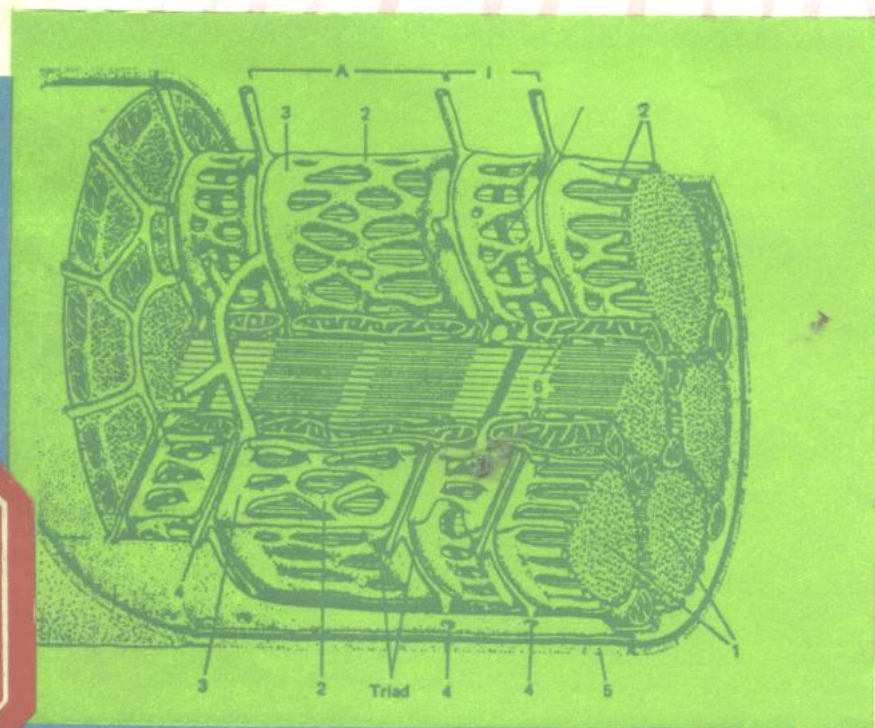# 细胞生理学

——大学生物及医学专业英语教材

吕国蔚

细 胞 生 理 学
CELL PHYSIOLOGY
——大学生物及医学专业英语教材

吕 国 蔚

LU GUO WEI

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内 容 简 介

细胞生理学是生理学的重要组成部分,在了解动物或人体生理活动规律中占有重要位置。本书主要根据国外 80 年代出版的有关书籍编写而成,对细胞的基本机能作了较详细的介绍,并反映当前的发展。

全书共分六章,全部用英文写成,依次叙述生物膜的基本构造、膜电位的形成和维持、膜兴奋的生理过程和发生机理、突触或接头的传递过程与机理,以及肌肉收缩基本过程和机理。

本书除可作为大学生物及医学系本科生和有关专业研究生的专业英语教材外,还可供生物医学工作者学习参考。

PREFACE

Human physiology has its base in cell physiology and biophysics. The topic of cell physiology occupies thus a central position in physiology. The physiology deals with issues of the interaction between large population of cells, organs, systems, and finally, the integrated function of an entire body. To understand the physiology, it is essential to first understand the basic organization of the cell and the function of its component parts and membrane in particular.

This book is intended primarily to serve as a supplementary reading material for the physiology course for medical students, graduate students, or other health professionals as well as a source for review and update of cell physiology for residents and practicing physicians. The book is designed to provide a relatively comprehensive description which readers can supplement with readings in current textbook of physiology. The context of the book may also be considered as an elementary introduction to the physiology.

It has been believed that a good textbook should tell what is known, what is unknown, and how it might come to be known. With that admonition as a guide, I have tried to make it my goal in this work to present an introduction to the present state of our knowledge regarding cell physiology and a framework into which new facts will fit as they became available. The context of the book has been engaged in a manner that is most useful to the readers, going from simple to more complex matters. To keep the book within reasonable limits of size, I have incorporated the new material at the expense of older and less essential one.

With the appearance of this book, I am very grateful to those authors and publishers whose publications I have taken passages and reproduced illustrations from. The sources of each of these will be found in the legends of relevant figures and the reference list at the end of the book. I am happy to have the opportunity to express appreciation for all assistance that made publication possible. My gratitude is indebted especially to Dr. Zhang Zhenghua(张正华) and Dr. Xu Yufang (须育方) and my editors at the Peking University Press for their enthusiam, encouragement, and help through the later stage of this work.

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Lu Guowei(吕国蔚)

June 1,1989
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CONTENTS

Physiology is concerned with the overall functioning of the tissues and organs of which the body is composed. Essentially, these tissues and organs are organized assemblies of large numbers of cells, often of several different types. Through the operation of various control systems, these complex assemblies respond to specific stimuli in an integrated fashion. The cell is, in a very real sense, a functional unit in relation to the body as a whole, and the behavior of single cells is a logical takeoff point for the study of more complex physiological systems.

