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Foreword

In 1961, Ke Xu and collaborators found that the conduction velocity of the nerve impulse in the giant nerve fiber of the shrimp abdominal nerve cord was about 200 m/s. This was the highest speed of information transmission ever observed among living organisms. Elucidating the foundation of this phenomenon turned out to be Xu's life work. This he accomplished in spite of the interruption of his research activity for 20 years due to political intervention.

I have never worked on the shrimp nerve myself, but thanks to rare chances I witnessed two major steps of the development of this research. It is probably the reason why the authors asked me to write Foreword. It seems therefore appropriate to describe here what happened around me during the course of the research. The process has been most unusual and I think it is worth recording.

In 1963, when I was a professor in the Department of Physiology at Tokyo Medical and Dental University in Tokyo, a young physiologist, Kiyoshi Kusano, returned from the United States to join our laboratory. I asked him what he was going to study. He answered that he wanted to work on the shrimp giant nerve fiber, because there was a report that a Chinese group found that it displayed an extraordinarily high conduction velocity. He probably got that information from Dr. T. H. Bullock, a prominent neurobiologist of the University of California, San Diego. Dr. Bullock later wrote to me that he heard of that report when he visited Moscow and spoke to a Russian who had just returned from China. Dr. Bullock was impressed, and after returning to the United States convinced his friends that the report must be confirmed, and, if true, its mechanism elucidated.

Kusano's plans were quite interesting to me. He soon confirmed that the conduction velocity of the shrimp giant nerve fiber was 210 m/s. Kusano then used the Ling–Gerard type microelectrode to record the action potential intracellularly. The action potential was large (about 100 mV), but, to his big surprise, the resting potential was not recorded with the action potential. The examination of the structure of the giant nerve fiber with the aid of the electron microscope showed that the surface of the nerve fiber was coated in a thick myelin sheath. Inside the sheath, there was a large space, in which the small diameter axon floated. Therefore, the tip of his

microelectrode must have been inside the nerve fiber but outside the axon. Because the electric insulation of the myelin sheath was very high, Kusano could still record a large action potential, even though the tip of the electrode was outside the axon. A similar phenomenon had already been described in the vertebrate myelinated nerve fiber. Presumably, the conduction of the impulse along the shrimp giant fiber was saltatory in a way similar to that of the vertebrate myelinated nerve fiber. The site of excitation, corresponding to the node of Ranvier in the vertebrate myelinated nerve fiber, could not be definitely identified in the shrimp. Probably, the membrane around the giant fiber–giant motor nerve synapse was excitable and functioned in a way similar to that of the node of Ranvier in the vertebrate nerve fiber. Kusano published a preliminary note in 1965 and then a full paper in 1966. I was pleased that my laboratory had produced good papers.

In his 1966 paper, Kusano did not cite the report of the Chinese researchers who had found a large conduction velocity in the shrimp nerve fiber. Nobody outside China seemed to know who they were. Moreover, at that time, the political relationship between China and Japan was at its worst. Communication between the two countries was not possible. After completing the work on the shrimp nerve fibers, Kusano left our laboratory to return to the United States.

In 1979, I also moved to a new laboratory in Okazaki, a city some 250 km southwest of Tokyo. One day, I had a visitor from China by the name of Ke Xu. He spoke fluent Japanese, so there was no language barrier. I was totally astonished when I heard that he was one of the anonymous Chinese researchers who had first found the rapid conduction velocity of the shrimp nerve fiber. He gave me several reprints, all written in Chinese. I examined them and was convinced that they were the first discoverers. I further found that they had also used the Ling–Gerard type electrode, and found a full-sized action potential without any resting potential. Finally, they had also examined the preparation with the electron microscope and found the thick myelin sheath on the surface of the giant fiber with the small axon floating inside the sheath. They had proposed that the saltatory conduction was present in this nerve fiber and that the giant fiber–giant motor nerve synapse was a possible functional node. These papers were published between 1961 and 1964.

I, however, thought that Kusano's 1966 paper should remain the paper to be cited on this subject, because Kusano's paper was written according to the internationally accepted format, containing a detailed description of the experiment and 15 figures of high quality. Xu et al.'s 1964 paper, which reported the electrophysiology using the microelectrode, was a conference abstract consisting of just one page without any figures. Nevertheless, the description showed that the authors had indeed found most of the results Kusano published the following year and 2 years later.

Xu told me that at that time China was undergoing the Cultural Revolution. Red Guards regarded Xu's research as "useless" and prohibited him from pursuing it any further. Xu had to interrupt his research on the shrimp nerve for 20 years. He recalled that, as he was reading Kusano's 1966 paper, he was looking at the Red Guards marching in the street outside the window.

Thus, he was the igniter. He found the rapid conduction velocity of the shrimp nerve. The information was transmitted strictly westward, from China to Moscow, then to San Diego, and finally to Tokyo, to produce Kusano's paper.

Remarkably, Xu remained the igniter even after the long interruption. S. Terakawa, an associate professor in our laboratory, and Xu started a joint research project on the shrimp giant fiber in our laboratory at Okazaki. The research turned out to be highly productive. First, they showed that the appearance of the resting potential depended on the location of the tip of the microelectrode. The location of the electrode tip was determined without ambiguity by filling the microelectrode with fluorescent dye. When the electrode tip was inside the myelin sheath but outside the axon, action potentials without a resting potential were recorded. When the electrode tip was inside the axon, action potentials with the resting potential were recorded. Second, they used the sucrose gap method to voltage clamp the membrane at the giant fiber–motor nerve synapse. They showed that this part of the membrane was indeed excitable, since an inward current appeared when the clamping pulse was depolarizing. They characterized the channels and estimated the channel densities at this part of the membrane. Third, they found a completely novel type of functional node in the giant fiber which they designated as the fenestration node. In portions of the fiber where the synapse was lacking, the fenestration node furnished the site of excitation. They published these results between 1991 and 1996.

Thus, the joint research clarified ambiguities that had remained in the classic paper of Kusano. The electrophysiology of the shrimp giant fiber was completed at Okazaki. Xu spent almost 35 years to get to this goal. Apart from the definite scientific significance of the achievements, the joint research brought us additional rewards: mutual understanding, trust, and friendship between scientists from China and Japan. I believe we should never underestimate the significance of the latter. (I wish to thank Dr. Charles Edwards for correcting the English of this Foreword.)

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Preface

In the mid-twentieth century A.L. Hodgkin published the epoch-making work on the study of the mechanism of nerve excitation and conduction function in the giant axon of *Loligo*. In the autumn of 1959, Prof. De-Pei Feng, director of the Shanghai Institute of Physiology, Chinese Academy of Sciences (CAS), appointed Ke Xu to set up a new laboratory to study the peripheral nervous system of marine invertebrates at the institute. At that time, in order to get appropriate living marine animals we occasionally had to work at the Institute of Oceanology, CAS, in Qingdao. There, while searching for living sea animals with giant nerve fibers or giant neurons, F. S. Chen and K. Xu noticed that the nerve impulse in the pair of medial giant fibers in the ventral nerve cord of *Penaeus orientalis* conducted at a very high velocity. Eventually, S. F. Fan, K. Xu, F. S. Chen, and B. Hao from the two institutes cooperatively established that the conduction velocity of the giant fibers was as high as 80–200 m/s. The experimental results were first published in the Chinese journal *Kexue Tongbao* (Chinese Science Bulletin) in 1961. Since then, the nerve fibers of *Penaeus* shrimp have been one of the main research subjects in Xu's laboratory.

In 1964, Hodgkin published his classic book *The Conduction of the Nervous Impulse*, which was used as the main reference material in Xu's laboratory until 1998, when he retired. D. P. Tang and K. Xu translated *The Conduction of the Nervous Impulse* into Chinese, and the Chinese version of the book was published in the following year. Another main reference book used for their work was I. Tasaki's *Nervous Transmission* (1953a), in which the experimental evidence in support of the saltatory conduction hypothesis in the myelinated nerve fibers of vertebrates was perfectly described and established. Besides that, the famous book of comparative neurobiology *Introduction to the Nervous System* by Bullock et al. (1977) was referred to in their work as a valuable guide.

Xu's research in China, including the study of shrimp nerve fibers, was interrupted completely for about 10 years by the Cultural Revolution which started in 1966. Unfortunately, although ordinary laboratory work resumed after this political event, the study of the nerve fibers of the *Penaeus* shrimp could not recommence

owing to the lack of living specimens in Shanghai at that time. Xu's research had to aim more at applied sciences in searching for useful substances affecting the basic nervous processes, such as nervous conduction and nervous transmission, from the natural resources of China. Thus, the study of shrimp nerve fibers in his laboratory was totally interrupted for more than 20 years.

Xu's research on the nerve fibers of the *Penaeus* shrimp had a turn of fortune when he received an invitation from Prof. Akira Watanabe of the National Institute for Physiological Sciences in Okazaki, Japan. Xu carried out research on the giant fibers of the *Penaeus* shrimp in Watanabe's laboratory for a few months each year from 1980 to 1985. At the institute, technicians ran an excellent aquarium where they always kept Xu's favorite experimental animal, *Penaeus japonicus*, which was slightly larger in size even than the *P. orientalis* of Qingdao, China. Xu also came across Assoc. Prof. Susumu Terakawa, who was a young physiologist just returned from the United States to join Watanabe's laboratory. Terakawa was attracted by the fact that the shrimp giant nerve fiber has the highest conduction velocity among all animals, and he was fascinated by the unusual morphological properties of the shrimp nerves which might be related to that fact. As questions were discussed and hypothesized and put to experimental tests, our collaboration became gradually tighter. Xu was glad to be given a chance to conduct research on the nerve fibers of the *Penaeus* shrimp and Terakawa was content with studying the unknown characteristics of the shrimp nerve as well as with contributing to the development of science in China.

After Xu's work during visits to Japan, he resumed his research on the *Penaeus* nerve fibers in his laboratory in Shanghai, because living specimens of some species of the *Penaeus* shrimp were already available even in the local food markets at that time. His work continued until he retired in 1998.

In 1999, we published a review entitled "Fenestration nodes and the wide submyelinic space form the basis for the unusual fast conduction of shrimp myelinated axons" in *The Journal of Experimental Biology.* In this paper, several basic findings on the anatomical structures and the conduction functions of the nerve fibers of *Penaeus* shrimp were fully described and discussed. Because the findings revised some of the traditional conclusions on the basic structures and functions of nerve fibers described in textbooks and common reference books on physiology and neuroscience, we decided to present this monograph of experimental data and findings on the specific structural and functional characteristics of the *Penaeus* shrimp more systematically, and to discuss the similarities and differences in myelinated nerve fibers between vertebrates and invertebrates.

We are especially indebted to Prof. T. P. Feng and Prof. A. Watanabe for their support for our work. We would like to extend our thanks to S. F. Fan as well as S. K. Huang and R. Yeh for their joint efforts at the very beginning of this work and to our other co-authors of the papers cited in this monograph. We extend our thanks also to the Japan Society for the Promotion of Science for supporting K. Xu's stay at the National Institute for Physiological Sciences in 1980, and to the Ministry of

Education and Science for providing an overseas research grant supporting S. Terakawa's stay at the Shanghai Institute of Physiology in 1989 and 1990. We are indebted to many technicians who maintained a steady supply of shrimp at the laboratories of Qingdao, Okazaki, and Shanghai. Without the help from both sides, both countries, the research described in this monograph would not have been possible.

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About the Authors

Ke Xu (Ke Hsu) *(right)* was a professor of physiology at the Shanghai Institute of Physiology, Chinese Academy of Science until his retirement in 1998. From 1982 to 1986 he was Visiting Scientist in the Department of Cell Physiology, National Institute of Physiological Sciences, Okazaki, Japan. His research interest is comparative neurophysiology and neuropharmacology. One of his works has focused on the structure and function of the nerve fibers of *Penaeus* shrimp. In addition, his research has contributed to the study of neurotoxins of animal and plant origin and to the establishment of specific characteristics of nerve conduction and synaptic transmission in the snake and scorpion.

Susumu Terakawa *(left)* has been a professor at the Photon Medical Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan, since 1993, where he has been engaged in physiological research on cells using photonic technologies. The research tools have included birefringence, scattering, interference, and evanescent wave illumination, and the subjects of his research have been neurons, peripheral nerves, secretory cells, sensory cells, and tumor cells as well as the giant nerve fibers of the shrimp. He traveled to China many times for collaboration with Ke Xu and for presenting lectures on light microscopy at more than 30 institutions. His collaboration with Ke Xu once led him on a great expedition to the Takuramakan Desert in search of a mysterious green scorpion.

Contents

Chapter 1
Introduction

Textbooks and common reference books of physiology and neurobiology often start with the description of the basic morphological features of nerve fibers in their corresponding chapter. For example, in the beginning of the second chapter of the classic monograph *The Conduction of the Nervous Impulse* by Hodgkin (1964, p. 20), the classification of nerve fibers was briefly outlined with a diagram as follows:

"On the basis of their appearance under the microscope nerve fibres may be divided into two classes. In the myelinated nerve fibres which, in vertebrates, include all except the smallest axons, the axoplasmic core of the fibres is surrounded by a sheath of fatty material known as myelin." and "In unmyelinated fibres there is no fatty sheath…."

In the citation it was indicated that:

1. Nerve fibers are divided into unmyelinated and myelinated according to whether the nerve axons are surrounded by a myelin sheath.
2. Thicker axons of vertebrates are surrounded by a sheath and are called myelinated fibers.
3. Myelins are regularly interrupted by nodes of Ranvier.

