1 Introduction

Ion channels are crucial components in the activity of living cells. They sit in the membranes of the cell and allow particular ions to pass through them from one side of the membrane to the other. Since they may be either open or closed, they can exert control over this ion movement by switching it on or off. Their study is one of the major endeavours of modern cell biology.

Most animal cells are in contact with moderately salty solutions such as sea water or the body fluids of the blood or the intercellular spaces. These extracellular solutions usually contain a relatively high concentration of sodium and chloride ions, and much lower concentrations of potassium, calcium and other ions. The plasma membrane acts as a barrier separating the cell contents from the outside, so that the ionic concentrations inside the cell can be maintained at levels appreciably different from those in the extracellular fluids. There is also an electrical potential difference between the cytoplasm and the external medium. This combines with the ionic concentration gradients to make an electrochemical gradient across the plasma membrane for each ion species. Cells make considerable use of these electrochemical gradients in their signalling and control systems: when the appropriate ion channels are open, the ions will flow down the gradients into or out of the cell.

The plasma membrane is composed of two layers of tightly packed lipid molecules (fig. 1.1). This structure is not readily permeable to polar molecules such as sugars or amino acids or to charged particles such as sodium or chloride ions. Such substances can pass through the membrane only via special protein molecules embedded in it. Ion channels form one group of these proteins; they permit rapid flow of ions across the membrane. Other membrane transport proteins may act as carriers for ions or other substances, transporting them at much lower rates than do channels, and sometimes up an electrochemical or concentration gradient.

An ion channel is usually composed of merely a few protein molecules,

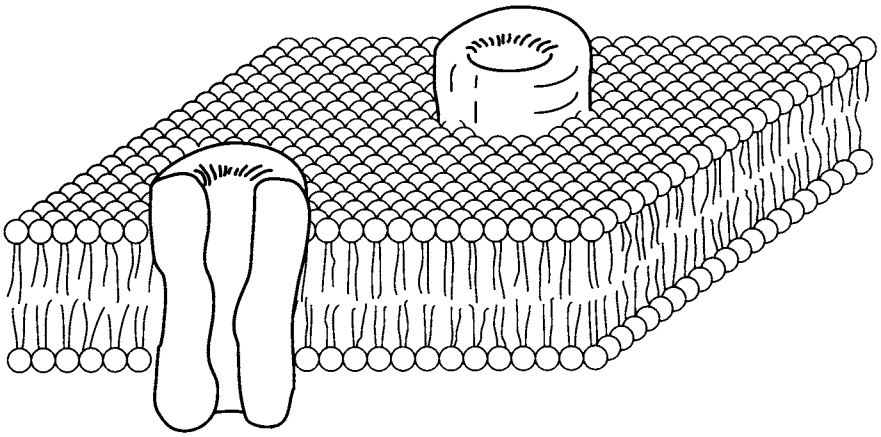

Fig. 1.1. Ion channels in the plasma membrane. The membrane phospholipids are arranged in a bimolecular layer with their polar heads on the outside and their hydrophobic tails inside. The bilayer is about 30 Å thick. Sitting in it are various intrinsic proteins, including the channels shown here.

sometimes of only one. It contains a central aqueous pore that can be opened by conformational change to allow ions to flow from one side of the membrane to the other. This arrangement allows ions to flow through the channel at rates up to 100 million ions per second when it is open. This ion movement forms an electric current that is sufficient to be measured by suitable techniques; hence we can observe the activity of an individual channel, and so of an individual molecule or molecular complex, just as it happens.

Ion channels vary considerably in their *gating*, by which we mean the factors that make them open or close. Some channels are opened by combination with particular chemicals outside or inside the cell, such as neurotransmitters or cytoplasmic messenger molecules. Others are opened by changes in the voltage across the membrane, and yet others by sensory stimuli of various kinds.

Channels show *selectivity* in the ions to which they are permeable. Some of them will permit only particular ions to pass through, such as sodium, potassium, calcium or chloride ions; others are selective for broader groups of ions, such as monovalent cations, or cations in general. Channel scientists have been much concerned with measuring this selectivity and with looking for explanations as to why it exists.

