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Brief Review

Microbial K^+ Channels

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A survey of the currently available genomes shows that K^+ channels are found in most free-living bacteria, archaea, and protists, indicating early evolution before the divergences of the three major domains of life. Though vertically descended from the primordial K^+ channels, more recent lateral transfers of K^+ channel genes must also have occurred. Variations in the motifs of transmembrane or cytoplasmic domains, S4 features, gating mechanism, and even the putative K^+ filter sequence are observed. Limited experimentation and sequence survey of free-ling versus parasitic bacteria show that K^+ channels are not essential for survival in ordinary circumstances. The lone K^+ channel in *Escherichia coli* is probably not for K^+ uptake but for resting-potential regulation in vivo. Protists (eukaryotic unicells) vary greatly; the fission yeast has no recognizable K^+ channel gene while paramecium has 298, some three times the number found in the human genome.

JGP readers are familiar with the satisfaction of using choice microbial channels to resolve structures at atomic resolutions. However, cherry picking does not provide a view of the tree, let alone the expanding forest of "completed" genomes of 270 prokaryotes (bacteria and archaea), 15 fungi, and 25 protists searchable in the public domain at this writing. Plants and animals constitute only a very small slice of life's diversity, as evident from objective analyses of molecular markers, such as the small subunit ribosomal RNAs (ssrRNAs). These ssrRNAs are universal and the degree of their nucleotide sequence similarity is used to gauge relatedness (Fig. 1). The following is a summary on microbial K^+ channels, as gleaned from genome survey and limited experimentations.

K^+ Filter Evolved Early

Using the canonical K^+ filter sequence as a query, putative genes (open reading frames) corresponding to K^+ channels are found in many of the 270 completed genomes, distributed in nearly all the major taxa of Bacteria and Archaea (formerly archebacteria). The flanks of the filters reveal channel subunits with 2, 4, or 6 transmembrane α-helical domains (abbreviated as 2, 4, 6TM below). The crystal structures of some of them have been solved (Fig. 1). Comparisons of these putative genes show that similar sequences are clustered in groupings largely consistent with groupings based on ssrRNAs, suggesting vertical descent of these channel sequences. For example, prokaryotic 2TM K^+ channels that also bear a domain with a Rossmann fold (the K^+ transport, nucleotide-binding domain [KNT], Roosild et al., 2002; and the related regulator of K^+ conductance domain [RCK]; Jiang et al., 2002). With one exception, all nine 2TM-RCK K^+ channels in one closely related sequence cluster all belong to the γ division of proteobacteria, as defined by ssrRNA and other molecular criteria. Furthermore, no known γ proteobacteria have channels that fall into a different 2TM-RCK cluster (see Kuo et al., 2005). *Aquifex aeolicus*, a bacterium that branched off near the root of the ssrRNA tree of life (Fig. 1), has K^+ channel genes. The simplest interpretation of these observations is that ancestral channels with K^+ filters appeared some 3 to 4×10^9 years ago before the divergence of Bacteria and Archaea. For comparison, the oldest fossils of protist, animal, and plant are ∼1.2, ∼0.6, and $\sim 0.4 \times 10^9$ years old, respectively.

The cytoplasms of all extant organisms studied are rich in K^+, where it serves as a major osmolyte and is considered the cation compatible with macromolecules. Given the presumed employment of K^+ in the primordial chemical evolution, the early appearance of a K^+ filter in later cellular evolution, as deduced from sequence analysis, is not surprising. However, lateral gene transfers among established species at still later times must have also occurred. For example, even though *Escherichia* and *Salmonella* are considered closely related by other criteria, 18% of the *E. coli* genes have been acquired through >200 such lateral transfers since *E. coli* diverged from *Salmonella* $\sim 10^8$ years ago (Doolittle, 1999). Kch, the K^+ channel of *E. coli*, does not appear to be directly related to those in *Salmonella*. One or both of the genes for these two channels must

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Abbreviations used in this paper: PMF, proton motive force; ssrRNA, small subunit ribosomal RNA.

