Chapter 1
Microbial Senses and Ion Channels

Ching Kung(✉), Xin-Liang Zhou, Zhen-Wei Su, W. John Haynes, Sephan H. Loukin, and Yoshiro Saimi

1.1 The Microbial World ... 2
1.1.1 Microbial Dominance ... 2
1.1.2 Molecular Mechanisms Invented and Conserved in Microbes ... 3
1.2 Microbial Senses ... 5
1.3 Microbial Channels ... 6
1.3.1 The Study of Microbial Ion Channels ... 6
1.3.2 The Lack of Functional Understanding of Microbial Channels ... 7
1.4 Prokaryotic Mechanosensitive Channels ... 8
1.5 Mechanosensitive Channels of Unicellular Eukaryotes ... 9
1.5.1 A Brief History of TRP Channel Studies ... 9
1.5.2 Mechanosensitivity of TRP Channels ... 10
1.5.3 Distribution of TRPs and their Unknown Origins ... 11
1.5.4 TRP Channel of Budding Yeast ... 13
1.5.5 Other Fungal TRP Homologs ... 16
1.5.6. The Submolecular Basis of TRP Mechanosensitivity – a Crucial Question ... 16
1.6 Conclusion ... 18
References ... 19

Abstract The complexity of animals and plants is due largely to cellular arrangement. The structures and activities of macromolecules had, however, evolved in early microbes long before the appearance of this complexity. Among such molecules are those that sense light, heat, force, water, and ligands. Though historically and didactically associated with the nervous system, ion channels also have deep evolutionary roots. For example, force sensing with channels, which likely began as water sensing through membrane stretch generated by osmotic pressure, must be ancient and is universal in extant species. Extant microbial species, such as the model bacterium *Escherichia coli* and yeast *Saccharomyces cerevisiae*, are equipped with stretch-activated channels. The ion channel proteins MscL and MscS show clearly that these bacterial channels receive stretch forces from the lipid bilayer. TRPY1, the mechanosensitive channel in yeast, is being

305 R.M. Bock Laboratories, 1525 Linden Drive, University of Wisconsin Madison, WI 53706, USA, ckung@wisc.edu

B. Martinac (ed.), *Sensing with Ion Channels. Springer Series in Biophysics 11*

developed towards a similar basic understanding of channels of the TRP (transient-receptor-potential) superfamily. TRPY1 resides in the vacuolar membrane and releases Ca^{2+} from the vacuole to the cytoplasm upon hyperosmotic shock. Unlike in most TRP preparations from animals, the mechanosensitivity of TRPY1 can be examined directly under patch clamp in either whole-vacuole mode or excised patch mode. The combination of direct biophysical examination in vitro with powerful microbial genetics in vivo should complement the study of mechanosensations of complex animals and plants.

1.1 The Microbial World

The game of 20 questions teaches children that the world consists of animals, vegetables, and minerals. Children become adults who continue to ignore the bulk of the biological world – the microbes. The origin of this ignorance is deeper than childhood indoctrination: we came from animals that deal with predators, prey, parents, progeny, peers, and possessions, all about our own size. Although science has revealed invisible microbes, to most people they are but parasites, pathogens, and pests. Even for scientists, the hard-wired animal-plant-mineral illusion, like the geocentric illusion of sunrise and sunset, is hard to dispel in daily life.

1.1.1 Microbial Dominance

Our ignorance and bias notwithstanding, microbes reign supreme on this planet in diversity, in number, and in mass (Woese 1994). This is true in the past, the present, and, in all likelihood, also in the future. Earth was formed ~4.6×10^9 years ago. Life began ~3.9×10^9 years ago at high temperatures, with liquid water, and under an anoxic reducing atmosphere. The appearance of ancestral cyanobacteria (~2.9×10^9 years) gradually built up O_2 in the atmosphere, predating the appearance of modern eukaryotes (~1.5×10^9 years). Thus a large part of our planetary history (from ~3.9×10^9 years ago to 1.5×10^9 years ago) saw forms that we would now classified as bacteria, archaea, and the microbial ancestors of eukaryotes. There were no animals or plants, let alone the human species, which is only 10^5 years old. The microbial way of life has continued to be successful to the present. Even a casual look at the current tree of life reveals that the greatest diversities are among microbes (Woese 2000) (Fig. 1.1). Contrary to the notion of evolution being a progression, with new forms replacing old, plants and animals are add-ons pasted onto the microbial diversity. Today, besides the niches occupied by animals and plants, microbes continue to thrive deep underground, in arctic waters, in hydrothermal vents, in the "Dead Sea". They are also present on, and in, just about every animal and plant. [There are more that 500 kinds of bacteria in our oral cavity! (Becker et al. 2002)]. As to the future, this planet will not become sterile with yet