It is now obvious that nearly all tissues and organs in the body are composed of cells, held together by various intercellular supporting substances. Figure 1 presents a comparison of cells in different types of tissues, using some of the common stains for light microscopy. Note that in most tissues the cells have simple shapes that reflect in large part their particular function. Thus, the cells of the skin form layers, the cells of the kidney form tubules, the cells of the glands form ducts to carry their secretions, and muscle cells form fibers that can contract and elongate. Nerve cells, however, give off fibers, trunks, and branches in various direction, and these processes seem to disappear in the surround. Very little, therefore, can be deduced about nerve cell function from the structures seen with routine staining procedures. Although the various cells of the body have such diverse functions and structures, they all consist basically of a fluid system, protoplasm, and are surrounded by a membrane which acts as a barrier between the fluid within the cell and the fluid outside it.

SKIN SMOOTH MUSCLE BONE

CONNECTIVE TISSUE RED BLOOD CELLS

GLANDS NERVE CELLS KIDNEY TUBULE
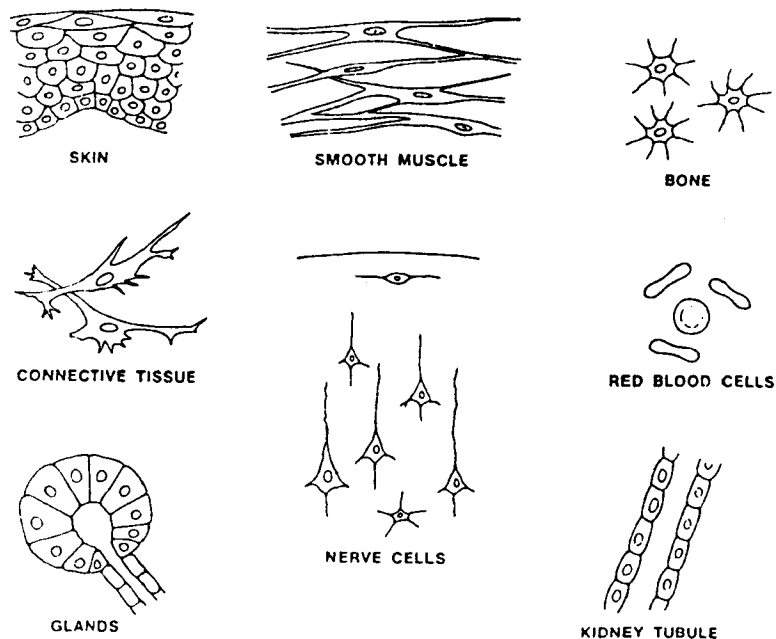
Fig. 1 Arrangements of cells to form tissues in different parts of the body.

Living cells are themselves complex and highly organized. In addition to a fluid phase (intracellular fluid or cytoplasm), the interior of a typical cell contains a number of inclusions or organelles (Fig. 2). It should be noted that here each of these subcellular compounds, i. e. , the nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondria, is a physiologic system, since translocation of information (genetic

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code) or material(biochemical substances) is associated with function. Indeed, the total unitary cell system is the integrated performance of all these functional elements, and, accordingly, this area of function is termed cell physiology.

Modern research has shown that these subcellular particles are again highly organized structures that play a vital role in the overall activity of the cells. For didactic purposes the detailed study of these structures is, nowdays, to a large extent, included in the formal disciplines of biochemistry and cell biology. Physiology proper is often considered to begin at the point where the cell as a whole interacts with its external environment or with neighboring cells, i. e. , with the exchanges of mater and energy that take place across its outer limiting membrane or plasma membrane.
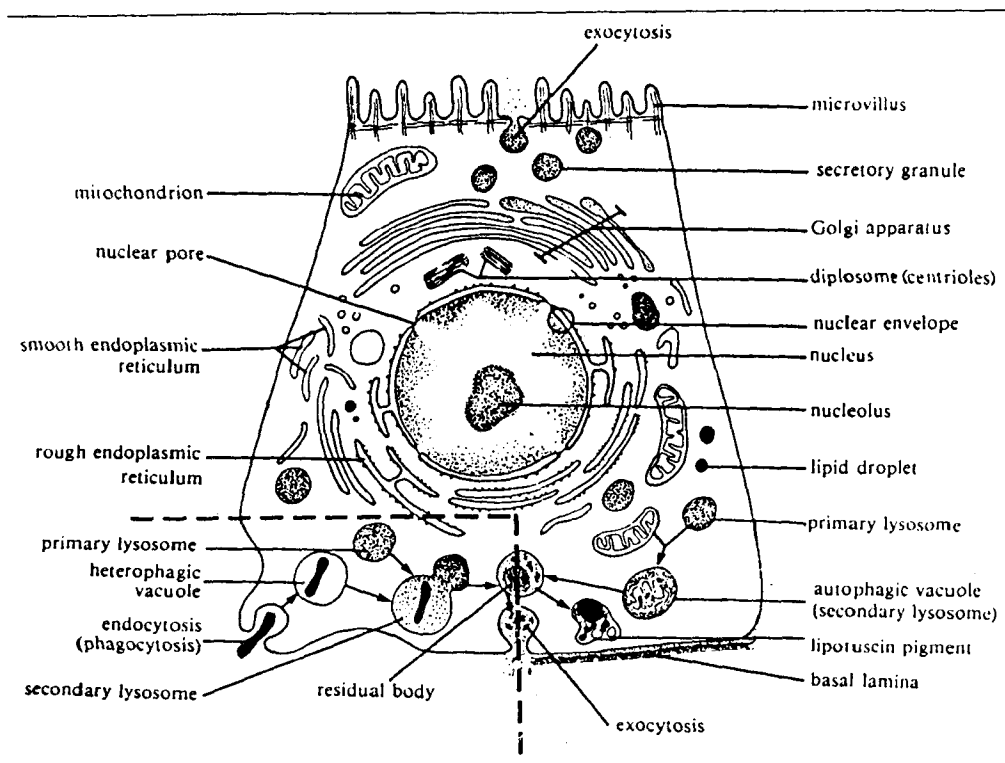
Fig. 2 A cell. The major organelles and some inclusions are shown. The exocytosis of a secretory granule is portrayed at the apex of the cell, while the cellular digestive system is depicted at the base of the cell.