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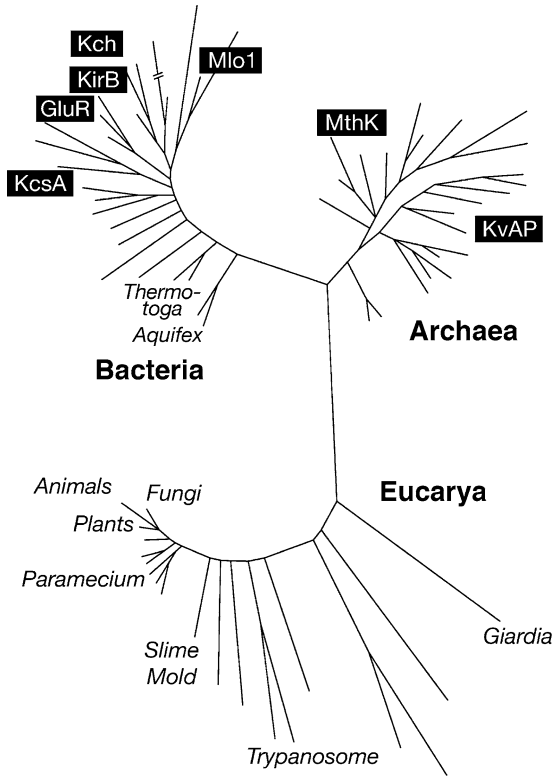

FIGURE 1. A universal phylogenetic tree based on ssrRNA sequences, showing the three domains of life. Note that animals, plants and fungi constitute only a small portion of biological diversity, even among the eukaryotes. Some of the organisms mentioned in the text are marked. Marked are also the K^+ channels, the crystal structures of which have been fully or partially solved: KvAP (6TM, voltage sensitive) from *Aeropyrum pernix*, a thermophilic archaeon, member of *Crenarchaeota*; MthK (2TM + RCK, Ca^{2+} binding, with a gating ring) from the methanogen *Methanobacterium thermoautotrophicum*, a member of *Euarchaeota*; Mlo1 (6TM + cyclic nucleotide–binding domain) from the soil bacterium *Mesorhizobium loti*, an α-proteobacterium (Gram negative); Kch (6TM + RCK) from *Escherichia coli*, a γ-proteobacterium; KirB (KirBAC1.1) a 2TM inward rectifier from *Burkolderia pseudomalli*, a rice pathogen of the β proteobacterium subdivision; GluR (GluR0) from the photosynthetic bacterium *Synechocystis sp* (cyanobacterium); KcsA (2TM) from *Steptomyces lividans*, an actinobactium (Gram positive). Lengths of the branches indicate differences in the nucleotide sequence of the small subunit ribosomal RNAs as a measure of the relatedness of different organisms. Modified from Pace (1997).

have arrived before the species divergence. Even within the genus *Salmonella*, *S. typhi*, *S. paratyphi A*, and *S. typhimurium* each has a clearly related 6TM K^+ channel, but the last alone has an additional 2TM-RCK type K^+ channel. Thus, among the organisms of common descent, the vertical descent from ancient K^+ channels is complicated by later lateral transfers. Small variations in the canonical filter sequence are found in several sequence clusters. The bacterium *Thermotoga maritima*, with a bacteria–archaea hybrid genome, judged to have branched off early from the root and evolved slowly (Nelson et al., 1999), has a 2TM channel with a TVGYSI filter sequence. Similarly, the archaeon *Sulfolobus solfataricus* has a TVGLYS and *S. tokodaii* a TVGLYA filter sequence. If these are indeed functional channels, they could be evolutionary relics before TXGy/fGD became canonized and it would be interesting to directly test their ion selectivities. Details on the numbers, kinds, domain features, as well as entire channel sequences collected from the 270 genomes with annotations can be found in Kuo et al. (2005).

Core Design and Variations

The smallest K^+ channel subunit known is a 94–amino acid peptide from a virus (PBCV-1) in a green alga (*Chlorella*), which, in turn, lives within a paramecium (*P. bursaria*). This channel and its relatives have been expressed in oocytes and showed K^+ permeation, selectivity, and gating (Kang et al., 2004). This and other 2TM subunits presumably configure like the S1-P-S2 core of KcsA of *Streptomyces lividans* (Doyle et al., 1998). The ancestral K^+ channels are presumably such tetramers with the four S2s converging to form the gate. The 2TM motif is found in most prokaryotic taxa and is more common than the 4 or 6TM motifs. Most of the 2TM subunits have additional cytoplasm domains added to the NH_2 and/or the COOH terminus. The most common addition is the RCK and related KTN domain, which presumably configures like the one in MthK channel of *Methanobacterium thermoautotrophicum* (Jiang et al., 2002) or Ktr K^+ import systems of *Methancaldococcus jannaschii* and *Bacillus subtilis* (Roosild et al., 2002). Homologues of animal inwardly rectifying IRK-type channels are found in the cyanobacteria, α proteobacterium, and the β proteobacterium (Durell and Guy, 2001) such as KirBac1.1 of *Burkholderia pseudomallei* (A. Kuo et al., 2003). 6-TM K^+ channel subunits are found in many prokaryotes, often with RCK, KTN, or other cytoplasmic domains. Less common are 6TM channels linked to cyclic nucleotide–binding domains. The crystal structure of such a domain from *Mesorhizobium loti*, a N_2-fixing soil bacterium, has recently been solved (Clayton et al., 2004). The prokaryotic glutamate receptor has the appearance of a fusion of the ligand-binding core of glutamine-binding protein and a KcsA-type K^+ channel. Only five such receptors are known thus far, all in cyanobacteria. The crystal structure of the binding domain in GluR0 from *Synechocystis sp.* has been solved (Mayer et al., 2001).