These two aspects of channel functioning – gating and selectivity – are conceptually distinct from one another. Molecular structure studies confirm this view: parts of the channel molecule concerned with gating seem to be separate from those concerned with selectivity.

We can illustrate what channels do with the example of the pancreatic β cell, as is shown in fig. 1.2. The β cells are concerned with the production and secretion of insulin, the hormone that controls blood sugar levels. Two

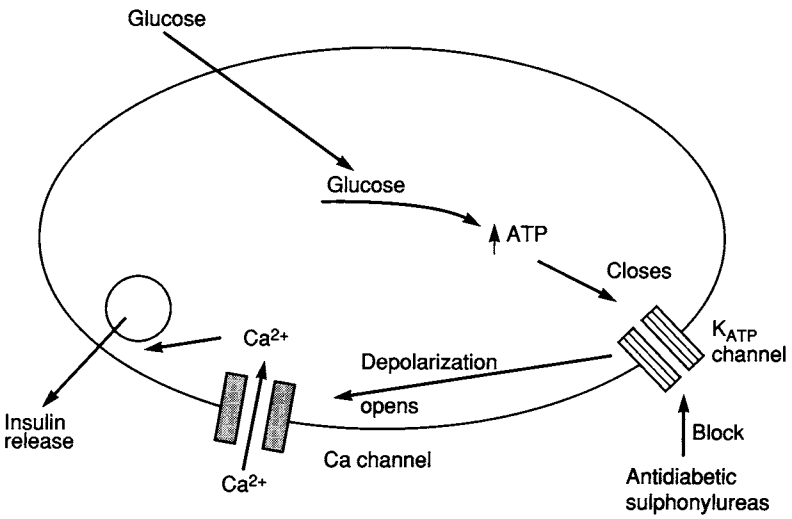

Fig. 1.2. How ATP-sensitive potassium channels and voltage-gated calcium channels are involved in the secretion of insulin from pancreatic β cells. (Courtesy of Professor N.B. Standen.)

types of ion channel in the plasma membrane are involved in this. The ATP-sensitive potassium channels close when the internal concentration of ATP (adenosine triphosphate) rises to an appropriate level. The voltage gated calcium channels open when the membrane potential of the cell becomes less negative by a sufficient amount.

When blood glucose levels are low, the ATP-sensitive potassium channels in the β cell are open so that potassium ions can flow out of the cell, and this ensures that the membrane potential is kept at a negative value. When blood glucose rises after a meal, uptake of glucose into the cell leads to a rise in its ATP levels, and this makes the potassium channels close. This leads to a change in the membrane potential to less negative values, and this in turn promotes the opening of voltage-gated calcium channels. The resulting inflow of calcium ions raises the internal calcium ion concentration, which then acts as a trigger for the release of insulin from the cell.

Knowledge of how the β cell and its channels work not only is of interest in itself and as part of our understanding of how the body works, it also helps greatly in the search for cures for diabetes, the disease in which the insulin control system does not work properly. One approach for some patients is to use drugs that will block the ATP-sensitive potassium channels and so promote opening of the calcium channels.

The discovery of ion channels

The concept of ion channels was around for many years before it was possible to examine their properties as individual entities. In their classic study of

the nature of the nerve impulse in 1952, Alan Hodgkin and Andrew Huxley provided a mathematical description of the flow of sodium and potassium ions through the nerve axon membrane. They found that the relations between the membrane potential and the ionic currents were very steep. It seemed likely that the changes in ionic permeability were associated with the movement of some electrically charged particles within the membrane. They could not detect this charge movement at that time whereas the currents produced by ion movement were readily observed, so they deduced there must be many ions moving across the membrane for each movable membrane charge. They therefore proposed that the ionic currents were localized at particular sites ('active patches', as they called them) in the membrane. It is these sites that later became known as voltage-gated sodium and potassium channels.