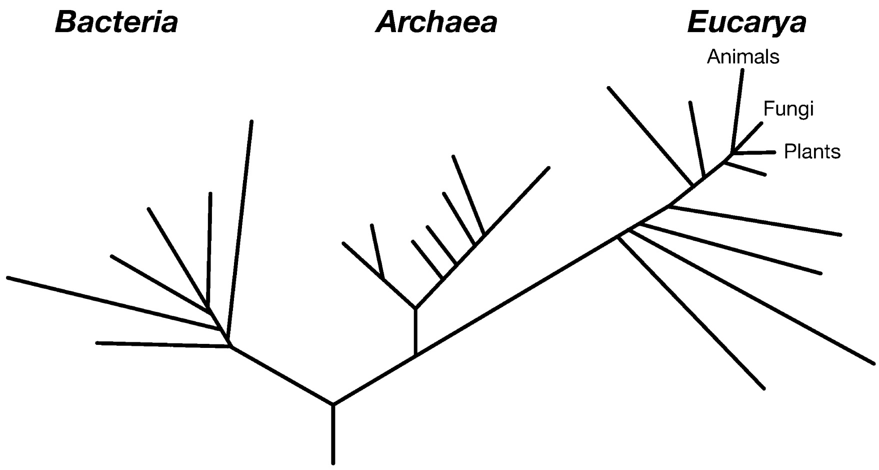

Fig. 1.1 The tree of life showing the relatedness of the three branches: *Bacteria*, *Archaea*, and *Eucarya*. Note that even among eukaryotes, multicellular macrobes are the minority (see Woese 2000)

another "wave of mass extinction", natural or man-made. Given the variety of ways in which microbes extract energy and the variety of niches they currently occupy, there is little doubt that microbes will survive such "disasters" and carry on for billions of years to come.

Another common misconception is that eukaryotes mean animals and plants. Though the visible animals and plants loom large in our mind, they are in fact a small part of the eukaryotic diversity (Embley and Martin 2006). Currently, taxonomists divide *Eukarya* into six clusters (Adl et al. 2005), one of which comprises both animals and fungi. The nondescript term "protists" in the common currency of scientific discourse in fact comprises the greatest variations Nature has devised for eukaryotes. The animalcentric, if not anthropocentric, view of physiology often overlooks this true diversity. For example, description and classification of transient-receptor-potential (TRP) channels usually deal only with those in mammals, with those of the fly and the worm thrown in as honorary guests. However, in reality, TRP channel genes are found in fungal genomes as well as those of ciliates, flagellates, slime molds, Trypanosome, *Leishmania*, etc., indicating an early origin (see Fig. 1.2 and Sect. 1.5.3 below).

1.1.2 Molecular Mechanisms Invented and Conserved in Microbes

We commonly speak of higher and lower organisms. Our self-appointed "higher" status can be defended only on the grounds of complexity. Complexity in biology has to do with the arrangement of cells and does not correlate with the plurality of