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I . THE CELL MEMBRANE

Cell function depends on the integrity of the cell membrane and its property of allowing certain molecules and ions to cross it while preventing the passage of others. In all cells of the body, the plasma membrane controls the interchange of substances between the cell and its environment. In nerve cells it is especially important for several reasons. First, the membrane controls movements of substances that directly affect nerve signaling. Second, the membrane is the site of the electrical activity that is the basis of rapid nerve signaling. Third, it is the site of action of peptides and hormones. Finally, it provides the sites for synapses, where signals are transmitted from one cell to another. Thus, much of the study of physiology is actually concerned with the organization and properties of the neuronal plasm membrane, and we will learn much more about those in the following chapters.

1. Fluid Mosaic Model

(1) Lipid bilayer

In cross sections of electron micrographs at low magnification the membrane appears as a single dark line, about 8 nm thick. At high magnification the membrane appears to have a triple-layered structure, an
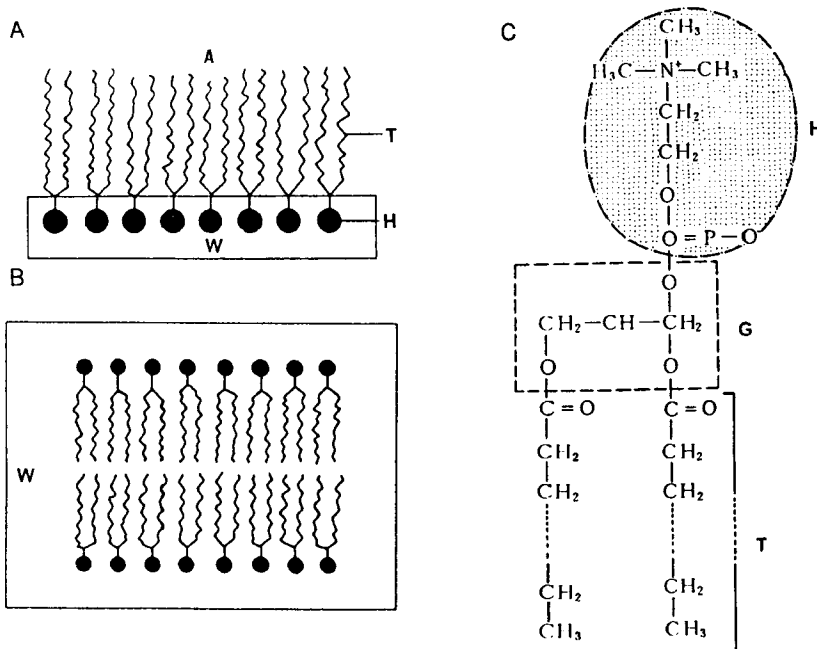
Fig. 3   The lipid mono(A)-and bilayer(B) and the lipid molecule(C).

A: air, W: water; H: polar head, T: nonpolar tail, G: glycerine.

inner and outer dense layer being separated by a less dense region between them. The outer layer is polysaccharide and the inner one is protein, both being one molecule thick. The central region is a bimolecular ar-

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rangement of phospholipid. In Figure 3, the black dots are the polar groups of the molecule which are hydrophilic (attracted to water) and consist of the nitrogen-carbon-phosphorus groupings of the phospholipid. The rest of each molecule, a fatty acid (long-chain hydrocarbon), is hydrophobic. Those long molecules are arranged at right angles to the membrane surface, in pairs, as shown in the Figure 3.