Hodgkin and Richard Keynes investigated the potassium permeability of nerve axons in 1955. They found that the movements of radioactive potassium ions across the axon membrane could best be explained if the ions passed through narrow pores in single file, and they used the word 'channel' to describe these ideas.

At the neuromuscular junction, the electrical changes following the action of the neurotransmitter substance acetylcholine on the muscle cell were investigated by Paul Fatt and Bernard Katz in 1951. It was reasonable to suppose that the ionic currents involved passed through particular sites in the muscle cell membrane, channels that were activated by acetylcholine. Both here and with the nerve impulse one could at that time measure only the currents produced by flow through some hundreds or thousands of channels at once.

The 1960s saw the discovery of a number of specific channel-blocking agents. Tetrodotoxin, for example, from the fugu puffer fish, specifically blocks voltage-gated sodium channels. This provided very convincing confirmation that the sodium and potassium channels of nerve axons really are separate from each other. It also allowed nerve potassium channels to be studied on their own, allowed estimates of channel densities in the membrane to be made, and ultimately proved crucial in the biochemical isolation of sodium channels.

The 1960s and 1970s also saw increasingly sophisticated approaches to the investigation of ion channels in nerve and muscle. Clay Armstrong used quaternary ammonium ions as blocking agents to probe the nature of potassium channels. Bertil Hille measured the permeability of channels to ions of different sizes, and so was able to estimate the minimum dimensions of the channel pore. These indirect methods gave some idea of channel properties and were highly influential in the conceptual models they produced, but it was still not possible to examine the activity of the individual channels directly.

Around 1970 some clues as to individual channel action were emerging.

Katz and Ricardo Miledi discovered that the end-plate membrane potential becomes markedly 'noisy' in the presence of acetylcholine, and they interpreted this as a series of 'elementary events' produced by the opening and closing of individual channels as they bound and released acetylcholine molecules. This led to a series of studies by Charles Stevens and others using the techniques of fluctuation analysis to gain information about the size and duration of these events.

A parallel development came from studies on artificial lipid bilayer membranes. Stephen Hladky and Denis Haydon found that when very small amounts of the antibiotic gramicidin were introduced into such a membrane, its conductance to electrical current flow fluctuated in a stepwise fashion. It looked as though each gramicidin molecule contained an aqueous pore that would permit the flow of monovalent cations through it. Could the ion channels of natural cell membranes act in a similar way? To answer this question it was first necessary to solve the difficult technical problem of how to record the tiny currents that must pass through single channels.

The breakthrough came in 1976 with the development of the patch clamp technique by Erwin Neher and Bert Sakmann. They used a glass microelectrode with a polished tip that could be applied to the surface of a cell so as to isolate a small patch of membrane. The voltage across this patch was held steady ('clamped') by a feedback amplifier so that they could measure the currents flowing through the individual ion channels in it. This revolutionary technique proved to be increasingly productive, especially after further technical improvements, so that now in each year thousands of scientific papers report results acquired by using it. In 1991 Neher and Sakmann were awarded the Nobel Prize for Physiology and Medicine for their work (see Neher, 1992; Sakmann, 1992).

Figure 1.3 shows the sort of record that is obtained by the patch clamp technique. The channel is closed for much of the time, so no current flows across the patch of membrane that contains it. But at irregular intervals the channel opens for a short time, producing a pulse of current. Successive current pulses are always of much the same size in any one experiment, suggesting that the channel is either open or closed, and not half open (although we shall see later that there are exceptions to this rule). The durations of the pulses, however, and the intervals between them, vary in an apparently random fashion from one pulse to the next. Hence the openings and closings of channels are *stochastic* events. This means that, as with many other molecular processes, we can predict when they will occur only in terms of statistical probabilities. But one of the great features of the patch clamp method is that it allows us to observe these stochastic changes in single ion channels as they actually happen: we can watch individual protein molecules in action.

