/jbc.M110.199695
Paynter, J. J., P. Sarkies, I. Andres-Enguix, and S. J. Tucker, 2008 Genetic selection of activatory mutations in KcsA. Channels 2: 413–418.
Paynter, J. J., L. Shang, M. K. Bollepalli, T. Baukrowitz, and S. J. Tucker, 2010 Random mutagenesis screening indicates the absence of a separate H + -sensor in the pH-sensitive Kir channels. Channels 4: 390–396.
Périer, F., K. L. Coulter, H. Liang, C. M. Radeke, R. F. Gaber *et al.*, 1994 Identification of a novel mammalian member of the NSF/CDC48p/Paslp/TBP-1 family through heterologous expression in yeast. FEBS Lett. 351: 286–290.
Persson, B. L., A. Berhe, U. Fristedt, P. Martinez, J. Pattison *et al.*, 1998 Phosphate permeases of Saccharomyces cerevisiae. Biochim. Biophys. Acta 1365: 23–30.
Petrezselyova, S., J. Zahradka, and H. Sychrova, 2010 Saccharomyces cerevisiae BY4741 and W303–1A laboratory strains differ in salt tolerance. Fungal Biol. 114: 144–150.
Plemenitaš, A., T. Konte, C. Gostinčar, and N. G. Cimerman, 2016 Transport systems in halophilic fungi. Adv. Exp. Med. Biol. 892: 307–325.
Plugge, B., S. Gazzarrini, M. Nelson, R. Cerana, J. L. Van Etten *et al.*, 2000 A potassium channel protein encoded by chlorella virus PBCV-1. Science 287: 1641–1644.
Priest, B. T., I. M. Bell, and M. L. Garcia, 2008 Role of hERG potassium channel assays in drug development. Channels (Austin) 2: 87–93.
Rachidi, M., and C. Lopes, 2007 Mental retardation in down syndrome: from gene dosage imbalance to molecular and cellular mechanisms. Neurosci. Res. 59: 349–369.
Rodríguez-Navarro, A., and F. Rubio, 2006 High-affinity potassium and sodium transport systems in plants. J. Exp. Bot. 57: 1149–1160.
Ros, R., G. Lemaillet, A. G. Fonrouge, P. Daram, M. Enjuto *et al.*, 1999 Molecular determinants of the Arabidopsis AKT1 K+ channel ionic selectivity investigated by expression in yeast of randomly mutated channels. Physiol. Plant. 105: 459–468.
Roux, B., and R. MacKinnon, 1999 The cavity and pore helices in the KcsA K+ channel: electrostatic stabilization of monovalent cations. Science 285: 100–102.
Sadja, R., K. Smadja, N. Alagem, and E. Reuveny, 2001 Coupling Gβγ-dependent activation to channel opening via pore elements in inwardly rectifying potassium channels. Neuron 29: 669–680.
Sanguinetti, M. C., and M. Tristani-Firouzi, 2006 hERG potassium channels and cardiac arrhythmia. Nature 440: 463–469.
Schachtman, D. P., and J. I. Schroeder, 1994 Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. Nature 370: 655–658.
Schachtman, D. P., J. I. Schroeder, W. J. Lucas, J. A. Anderson, and R. F. Gaber, 1992 Expression of an inward-rectifying potassium channel by the Arabidopsis KAT1 cDNA. Science 258: 1654–1659.
Schwarzer, S., L. Kolacna, H. Lichtenberg-Fraté, H. Sychrova, and J. Ludwig, 2008 Functional expression of the voltage-gated neuronal mammalian potassium channel rat ether à go-go1 in yeast. FEMS Yeast Res. 8: 405–413. https://doi.org/10.1111/j.1567-1364.2007.00351.x
Sentenac, H., N. Bonneaud, M. Minet, F. Lacroute, J. M. Salmon *et al.*, 1992 Cloning and expression in yeast of a plant potassium ion transport system. Science 256: 663–665.
Shikano, S., B. Coblitz, H. Sun, and M. Li, 2005 Genetic isolation of transport signals directing cell surface expression. Nat. Cell Biol. 7: 985–992.
Stemmer, W. P., 1994 DNA shuffling by random fragmentation and reassembly: in vitro recombination for molecular evolution. Proc. Natl. Acad. Sci. USA 91: 10747–10751.
Tang, W., A. Ruknudin, W. P. Yang, S. Y. Shaw, A. Knickerbocker *et al.*, 1995 Functional expression of a vertebrate inwardly rectifying K+ channel in yeast. Mol. Biol. Cell 6: 1231–1240.
Tempel, B. L., D. M. Papazian, T. L. Schwarz, Y. N. Jan, and L. Y. Jan, 1987 Sequence of a probable potassium channel component encoded at Shaker locus of Drosophila. Science 237: 770–775.
Tong, A. H. Y., and C. Boone, 2006 Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods Mol. Biol. 313: 171–192.
Vacata, V., A. Kotyk, and K. Sigler, 1981 Membrane potentials in yeast cells measured by direct and indirect methods. Biochim. Biophys. Acta 643: 265–268.
Van Goor, F., S. Hadida, P. D. J. Grootenhuis, B. Burton, J. H. Stack *et al.*, 2011 Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc. Natl. Acad. Sci. USA 108: 18843–18848.
Vembar, S. S., and J. L. Brodsky, 2008 One step at a time: endoplasmic reticulum-associated degradation. Nat. Rev. Mol. Cell Biol. 9: 944–957. https://doi.org/10.1038/nrm2546
Vergara, C., R. Latorre, N. V. Marrion, and J. P. Adelman, 1998 Calcium-activated potassium channels. Curr. Opin. Neurobiol. 8: 321–329.
Volkov, V., 2015 Quantitative description of ion transport via plasma membrane of yeast and small cells. Front. Plant Sci. 6: 5–22.
Wang, K., W. Lu, J. Chen, S. Xie, H. Shi *et al.*, 2012 PEDV ORF3 encodes an ion channel protein and regulates virus production. FEBS Lett. 586: 384–391. https://doi.org/10.1016/j.febslet.2012.01.005
Wang, W.-H., P. Yue, P. Sun, and D.-H. Lin, 2010 Regulation and function of potassium channels in aldosterone-sensitive distal nephron. Curr. Opin. Nephrol. Hypertens. 19: 463–470. https://doi.org/10.1097/MNH.0b013e32833c34ec
Wangemann, P., 2006 Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential. J. Physiol. 576: 11–21.
Weir, M., and J. B. Keeney, 2014 PCR mutagenesis and gap repair in yeast, pp. 29–35 in *Yeast Genetics*, edited by J. S. Smith, and D. J. Burke. Humana Press, New York.
Welling, P. A., and K. Ho, 2009 A comprehensive guide to the ROMK potassium channel: form and function in health and disease. Am. J. Physiol. Renal Physiol. 297: F849–F863. https://doi.org/10.1152/ajprenal.00181.2009
Wolfe, D. M., and D. A. Pearce, 2006 Channeling studies in yeast: yeast as a model for channelopathies? Neuromolecular Med. 8: 279–306.
Wright, M. B., J. Ramos, M. J. Gomez, K. Moulder, M. Scherrer *et al.*, 1997 Potassium transport by amino acid permeases in Saccharomyces cerevisiae. J. Biol. Chem. 272: 13647–13652.
Wulff, H., N. A. Castle, and L. A. Pardo, 2009 Voltage-gated potassium channels as therapeutic targets. Nat. Rev. Drug Discov. 8: 982–1001. https://doi.org/10.1038/nrd2983
Ye, Y., H. H. Meyer, and T. A. Rapoport, 2001 The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. Nature 414: 652–656.