The three-layered structure was originally termed the unit membrane, and was thought to consist of oriented lipid and protein complexes. The present conception is that the bilayer is due to oppositely oriented lipids that form a matrix within which protein may completely contained, or may be partially, sticking out on one side or the other. Both lipid and protein components are in fact in a fluid state, as illustrated by fluid mosaic membrane model in Figure 4.
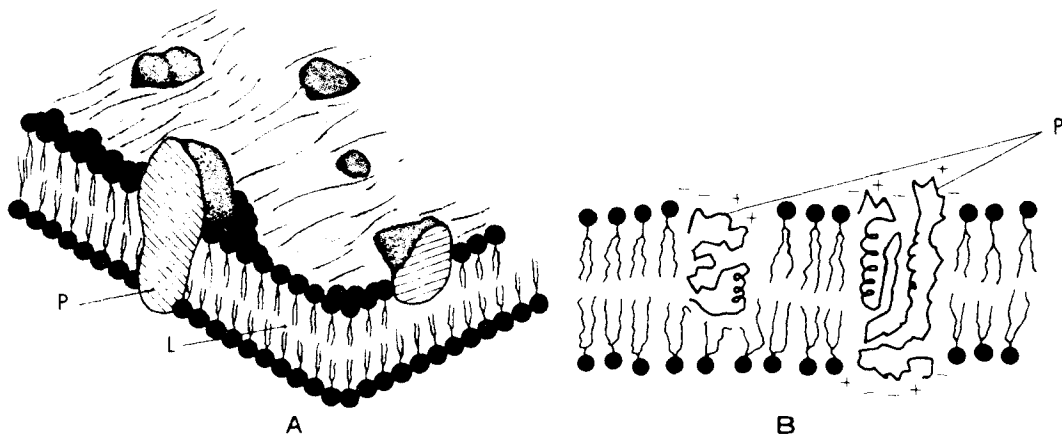
Fig. 4 The fluid mosaic model of the plasma membrane, showing its stereoscopic view (A) and section (B). Note the polar portions of proteins extrude to the outside of membrane and contact to water.

P: protein, L: lipid bilayer.

Lateral movements of lipid and protein components are in fact relatively rapid. However, they are constrained by attachments to the immediately underlying cytoplasm; this provides the basis for regional specializations of the membrane, which are important in nerve cells, as seen in the differing structures of axons, dendrites, and synapses. Since the membrane is largely lipid in composition, fat-soluble substances penetrate into cell easily, whereas non-fat-soluble ones enter only slowly or not at all. Lipids are good electrical insulators, a fact that we will discuss later in connection with the high electrical impedance of membranes, characteristic of all cells.

(2) Membrane proteins

Many of the membrane proteins are glycoproteins, with polysaccharide chains that protrude from the external surface as shown in Figure 5. Together with other carbohydrate molecules they form a thin layer covering the cell surface, called a glycocalyx. The glycocalyx fills the extracellular space, and in this position has several important roles to play. The glycoproteins are believed to function as "cell recognition molecules", which help to guide migrating neurons to their targets. They may also serve as "cell adhesion molecules", which help to bind cells together. The composition of the glycocalyx may be important in regulating the diffusion of molecules in the extracellular space. It has also speculated that the membrane glyco-

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proteins may be sensitive to weak electric currents that flow around active neurons.

It has been found in freeze-fracturing studies that all cell membrane have a common basic appearance: a smooth surface from which the globular proteins protrude as small bumps 6—19 nm in diameter(Fig. 5). These so called intramembranous particles are more numerous in the inner leaflet(the P-face) than in the outer leaflet(the E-face) and may assume specific configurations in the region opposed to another cell. It has been suggested that they represent ionic transport sites, but it is likely that there are particles with different functions. In the postsynaptic junctional membrane, the particles are most probably related to receptor functions. The particle distribution may change as a result of changes in activity.
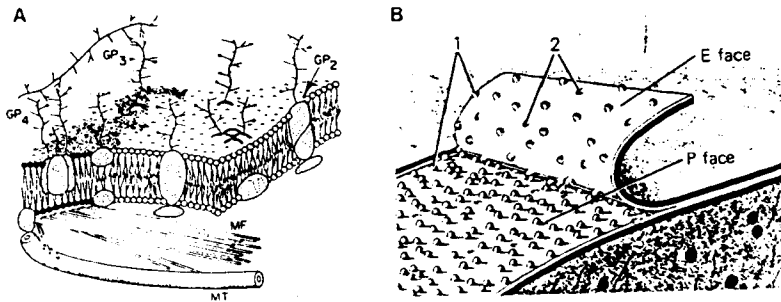
Fig. 5 A. Transmembrane control of the distribution of cell surface receptors by extracellular and membrane-associated cytoskeletal components. GP2, GP3 and GP4 indicate integral glycoprotein complexes; MF, microfilaments; MT, microtubule. (From Nicolson, *Biochim. Biophys. Acta*, 457, 1976.) B. Membrane cleavage occurs when a cell is frozen and fractured (cryofracture). Observe that most of the membrane proteins (1) remain bound to the outer surface of the layer lying closest to but facing away from the cytoplasm (P face), whereas fewer proteins are found in association with the layer farthest from but facing towards the cytoplasm (E face). For every protein particle that bulges on one surface, a corresponding depression (2) appears within the opposite surface. In the frozen tissue, hydrophilic bonds are more rigid and stable, while the hydrophobic lipid interactions remain more fluid. Consequently, cleavage occurs in this region because the lipid layers are bound by the weaker hydrophobic interactions. The study of these protein particles by cryofracture contributed significantly to our knowledge of cell membranes. (Modified and reproduced from Krstic RV: *Ultrastructure of the Mammalian Cell*. Springer-Verlag, 1979.)