The other great technical advance in the study of ion channels has been the

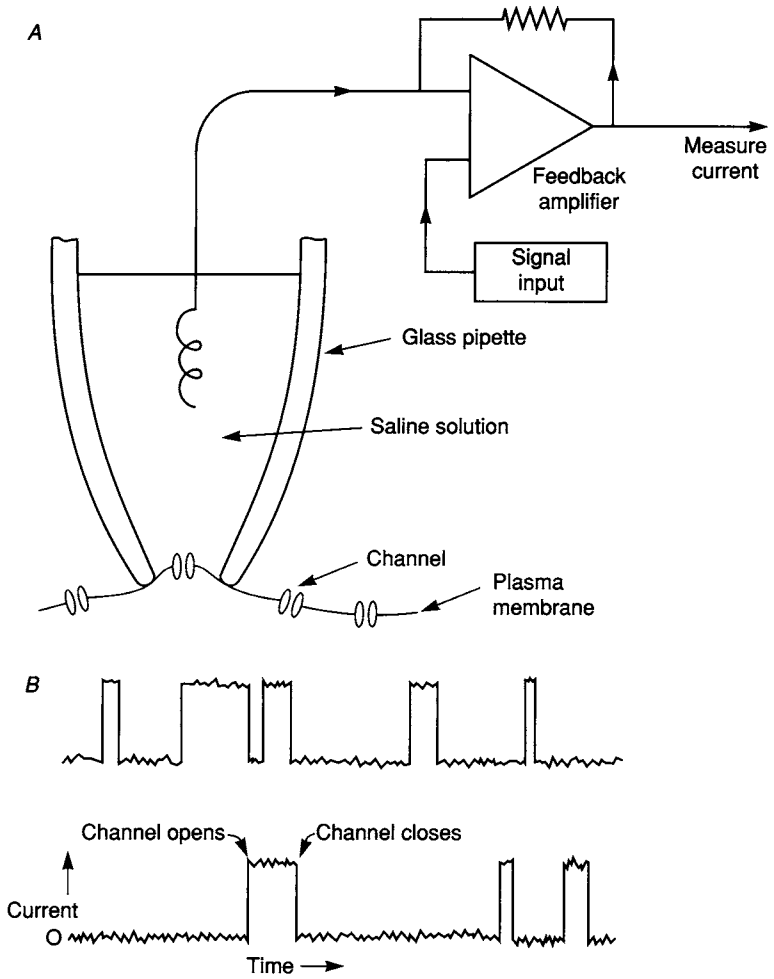

Fig. 1.3. Patch clamp records of channel opening and closing. The patch clamp technique (*A*, not to scale) allows the current flowing through an individual ion channel to be measured. The records (*B*) show short bursts of outward current while the channel is open. Currents are typically in the range of 1 to 5 pA and might last for a few microseconds or milliseconds. Notice that the current pulses are constant in amplitude but variable in duration. It is conventional to show current passing inward through the plasma membrane (i.e. from the extracellular to the cytoplasmic side) as downward deflections.

use of the methods of molecular biology to investigate their structure. The amino acid sequences of the subunits of the nicotinic acetylcholine receptor channel were determined in 1982 by teams led by Shosaku Numa, Jean-Pierre Changeux and others, and that of the voltage-gated sodium channel followed two years later. More and more sequences have been published every year since then. Sequences give us strong clues about structures, and knowledge of the structures of channels gives us an increasing understanding of the way they work.