The different types of impulse conduction in unmyelinated and myelinated fibers are another essential subject in these textbooks and reference books of physiology and neurobiology. The experimental materials for analyzing the specific manner of impulse conduction in the myelinated fibers of vertebrates were systematically described in the famous book *Nervous Transmission* by the Japanese physiologist I. Tasaki (1953a). In this book, it was concluded that nerve impulse conduction in the myelinated nerve fibers of vertebrates occurs in a saltatory manner through one node (the node of Ranvier) to another, in contradistinction to conduction in a continuous manner in unmyelinated fibers, which are found in the nervous system of both invertebrates and vertebrates.

In the authoritative book on comparative neurobiology *Introduction to Nervous Systems* by Bullock et al. (1977, p. 61), it was concluded that "True myelin is peculiar

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to vertebrates. Even in the lowest vertebrates, the cyclostomes, none has been found." In view of the fact that the myelin sheath had never been found in the nervous system of invertebrates, naturally neither the node of Ranvier nor the phenomenon of saltatory conduction could be found there. Consequently, it was common to find that the myelin sheath, node of Ranvier, and phenomenon of saltatory conduction were generally described to be peculiar to the nervous system of vertebrates. However, in recent years a myelin sheath that structurally and functionally compares to that of the nerve fiber of vertebrates was unexpectedly found in the nervous system of the *Penaeus* shrimp, an invertebrate in the family Penaeidae (of Crustacea). Moreover, both the common type of node and the phenomenon of saltatory conduction were established, step by step, in the myelinated fibers of this shrimp.

The findings of structural and functional characteristics of the myelinated fibers of the *Penaeus* shrimp were concisely published in a review by Xu (Hsu) and Terakawa (1999). However, these findings are not yet properly accepted by neurobiologists, except for the finding of ultrahigh conduction velocity of the medial giant fibers of *Penaeus chinensis* and *Penaeus japonicus*, which has been mentioned in some neurobiology reference books. The reason is probably that these findings would require a revision of well-established views of the basic structure and function of nerve fibers in neurobiological sciences, and they are subject to confirmatory experiments beyond any uncertainty by other researchers. Also, as a matter of fact, the published materials on these findings experienced some complications. Thus, in this monograph the experimental data on the specific structural and fundamental characteristics of the myelinated fibers of the marine shrimp are presented, as systematically as possible, and discussed in comparison with those of the vertebrate nerve fibers (Fig. 1.1).

Generally speaking, the following main subjects covered in this monograph are as follows:

1. Until the present, description of the myelin sheath as a tissue in the nervous system of vertebrates only is commonly found in the various textbooks of neurobiological science. Some citations, for example, taken from these books, which were published in recent years, follow:

   "In both vertebrates and some invertebrates, glial cells accompany axons along their length, but specialization of these glial cells to form myelin occurs predominantly in vertebrates." (Lodish et al. 2004, p. 286)

   "Axon may be either myelinated or unmyelinated. Invertebrate axons or small vertebrate axons are typically unmyelinated, whereas larger vertebrate axons are often myelinated." (From Byrne and Roberts 2005, p. 125)

   In dictionaries, a description such as "Myelin is a unique adaptation of vertebrates formed by Schwann cell in PNS and by oligodendrocytes in central nervous system" is used (Fullorlove and Robertson 2002), and a similar statement is found elsewhere (Cammack 2006).

   From these citations it is evident that the lack of myelinated fibers in the nervous system of invertebrates is a widespread view, not only in books on physiology and neurobiology, but also in biological dictionaries, when it comes

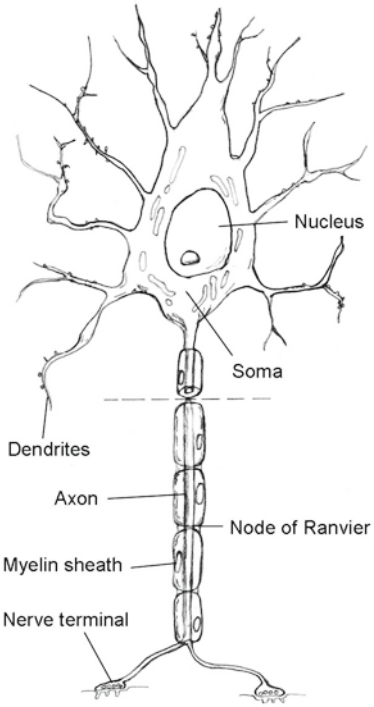

Fig. 1.1 Schematic diagram of main structures of motor nerve fiber in vertebrates

to the morphology of nerve fibers. However, in the past 20 years, discovery of a myelin sheath was established in the nerve fibers of the *Penaeus* shrimp. The experimental materials used for the studies of such a myelin sheath are described in Chap. 5 of this monograph. There, the myelin sheath of shrimp is discussed in comparison with that of vertebrates.

2. By light microscopic observation, it was established that the nerve axon is tightly enclosed by the myelin sheath in the myelinated fibers of vertebrates. Actually, under electron microscopic observation, there is a narrow extracellular gap of about 20 nm in width between the axon and the myelin sheath, as shown in Fig. 1.2.

   The more exact range of the width of the extracellular space between the axon and the myelin sheath of the vertebrate myelinated fibers was described to be 150–300 Å in the book by Hodgkin (1964, p. 24). The myelin sheath tightly enclosing the axon was even considered as a necessary condition of the myelin sheath for its functioning. However, that conclusion is now found to be true only for the myelinated fibers of vertebrates, because two unique structures designated as "submyelinic (gap) space" and "microtubular sheath" were discovered between the myelin sheath and the axon of the myelinated fibers of the *Penaeus* shrimp. The experimental materials on these discoveries are described and discussed in Chap. 6 of this monograph.

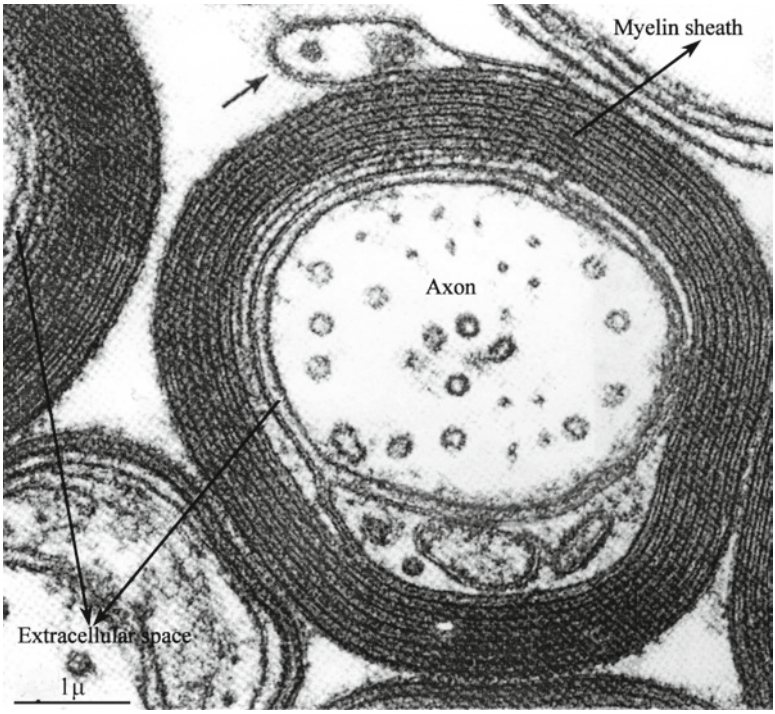

Fig. 1.2 Electron micrograph of cross section of myelinated nerve fiber of dog showing *extracellular space* between *axon* and *myelin sheath*. *Arrow* points to outer loop. *Bar* 1 μm. (Modified from Raine 1977, with permission by Plenum as a member of Springer Link)

3. Because the myelin sheath had never been found in the nervous system of invertebrates, there may be no common nodal structure, such as the node of Ranvier. However, in connection with the finding of the myelin sheath on the nerve fibers of the *Penaeus* shrimp, a few kinds of functional nodes and especially a regularly distributed novel common-type node, which is fully comparable with the node of Ranvier both in structure and in function, were established in the myelin sheath of the nerve fibers of that species. The experimental data on these findings are described and discussed in Chaps. 7 and 8 of this monograph.
4. The basic function of nerve fibers is to conduct an electrical signal, the action potential, for fast communication within a living organism. It was established that the action potential is conducted by a mechanism of regenerative local action current in both the unmyelinated and the myelinated nerve fibers, but the manner of local action current flow in the two kinds of nerve fibers differs. In unmyelinated fibers, the flow of local action current is continuous, as shown in Fig. 1.3a, whereas it is self-generated only in nodal regions and flows from one node to the next along myelinated nerve fibers. The action potential is conducted in a

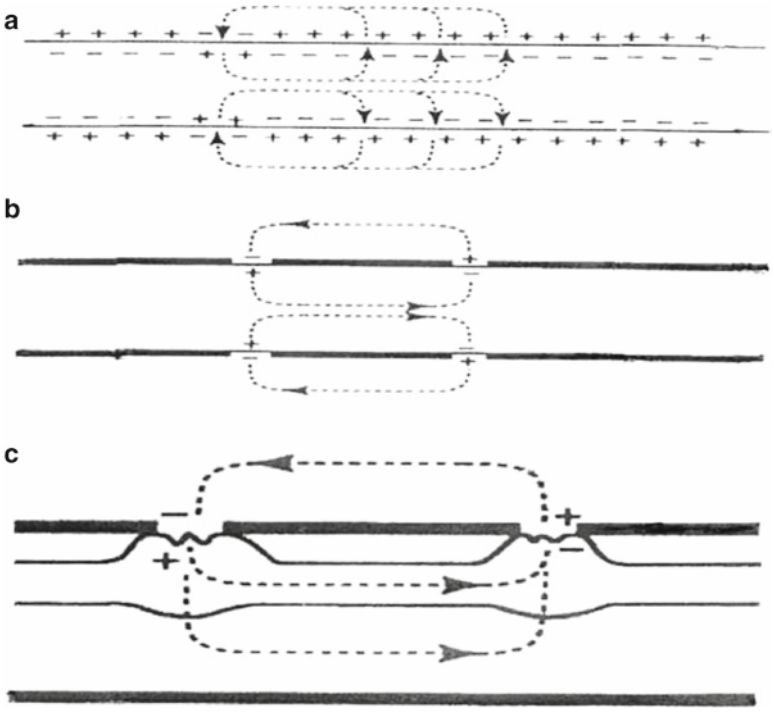

Fig. 1.3 Three types of action current flow during impulse conduction in nerve fibers. (**a**) Continuous conduction in unmyelinated fibers. (**b**) Saltatory conduction in vertebrate myelinated fibers. (**c**) Saltatory conduction in myelinated fibers of *Penaeus* shrimp. (From Xu (Hsu) and Terakawa 1999)

saltatory manner through nodes of Ranvier in the myelinated fibers of vertebrates (Fig. 1.3b). It has now been established that a specific manner of saltatory conduction occurs in the myelinated fibers of the *Penaeus* shrimp (Fig. 1.3c). The similarities and differences between the two manners of saltatory conduction are analyzed and described in several chapters of this monograph.

5. The rapid signal propagation of nerve fibers is functionally important for living organisms. The conduction velocity of nerve fibers in various living organism varies in a range from several millimeters to about 100 meters per second. From these data and the morphology of nerve fibers, two rules of nerve impulse to increase the conduction velocity were deduced:

   (a) The conduction velocity of unmyelinated nerve fibers generally increases with their axon diameter.
   (b) The conduction velocity of myelinated nerve fibers is significantly higher than that of the unmyelinated fibers of the same diameter.

Based on these experimental observations, it was concluded that two distinct mechanisms or strategies for increasing the conduction velocity of nerve fibers evolved in organisms. This subject is commonly stated in texts and reference books of physiology and neurobiology (Kandel et al. 2000; Squire et al. 2008).

As a matter of fact, in recent years a new mechanism or strategy of nerve fibers for increasing conduction velocity was found in the nervous system of the *Penaeus* shrimp. The experimental data on this finding are presented, and the characteristics of the new strategy of the myelinated nerve fibers of the *Penaeus* shrimp are discussed, in Chap. 10 of this monograph.

6. In texts and reference books of physiology and neurobiology, the highest conduction velocity of nerve fibers had been described to be about 120 m/s, the conduction velocity of the thickest of the myelinated fibers, namely the Aα group of mammalian nerve fibers. However, it was reported that the upper limit of conduction velocity of a pair of the medial giant fibers of the largest *Penaeus chinensis* and *Penaeus japonicus* was as high as 200 and 210 m/s, respectively. Although this unusually high conduction velocity was mentioned in some reference books of neurobiology, the mechanism of such high-speed conduction should be analyzed in more detail. The factors decisive to the ultrahigh velocity of nerve conduction are described and discussed in Chap. 11 of this book.
7. Finally, on the basis of the experimental findings obtained in the nerve fiber of the *Penaeus* shrimp, some ideas on the evolution of the myelin sheath, nodal structure, and conduction function of nerve fibers are discussed in the last chapter of this monograph.

Chapter 2
The *Penaeus* Shrimp as an Experimental Marine Animal for Neurobiological Study

Searching for specific experimental material is especially important in studies on the mechanism of the basic elements of the nervous system, such as nerve fibers, synapses, and neurons. For example, it is well known that Young (1936) found that there are a few unmyelinated giant fibers in the nerve trunks of *Loligo*. The diameter of the largest giant fiber is as large as about 0.5 mm. Owing to the finding of the giant axon preparation, electrophysiological analysis and biochemical studies of basic nerve activity have been greatly facilitated and promoted. Besides, the findings of the giant synapse and giant neuron in the nervous system of some marine invertebrates also made it easier to analyze the processes of basic nervous activities.

