involved in stromatolite growth are unknown. However, microbial mats have been identified in 3.42-billion-year-old rocks from South Africa[5], and the chemistry of the rocks indicates that anoxygenic phototrophs (photosynthetic organisms that do not produce oxygen) were probably the dominant mat-forming organisms.

What about oxygen? An aerobic biosphere was not possible before the evolution of oxygen-producing cyanobacteria, and oxygen-producing photosynthesis accomplishes the vast majority of the primary production today. So whether or not cyanobacteria were present is crucial to our understanding of the nature of the biosphere 3.5 billion years ago.

To explore this issue, we return to Western Australia and to the Apex Chert, which is slightly younger than the Dresser Formation. Here, a series of organic remains has been interpreted as possibly being those of cyanobacteria[6]. Unfortunately, a cyanobacterial affiliation cannot be decided by morphology alone, and the interpretation of these remains as biogenic is controversial[7]. We cannot, therefore, be confident that oxygen-producing organisms existed 3.5 billion years ago. But we can be certain that an active biosphere was in place. It was probably quite complex, including many of the components of modern anaerobic ecosystems. Of these, we can identify anoxygenic phototrophs, sulphate reducers and methanogens.

Another intriguing aspect to Ueno and colleagues' research[2] is its geological setting. The silica dykes housing the methane originated from deep in Earth's crust — maybe as much as 1 kilometre below the surface[8]. This is not the normal bubbly lake or coastal marine sediment usually associated with methanogenesis. These results, therefore, point to an active subsurface biosphere. A deep subsurface biosphere exists today, and by some estimates[9] it is as massive as the one on the Earth's surface. The results of Ueno *et al.* do not tell us anything about the magnitude of the ancient subsurface biosphere. But the presence of life within Earth's crust, combined with good evidence for life in surface environments, suggest that by 3.5 billion years ago the Earth was teeming with microorganisms. ■

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MEMBRANE BIOLOGY

Permutations of permeability

William N. Zagotta

The first glimpse into the molecular basis of how sodium ions are transported across cell membranes by ion channels shows that cation-selective channels are variations on potassium channels.

Ion channels are nature's gatekeepers: by allowing certain ions to pass across the membrane and excluding others, these proteins control the electrical state of the cell. Channels selective for sodium ions allow Na^+ to enter the cell, causing depolarization of the membrane. In the case of neurons, this can trigger the cell to fire an action potential or release a neurotransmitter. Channels selective for potassium ions allow K^+ to exit the cell, causing hyperpolarization of the membrane and decreasing the cell's electrical excitability[1]. The structural basis for K^+ selectivity is well established, mainly from work on the bacterial K^+ channel KcsA (refs 2–4), but the basis for Na^+ permeability has been unclear. In this issue, Jiang and colleagues (page 570)[5] reveal the first atomic structure of a channel with both Na^+ and K^+ permeability, the NaK channel from *Bacillus cereus*.

Given their opposite roles, it is surprising that many Na^+ channels and K^+ channels are structurally related. Both are tetrameric (with four subunits) or pseudotetrameric channels with a 'selectivity filter' in the narrowest part of the pore, formed from the P-loop between the inner and outer transmembrane helices (Fig. 1). This motif is shared by many other ion channels that range in selectivity from highly K^+-selective to highly Na^+- or Ca^{2+}-selective (Fig. 2, overleaf). Highly K^+-selective channels almost all possess the amino-acid signature sequence TVGYG in their selectivity filter. Together with the hydroxyl group of the threonine (T), the backbone carbonyls from the signature sequence form four K^+-binding sites in the selectivity filter (Fig. 2). The approximately 1,000:1 selectivity for K^+ over Na^+ of these channels arises from the K^+ selectivity of these sites and mutual repulsion between the K^+ ions bound to the sites[6].

The similarity of selectivity filter sequences to the signature sequence, however, breaks down as channels become more Na^+ and Ca^{2+} permeable (Fig. 2). The hyperpolarization-activated HCN channels exhibit only about a 4:1 selectivity for K^+ over Na^+, and the transient receptor potential (TRP) channels and cyclic nucleotide-gated (CNG) channels are equally selective for K^+ and Na^+ (refs 7, 8).