Most of the 6TM subunits, like *Shaker*, have S4s that bear the positively charged arginines or lysines (rarely histidines) at every third residue. The number of the (+XX) repeats in S4s varies greatly among prokaryotes. Those in *Crocosphaera watsonii* and *Streptococcus mutans* have eight such consecutive repeats. KvAP, the voltage gate K^+ channel from the archaeon *Aeropyrum*

pernix, has been scrutinized with crystallography (Jiang et al., 2003), EPR spectroscopy (Cuello et al., 2004), and other methods. The current rigorous debate on KvAP structures is no doubt familiar to most *JGP* readers. The 4TM S1-S2-S3-P-S4 subunits are found only in two species of *Actinobacteria* and in one α *Proteobacteria* so far. It should be interesting to see if they alone form functional channels and of what characteristics. Occasionally, ORFs predicting K^+ filter sequence attached to only one TM are encountered in both archaeal and bacterial genomes. It is not known whether these are sequencing errors, pseudogenes, or encoders of peptides that noncovalently assemble with other peptides to form functional channels. Two-pore-domain K^+ channels that are found in paramecium (with 12 TMs), fungi (8 TMs), and animals (4 TMs) have not been encountered in the 270 prokaryotic genomes examined. In sum, most major motifs of K^+ channels found in animals are also found in extant prokaryotes.

K^+ Channels Are Probably Not "Essential"

Prokaryotes are found in ordinary as well as many extreme environments unfamiliar to us. DNA analyses after amplifications show huge varieties of organisms, most of which cannot be cultivated yet. The 270 genomes available are a sample that is not only very small but is also biased toward laboratory feasibility as well as human interests, intellectual, agricultural, industrial, military, and mostly medical. Parasitism of the microbes and pathogenicity to the hosts have evolved in many different bacterial lineages. From free-living species, community members, commensals, opportunistic parasites, obligate-extracellular, to obligate-intracellular parasites or organelles such as mitochondria and chloroplasts, the trend is clearly toward genome downsizing (Bentley and Pasrkhill, 2004). Along the way, genes for functions needed for environmental adaptations are jettisoned. Some are apparently discarded early such as those for motility, and some are discarded late such as those for central metabolism. Most, but not all, parasitic prokaryotes have no K^+ channel genes. Although most free-living prokaryotes have recognizable K^+ channel genes, three known species have none. Among the free-living species, those that are metabolically versatile (able to use different energy sources, carbon sources, with and without oxygen) and can thrive in different conditions tend to have multiple K^+ channel genes. With a few exceptions, most parasites from different lineages that have forgone their K^+ channels nonetheless retained their K^+ uptake pumps or exchangers (Kdp, Kup, Trk, or Ktr homologues; for detail see Epstein et al., 1993; Epstein, 2003). It seems that K^+ channels may play less central a role than these active uptake mechanisms.

In the Gram-negative *E. coli*, knocking out its only K^+ channel gene (*kch*) has no detectable effects on growth in various media (Kuo et al., 2005) or on their survival under various stresses applied so far (unpublished data). A recent high-throughput functional analysis also showed that deleting the sole K^+ channel gene of the Gram-positive *Bacillus subitlis* resulted in no detectable phenotypes (see Micado Database). Thus both the evolutionary trend and deletion experiments suggest that K^+ channels are not central to survival in the laboratory. It is currently fashionable among genomicists to define the "minimal gene set", i.e., the housekeeping genes needed to support "life." The subtext, however, is that this is life in pampered laboratory conditions that do not much change. By such a criterion, K^+ channel genes are probably not members of the minimal set. In the reality of the wild, however, free-living organisms encounter environmental changes and need to thrive and not just to survive. The widespread occurrence of K^+ channel genes in the streamlined genomes of most free-living prokaryotes strongly argues that K^+ channels provide adaptive advantages in the wild, even though such advantages are obscure to laboratory science at present. In concluding that K^+ channel is not essential to prokaryotes, we wish to again caution the readers that the experimental evidence is very limited and the genome survey is on a small and biased sample from a vast population.