It happened to be in the 1960s that a group of Chinese young scientists (Fan et al. 1961; Huang et al. 1963; Yeh et al. 1963; Hao and Hsu 1965) and two Japanese scientists (Kusano 1966, in the laboratory of Watanabe; Hama 1966) independently reported that most of the nerve fibers in the ventral nerve cord of *Penaeus orientalis* and *P. japonicus* are enclosed by a thick myelin sheath. It is especially interesting that there is a myelinated giant fiber system in their ventral nerve cord. The myelinated giant fibers were considered to be good experimental preparations for comparative study with the unmyelinated giant fiber, which was known with respect to the structure and function. At the same time, it had been found that the myelinated nerve fibers of marine invertebrate animals have some unique structural and specific functional characteristics, which can be interesting to some neurobiologists for some particular studies on the functional relationship of the nerve axon and myelin sheath.

In the myelinated giant fiber system of the *Penaeus* shrimp, several pairs of giant synapses were found between two myelinated giant fibers. Moreover, several giant neurons were observed in the ganglia of the ventral nerve cord of the shrimp. All these basic neural structures have specific characteristics, which made them useful as an experimental preparation. Nowadays living specimens of the higher species of *Penaeus* shrimp are commonly available from local markets of large cities

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throughout the world. Moreover, the shrimp can be easily kept in a seawater tank in the laboratory for a long time for experimental use.

All experimental materials used for comparative studies on the specific structure and function characteristic to the myelinated nerve fibers of *Penaeus* shrimp described in this monograph were taken from the following species, which belong to three genera of family Penaeidae:

1. Genus *Penaeus*
   (a) *P. orientalis* Kishinouye (more specifically, *P. chinensis* Osbeck)
   (b) *P. japonicus* Bate
   (c) *P. monodon* Fabricus
   (d) *P. semiculcatus* De Haan
   (e) *P. aztecus*
   (f) *P. duorarum*
   (g) *P. setiferus*
2. Genus *Trachypenaeus*
   (a) *T. curvirostris*
3. Genus *Metapennaeopcis*
   (a) *M. barbata*

For morphological study on the myelinated nerve fibers, all the aforementioned species of *Penaeus* shrimp were used, whereas in the experiments for electrophysiological study, the medial giant fiber and motor giant fiber of *P. chinensis* and *P. japonicus* were mainly used. Although the specific functional characteristics of the myelinated nerve fibers of the *Penaeus* shrimp were studied mostly in the preparations from these two species, it could be supposed that they should share common functional characteristics with the rest of the aforementioned species of the *Penaeus* shrimp, because the nerve fibers of the *Penaeus* shrimp have the same specific structural characteristics.

2.1 Classification of Some Higher Species of *Penaeus* Shrimp

It could be further supposed that both the specific structure and functional characteristics of nerve fibers should be shared by other higher species of the shrimp family. For example, there are about 50 higher species from the following eight genera in family Penaeidae.

1. Genus *Penaeus*: about 10 species (7 were studied)
2. Genus *Penaeopsis*: 2 species
3. Genus *Parapenaeus*: 5 species
4. Genus *Metapenaeus*: 5 species (1 was studied)

5. Genus *Atypenaeus*: 1 species
6. Genus *Trachypenaeus*: 5 species (1 was studied)
7. Genus *Parapenaeopsis*: 7 species
8. Genus *Metapenaeopsis*: 13 species

These species of *Penaeus* shrimp live widely in different parts of the sea and are available for experimental use in some localities. It is expected that they will be used for neurobiological experiments and their nervous system will be further studied in future.

2.2 Morphology of the Nervous System of the *Penaeus* Shrimp

The nervous system of the *Penaeus* shrimp belongs to the ganglionic nerve system, which is usually divided into the central and peripheral systems. The former is termed the ventral nerve cord, which is located close to the ventral surface of the body along the median line. It is surrounded by a connective tissue sheath. The thoracic and abdominal segments of the ventral nerve cord can be easily excised out for experimental use.

The anatomical structure of the ventral nerve cord with the peripheral nervous system of *P. chinensis* is shown schematically as an example in Fig. 2.1. The ventral nerve cord consists of segmental ganglia and connectives between them. From head to tail there are a head (brain) ganglion, a subesophageal ganglion, five thoracic ganglia, five abdominal ganglia, and a tail (or telson) ganglion. A pair of circumesophageal connectives separately links the right and left hemisphere of head and subesophageal ganglia, respectively. A commissure nerve connects a pair of subesophageal connectives. Other connectives lie between other adjacent segmental ganglia of the ventral nerve cord.

From the head, subesophageal, and tail ganglia of the ventral nerve cord originate three, five, and four pairs of nerve trunks, respectively. Each thoracic and abdominal ganglion sends out two pairs of nerve trunks, which are named the first and the second root. A pair of the third root is sent out from each abdominal connective. Also, a pair of nerve roots branch from the subesophageal connective to innervate a pair of teeth. All nerve trunks and nerve roots comprise the peripheral nervous system of the *Penaeus* shrimp.

In the ventral nerve cord of the *Penaeus* shrimp there is a myelinated giant fiber system, which consists of a pair of medial giant fibers, a pair of lateral giant fibers, six Y-shaped motor giant fibers, and 12 pairs of the axon–axonal giant synapses formed between the medial and lateral giant fibers as well as the motor giant fibers. The medial and lateral giant fibers parallel and symmetrically run through the ventral nerve cord from the head to the tail ganglion. As to each motor giant fiber, it originates from the last thoracic ganglion or every abdominal ganglion and leaves from the corresponding connective. The giant fiber system as a whole is responsible for the fast escape reflex of the body.

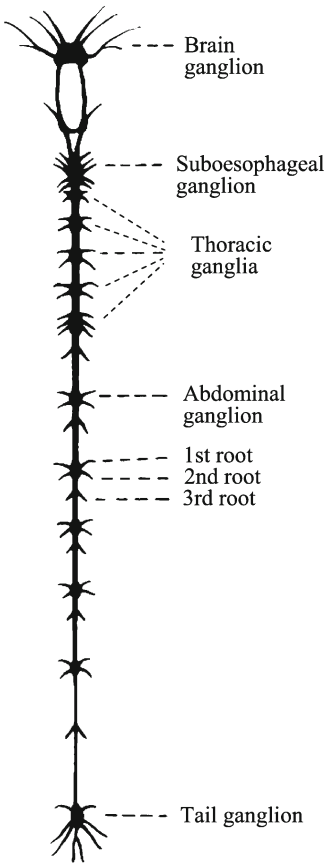

Fig. 2.1 Schematic diagram of ventral nerve cord of *Penaeus chinensis*. (Modified from Fan et al. 1961, taken by open access)

2.3 Myelinated Giant Fiber Preparation

Among the myelinated giant fibers in the ventral nerve cord of *P. orientalis (chinensis)* and *P. japonicus*, a pair of the medial giant fibers is the largest. The larger one is more than 150 μm in diameter. The medial giant fiber, especially its abdominal portion, was usually chosen to make the single nerve fiber preparation for electrophysiological experiments. Two medial giant fibers together with a thick blood vessel lie superficially in the dorsal side of the ventral nerve cord and are surrounded by a thick connective tissue sheath, which separates them from the other nerve fibers in the ventral nerve cord. Therefore, the medial giant fiber is easily excised out for experimental use.

Passing through each thoracic and abdominal ganglion, each medial and lateral giant fiber provides a thin branch, respectively. In a ganglion, the two branches lose their myelin sheath and mutually cross to opposite sides (Fig. 2.2a). The branch seems to be the axon of the neuron(s) located in the abdominal side of the ganglion,

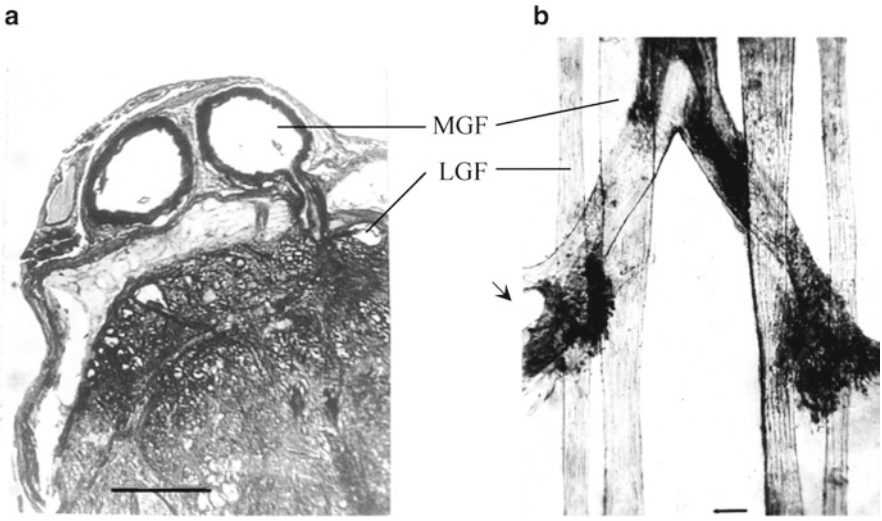

Fig. 2.2 Functional node. (**a**) Cross-sectional micrograph of abdominal ganglion of *P. chinensis* shows unmyelinated branches of a medial and a lateral giant fiber. (**b**) Micrograph of four giant synapses formed between a pair of medial and a pair of lateral giant fibers with a Y-shaped motor giant fiber of *P. chinensis*. *MGF*, medial giant fiber; *LGF*, lateral giant fiber; *arrow*, giant synapses. *Bars* 100 μm. (From Xu, unpublished data)

because there is no synaptic delay mentioned during the impulse conduction along the giant fibers. If this consideration is true, the medial giant fiber should be a common axon of neurons located in the head ganglion and each thoracic and abdominal ganglion.

A Y-shaped motor giant fiber originates from the last thoracic and every abdominal ganglion. It runs backward for a little distance along the ventral nerve cord and divides into two branches, which form two pairs of giant synapses with both the medial and the lateral giant fibers, respectively, before leaving the ventral nerve cord through the third root (Fig. 2.2b).

The motor giant fiber is also a syncytium. In the last thoracic ganglion and each abdominal ganglion there are two groups of neurons, which are located in the abdominal side of a ganglion symmetrically along the middle line of the ventral nerve cord (Fig. 2.3). The two groups of neurons possess their axons, respectively, but they once more fuse to a common axon as they leave the ganglion. The commonly fused motor giant axon finally divides again into two branches before leaving the connective through three roots (Fig. 2.2b).

The myelinated giant fibers of the *Penaeus* shrimp can be also used for biochemical study. For example, the free amino acid (FAA) contents of the medial and the motor giant fibers were determined, respectively, using single fiber preparations obtained from *P. chinensis*. It was found that the concentrations of aspartic acid, glutamic acid, and total neutral amino acids in the motor giant fiber are 7.5, 3, and 3.3 times higher than those in the medial giant fiber, respectively. The higher

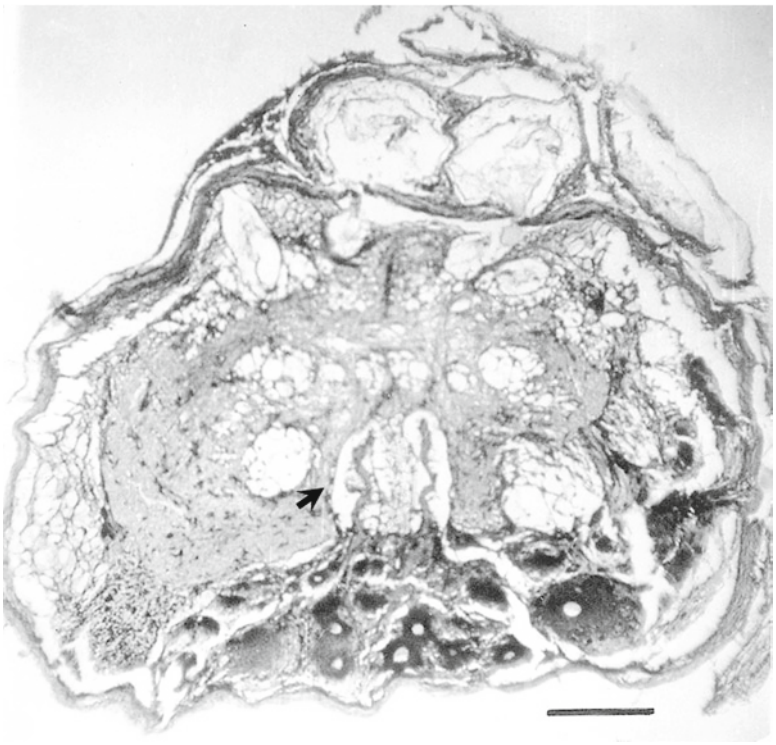

Fig. 2.3 Cross-sectional micrograph of abdominal ganglion of ventral nerve cord of *P. chinensis*. A pair of motor axons formed by two groups of motor neurons is shown in *bottom part*. *Arrow* indicates two common axons of two groups of motor neurons. *Bar* 100 μm. (From Xu, unpublished data)

concentration of FAAs in the motor giant fiber is certainly because the axon diameter of the motor giant fiber is thicker than that of the medial giant fiber. What is not explained by the diameter is that the concentration of aspartic acid in the motor giant fiber was two times higher than that of glutamic acid, whereas no such difference was found in the medial giant fiber (Shih and Hsu 1979). The difference in proportion of aspartic acid to glutamic acid is probably the result of the distinctive function of the motor axon and the axon of the interneuron of the shrimp.

Recently, the myelin sheath of the peripheral and especially the central nervous systems of vertebrates became a field of active study; thus, the new variation of the myelin sheath of invertebrate nerve fibers should be a useful subject for comparative studies.