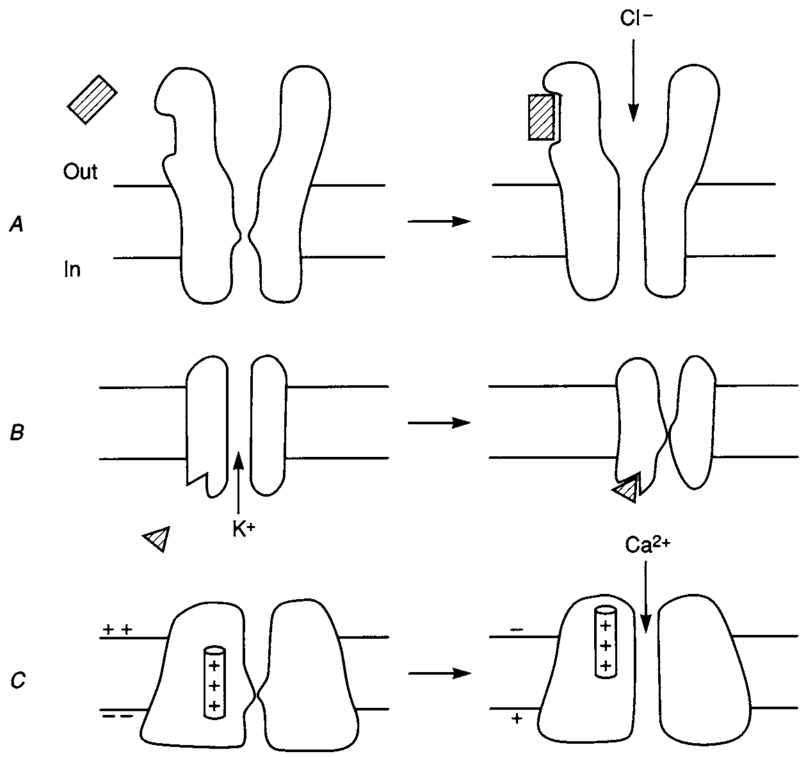

Fig. 1.4. Gating and selectivity in various types of channels. *A* shows a neurotransmitter-gated channel that is selective to anions. *B* shows a channel selective for potassium ions that is closed by the binding of an internal ligand such as ATP. *C* shows a calcium-selective voltage-gated channel; part of the internal structure of the channel is charged and moves when the membrane potential becomes more positive inside, and this acts as the trigger for opening the channel. *A* is based loosely on the glycine receptor channel, which mediates synaptic inhibition in the spinal cord, *B* on the ATP-sensitive potassium channel of pancreatic β cells, and *C* on the voltage-gated calcium channels of neurons and secretory cells. The diagrams are much simplified: channels of the *A* and *B* types often require more than one ligand molecule for gating, and channels like *C* usually have four internal gating sections.

Different types of channel

We now know that there are many more different types of ion channel than was first supposed. New varieties are constantly being reported. This diversity leads to problems in their classification and nomenclature, both of which may need to be revised from time to time as the relations between different channels become clearer.

Channels are commonly described in terms of their ionic selectivity and their gating properties. Figure 1.4 gives an impression of the range of different types in these respects. The nerve axon, for example, possesses voltage-gated channels, i.e. channels that are opened by a change in the membrane potential, and these are of two main types – sodium channels and potassium channels. Voltage-gated calcium channels also occur, particularly at the nerve terminals. We can refer to all these voltage-gated channels as a group distinct from others.

Another major group of channels consists of those that are directly activated by neurotransmitters. These include the nicotinic acetylcholine receptor channel, the γ-aminobutyric acid (GABA) receptor channel, and the glycine

receptor channel. A third group includes all those gated by combination with internal ligands, such as calcium ions, ATP, cyclic nucleotides and so on. Sometimes these two groups are themselves combined together as ligand-gated or chemically gated channels, to distinguish them from the voltage-gated channels.

There are other channels that fall outside these definitions. Gap junctions form channels between the cytoplasmic compartments of adjacent cells by crossing two plasma membranes. Gramicidin channels are apparently formed spontaneously when two monomers come together in the membrane into which they have been introduced.

An alternative approach is to use the molecular structure of the channels as the basis for their classification and nomenclature. Exciting discoveries have been made in this respect. The different voltage-gated channels of neurons and other cells have markedly similar structures and show appreciable similarity in their amino acid sequences. We can therefore describe them as a family of related proteins, with the implication that they have a common evolutionary origin. Further sequence determination has shown that some cyclic nucleotide-gated channels and some plant potassium channels should also be included with them to form a superfamily of related sequences.