Kch Probably Regulates V_m of E. coli In Vivo

There are two possible physiological roles of Kch: bulk K^+ uptake or V_m regulation. K^+ uptake pumps and exchanges mentioned above (Kdp, Kup, Trk, or Ktr) (Epstein et al., 1993; Epstein, 2003), but not the Kch channel, were discovered by selecting and analyzing K^+ auxotrophs, mutants that fail to grow unless various amount of K^+ is supplied. Since forward-genetic searches for these K^+ auxotrophs after random mutageneses have no preconceived bias, Kch should have been discovered, had it been one of the uptake mechanisms.

Analysis of K^+-sensitive mutants also shows that Kch is not for bulk K^+ uptake. After a random mutagenesis of the *kch* gene, mutants have been isolated that failed to grow in medium enriched with K^+, but not Na^+ or sorbitol. This K^+-specific sensitivity can be suppressed by a second mutation engineered into the K^+ filter of Kch. Thus, growth inhibition is likely due to K^+ leakage through Kch channels that open uncontrollably due to the first mutations. All seven of these randomly selected "loose cannon" mutations are found to be in the RCK domain, befitting its proposed role in gating regulation. Direct measurement showed no difference in the amount of cytoplasmic K^+ of the wild type and these mutants (M.M.-C. Kuo et al., 2003). Bacteria require a proton motive force (PMF) for nutrient uptake and other processes during growth. Their membrane

potential (V_m) is largely determined by the electrogenic H^+ extrusion and normally not by the resting permeabilities of any ions. The current model in bacteriology is that V_m is adjusted by an unknown mechanism according to the external pH, so that the PMF is maintained and the internal pH remains constant (Harold and Maloney, 1996). We hypothesize that the loose cannon mutations increase the passive K^+ permeability through Kch, locking the V_m at or near E_{K+}, the equilibrium potential of K^+. When $[K^+]_{out}$ is low, the V_m of such a mutant is deep enough to sustain the PMF for growth, but when $[K^+]_{out}$ is high, V_m becomes too shallow to sustain that PMF (M.M.-C. Kuo et al., 2003). Indeed, raising $[K^+]_{out}$ from $\sim 10^{-4}$ to $\sim 10^{-3}$ M stops the growth of such a mutant. Interestingly, we also found that a concomitant increase of $[H^+]_{out}$ from $\sim 10^{-7}$ to $\sim 10^{-6}$ M in the medium restores its growth at 10^{-3} M $[K^+]_{out}$. This Nernstian relationship is consistent with the primary importance of PMF and that the mutant Kch channel indeed locks the V_m at or near E_{K+}. By extension, these results support the notion that the wild-type Kch can open to force the V_m toward E_{K+} under certain appropriate circumstances, since the loose cannon mutant Kch is able to do so at all times.

Roles of K^+ Channels in Other Prokaryotes?

Caution should be exercised in extending from what little we have learned from *E. coli* above. The activities of nine prokaryotic K^+ channels have been shown by their K^+ currents upon reconstitution or heterologous expression (KcsA, LctB, MthK, GluR0, and KvAP), by Rb^+ fluxes upon reconstitution (KirBac1.1, MloK1), by complementation of a triple K^+ auxotrophic mutant (Mjk1), or by K^+-sensitive mutants (Kch) (details on KcsA, LctB, MthK, GluR0, KvAP, KirBac1.1, MloK, Mjk1, and Kch can respectively be found in Schrempf et al., 1995; Wolters et al., 1999; Jiang et al., 2002; Chen et al., 1999; Ruta et al., 2003; Enkvetchakul et al., 2004; Nimigean et al., 2004; Hellmer and Zeilinger, 2003; M.M.-C. Kuo et al., 2003). None of these nine channels were studied in their native membranes, so their roles in the original bacteria or archaea are unknown. Insight into their in vivo functions will be difficult to come by since some have special niches and several are extremophiles. Although K^+ channels are well known to stabilize V_m in excitable cells in animals, those in the adsorptive or secretory epithelia recycle K^+ with the Na^+/K^+ ATPase to sustain the transcellular flux of Na^+ in bulk. In plants, where the H^+ATPase-driven V_m can be more negative than E_{K+}, the AKT1 K^+ channel has been shown to take up K^+ in the roots (Hirsch et al., 1998). Fungi and prokaryotes also use H^+ extrusion instead of the Na^+/K^+ATPase as the primary builder of electrochemical gradients. That Mjk1 (Hellmer and Zeilinger, 2003) or certain KcsA mutants (Irizarry et al., 2002) support the growth of *E. coli* K^+ auxotrophs shows that these channels are capable of taking up K^+ for uptake mutant *E. coli* in dire need of K^+, although it is not known whether they serve as physiological K^+ uptake mechanism in their native cells. In their vast span of habits and habitats, some prokaryotes are likely to use K^+ channels to take up K^+ even though it has not been shown. Until we know more on what K^+ channels do for the living microbes, it would be futile to speculate on what drives the appearance of K^+ channels in the primordial cells.