Incidentally, in all myelinated nerve fibers of the *Penaeus* shrimp there is a unique axon sheath, which consists of the longitudinal bundles of microtubules. The nerve tissue of the *Penaeus* shrimp should be a good preparation for a comparative biochemical study of microtubules in the nerve tissue of a living organism. Actually, Jiang et al. (1987) purified tubulin, a basic protein of microtubules, from

the ventral nerve cord of *P. chinensis*, and showed that the protein is rather conservative, because its molecular characteristics are similar to that prepared from rabbit brain. For example, the tubulin extracted from the ventral nerve cord of *P. chinensis* was dissociated also into two subunits with the molecular weight of about 55,000. The microtubules reconstructed from tubulin of *P. chinensis* nerve tissue were similar to those extracted from rabbit brain. The antiserum containing anti-tubulin against chicken brain was prepared from rabbit. It was shown that the microtubular sheath in the myelinated nerve fiber of *P. chinensis* positively reacted with the fluorescently labeled goat anti-rabbit IgG. These results showed that the microtubules in *P. chinensis* nerve tissue are molecular homologs to those in vertebrate nerve tissues.

2.4 Axon–Axonal Giant Synapse Preparation

Two pairs of giant synapses in the connective between the last thoracic and the first abdominal ganglia were dissected out from the ventral nerve cord of *P. chinensis* (see Fig. 2.2b). These giant synapses are formed between a pair of medial giant fibers and a pair of lateral giant fibers with a Y-shaped motor giant fiber. These axon–axonal giant synapses of the medial giant fiber to the motor giant fiber are larger than others, which are located in other connectives between two adjacent abdominal ganglia of the ventral nerve cord of the shrimp, and are more useful for the experimental study of the synapse.

The morphology of the giant synapse was demonstrated by the dye-injection method. In the experiments, a giant synapse preparation consisting of a piece of the medial giant fiber with a piece of the connected motor giant fiber of *P. japonicus* was used. A dye (Lucifer yellow) was injected through a microelectrode mostly into the axon of the postsynaptic motor fiber, because its diameter is thicker than that of the medial. In a few cases, the injected dye leaked through the synaptic membranes into the presynaptic axon (Fig. 2.4a). The result showed that there are gap junctions between the presynaptic and postsynaptic membranes that permit the dye molecules to pass through. The gap junction was also demonstrated by electron microscopic technique in the synaptic membranes of the giant synapses of both *P. japonicus* and *P. chinensis* (Kusano 1966; Xu and Zhao 2000). Consequently, it could be concluded that in the giant synapse there is an electrical transmission that plays the functional role in saltatory conduction of the giant fiber.

According to Fig. 2.4a, the presynaptic axon of the giant synapse sends a thin branch to connect with a thick side hillock of the postsynaptic (motor) axon. Although there is a significant difference in size between the presynaptic and postsynaptic axons, the giant fibers form a giant synapse of a certain area, which is a morphological characteristic of the axon–axonal giant synapse of the shrimp. In a rare case, the pre- and postsynaptic axons are connected directly without a connection through the thin branch, as was shown in the paper by Hsu and Terakawa (1984). In some cases, the dye injected into the postsynaptic axon did not leak into the presynaptic

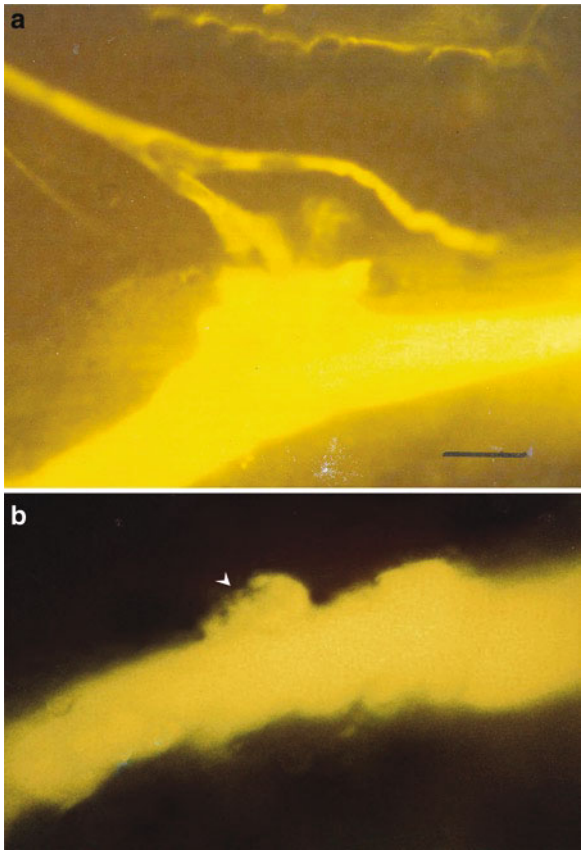

Fig. 2.4 Micrographs of giant synapse of medial giant fiber to branch of Y-shaped motor giant fiber of *Penaeus japonicus* dyed with Lucifer yellow. (**a**) Axon of postsynaptic motor giant fiber injected with the dye shows a diffusion into the axon of presynaptic medial giant fiber. (**b**) Dye injected into axon of this postsynaptic motor giant fiber did not diffuse into the axon of presynaptic medial giant fiber. *Arrowhead* indicates postsynaptic uneven side hillock. *Bar* (**a**) 50 μm. (From Xu and Terakawa, unpublished data)

axon (Fig. 2.4b). This preparation showed that the side hillock of the postsynaptic axon came into contact with the presynaptic ending by uneven invasions.

The fine structure of the giant synapse was primarily studied by the technique of electron microscopy. Several pictures showed that in the region of the giant synapse the presynaptic axon has many small buttons before contacting the postsynaptic membrane of the uneven side hillock of the motor giant fiber (Fig. 2.5a). Thus, the total area of the synaptic membranes of the giant synapse is greatly increased by the ending buttons, in which abundant vesicles of 30–50 nm in diameter (Fig. 2.5b) are present. In the synaptic membrane of the buttons are usually also displayed presynaptic densities besides the structure of gap junction. Postsynaptic membrane densification was sometimes observed (Kusano 1966). According to these facts, it could be preliminarily concluded that the giant synapse is an electrical and chemical mixed synapse, although the synaptic membrane densification does not appear to be identical to conventional chemical synapses. Having both electric and chemical transmission, the giant synapse should be another morphological characteristic of the axon–axonal giant synapse.

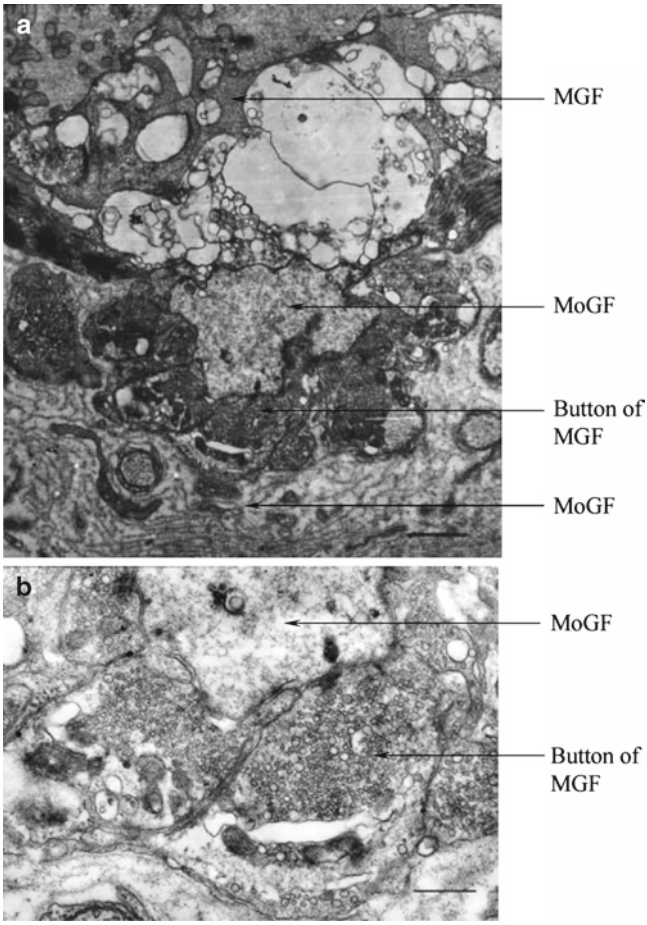

Fig. 2.5 Electron micrographs of giant synapse of medial giant fiber to motor giant fiber of *P. chinensis*. (**a**) Presynaptic axon ends with *buttons* around a lateral process of the postsynaptic axon to make the synaptic contacts. (**b**) Synaptic vesicles in presynaptic buttons of giant synapse. *MGF*, medial giant fiber; *MoGF*, motor giant fiber. *Bars* (**a**) 1 μm; (**b**) 0.5 μm. (From Xu, unpublished data)

Synapses are usually divided into two classes: the electrical and the chemical. Concerning the electrical synapses, two examples are usually given in texts and reference books of neurobiology (e.g., Kandel et al. 2000, p. 177). The bilateral electrical synaptic transmission occurs at the septa of the lateral giant fiber of crayfish (Watanabe and Grundfest 1961) and the unilateral electrical synaptic transmission occurs at the giant synapse of the lateral giant fiber to the motor giant fiber of crayfish (Furshpan and Porter 1959). Subsequently, Giaume and Korn (1983) found that the electrical transmission of the giant synapse of the lateral giant fiber to the motor giant fiber of crayfish is really bilateral: the rectifying mechanism of the synapse is caused by the difference in resting membrane potential between the

presynaptic giant fiber and the postsynaptic motor giant giber (by about 15 mV). However, according to experimental data available, the transmission of the giant synapses of both the lateral and the medial giant fiber to the motor giant fiber is bilateral (Ma et al. 1998).

It is evident that the giant synapses of the medial and the lateral giant fibers to the motor giant fiber of the *Penaeus* shrimp should be a useful preparation for analyzing the transmission mechanism of electrical and chemical mixed synapses.

2.5 Giant Neuron Preparation

The giant neurons in the ganglia are assembled in the abdominal side of the ventral nerve cord of the *Penaeus* shrimp. Most of the neurons are unipolar. Among them, several giant neurons about 200 μm in diameter lie superficially in the dorsal side of the ganglion (see Fig. 2.3). The giant neurons can be easily removed from the ganglion for experimental use. Thus, the giant neurons should be a convenient preparation for both physiological and biochemical studies. For example, several (from 7 to 17) fresh giant neurons 80–270 μm in diameter were collected from the abdominal ganglia of *P. chinensis* as a sample, whereby their FAA content was determined. It was found that a considerable amount of γ-aminobutyric acid was present in the ganglionic neurons, but no trace of it could be detected in either the medial giant fiber or the motor giant fiber of the shrimp (Shih and Hsu 1979).

2.6 Stretch Receptor Preparation

It is well known that in crayfish there is a pair of stretch receptors with distinct structural and physiological characteristics occurring on both sides of the dorsal musculature in each abdominal segment: one is a rapidly adapting and the other a slowly adapting receptor. In the sea shrimp *Penaeus chinensis*, a similar pair of stretch receptors was found, which is easily isolated to become a good preparation for electrophysiological experiments. Yang and Xu (1984) interestingly observed that during maintained stretch of the two receptor muscles, both slow- and rapid-adapting stretch receptors of the shrimp showed a continuously rhythmic or tonic discharge pattern when seawater was replaced with Mg^{2+}-free medium. In contrast, when the Mg^{2+} concentration of the perfusion seawater was doubled, both types of stretch receptor showed the rapid adapting or phasic discharge.

2.7 Cardiac Ganglion Preparation

In the cardiac ganglion of lobster, it was found that the large follower cells are connected together by pathways that are very effective for electronic spread, but not for transmission of brief potential changes such as spikes (Watanabe 1958; Watanabe

and Bullock 1960). It is worth noticing that the neurons in the cardiac ganglion of the *Penaeus* shrimp could be a preparation for study of the specific neurons innervating the shrimp heart, which is located close to the dorsal surface along the median line under the head crest. Especially, the rhythmic contraction of the heart, clearly visible to the unaided eye, would reveal its electrical function.

2.8 Overall View

In the nervous system of the *Penaeus* shrimp, there is a myelinated giant fiber system that can be easily made into preparations of the myelinated giant fiber, giant synapse, and giant neuron. The shrimp is widely distributed in the sea and easily survives in the laboratory. It should be a convenient experimental animal for neurobiological study.

Chapter 3
Morphological Studies on the Myelin Sheath of Nerve Fibers

3.1 Some Significant Events in the Morphological Studies of Nerve Fibers by Light Microscopy

The first description of the peripheral myelinated fibers in the nervous system of vertebrates is believed to have appeared as early as in 1719 in the book *Epistolae physiologicae super compluribus naturae arcanis* by Antonie van Leeuwenhoek. Historically, the myelinated nerve fiber was regarded as a tubular structure consisting of a dark core and a light shell (see Sotnikov 1976). The dark core was then established to be connected with the body of nerve cells, and thus was called the axon. The light shell surrounding the axon was shown to consist of lipids and lipoproteins, and was suggested to be named myelin. Actually, myelin, considered as a structural entity of nerve fibers, has been known since the mid-19th century, and its name was believed to be generally attributed to Virchow who reported the presence of sheaths around the nerve fibers in 1854 (see Raine 1977).

Myelin Sheath Formation of the myelin surrounding the peripheral nerve axon of vertebrates was shown to relate to the Schwann cells. In 1838, it was R. Remak who for the first time mentioned those elements that ever since have been known as Schwann cells. The author stated that the nerve fibers in the sympathetic nervous system (which had been termed "Remak fibers") bear small, oval, or round corpuscles. In the following year, T.A. Schwann correctly confirmed that these corpuscles are cell nuclei, and the cells were then called after his name, Schwann cells. Considering the relationship between the myelin sheath and Schwann cells, Ranvier had considered in 1854 that the myelin sheath was a fluid or nearly fluidic fat produced by and contained within the Schwann cells. Other workers, on the other hand, regarded the myelin sheath as an extracellular material secreted by the axon and lying between it and the Schwann sheath, which otherwise was called the neurilemma at that time (see Pannese 1994).