(3) Cytoskeletons

Early notions of cell structure envisaged the plasma membrane as having no structural connections with the cell's interior. Recent findings suggest, however, that there exists a system of membrane associated intracellular filaments linked to the membrane proteins(Fig. 5). These filaments are of at least three types: thin microfilaments which probably contain actin; thick filaments similar to polymerized myosin; and microtubules composed of a protein, tubulin. The filaments appear to provide for movements of the proteins and the glycoproteins of the membrane. Microfilaments and microtubules appear to have opposite roles in maintaining the distribution and motility of cell surface proteins. The microfilaments probably mediate translocation of membrane proteins, while the microtubules represent a system by which the proteins are anchored to the cytoplasm. Further, various external agents may cause the membrane proteins to be redistributed from a dispersed state to clusters and patches. Myosin seems to be a component of the outer cell surface. It thus appears that there is a contractile system on each side of the membrane which presumably in-

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fluences the display of certain membrane components.

2. Ionic Channels

(1) Channel configuration

Cell membranes are practically impermeable to intracellular protein and other organic anions, but moderately permeable to Na^+ and rather freely permeable to Cl^- and K^+. Particle size affects the movement of ions across cell membranes, and it should be noted that the ions in the body are hydrated. Thus, although the atomic weight of potassium(39) is greater than the atomic weight of sodium(23), the hydrated sodium ion i. e. , Na^+ with its full complement of water, is larger than the hydrated potassium ion. However, it is clear that ions cross membranes via ion channels rather than simple pores. The channel is so large that it allows large cations, such as Ca^{2+}, NH_4^+, and even certain organic cations, to pass. Anions such as Cl^- are excluded, however. This cation selectivity suggests that the channel has a negative charge at its mouth that attracts a variety of cations bellow a certain size and repels anions because of their charge (Fig . 6) .
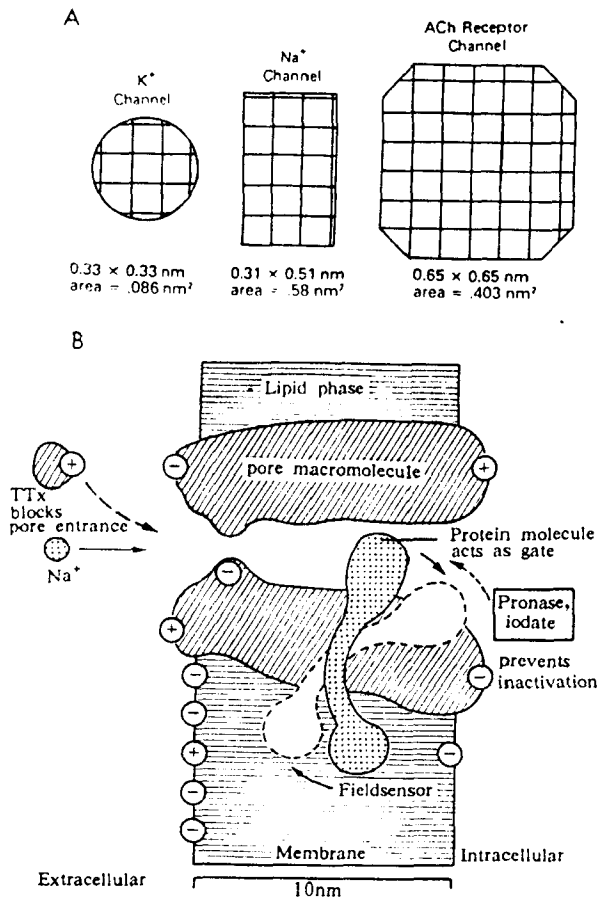
Fig. 6   A. A comparison of the dimensions of ionic selectivity filters for the K^+, Na^+ and ACh receptor channels. This sketch illustrates the minimum size of the pore that will pass the known permeant ions for these three channels in the nerve and muscle of the frog. Grid marks in 0. 1-nm(1-Å) steps. Sizes were evaluated from space-filling models of the permeant and impermeant ions. (From Hille, 1984.) B. Model of a Na^+ channel in the membrane, diagrammatic. The membrane components and the ions are drawn approximately to scale. The Na^+ ions can pass through the pore; the dashed arrows indicate the inhibitors tetrodotoxin (TTX, which blocks the pore entrance) and pronase or iodate (which prevents inactivation).

B. Hille and his coworkers have estimated that the channel activated by acetylcholine is substantially larger in diameter than the Na^+ or K^+ channel. At its narrowest point in cross section, the dimensions of the channel pore are approximately 0. 65 nm(6. 5 Å). In contrast, the Na^+ channel is 0. 31 nm $\times$ 0. 51 nm

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and the K^+ channel is only 0.33 nm $\times$ 0.33 nm(Fig. 6).

The relative ease with which ions with good hydrogen-bonding characteristics pass through the channel led Hille to suggest that part of the inner wall of the protein channel is made up of amino acids that are rich in oxygen atoms. A. Woodhull also found that when the pH of the fluid surrounding the cell is lowered, the conductance of the open channel is reduced gradually, and this reduction exactly parallels the titration curve for the carboxylic groups of amino acids. On the basis of these results, Hille proposed the following mechanism by which the channel selects for Na^+ ions. There are negatively charged carboxylic acid groups located at the outer mouth of the pore that perform the first step in the selection process by attracting cation and repelling anions. Cations that are larger than 0.3 $\times$ 0.5 nm in diameter are too large to pass through the pore but only after losing most of the waters of hydration they normally carry in free solution. The negative carboxylic acid group, as well as the oxygen atoms that line the pore, can substitute for these waters of hydration, but the degree of effectiveness of this substitution varies for different ions. The greater the effectiveness of this substitution for a given ion species, the more readily that ion permeates the Na^+ channel.