Not all channel amino acid sequences, however, are related. Transmitter-gated channels are quite separate from the voltage-gated channels and their relatives, showing little or no sequence similarity with them and having distinct structures. Within this group, the receptor channels activated by acetylcholine, glycine, GABA and 5-hydroxytryptamine are clearly related to each other, whereas the glutamate receptors are rather different. The ABC (ATP binding cassette) superfamily forms another distinct group of proteins, only some of which are ion channels.

Knowledge of molecular structures leads to finer distinctions between channels as well as broader groupings of them. The acetylcholine receptor channels of the mammalian nervous system, for example, are of several different types, all rather different from those found at the neuromuscular junction. There are many different subvarieties of voltage-gated potassium channels, and often a number of these may be found in the same cell. Different organisms may possess similar channels with just minor differences in their amino acid sequences.

All this diversity provides a challenge for our comprehension. We need to be precise in our descriptions: it is no use talking about '*the* potassium channel' unless the context is clear and circumscribed. But the very diversity of channels demonstrates their importance to living organisms and makes them a fascinating subject for study.

2 Ions on the move

The existence of electric charge is one of the fundamental features of the universe. Single charges are associated with single subatomic particles: they are unitary and either positive or negative. Charges of the same sign repel each other and those of opposite sign attract each other. The attractive force between positive and negative charges means that it requires an appreciable amount of energy to separate them, and thus energy is released when they are allowed to come together again. Consequently we find that the normal state of matter is to be electrically neutral, with equal numbers of positive and negative charges.

The reasons for this state of affairs can be fruitfully discussed only by theoretical physicists, but the consequences are evident to all of us. When we boil water in an electric kettle, the charges that were separated some time ago at considerable energy cost in some distant power station are finally allowed to come together again, and their flow through the resistance of the kettle's heating element releases energy that we can use to make a cup of coffee. Living cells can also utilize the energy available from the attractive force between electric charges of opposite sign, and this may be evident ultimately in the flow of ions through ion channels.

We begin this chapter with a short refresher course on some aspects of the physics and chemistry of ions. Readers whose physical chemistry is in good shape may wish to skip the first few sections.

Electricity

Static electricity is the accumulation of excess positive or negative *electric charge* in some region, produced ultimately by the separation of electrons from their atoms. Quantities of charge Q are measured in coulombs, C. The positive

Table 2.1. *Some electrical quantities and their units*

Quantity	Symbol for quantity	Unit	Symbol for SI unit	Equivalent form of SI unit
Charge	*Q*	coulomb	C	A s
Current	*I*	ampere	A	$C\ s^{-1}$
Potential difference	*V*, *E*	volt	V	$J\ C^{-1}$
Energy (work)		joule	J	C V
Power		watt	W	$J\ s^{-1}$, A V
Resistance	*R*	ohm	Ω	$V\ A^{-1}$
Conductance	*G*	siemens	S	Ω^{-1}, $A\ V^{-1}$
Capacitance	*C*	farad	F	$C\ V^{-1}$

It is conventional to write the symbols for quantities in italics and the symbols for units in roman type. Notice particularly the difference between *C*, the symbol for the quantity capacitance, and C, the symbol for the coulomb, the unit of charge. The unit of time is the second, s.

Table 2.2. *Some prefixes for multiples of scientific units*

Multiple	Prefix	Symbol
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{3}	kilo	k
10^{6}	mega	M
10^{9}	giga	G

charge on a single sodium or potassium ion (the elementary charge, e_0) is 1.602×10^{-19} C, so one coulomb corresponds to the charge on 6.24×10^{18} univalent ions. The charge on one mole of univalent ions is given by the Faraday constant F, which is equal to Avogadro's number N_A (the number of ions, atoms or molecules in a mole, 6.022×10^{23}) multiplied by the charge on each of them, i.e.

$$\begin{aligned} F &= N_A e_0 \\ &= 6.022 \times 10^{23} \times 1.602 \times 10^{-19} \\ &= 96\,500 \text{ coulombs mol}^{-1} \end{aligned}$$

Tables 2.1 to 2.3 collect together some of the physical quantities, units and constants used in ion channel work.