It is evident that the nature of the myelin sheath was not yet clarified at that time. Thus, for a long time, the layer called the neurilemma or Schwann sheath and the

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myelin sheath itself had been successively considered to be associated with the nerve axon. Accordingly, depending on whether these two components were present, the nerve fibers were generally divided into four groups in the literature on the nervous system. For example, in the classic book on *The Anatomy of the Nervous System*, by Ranson (1922, p. 47), the nerve fibers were classified as follows:

1. Myelinated fibers with a neurilemma found in the peripheral nervous system, especially in the cerebrospinal nerves
2. Myelinated fibers without a neurilemma, found in the central nervous system
3. Unmyelinated fibers with nuclei (Remak fibers), especially numerous in the sympathetic system
4. Naked axons, abundant in the gray matter of the brain and spinal cord

As a matter of fact, the undecided question on the relationship of neurilemma to the myelin sheath of nerve fibers was finally settled by using the electron microscopic method to study the fine structure of nerve fibers.

Node of Ranvier As early as 1871, Ranvier observed that the myelin sheath of the peripheral nerve axons of vertebrates was regularly interrupted by narrow gaps. The author further investigated the structure of these gaps in living frog nerve fibers and in nerves of several mammals by various techniques. He finally concluded that the intermittent gaps represented the regions of contact, which he called the node. Since then, the gap structures were designated after his name as "nodes of Ranvier" (Pannese 1994). As for the existence of nodes of Ranvier in the central nervous system of vertebrates, debates continued for more than half a century. In the 1940s, their presence in the central nervous system of vertebrates was established by microscopic studies on teased nerve fibers by Hess and Young (1949).

Schmidt–Lanterman Incisures In 1874–1877, H.D. Schmidt and A.J. Lanterman consecutively reported that in the sheath of the peripheral myelinated fibers of vertebrates there were intermittent funnel-shaped incisures, which then were named after them as Schmidt–Lanterman incisures (S-L incisures) or S-L clefts in the literature. A micrograph of S-L incisures is shown in Fig. 3.1. Actually, the real entity of S-L incisures had been doubted for a long time, until the next century, when the reality of the structure was elucidated by electron microscopy technique. The fine structure and the mechanism of formation of S-L incisures were finally established by the work of Robertson (1958).

By light microscopy studies, it was established that the myelin sheath is present on the thick nerve axons in the nervous system of all vertebrates as well as some lower species (see Bullock et al. 1977, p. 61). Study with a polarized light microscope revealed that the myelin sheath of living nerve fibers is distinctively birefringent, indicating that it consists of a laminated structure (Schmitt and Bear 1937). A connective tissue sheath outside the myelin sheath of nerve fibers has a low birefringence and can be clearly differentiated from the myelin sheath under the polarized light microscope.

The Sheaths of the Invertebrate Nerve Axons As for the sheaths of the nerve axons of invertebrate animals studied by light microscopy, there were just a few reports indicating that the developed sheaths of nerve axons were found only in limited

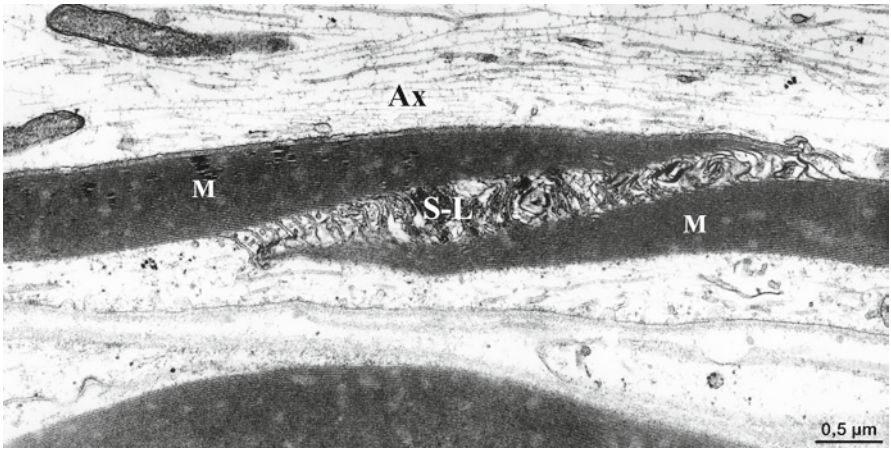

Fig. 3.1 Electron micrograph of a Schmidt-Lantermann incision *(incisure)* in the rat myelinated fiber (*S-L*). *M*, myelin sheath. *Ax*, axon. *Bar*, 0.5 mm. (From a web site by H. Jastrow with permission)

species belonging to the Annelida and the Arthropoda. Namely, it was reported that some nerve fibers of earthworms, prawns, shrimps, and crabs have a sheath resembling the myelin sheath of vertebrates. To put these observations concretely:

1. A well-developed nerve axon sheath was found in a number of species belonging to three families of polychaetes (Nicol 1948).
2. Many nerve fibers in the decapod crustaceans (shrimp, prawns, and crabs) possess a sheath that bears a striking similarity, under the light microscope, to the myelin sheath of vertebrates, including the interruption by nodes (Johnson 1924; Holmes 1942; see Roots 1984).

The myelin-like sheath of invertebrates observed under the polarized light microscope showed a distinct birefringence property, indicating that it should consist of the laminated structure (Bear and Schmitt 1937; Taylor 1941). However, a structure similar to S-L incisures in the myelin-like sheaths of the nerve axons of the aforementioned invertebrate animals was not reported.

3.2 The Myelin Sheath of the Nerve Fibers of Vertebrates Studied by Electron Microscopy

The fine structures of the myelin sheath, node of Ranvier, and S-L incisures in the nerve axon of vertebrates defined by the light microscopic method were further studied by the technique of X-ray diffraction and especially by electron microscopy methods.

Myelin Sheath The polarization microscopic studies by Schmitt (1936), Schmitt and Bear (1937), and especially the X-ray diffraction studies by Schmitt and his colleagues (Schmitt 1936; Schmitt et al. 1936), provided the first suggestions that the peripheral myelin had a concentric lamellar structure with a periodicity of about 17–18 nm (see Raine 1977, p.2).

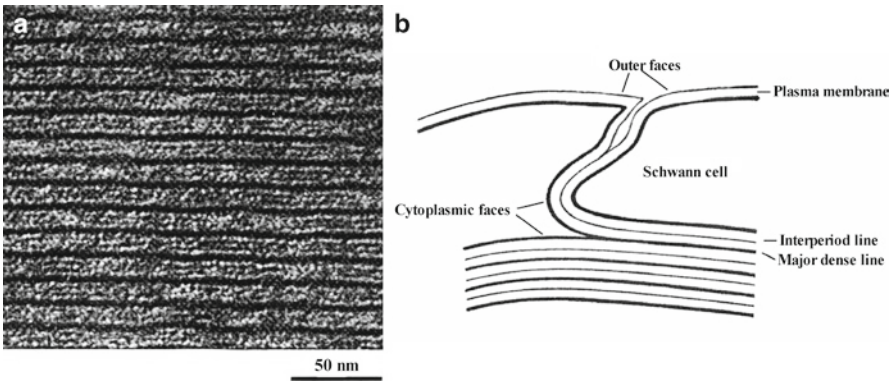

Fig. 3.2 Multiple layers of myelin sheath. (**a**) Electron micrograph of part of myelin sheath of dog nerve fiber shows alternately repeated *major dense lines* and *interperiod lines* in the myelin sheath. (From Raine 1977.) (**b**) Schematic diagram shows *major dense lines* and *interperiod lines* formed by two *cytoplasmic faces*, respectively, of extended membrane of *Schwann cell*. (From Schmitt and Geschwind (1957). Permission from Elsevier

The first visualization of the "concentric lamellae" in the myelin sheath of vertebrate nerve fibers suggested by X-ray diffraction study was reported by Fernándes-Morán (1950) and Sjostrand (1953). By electron microscopic observations, the compact layers of myelin sheath in transverse sections of the fixed nerve fibers were shown to be an alternating repetition of major dense and intraperiod lines, and the period of the two major dense lines was measured to be about 10 nm (Fig. 3.2a). The mechanism of formation of the compactly multilayered myelin sheath from the Schwann cell was clearly established by Geren (1954) in the peripheral nerve fiber preparation of chick embryos. It was shown that the major dense and intraperiod lines are formed by the extended cell membrane of the Schwann cell (shown schematically in Fig. 3.2b).

Before myelination, an axon lies in an invagination of the Schwann cell (Fig. 3.3a). The membrane of the cell then surrounds the axon and joins to form a double membrane structure that communicates with the cell surface. This structure, called the mesaxon, then elongates around the axon in a spiral fashion (Fig. 3.3b). After a few turns are completed, the cytoplasm of the Schwann cell disappears, leaving the insulating membranes piled on top of one another (Fig. 3.3c). The mesaxon accordingly winds around the axon. The cytoplasmic surfaces of the extended plasma membrane of Schwann cell are condensed to form the major dense line, and its two external surfaces form the intraperiod line (see Fig. 3.2b).

Generally speaking, the myelin sheath of peripheral nerve fibers is formed with the plasma membrane of a Schwann cell flattened by emptying its cytoplasm and wrapping the axon spirally. Thus, the myelin was finally clarified to be an appendage of the Schwann cell, and the myelin sheath is dependent upon intercellular interactions with the axon of nerve cell during its formation. The myelin sheaths of the peripheral and the central nerve fibers were established as being formed by the

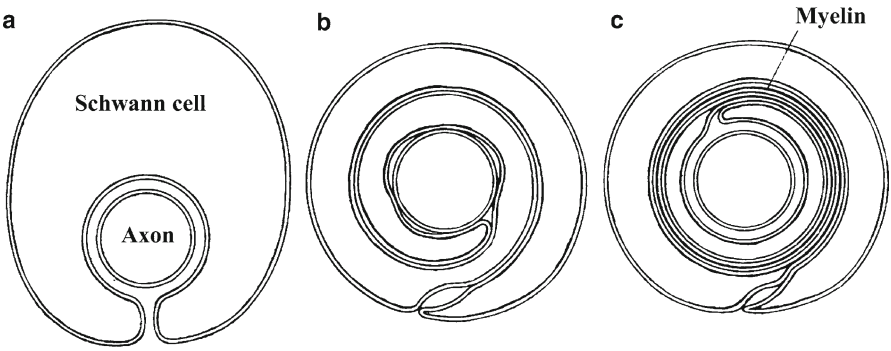

Fig. 3.3 Schematic diagram shows spiral wrapping of extended membrane of *Schwann cell* around nerve *axon*. (From Robertson 1960. Permisson from Elsevier)

Schwann cell and oligodendrocyte, respectively. The apparent morphological difference between the two kinds of glial cells is that a Schwann cell forms an internodal segment of myelin sheath for a peripheral nerve fiber only, whereas an oligodendrocyte forms a segment of the myelin sheath at the same time for a number of central nerve fibers.

Fine Structures of the Nodal Area The fine structures of the Ranvier node and its nearby region were studied by Uzman and Nogueira-Grag (1957), Robertson (1959), and others. In the longitudinal section of myelinated fibers including a node of Ranvier, it was shown that every major dense line, as well as the intraperiod line, of the myelin sheath regularly ends at the edge of the nodal region with a lateral loop, which is attached tightly to the axonal membrane next to the Ranvier node. The structure of the lateral loops tightly connected to the axoplasmic membrane is specifically called the paranodal region. An obvious difference in the morphology of the nodal region of the peripheral and the central myelinated fibers of vertebrates is that the nodal region of the peripheral myelinated fibers is covered with loose processes of astrocytes, another kind of glial cell (Fig. 3.4a, b).

Fine Structures of S-L Incisures S-L incisures and lateral loops in the paranodal region were supposed to be formed by a remaining cytoplasmic canal or tube of the fused cytoplasmic surfaces of Schwann cell or oligodendrocyte membrane. The formation of the lateral loops and of the S-L incisures is schematically shown in Fig. 3.5. If a myelin sheath was unrolled from the axon, it would be a flat, spade-shaped sheet surrounded by a canal or tube filled with cytoplasm. The major dense line formed by apposition of the cytoplasmic faces opens up at the edges of the sheet, enclosing the cytoplasm within a lateral loop. If the spade-shaped sheet was rolled on the axon, the lateral loops should regimentally be arranged at the paranodal region.