(2) Channel distribution

Characterization of the Na^+ channel has been aided greatly by the availability of several naturally occurring neurotoxins——tetrodotoxin(TTX) from the puffer fish, saxitoxin(STX) from paralytic shellfish, batrachotoxin from South American poisonous frogs, and the venom from the North African scorpion. These toxins bind tightly to the channel and therefore can be used as specific probes for localizing the channel molecules. The binding of radiolabeled TTX molecules to axon membrane has been studied to obtain an estimate of the density of voltage gated Na channels per unit area of axon membrane. These studies indicate that TTX binds to a small number of specific sites on the membrane. These specific sites are thought to represent the Na^+ conductance channels, because the binding constant and the kinetics of TTX binding to these sites correspond to the values determined by physiological measurement of the TTX blockade of Na^+ conductance.

M. Ritchie and his colleagues estimated the number of Na^+ channels by measuring the total amount of TTX that was bound when these specific binding sites were saturated. They found that the greater the density of Na^+ channels in the membrane of an axon, the greater the velocity at which the axon conducts action potentials. This result is to be expected. A greater density of Na^+ channels allows more current to flow through the active membrane and along the axon core to discharge the membrane capacitance of the unexcited membrane downstream. Depending on cell type, the values obtained for nonmyelinated axons range from 35 to 500 channels per square micrometer of axon membrane.

Even at 500 channels/μm^2, the density of Na^+ channels is quite low——about one channel in 4,000 membrane molecules. Despite this small number, quite large Na^+ currents can flow during the action potential. The current density through each channel must therefore be high. By dividing the total Na current that flows during a voltage clamp pulse(see below) by the number of Na^+ channels in the membrane, it is possible to calculate that a single Na^+ channel passes up to 10^7 Na^+ ion/sec. Both empirical data and theoretical calculations indicate that carrier molecules cannot transport ions at this rate. The only plausible mechanism for such a high rate would be the flow of Na^+ ions through an aqueous channel.

(3) Channel classification

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These channels are usually passages through protein molecule(Fig. 6). Ion channels, which span the membrane , thus fall into a class of molecules called intrinsic membrane proteins . Charge configurations
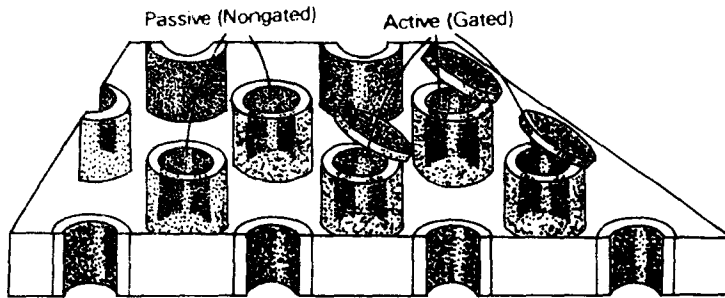
Fig. 7   Two types of channels provide pathways for ions to permeate the membrane: passive channels are always open; active, or gated, channels have the ability to open or close in response to one of a variety of chemical, electrical, or, in some cases, physical stimuli. If this diagram were drawn to scale, inner diameters of pores would be about one-tenth of the membrane thickness, and the distances between neighboring channels would be about ten times greater than the thickness of the membrane.
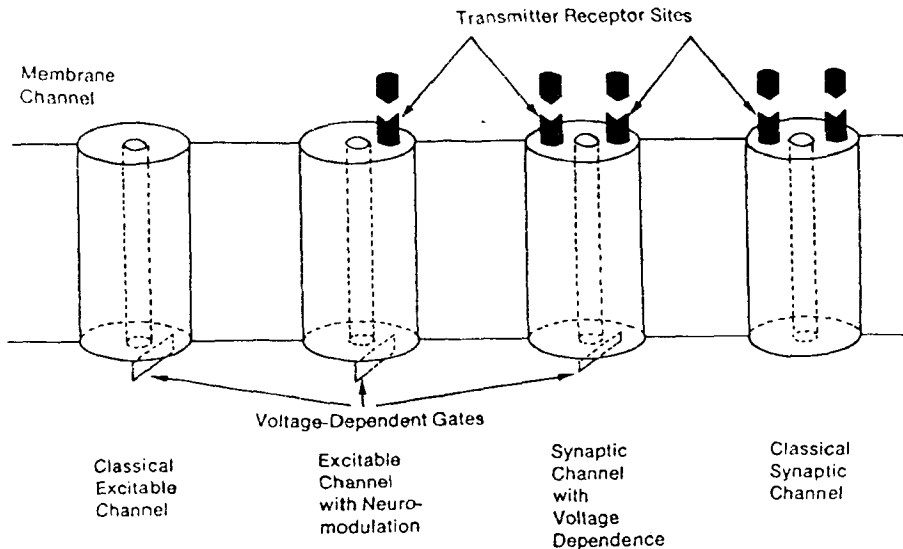
Fig. 8   Simple models to illustrate different types of voltage-sensitive and ligand
(neurotransmitter) sensitive channels.