$\Delta\mu K^+$ Gates TOK1 of Yeast

Fungi constitute the third major eukaryotic kingdom. The genomes of 11 ascomycetes (e.g., yeasts, smuts, and molds) and 4 basiomycetes (e.g., mushrooms) have been completely sequenced. Each usually encodes only one recognizable K^+ channel subunit with a membrane topology of S_1- S_2- S_3- S_4- S_5- P_1- S_6- S_7-P_2-S_8. This 8TM two-pore-domain topology has not been encountered outside the fungal kingdom. The S_1- P_1- S_2- S_3-P_2-S_4 two-pore-domain K^+ channels, common in animals, and the S_1- S_2- S_3- S_4- S_5- P_1- S_6- S_7- S_8- S_9- S_{10}- S_{11}-P_2-S_{12} found in the *Paramecium* genome (unpublished data) are not found among fungi. No known prokaryotes harbor two-pore-domain channels. What segregates these topological types into different taxa is obscure.

Gustin et al. (1986) patch clamped yeast spheroplasts of the budding yeast *Saccharomyces cerevisiae* and discovered the activities of a 20-pS outwardly rectifying K^+ channel, the gene of which was recognized after the completion of the yeast genome by four groups independently and is now called *TOK1* by priority (Ketchum et al., 1995). The outward rectification is not caused by a simple voltage sensitivity as originally thought, but is apparently a function of $\Delta\mu K^+$, the total K^+ motive force, where $\Delta\mu K^+ = V_m - E_k$. This outward rectifier was previously analogized to an inward rectifier inserted backward (Ketchum et al., 1995). This notion is no longer tenable because the previously reported blockage by external Mg^{2+} could not be replicated (Zhou et al., 1995; Lesage et al., 1996). Q_{10} and other analyses indicate that this rectification possibly reflects a rapid collapse of the K^+ filter when $\Delta\mu K^+$ becomes inward (Loukin and Saimi, 1999). In addition to this rapid gating, TOK1 also exhibits a slow gating with temperature dependence typical of gating in other K^+ channels. Growth-stopping mutations that remove the latter gating state were found to be located at the cytoplasmic end of S_6 and S_8 (Loukin et al., 1997), which is now modeled to be at or near the inner gate by analogy to the KcsA structure. Many second-site mutations that subdue the above overly active channels were found to be deletions of the COOH-terminal cytoplasmic domain trailing S_8. Kinetic analyses best fit a model in

which the cytoplasmic domain acts as a "foot in the door" that keeps the inner gate locked to stabilize the open state (Loukin and Saimi, 2002). Interestingly, this domain, when separately produced in addition to the channel body without its COOH tail, still acts as the "foot" to the channel gate (Loukin et al., 2002). The current detailed model of TOK1 gating also describes the filter collapse as a prerequisite for the inner gate movement (Loukin et al., 2002). Deleting TOK1 leads to no detectable growth or tolerance phenotypes in the laboratory, although it has been shown to take up K^+ in a certain mutant context (Fairman et al., 1999).

A Large Variation of K^+ Channel Gene Numbers

Even among eukaryotes, the greatest diversity as judged by molecular markers remains among the unicells, loosely referred to as protists (flagellates, ciliates, diatoms, amoebae, etc.) (Fig. 1). This diversity is reflected in the numbers of K^+ channel genes in their genomes. Plants and multicellular animals typically have tens of K^+ channel genes each; vertebrates have ∼100 each. Table I summarizes the number of recognizable K^+ channel gene in the completely sequenced protist genomes and their genome sizes. Like the prokaryotes discussed above, these organisms are vastly different in their anatomy, lifestyle, and niches. The number of recognizable K^+ channel genes ranges widely among these genomes. One may be surprised that the cellular slime mold, with its complex life cycle, which entails amoebae chemotaxis and aggregation into multicellular slugs and fruiting bodies, has only three K^+ channel genes. Even more puzzling, no K^+ channel genes can be found in the genome of the fission yeast *Schizosaccharomyces pombe*, a free-living organism well studied as a model for eukaryotic cell cycle progression.