By electron microscopic study, the S-L incisures were shown to be common in the peripheral myelinated fibers of vertebrates, but rare in the central ones. As is shown in the scheme (Fig. 3.5), they are the regions where the two cytoplasmic

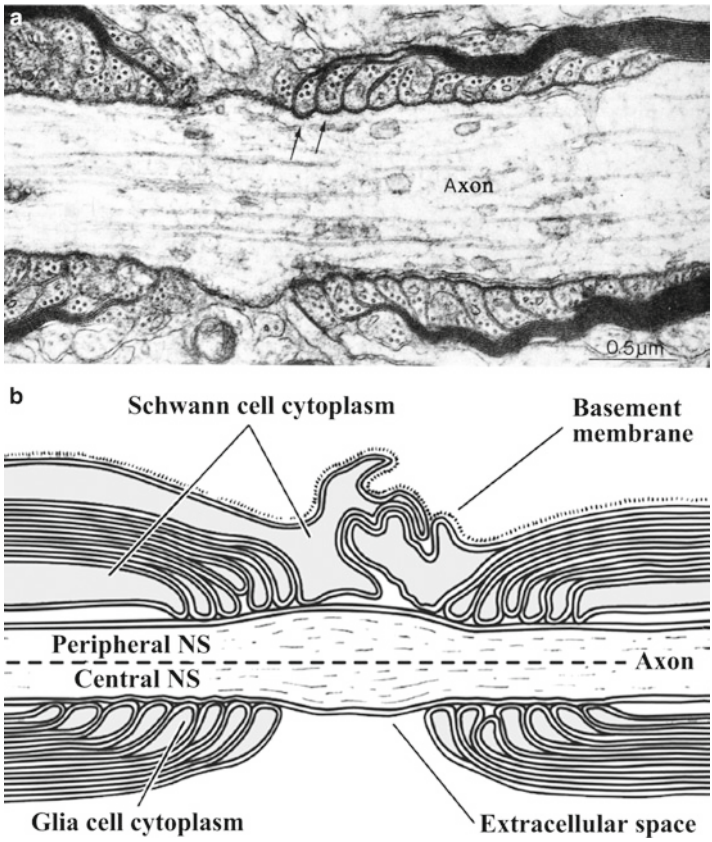

Fig. 3.4 Node of Ranvier. (**a**) Electron micrograph of paranodal region of central myelinated fiber of rat. Myelin layers ended with lateral loops in the paranodal region (*arrows*). (**b**) Schematic diagram of fine structures of paranodal region of vertebrate myelinated fiber illustrates lateral loops in paranodal regions of both *peripheral* nervous system (*NS*) and *central NS* myelinated nerve fibers. (From R. P. Bunge 1968)

surfaces of the extended cytoplasmic membrane of a Schwann cell or oligodendrocyte are not piled to form the major dense line, with cytoplasm remaining between them.

As a result of the establishment of the fact that the myelin sheath is simply formed with the extended cytoplasmic membrane of a Schwann cell or oligodendrocyte, the use of the term “neurilemma” or “Schwann sheath” disappeared from the literature, or it is seldom used to express the cytoplasmic layer of Schwann cell in the myelin sheath of vertebrate nerve fibers.

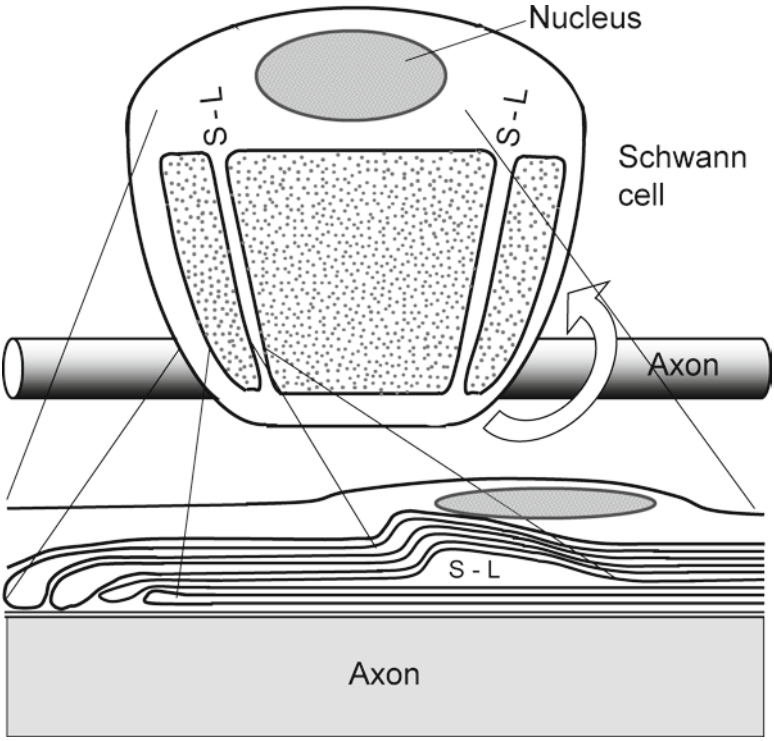

Fig. 3.5 Schematic diagram shows formation of *Schmidt–Lanterman incisures* (incisions) and *lateral loops* in *paranodal region* of a vertebrate myelinated fiber. Modified from (Hirano and Dembitzer 1967), with permission by Rockefeller University Press

3.3 The Myelin-Like Sheath of the Nerve Fibers of Some Invertebrates Studied by Electron Microscopy

The sheath of nerve axons of invertebrates was first studied under the electron microscope in the brain ganglion of crab (*Cancer irroratus*) by McAlear et al. (1958). They showed that the sheath is strikingly similar to the myelin sheath of vertebrates, and that it also bears two associated structures, which resemble the node of Ranvier and S-L incisures, respectively, of the myelinated fibers of vertebrates. Today, this conclusion of the early electron microscopic work on the fine structure of the crustacean nerve sheath should be enforced by a detailed comparison of it with the vertebrate myelin sheath, because up to now, the widely adopted view that the true myelin sheath and true nodal structure do not exist in invertebrates has not yet been changed in the neurobiological literature. As to the S-L incisures, there has been no subsequent report describing their participation in the ensheathment of the invertebrate nerve fibers.

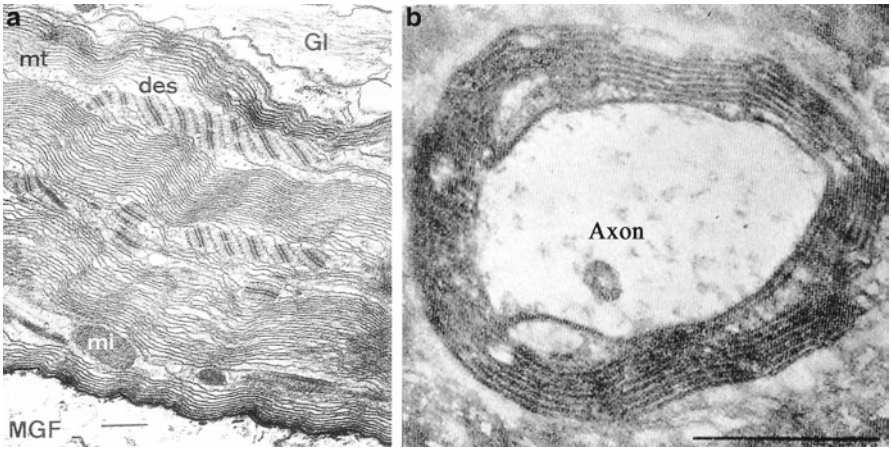

Fig. 3.6 Comparison of myelin-like sheaths. (**a**) Electron micrograph of myelin-like sheath of median giant fiber of earthworm (*Lumbricus terrestris*). *des*, desmosomes; *mi*, mitochondria; *mt*, microtubules; *Gl*, glial cells; *MGF*, median giant fiber. (From Günther 1976, with permission) (**b**) Electron micrograph of cross-sectioned nerve fiber of shrimp (*Macrobrachium nipponensis*) shows circular-type layers in myelin-like sheath. (From (Yeh and Huang 1962), with permission by the Biophysica Biochemica Acta Sinica journal office). *Bars* 1 μm

Later on, the sheaths formed by the multilayered membranes of glial cells were observed in some other species of invertebrates, but they are not completely devoid of cytoplasm. The fine structures of the myelin-like sheath enveloping the median and two lateral giant nerve axons were reported in two species, *Eisenia foetida* (Hama 1959) and *Lumbricus terrestris* (Coggeshall 1965; Günther 1973, 1976), of earthworms (Lumbricidae). It was concluded that the sheaths are a mixture of compacted and noncompacted portions, or both of these arranged concentrically. As an example, the fine structure of the myelin-like sheath of the median giant fiber of the earthworm *L. terrestris* is shown in Fig. 3.6a. Moreover, the median giant fiber is more heavily "myelinated" than a pair of the lateral giant fibers. It is interesting to mention that the median giant fiber of the earthworm was proved to conduct nerve impulses in a saltatory manner, and the functional nodes are an unmyelinated branch and a short opening in the loosely multilayered sheath (Günther 1976). This subject is described and discussed later in more detail.

The loosely multilayered sheath of the nerve axons of the prawn (*Macrobrachium niponensis*) was reported by Yeh and Huang (1962). The repeating units of the sheath layers were measured to be 24 and 42 nm. It is evident that the fine structure of the sheath of the nerve axon in the prawn is also different from that of vertebrates. It is interesting to mention that the layers of the prawn sheath having the repeating units of 42 nm are the circular type (Fig. 3.6b). Actually, the circular-type multilayered structure was also found in the compact multilayered sheath of the nerve axons of *P. chinensis* (see Fig. 5.4b, later in this volume).

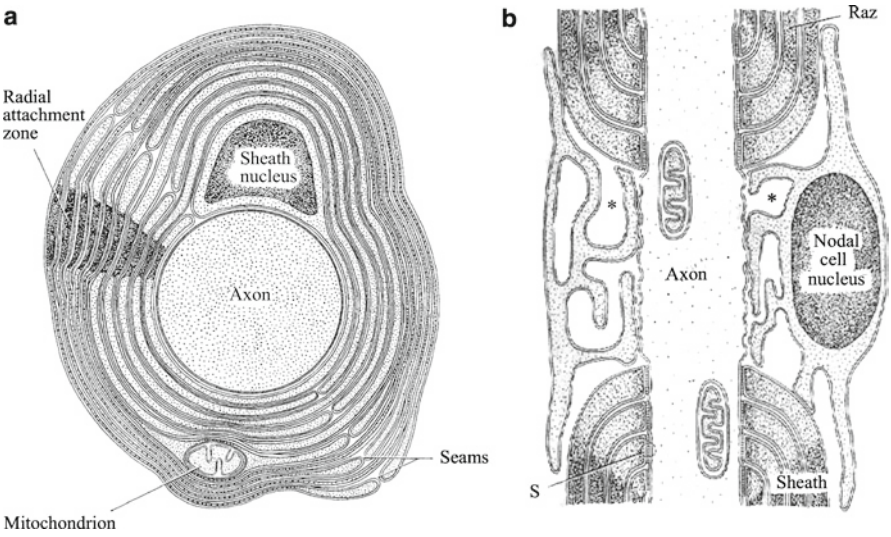

Fig. 3.7 Schematic diagrams show fine structures of myelin-like sheath (**a**) and nodal region (**b**) of prawn (*Palaemonetes vulgaris*) nerve fibers. *Raz*, radial attachment zone; *S*, septate structure formed by laminae of myelin sheath. (From Heuser and Doggenweiler 1966, with permission by Rockefeller University Press)

The fine structure of the myelin-like sheath of the prawn (*Palaemonetes vulgaris*) nerve axons was minutely studied by Heuser and Doggenweiler (1966). The authors pointed that the loosely laminated sheath of the prawn nerve axons is significantly different from the compactly laminated sheath of the vertebrate nerve axons. Moreover, the nodal structure of the prawn differs from the Ranvier node of vertebrate nerve axons in several aspects. It was concluded that these loose structural features might make the prawn sheath with its nodes less effective than vertebrate myelin in speeding up impulse conduction. In their paper, schemes show the fine structure of the myelin-like sheath and the node of the prawn nerve axon. As shown in Fig. 3.7a, the sheath is composed of many lamina, each an isolated cellular process that contains a cytoplasm and surrounds the axon concentrically to form a simple cellular seam. The seams are arranged in a highly organized manner, as the seams of alternate laminae are in close register and are on the side of the axon opposite to the seams of adjacent laminae. The question of the formation of this sheath, unfortunately, as the authors indicated, is as yet unexplained. The paranodal region of the prawn sheath is closely connected with the axon but is separated from it by a gap of about 200 Å (Fig. 3.7b).

Recently, an interesting experimental result was obtained in studies of the ensheathments of the nerve axons of copepods, small planktonic crustaceans with very quick reaction time (Weatherby et al. 2000). The myelin-like sheath of the nerve axons of the small planktonic crustaceans is also loosely laminated.

Indeed, among the ensheathments of the invertebrate nerve fibers, a sheath composed of compact layers apparently similar to the myelin sheath of the nerve axons of vertebrates has been reported in nerve fibers of *P. chinensis* (Huang et al. 1963; Yeh et al. 1963; Hao and Hsu 1965), as well as in that of *P. japonicus* (Kusano 1966; Hama 1966). However, there were some complications in the reported fine structure of the *Penaeus* shrimp nerve fibers. For example, in the paper of Huang et al. (1963), it was indicated that the myelin sheath of *P. chinensis* nerve axons is mixed with a radial narrow thick-layer zone. Especially, Kusano (1966) and Hama (1966) reported that the sheath of *P. japonicus* nerve axons is separated by a wide gap into an outer compactly laminated and an inner loosely laminated component. Therefore, the sheath naturally could not be considered as the true myelin sheath by the authors themselves and in the neurobiological literature. This question is described and discussed in the next two chapters.

3.4 The Myelin Sheath Was Concluded to Have Evolved in Vertebrates

In the book *Introduction to Nervous Systems* by Bullock et al. (1977, p. 59) it was described in the classification of nerve fibers that the nerve axons usually are surrounded by a sheath, but many smaller fibers are naked axons, which are not immediately surrounded by a glial cell. In sheathed axons, the glial cell may surround an axon as a single layer (Fig. 3.8a, b) or in loose folds (Fig. 3.8c–e). Collectively, the foregoing varieties of nerve axons are called unmyelinated because they lack the following feature. The glial cell may instead form a tightly wrapped spiral around the axon, and such fibers are called myelinated (shown in Fig. 3.8f).

The "medullated" sheath consisting of the external compactly multilayered and inner loosely layered components of the nerve axons of *P. japonicus* was reported by Kusano (1966, 1971) and was cited in the book of Bullock et al. (1977, p. 58). In Bullock et al., the sheath as a whole was clearly classified into the category of loosely laminated, and the nerve fibers of the shrimp were considered to be the unmyelinated category. Consequently, the common conclusion in their book was "True myelin is peculiar to vertebrates."