around them and related variables make them relatively specific. Thus, for example, there are separate Na$^+$ channels, K$^+$ channels, and Cl$^-$ channels. Ion channels themselves can also be subdivided into two categories: they are either passive or active(Fig. 7). Passive channels are always open. Active channels have a gate somewhere along their length that can be either open or closed. The gates of these active channels may be controlled by synaptic transmitters, by membrane potential, or, in the case of receptor cells,

by various physical stimuli. Most active channels are closed when the membrane is at rest. The ease with which ions pass through some of these channels is therefore controlled (gated) by voltage or by agents such as neurotransmitters(Fig. 8). Thus, for example, passage of Na^+ through the Na^+ channels in excitable tissues is greatly increased by a decrease in membrane potential, i. e. , they are voltage-gated. At synaptic junctions and elsewhere, many ion channels are chemically gated, i. e. , their ability to pass ions is increased by the binding of a given neurotransmitter or hormone to receptors associated with them. Passive channels are important in determining the resting membrane potential as well as in influencing synaptic integration. Active channels generate action, synaptic, and receptor potentials.

Experiments have made it clear that, at specific sites on an excitable cell, excitable channels may have receptors for neurotransmitters which permit modulation of the excitable properties. Complementing this work are the findings of those working on synapses that, in many cases, the responses to a neurotransmitter may depend on the level of the resting membrane potential. The situation is summarized in Figure 8. At the far left is the traditional impulse channel, with only a voltage-dependent property, while at the right is the traditional synaptic channel, with only transmitter receptor sites. Between are channels which contain some degree of the other property. These channels obviously provide the nervous system with much greater flexibility in modifying trains of impulses, on the one hand, or integration of synaptic inputs, on the other, depending on the ongoing state of the organism and level of activity in its neural circuits.

The number of ionic conductances identified has grown rapidly, and it is impossible in an introductory account to describe them adequately. In addition to the fast sodium, slow inward (Na^+ and/or Ca^{2+}) "bursting", transient inward(Ca^{2+} dependent nonspecific cation current) channels, calcium channels have also been found in many neurons and may be significant for mediation of action potentials. Among the outward currents, an interesting finding has been that at some site such as at the node of Ranvier the late conductance activated by the impulse in the Hodgkin-Huxley model may be absent. Among the outward currents, one of the most important findings has been a slow K^+ current that depends on an increase in intracellular free Ca^{2+}. Another K^+ current has been found to be present in some molluscan cells.

(4) Channel glycoprotein

W. Catterall has recently photoaffinity labeled the Na^+ channel from the rat brain by treating the rat brain membranes with a radioactively labeled azido nitrobenzoyl derivative of scorpion toxin. In the dark, this derivative binds reversibly to the same sites in the protein as does the toxin, but it can form a covalent bond with amino acid residues at the binding site when exposed to ultraviolet light. With this and related approaches, Catterall isolated three subunits that are thought to be present in the functional channel in equal proportions: one large glycoprotein with a molecular weight of 270,000 (α) and two smaller polypeptides with molecular weights of 39,000 (β_1) and 37,000 (β_2). Only the α and the β_1 subunits bind toxin, the β_2 subunit does not bind toxin but is linked to the subunit by a disulfide bond.

On the basis of the amino acid sequence, Numa and his colleagues have generated several hypotheses concerning the evolution and structure-function relationships of the Na^+ channel. For example, there are four similar sequences of about 150 amino acid residues each within the moleculae. This homology led Numa and colleagues to suggest that the channel may have evolved from a single ancestral DNA segment that was duplicated within the gene three times. By looking at the way in which individual amino acids are distributed along the entire peptide of 1820 residues, they were also able to identify several candidate domains

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concerned with the various functional properties of the Na^+ channel molecule. In particular, several long hydrophobic stretches are postulated to pass through the membrane; other regions possess a high density of charged amino acids that could serve in the gating process, in cation selectivity, and in binding positively charged blocking drugs (TTX). It will be possible to test these hypotheses in the future by altering the nucleotide sequence at specific sites within the clone, thereby changing one or more individual amino acids in the channel protein. With this procedure, called "site-directed mutagenesis," modified channels can be tested for functional changes; after synthesis, the modified protein can be inserted into artificial lipid bilayer membranes for voltage clamp analysis.