Also, as the K^+ selectivity decreases, a new feature emerges in these channels — Ca^{2+} permeability. Both the TRP and CNG channels are highly permeable to Ca^{2+}. Paradoxically, Ca^{2+} also blocks some of these channels at low concentrations. Ca^{2+} permeability is thought to arise at high concentrations of Ca^{2+} from the binding of multiple Ca^{2+} ions that exhibit mutual repulsion[1]. This property is finely honed in the voltage-gated Ca^{2+} channels to produce extremely high Ca^{2+}-selectivity.

Jiang and colleagues[5] reveal that the properties of Na^+ permeability and Ca^{2+} block also seem to be shared by the NaK channel from *B. cereus*. NaK contains a selectivity filter sequence (TVGD) similar to that of most CNG channels (TIGE). The structure of the NaK channel, therefore, gives us our first glimpse into how differences in the sequence of the selectivity filter can produce varying Na^+ and Ca^{2+} permeability in these channels.

Overall, the backbone structure of NaK is very similar to that of KcsA. It is composed of four subunits surrounding a central pore. Each subunit has two membrane-spanning helices, M1 (outer helix) and M2 (inner helix), with an intervening P-loop (Fig. 1). The pore is lined by the M2 on the intracellular side, and by the P-loops on the extracellular side.

The NaK structure diverges from KcsA in the selectivity filter. Molecular dynamics simulations implicated the first two binding sites in the selectivity filter of KcsA as the most K^+-selective sites[9]. In NaK, these two binding sites are replaced by a 'pore vestibule' that can accommodate just a single ion (Fig. 2). This

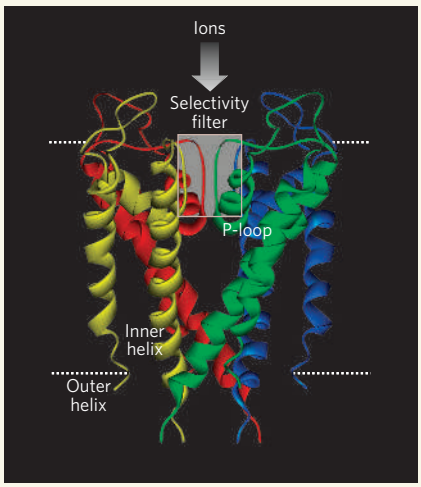

Figure 1 | Structure of the KcsA potassium ion channel. The four subunits of the channel are shown in different colours. They surround a central pore, guarded by the selectivity filter made up of the P-loops from each of the subunits. This filter determines the permeability of the pore to different ions. Dotted lines show the approximate position of the cell membrane.

CHEMISTRY

Perkin, the mauve maker

One hundred and fifty years ago this week, a teenager experimenting in his makeshift home laboratory made a discovery that in effect launched the modern chemicals industry. William Perkin was an 18-year-old student of August Wilhelm Hofmann at the Royal College of Chemistry in London, working on the chemical synthesis of natural products. In a classic case of serendipity, Perkin chanced on his famous 'aniline mauve' dye while attempting to synthesize something else entirely: quinine, then the only known remedy for malaria.

As a student of the renowned German chemist Justus von Liebig, Hofmann had made a name for himself by showing that a basic compound called aniline, obtained from coal tar, had the same properties as a compound distilled from raw, plant-derived indigo. Coal tar was the residue of coal-gas production, and there was intense interest in finding uses for the aromatic compounds, such as aniline, that could be extracted from it.

Working at home, Perkin tried to use an aniline derivative to make quinine by oxidation, based on the similarity of their chemical formulae (their molecular structures are very different). The reaction produced only a reddish sludge. But when the inquisitive Perkin tried the reaction using aniline instead, he got a black precipitate that dissolved in methylated spirits to give a purple solution. Textiles and dyeing being big business at the time, Perkin was astute enough to test the coloured compound on silk, which it dyed richly. Hitherto, almost all dyes were natural compounds extracted from plants and animals.

Boldly, Perkin persuaded his father and brother to set up a small factory with him to manufacture the dye, which he called mauve; the picture here is of a shawl, dyed with mauve, from 1862. The Perkins and others (including Hofmann) soon discovered a whole rainbow of aniline dyes, and by the mid-1860s aniline-dye companies included the nascent giants of today's chemicals industry, such as Bayer, Hoechst and BASF.