Since then, the lack of the myelin sheath in the nervous system of invertebrates has been accepted as a final common conclusion, and from the evolutionary point of view the myelin sheath was concluded to be uniquely evolved in the nervous system of vertebrates. For example, in a review of Stewart and Jessen (1995) it was concluded that:

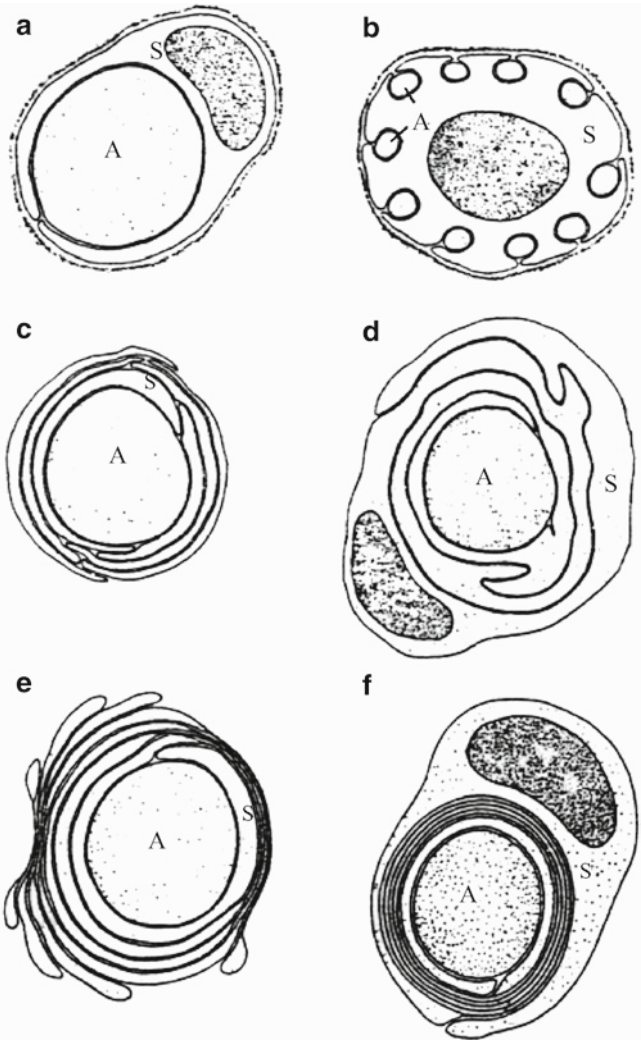

Fig. 3.8 Schematic diagrams show forms of ensheathment of nerve axons. (**a**) Large axon (*A*) surrounded by a single glial cell [as in insect peripheral nervous system (PNS)]. (**b**) Many axons invaded cytoplasmic trough of glial cell (as in vertebrate peripheral nerve). (**c**–**e**) Axon surrounded by multiple layers of glial cell processes [as in insect PNS, central nervous system (CNS), or earthworm giant fiber]. (**f**) Axon surrounded by compact multilayered sheath (myelin sheath) of Schwann cell (in vertebrate PNS). *A*, axon; *S*, glial sheath. (From Bunge 1968 with permission by Physiological Society of America)

"Although glial structures showing some similarities to myelin sheaths have been observed in the giant fibers of crustaceans (see Heuser and Doggenweiler 1966), true myelin sheaths are not apparent until the vertebrates."

Chapter 4
Biochemical Studies on Myelin of the Nervous System

Compact multilayered myelin, the true myelin, has been considered to be found in all vertebrate classes except for the primitive classes, such as lampreys and hagfish (Bullock et al. 1984; Koeppen and Stanton 2008). It appears throughout the nervous system, being formed by the extension of the cell membrane of oligodendrocytes in the central nervous system (CNS) and the Schwann cell in the peripheral nervous system (PNS), respectively. During the process of extension, the chemical composition of the membrane is modified.

Korey et al. (1958) were the first to dry and separate the white matter into crude glial and myelin fractions for their analytical study. A few years later, several groups of authors devised methods for isolating highly purified and better characterized preparations. It was established that when nerve tissue is homogenized in media of a low ionic strength, the myelin peels from the axons and turns into vesicles of the size of mitochondria or nuclei. Because of their high lipid content, these myelin vesicles have the lowest intrinsic density among any membrane fractions of the nervous system. For isolation of the myelin, investigators took advantage of its two properties: large vesicles and low density (see Norton and Cammer 1984).

4.1 Myelin Composition in Vertebrates

The most conspicuous characteristic of myelin relative to other cell membranes is its high ratio of lipid to protein. As is shown in Table 4.1, the ratio of lipid to protein in CNS myelin in the human, bovine, and rat is about 3:1.

CNS Myelin Lipids The lipids of brain myelin from the human, bovine, and rat are listed in Table 4.1.

It is evident that the lipid composition of CNS myelin from these mammalian species studied is very much the same except the myelin of the rat has less sphingomyelin than does that of bovines and humans. All the lipids assayed in the whole-brain preparation are also present in the myelin fraction; there are no myelin lipids

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Table 4.1 Myelin composition of the central nervous system

Substance	Human	Bovine	Rate
Protein	30.0	24.7	29.5
Lipid	70.0	75.3	70.5
Cholesterol	27.7	28.1	27.3
Total galactolipid	27.5	29.3	31.5
Cerebroside	22.7	24.0	23.7
Sulfatide	3.8	3.6	7.1
Total phospholipids	43.1	43.0	44.0
Ethanolamine phosphatide	15.6	17.4	16.7
Lecithin (choline PG)	11.2	10.9	11.3
Sphingomyelin	7.9	7.1	3.2
Phosphatidylserine (serine PG)	4.8	6.5	7.0
Phosphatidylinositol (phosphatidyl PG)	0.6	0.8	1.2
Plasmalogens	12.3	14.1	11.2

Source: Cited from Norton and Cammer (1984) and Siegel et al. (1994) (Permission by Wolters Kluwer Health/Lippincott Williams & Wilkins)

that are not also found in other subcellular fractions of the brain. Although there are no myelin-specific lipids, cerebroside is the most typical lipid of myelin. During development, the concentration of cerebroside in the brain is directly proportional to the amount of myelin present.

Besides the major components highly characteristic of myelin listed in Table 4.1, several classes of lipid are minor components of myelin: these include at least three fatty acid esters of cerebroside and two glycerol-based lipids, collectively called galactosyldiglyceride. The myelin from mammals also contains 0.1–0.3% of ganglioside. The proportion of the different gangliosides in the myelin is different (greatly enriched in monosialoganglioside, GM_1) from that in other brain membranes. Myelin from certain species contains an additional unique component, GM_4.

PNS Myelin Lipids Myelin from the PNS has the same lipids as in the CNS, although there are quantitative differences. The analyses show that PNS myelin has less cerebroside and sulfatide and considerably more sphingomyelin than CNS myelin. Of interest is the presence of the ganglioside LM_1 (sialosyl-lactoneotetraosylceramide) as a characteristic component of myelin in the PNS of some species.

CNS Myelin Proteins Before myelin isolation techniques were available, information on myelin proteins, as was that on myelin lipids, was necessarily indirect. It was the application of the technique of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) that permitted the separation of proteins on the basis of molecular weight and made analysis of proteins routine for discovering new proteins in the myelin. The protein composition of CNS myelin is simpler than that of other brain membranes, with the proteolipid protein (PLP)and basic proteins making up to 60–80% of the total in most species. Most other proteins and glycoprotein are present to lesser extents. With the exception of the myelin basic protein (MBP), myelin proteins are neither easily extractable nor soluble in aqueous media. However, similar to other membrane proteins, they may be solubilized in sodium dodecyl

sulfate solutions, and under this condition, they can be separated by electrophoresis in polyacrylamide gels. MBP and PLP are major components of all mammalian CNS myelin, and similar proteins are present in the myelin of many other lower species.

The myelin PLP is the major component of the organic solvent-extractable lipoprotein complex of the whole brain. The molecular mass (M_r) of the complex estimated by sequence analysis is about 30,000. The amino acid sequence suggests that there are several spanning domains. Presumably, the properties of this protein promote the formation and stabilization of the characteristic compact, multilayered structure of myelin. In addition to PLP, the myelin of the CNS has lesser quantities of another protein, DM-20 ($M_r = 20{,}000$), which has similar physical properties. The structure of this protein is related to that of PLP by a deletion of 40 amino acids. DM-20 appears earlier than PLP during development, and it is thought that it might have a role in oligodentrocyte differentiation in addition to a structural role in the myelin.

The major MBP has long been of interest; it is the antigen to elicit a cellular immune response that produces the CNS autoimmune disease called experimental allergic encephalomyelitis. The amino acid sequences of the major MBP for a number of species are similar (molecular weights are around 18,500). MBP is supposed to be located on the cytoplasmic face of the myelin membranes corresponding to the major line and to influence the close apposition of the cytoplasmic faces of the membrane.

In addition to the major MBP, most species of mammals contain various amounts of other basic proteins related to it in sequence. Besides PLP and MBP, there are many higher molecular weight proteins present in the gel electrophoretic pattern of myelin. There is also a minor quantity of the myelin-associated glycoprotein (MAG), which might have a function in cell–cell interactions.

PNS Myelin Proteins Very little information was available on these proteins before the application of SDS-PAGE. The gel electrophoretic analysis shows that a single protein, P_0 ($M_r = 30{,}000$), accounts for more than half of the PNS myelin protein. The protein has about 220 amino acids with an intracellular domain, a hydrophobic transmembrane domain, and a single Ig-like domain. The amino-terminal extracellular domain includes a single sequence for insertion of protein into the membrane and a glycosylation site. It is interesting to note that PLP and P_0 protein may have similar roles in formation of structures as closely related as myelin of the CNS and PNS.

The content of MBP of the PNS (often called P_1 protein) varies from approximately 5% to 18% of total protein in contrast to the CNS where it is of the order of 30%. The MBP of $M_r = 18{,}500$ is the most prominent component. MBP may not play so critical a role in myelin structure in the PNS as it does in the CNS.

PNS myelin also contains another positively charged protein referred to as P_2, with M_r of about 15,000. The large variation in the amount and distribution of the protein from species to species raises so far unanswered questions about its function (see Norton and Cammer 1984; Siegel et al. 1994).

4.2 Compositions of the Myelin Sheath in *Penaeus* Shrimp and Myelin-Like Sheath in Some Invertebrates

Myelin-like sheaths are considered to enclose the giant axons in some species of annelid and the thick axons of decapod crustacea, such as shrimps, prawns, and crabs.

Myelin-Like Sheath of Annelids and Decapod Crustacea Okamura et al. (1985b) analyzed the ventral nerve cord of the earthworm (*Lumbricus terrestris*) and established that the lipids are rich in cholesterol and phospholipids, but have neither galactocerebroside nor sulfatide, both of which are present in the myelin of vertebrates. Only traces of glucocerebroside were detected. In another paper, Okamura et al. (1985a) enumerated the concentration of glycophingolipids in the nervous system of some invertebrates and vertebrates determined by using high performance liquid chromatography. Among these invertebrate animals are shrimp (*Alpheus heterochaelis*; *Paraemonetes pugio*), lobster (*Homarus americanus*), crab (*Calinectes sapidus*, *Ocypode quadrata*, and *Clibanarrius vittatus*), and crayfish (*Procambarus* spp.). In the nervous system of the invertebrates, glucocerebrosides, but no galactocerebrosides, are present.

Composition of Myelin Sheath in the Penaeus *Shrimp* The true myelin with a specific fine structure was established in the nervous system of *P. chinensis.* Many shrimps from the *Penaeus* genus were studied morphologically and by electrophysiology. Among them, three species, *P. aztecus*, *P. duorarum*, and *P. setiferus*, were studied by Kusano and LaVail (1971). Their study showed that the nerve fibers of the *Penaeus* shrimp have common specific structural and functional characteristics. Shimomura et al. (1983) examined the lipid composition of the ventral nerve cord, brain ganglion, optic nerve, and antenna of the brawn shrimp, *P. aztecus*. High concentrations of glucocerebrosides were found in these tissues, but not in the nonneural tissue. Okamura et al. (1986) found a high concentration of glycosphingolipids in the nervous system of *P. setirus*. The lipid and protein compositions of the glial membrane ensheathing the axons of *P. duorarum* were determined by Okamura et al. (1986) to be rich in lipid with a 15:1 lipid to protein ratio, with cholesterol and phospholipids being the major constituents. No galactocerebroside was found, but there are substantial amounts of glucocerebroside, about the same proportion as galactocerebroside, in mammal myelin. Sphingomyelin is present, but its structure is different from that in mammals. The proteins in the myelin of the shrimp were also determined by Waehneldt et al. (1989). There are four major proteins, with apparent molecular weights of M_r = 21,500, 40,000, 78,000, and 85,000, and four minor components (M_r = 36,000, 41,500, 43,000, and 50,000). It seems certain that the myelin of the three species of *Penaeus* shrimp also contains these proteins, and it is evident that the characteristic difference between mammalian myelin and *Penaeus* shrimp myelin is that the former is rich in galactocerebroside and the latter contains the same portion of glucocerebroside.

Chapter 5
Myelin Sheath with a New Type of Fine Structure Found in the Nervous System of *Penaeus* Shrimp, an Invertebrate

It is necessary to recall some common understanding on the myelin sheath before discussing whether the sheath of the *Penaeus* shrimp can survive judgment as "the true myelin sheath."