3. Sodium-Potassium Pump

(1) Na-K ATPase

The sodium-potassium pump responsible for the coupled active transport of Na^+ out of cells and K^+ into cells is a unique protein in the cell membrane. This protein is also an adenosine triphosphatase(ATPase), i. e., an enzyme that catalyzes the hydrolysis of adenosine triphosphate(ATP) to adenosine diphosphate (ADP), and it is activated by Na^+ and K^+. Consequently, it is known as sodium - potassium - activated
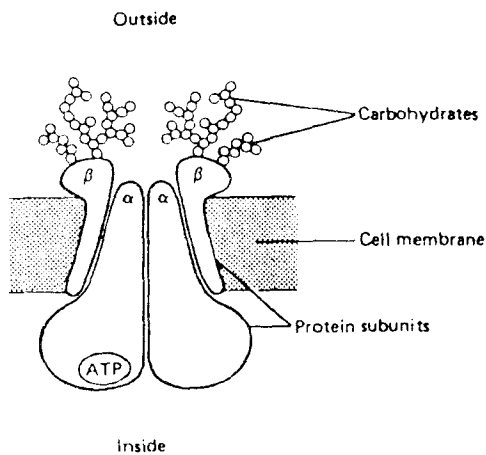
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Fig. 9   Proposed structure of Na^+-K^+ ATPase. The molecule is made up of two α and two β subunits. Each α unit has an intracellular catalytic site for ATP and an extracellular site to which cardiac glycosides bind. There are also 3 binding sites for Na^+ and 2 binding sites for K^+. The mechanism by which transport of the ions occurs is unknown but could involve conformation changes with resulting shifts in the relations of the subunits to each other. (Reproduced from Sweadner KJ, Goldin SM; Active transport of sodium and potassium ions; Mechanism, function, and regulation. *Engl J Med* 1980; 302; 777.)

Inside

adenosine triphosphatase(Na-K ATPase). The ATP provides the energy for transport. The pump extrudes three Na^+ from the cell for each two K^+ it takes into the cell, i. e., it has a coupling ratio of 3/2. Its activity is inhibited by ouabain and related digitalis glycosides used in the treatment of heart failure. It is made up of two α units, each with a molecular weight of about 95,000, and two β subunits, each with a molecular weight of about 40,000. Separation of the subunits leads to loss of ATPase activity. The alpha subunits contain binding sites for ATPase and ouabain, whereas the beta subunits are glycoproteins(Fig. 9). Application of ATP by micropipette to the inside of the membrane increases transport, whereas application of ATP to the outside of the membrane has no effect. Conversely, ouabain inhibits transport when applied to the outside but not to the inside of the membrane. Consequently, the alpha subunits must extend through the cell membrane. A proposed structure for Na-K ATPase is shown in Figure 9.

(2) Conformational states

The protein could exist in 2 conformational states. In one, three Na^+ bind to sites accessible only from

the inside of the membrane. This triggers hydrolysis of ATP, and the protein changes its conformation so that the three Na^+ are extruded into the extracellular fluid. In the second conformation, two K^+ bind to sites accessible only from the outside of the membrane. This triggers a return to the original conformation while extruding two K^+ into the interior of the cell. It appears that Na^+ binding is associated with phosphorylation of the protein and K^+ binding with dephosphorylation. As shown in Figure 10, at the inside of the membrane Na^+ becomes bound to a carrier Y, forming the molecule Na^+Y. Na^+Y diffuses through the membranes and splits apart spontaneously at the outside of the membrane. The concentration of Na^+Y is therefore small at the outer side, and the outflow of Na^+Y predominates over the inflow. This temporary binding to the carrier molecule Y thus enables Na^+ to diffuse outward, against its concentration and potential gradients. At the outer side of the membrane the carrier molecule Y is converted to a carrier molecule X, which binds to K^+ in the external solution. The resulting compound K^+X diffuses through the membrane, splitting into K^+ and X at the inner side. On the inside metabolic energy is used-ATP is split——to reconvert the carrier molecule X to the molecule Y. This is the only endothermic reaction in the cycle; the coupling of X to K^+ saves about half of the energy that would be required for uncoupled Na^+ transport. The existence of such a coupled Na-K pump can be demonstrated by the removal of K^+ ions from the external solution. When no K^+ is available to form the complex K^+X at the outer side of the membrane, the coupled pump is blocked and the outflow of Na^+ from the cell falls to about 30% of normal.
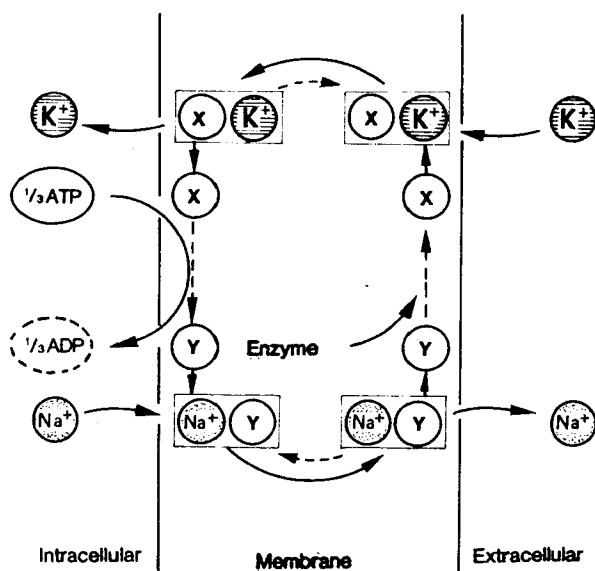

Fig. 10 Coupled Na^+-K^+ pump. Diagram of the transport of Na^+ and K^+ through the membrane by the carriers X and Y. Energy is provided by the breakdown of adenosine triphosphate (ATP) into adenosine diphosphate (ADP).