There has been a long tradition of electrophysiology of the ciliate *Paramecium* because it is a large cell with an excitable membrane and overt behaviors. Voltage clamp and other methods revealed at least six types of K^+ conductances, activated by de- or hyperpolarization, by Ca^{2+} upon de- or hyperpolarization, by posterior touch, and the resting leak K^+ conductance (Preston et al., 1991). Mutational analyses showed, among other findings, that some of these channels have calmodulin as a detachable subunit and are regulated by calmodulin in a lobe-specific manner (Saimi and Kung, 2002). The recently completely sequenced genome of *Paramecium tetraurelia* has several surprises. This 68-Mb genome of a unicell includes a stunning 298 recognizable K^+ channel genes, some three times the number of K^+ channel genes in the 2,650-Mb human genome. Most of these *Paramecium* genes correspond to the animal 6-TM CNG-type K^+ channels. 15 of them have been randomly chosen and found all to be transcribed (Haynes et al., 2003), and a type of genetic interference experiment even showed that some are correlated with known K^+ currents (Ling et al., 2001). A similarly large number of K^+ channel genes are found in the genome of another ciliate, *Tetrahymena pyriformis*. Speculations on this astounding channel plurality are beyond the scope of this brief review but can be found in Haynes et al. (2003).

TABLE I

Microbial K^+ Channel Genes[a]

Organism	Genome size (Mb)	K^+ channel genes
E. coli[b]	5	1
Fission yeast[c]	12	0
Budding yeast[c]	12	1
Giardia[d]	12	2
Cryptosporidium[e]	10	2
Trypanosoma[f]	35	2
Slime mold[g]	34	3
Plasmodium[h]	30	3
Leishmania[i]	35	3
Diatom[j]	34	18
Chlamydomonas[k]	125	25
Paramecium[l]	68	298

[a]For comparison, *Arabidopsis* (a plant), *Drosophila*, and human genomes are 120, 170, and 2,650 Mb and contain 15, 27, and ∼100 K^+ channel genes, respectively.
[b]Prokaryote genomes range from 0.5 to 9 Mb, containing 0–5 K^+ channel genes.
[c]Fission yeast, *Schizosaccharomyces pombe*; budding yeast, *Saccharomyces cerevisiae*. Fungal genomes are ∼10–15 Mb, having 0–3 K^+ channel genes.
[d]*G. lambria*, branched off near the origin of eukaryotes.
[e]*C. parvum*, the causative agent of cryptosporidiosis.
[f]*T. gambiense*, African sleeping sickness.
[g]*Dictyostelium discoideum*, a cellular slime mold.
[h]*P. falciparum*, malaria.
[i]*L. major*, leishmaniasis.
[j]*Thallassiosira pseudonana.*
[k]*C. reinhardtii*, a green flagellate, technically a plant.
[l]*P. tetraurelia*, a ciliate.

Prospective

Microbes continue to dominate our planet in diversity, in number, and in total mass (Gould, 1996; Pace, 1997; Nee, 2004). This fact, however, will not overcome our anthropocentricity. Our innate pride together with the goal to better human life, let alone to justify our research grants, focuses our attention on human biology. Some feel that we study other life forms, if we have to, only because they are "models" of human at some level. Even as models, microbes have taught us a lot of biology. We understand the central metabolism with the help of mitochondria and yeast, the central dogma through bacteria and phages, and now, ion filtration and hopefully voltage gating and mechanical gating through microbial channels. Some study other life forms simply because they are interesting. The microbial terra incognita is vast and cannot even be fully esti-

mated at present. Even in the most well-understood organism, *E. coli*, ~20% of its genes are still without functional assignments at this writing. Microbes pose many mysteries. For example, why is there a K^+ channel in *Sulfolobus solfataricus* with a positive resting membrane potential? Why do fungi monitor their $\Delta\mu K^+$ and not V_m? How come paramecium requires 298 K^+ channel genes while fission yeast get by with none? The natural history of molecules, such as K^+ channels, is fascinating on its own. Such fascinations have been a major driving force in the activities of the Society of General Physiologists since its inception.

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