SCIENCE MUSEUM

Philip Ball

ion is not bound by carbonyl oxygens, as it would be in KcsA, because of a rearrangement in the backbone of the selectivity filter, presumably caused by the change of tyrosine (Y) in KcsA to aspartate (D) in NaK. The third and fourth ion-binding sites are structurally very similar to their counterparts in KcsA, but can bind to K^+ and Na^+ with virtually no rearrangements in the protein. These differences might make the NaK pore seem shorter in electrophysiological and flux studies than the K^+ channel pore[1].

Another intriguing finding in the NaK structure was the presence of a divalent binding site, capable of binding Ca^{2+}, at the extracellular entrance to the NaK selectivity filter. Although an extracellular Ca^{2+}-binding site has been proposed in many Ca^{2+}-permeable channels, this site has always been thought to involve the carboxylate of an acidic residue in the selectivity filter. Surprisingly, in the NaK structure this acidic residue, the aspartate, is facing away from the pore, and the binding site for Ca^{2+} is formed by backbone carbonyls of the glycine residues at the extracellular end of the selectivity filter sequence. In CNG channels, this acidic residue has also been proposed to be involved in regulating a number of permeation and blocking properties, including ion selectivity, divalent block, proton block and state-dependent tetracaine block[10]. Although the sequence similarity is weaker between CNG channels and voltage-gated Ca^{2+} and Na^+ channels (Fig. 2), a similar role for an acidic residue has been suggested in those channels[1].

The finding that the aspartic-acid residue in the NaK structure does not bind directly to the ions raises some interesting questions. Can the charge on this residue exhibit a through-space electrostatic interaction with ions in the pore? Do mutations of this residue alter the backbone structure and so indirectly alter ion-binding sites? Could the position of this acidic residue be different in open channels? And, is the NaK channel pore structure representative of eukaryotic CNG channels and voltage-gated Ca^{2+} channels? As always, X-ray crystallography has answered many questions with astonishing clarity, but there are yet more to be addressed. ■

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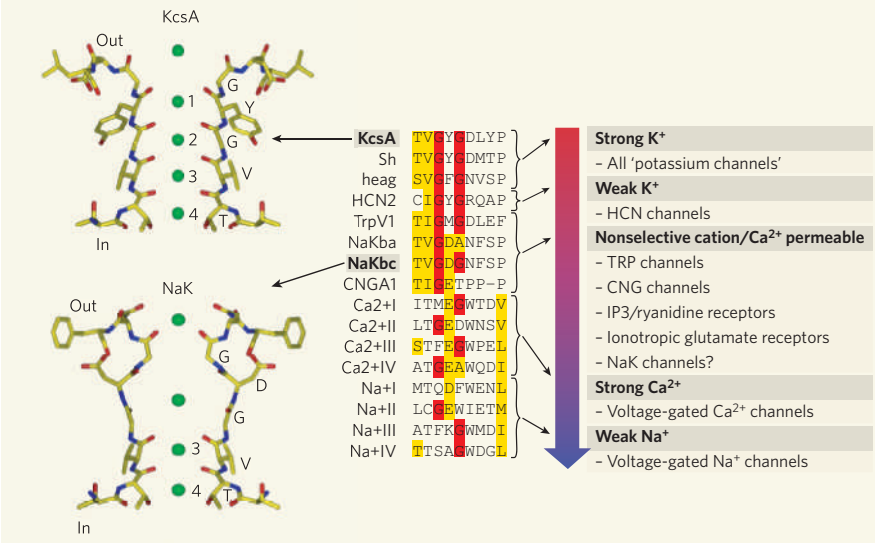

Figure 2 | Mechanism of ion selectivity. The amino-acid sequences from a range of ion channels (centre) show remarkable similarity in the region of the selectivity filter. Red highlights show the conserved glycines in the 'signature' sequence TVGYG found in the potassium ion channel KcsA. Yellow highlights show amino acids that are chemically similar. As the filter sequence becomes less conserved, the channels lose their selectivity for K^+ over Na^+ and become more permeable to Ca^{2+}. The structure of the nonselective cation channel NaK from *Bacillus cereus* has been solved by Jiang and colleagues[5]. Comparison of the selectivity filters of NaK and KcsA (left) provides structural clues to sodium-ion permeability.

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