Yi, B. A., Y. F. Lin, Y. N. Jan, and L. Y. Jan, 2001 Yeast screen for constitutively active mutant G protein-activated potassium channels. Neuron 29: 657–667.

Yoo, D., L. Fang, A. Mason, B.-Y. Kim, and P. A. Welling, 2005 A phosphorylation-dependent export structure in ROMK (Kir 1.1) channel overrides an endoplasmic reticulum localization signal. J. Biol. Chem. 280: 35281–35289.

Yu, L., X. Jin, N. Cui, Y. Wu, Z. Shi *et al.*, 2012 Rosiglitazone selectively inhibits K(ATP) channels by acting on the K(IR) 6 subunit. Br. J. Pharmacol. 167: 26–36. https://doi.org/10.1111/j.1476-5381.2012.01934.x

Zaks-Makhina, E., Y. Kim, E. Aizenman, and E. S. Levitan, 2004 Novel neuroprotective K+ channel inhibitor identified by high-throughput screening in yeast. Mol. Pharmacol. 65: 214–219.

Zaks-Makhina, E., H. Li, A. Grishin, V. Salvador-Recatala, and E. S. Levitan, 2009 Specific and slow inhibition of the Kir2.1 K+ channel by gambogic acid. J. Biol. Chem. 284: 15432–15438. https://doi.org/10.1074/jbc.M901586200

Zerangue, N., B. Schwappach, Y. N. Jan, and L. Y. Jan, 1999 A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane KATP channels. Neuron 22: 537–548.

Zuzarte, M., K. Heusser, V. Renigunta, G. Schlichthörl, S. Rinné *et al.*, 2009 Intracellular traffic of the K^+ channels TASK-1 and TASK-3: role of N- and C-terminal sorting signals and interaction with 14–3-3 proteins. J. Physiol. 587: 929–952. https://doi.org/10.1113/jphysiol.2008.164756

Communicating editor: J. Rine