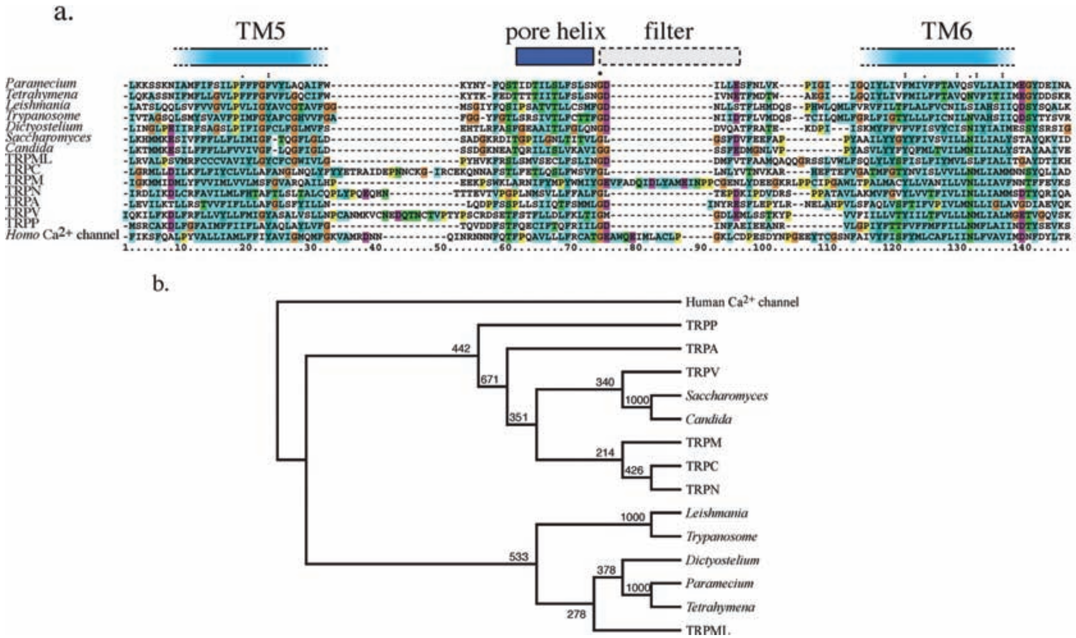

Fig. 1.2 An alignment and unrooted cladogram of the major family members of TRP (transient-receptor-potential) channels. **a** A Clustal W (Gonnet 250) alignment made using the program Clustal X. Several representative TRP genes found in various protists were aligned with a member of each major family of TRP channel along with a calcium channel (conserved in multicellular organisms) for comparison. The protist channels were found by BLAST searching genomic sequences currently available for each organism listed. The majority of these protist sequences were predicted by automated annotation procedures at the respective sequencing centers. The *Leishmania* sequence was recently described (Chenik et al. 2005). The color coding represents both a frequency of conservation and the chemical relatedness of residue side chains. **b** A single unrooted bootstrapped cladogram constructed using the neighbor joining method (Saitou and Nei 1987) drawn from the Clustal W (Gonnet 250) alignment (with all gapped sequence removed) shown above made using the program Clustal X. From a comparison of 1,000 possible trees, the numbers represent the number of trees in which the branches shown were present. The calcium channel was selected as the outgroup for the purpose of drawing this tree

molecular components. While the chimp and the mouse have the same number of genes as we do, the unicellular *Paramecium* actually has nearly twice that number (Aury et al. 2006)!

Not only do animals not have more types of molecules, their molecules are no more intricate than those of microbes. Students of modern biology are familiar with bacterial cytochromes, rhodopsins, ribosomes, Kreb's cycle, oxidative phosphorylation, photosynthesis, etc. Even the cytoskeleton, which is often cited as a hallmark of eukaryotes, is not exclusive. Distant homologs of actin, tubulin, etc., have been found to function in prokaryotes (Shih and Rothfield 2006). Ion channels – the focus of this volume – are clearly ancient and are almost ubiquitous among free-living microbes (see Sect. 1.3).

Of course, the universality of the structures and functions of different kinds of DNAs, RNAs, proteins, and lipids reflects their early evolutionary origin. Honed by selection among early cellular forms, they now continue to serve in all three branches of life. It therefore seems obvious that, if one is to study the basic physical and chemical working of these molecules, it makes little difference whether they are taken from a bacterium, a worm, or a human.

Unlike humans or worms, microbes can be cultivated in small spaces, in short time frames, and therefore with little expense. The cultures can be clonal, therefore having cells of the same genotype and phenotype. The streamlined genomes of most microbes obviate the need to deal with introns and other intervening sequences. Their smaller genomes make genetic and genomic exploration much simpler. The molecular tools collected through the last 50 years of the molecular biology revolution have made *Escherichia coli* and *Saccharomyces cerevisiae* convenient in vivo laboratories and factories. Moreover, there is little expressed ethical concern and therefore little risk of animal-rights objections when sacrificing billions of microbes. It is therefore no accident that much of the molecular insights into the workings of DNA, RNA, enzymes, and now ion channels, have come from investigation of microbial materials.

1.2 Microbial Senses

A large part of the bacteriology literature deals with microbial responses to various "environmental stresses", i.e., changes in temperature, hydration, pH, carbon or nitrogen source, and energy source. From this literature, such basic concepts as end-product feedback inhibition of enzymes, promoter regulation by transcription factors, and the concept of second messengers, such as cAMP, have been derived. Here, the sensors are often the enzymes and the promoters. In these cases, the routes from sensing to response do not involve ion channels, and are not further reviewed here.

Many bacteria and archaea are also capable of chemotaxis – the active seeking of attractants and avoiding of repellents. Here, the receptors are trimers of dimeric binding subunits that straddle the plasma membrane, and the binding signal is

http://www.springer.com/978-3-540-72683-8

Sensing with Ion Channels
Martinac, B. (Ed.)
2008, XXIII, 304 p., Hardcover
ISBN: 978-3-540-72683-8