5.1 Necessary Conditions for Identification of the Myelin Sheath

From the authoritative literature (Bullock et al. 1977, p. 60; Nicholls et al. 2001), it can be concluded that there are at least two conditions to be taken into consideration as sufficient for identification of the myelin sheath:

1. Morphologically, the sheath is composed of compact multilayers formed by the fused and extended cytoplasmic membrane of the glial cell devoid of its cytoplasm. Precisely speaking, a small amount of cytoplasm remains in the narrow canal expressed in the lateral loops and S-L incisures.
2. Functionally, the sheath has a high electrical resistance and a low capacitance, so that it speeds up axonal impulse conduction; namely, the sheath together with its nodal structure serves as the morphological basis for the saltatory conduction of the nerve impulse.

The spiraled form, in which the doubled neuroglial cell membrane tightly wraps the axon, was indicated in the literature cited and also in many texts and reference books of neurobiology. However, it should not be a sufficient condition for the identification of the myelin sheath, because it is enough to be considered as a myelin sheath if its compact multilayers are functional to offer a sufficiently high electrical resistance and a low capacitance, and together with the node to serve as a morphological basis for saltatory conduction, regardless of the compact multilayers formed in a concentric manner or in a spiral manner.

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In connection with collecting further experimental data on the fine structure of the myelin sheath of the *Penaeus* shrimp nerve fibers, it can be demonstrated that the myelin sheath meets all the conditions so as to be identified as the myelin sheath in both morphological and functional aspects, which are fully comparable to those of the myelin sheath of the vertebrate nerve axons.

5.2 Specific Morphological Structures of the Myelinated Fibers of the *Penaeus* Shrimp

For convenience of description, it is better beforehand to mention that the myelinated nerve fibers of the *Penaeus* shrimp have two specific structures that are not present in those of vertebrates. In brief, the axon is directly covered by a thin layer of Schwann cells (axon wall), and then is surrounded by a myelin sheath with a wide gap space between the Schwann cell layer and the sheath. Actually, it happened at the moment of initial discovery that these two specific structures had not been properly identified. First of all, two micrographs cited from the earliest papers of two groups, including Hsu Ke, described the specific structural characteristics of the myelinated nerve fibers of the *Penaeus* shrimp as follows.

In the left-hand micrograph of Fig. 5.1 (Fig. 5.1a), the thin axon with its unique thin wall in a medial giant fiber of *P. chinensis* is dyed black and is marked by an arrow. It is evident that the diameter of the axon, even with its unique thin wall together relating to that of the host giant fiber, is unimaginably small. As shown in both micrographs in Fig. 5.1, the axons in the medium-sized myelinated nerve fibers could hardly be recognized, but those in the small-sized myelinated fibers could not be deciphered, although they also have the same specific structural characteristics. As a mater of fact, the wide gap space and unique thin axon wall between the myelin sheath and the axon were shown in all myelinated fibers of all species of *Penaeus* shrimp observed. In the fixed preparation, the diameters of the axon together with its wall in the larger myelinated fibers of *P. chinensis* were measured to be as small as about one-tenth the diameter of the entire nerve fiber. However, as shown in the figures, the axons of a motor giant fiber and several medium-sized motor fibers usually are thicker, even though their diameters are as small as one-fourth of those of the entire fibers.

5.3 Identification of the Location of the Axolemma and the Sheath Component in the Myelinated Fibers of the *Penaeus* Shrimp

It was totally unexpected that we needed to identify the location of the axoplasmic membrane of a myelinated nerve fiber by experimental measure. Interestingly, the location of the membrane was not so obvious. In the very beginning, by light microscopic

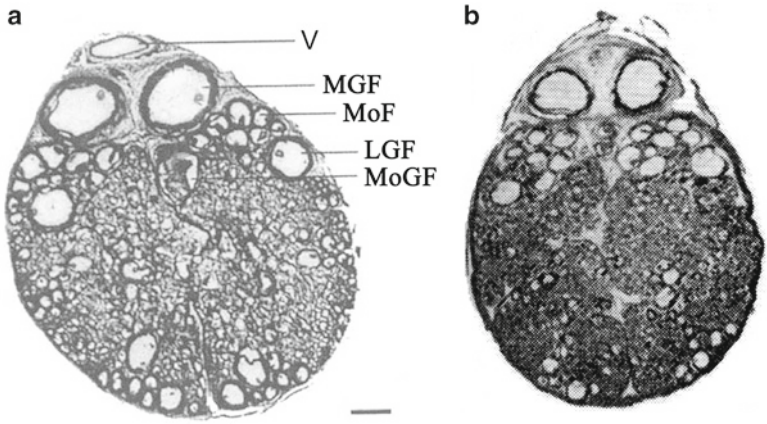

Fig. 5.1 Micrographs of cross-sectioned ventral nerve cord of *Penaeus* shrimp show a specific structure (a wide space under the myelin sheath) of myelinated fibers. (**a**) *P. chinensis* (modified from Huang et al. 1963). (**b**) *P. japonicus* (from Kusano 1966, with permission by John Wiley and Sons). *V*, blood vessel; *MGF*, medial giant fiber; *LGF*, lateral giant fiber; *MoGF*, motor giant fiber; *MoF*, middle-sized motor fibers. *Bar* 100 μm

observation, Fan et al. (1961) surprisingly found that many nerve fibers, including all the so-called giant fibers, in the ventral nerve cord of *P. chinensis* are coated with a relatively thick osmiophilic sheath. By using a polarized light microscope on the single nerve fiber preparations, Hao and Hsu (1965) observed that the sheath of the nerve fibers, similar to that of the myelinated fibers of vertebrates, is differentiated into two layers: the outer layer expressing the connective tissue sheath and the inner one displaying a distinct birefringence, showing that it is composed of a multilayered structure. By electron microscopic technique, Huang et al. (1963) and Yeh et al. (1963) found that not only is the osmiophilic sheath of *P. chinensis* nerve fibers composed of the compact multilayered structure, which should be the myelin sheath, but also under the myelin sheath there are two unprecedented specific structures (as shown in Fig. 5.1). Soon after that, the compact multilayered myelin sheath, as well as the two unprecedented specific structures, were reported by Kusano (1966) and Hama (1966) in the nerve fibers of *P. japonicus*, another species of *Penaeus* shrimp. Subsequently, it took several years to clarify the two specific structures in the myelinated fibers of the *Penaeus* shrimp.

When approaching the specific morphological structures of the myelinated fibers of *P. chinensis* and *P. japonicus*, both Chinese and Japanese groups of authors were confronted with the question of identifying the location of the axoplasmic membrane. Eventually, both groups independently and correctly recognized an inner component of the myelinated fibers of the *Penaeus* shrimp to be the axoplasmic membrane. Indeed, in the early days of the published papers of each group, the indefinable points of view were in question. In any case, the vagueness of the early work has to be discussed and clarified.

One point of concern in the papers of Huang et al. (1963) and Yeh et al. (1963) was to identify the axoplasmic membrane of the myelinated fibers of *P. chinensis*.

In their work, it had been clearly established that: "The myelin sheath is lamellae, the repeating unit of which measures 80 Å" (Huang et al. 1963), and the thin (axon) wall is composed of "distinct bundles of submicroscopic filaments" (later these were clarified to be microtubules) (Yeh et al. 1963). Thus, based on the obvious difference in the fine structures, the authors considered that the myelin sheath and the unique (axon) wall of *P. chinensis* nerve axon are different kinds of sheath. Consequently, they thought that the axoplasmic membrane of the nerve fibers should underlie the myelin sheath, as it is the case in the myelinated fibers of vertebrates. Then, the deduced judgment was strengthened by the experimental results showing that the action potential recorded from the wide gap space of the myelinated fibers is a positive and monophasic spike of an amplitude as high as 70 mV. This finding suggested that the recording should be the intra-axonal. On the other hand, such a recording of the action potential was not accompanied by the resting membrane potential, which suggested that the recording, on the contrary, should be the extra-axonal. Then, unfortunately, many attempts to insert the microelectrode directly through the thick myelin sheath, the wide gap space, and the axonal wall into the thin axon at the medial giant fiber preparation of *P. chinensis* ended in failure.

These facts further puzzled the authors to decide the location of the axoplasmic membrane of the myelinated fibers. Under these circumstances, the term "fiber-like structure" was temporarily used to express the axon in the published papers. Later on, using a histological method, it was found that there are many cell nuclei distributed in the unique thin wall, which tightly encloses the "fiber-like structure." This fact undoubtedly showed that the wide gap present under the myelin sheath morphologically is the extracellular space, and in fact the "fiber-like structure" is the axon of the myelinated nerve fibers of *P. chinensis*.

Another point of concern about the unique axon sheath as the loosely layered component of the "medullated or Schwann cell sheath" of *P. japonicus* nerve fibers was presented by Kusano (1966, 1971) and Hama (1966). They considered that the sheath of the nerve axon is separated by a wide gap into two layers, which are formed by the same Schwann cells. On the sheath of *P. japonicus* nerve axons, Kusano concluded that the axon is doubly coated directly by Schwann cells and indirectly by the myelin sheath layer that is produced by those Schwann cells. The arrangement of the inner sheath layers is similar to that in typical unmyelinated nerve fibers, whereas the myelin sheath layers located at the outer position show a spacing of about 9 nm between two osmiophilic layers, and a similar observation on the sheath of the nerve axons of *P. japonicus* was made by Hama also. The authors correctly identified the axoplasmic membrane under the inner component of the sheath of *P. japonicus* nerve axons, although they also did not successfully insert the microelectrode into the axon.

As already described, because of the complicated structure of the sheath and the inner loose-layered component of *P. japonicus* nerve fibers, the nerve fibers coated with this kind of sheath were considered to be unmyelinated. Thus, in the book by Bullock et al. (1977, p. 58), there are two diagrammatic drawings cited from the paper of Kusano (1966) to show the difference in the fine structures between the myelin sheaths of the vertebrates and *P. japonicus*. In the drawings the sheath of *P. japonicus* nerve axons was literally marked as unmyelinated (Fig. 5.2).

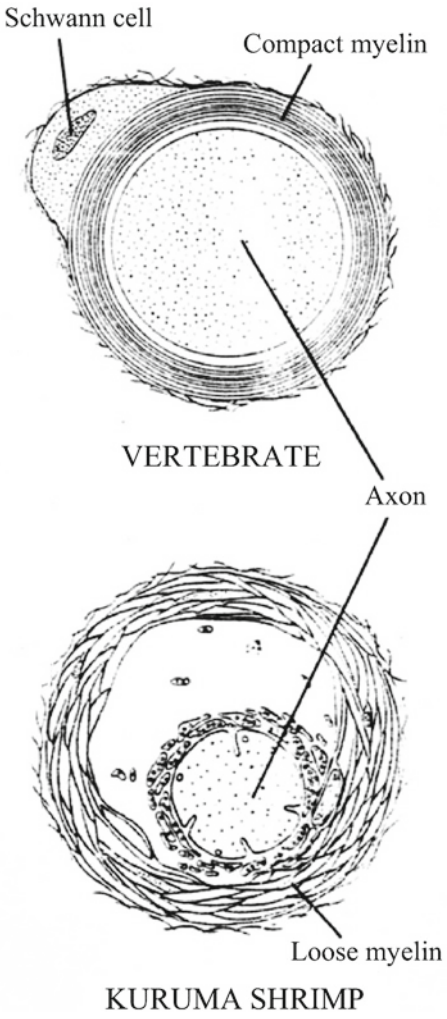

Fig. 5.2 Schematic drawings of fine structures of vertebrate nerve fiber (*upper*) and nerve fiber of Kuruma shrimp (*P. japonicus*) (*lower*). (From Bullock et al. 1977, with permission by Freeman)

Note: The question of the morphological nature of the unique axon wall of the *Penaeus* shrimp nerve axons is discussed in the next chapter.

5.4 Specific Characteristics of the Fine Structure of the Myelin Sheath of the *Penaeus* Shrimp

In fact, both the fine structure of the sheath of *P. chinensis* nerve fibers reported by Huang et al. (1963) and that of the outer component of the sheath of *P. japonicus* nerve fibers reported by Kusano (1966) were morphologically comparable to that of

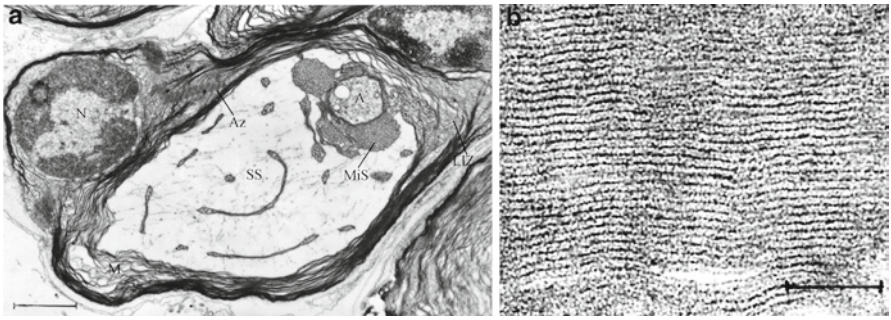

Fig. 5.3 Electron micrographs of middle-sized myelinated fiber of *P. chinensis* (**a**) and a magnified part of its myelin sheath (**b**). *A*, axon; *Az*, attachment zone; *LlZ*, lateral loop zone; *M*, myelin sheath; *MiS*, microtubular sheath; *N*, nucleus of myelin-forming cell; *SS*, submyelinic space. *Bars* (**a**)1 μm; (**b**) 0.5 μm. (From Xu, unpublished data)

the vertebrate nerve fibers. As to the unique thin axon wall of the *P. chinensis* nerve fibers described by Yeh et al. (1963) or the inner component of the myelin sheath of *P. japonicus* nerve fibers described by Kusano (1966) and Hama (1966), both should be a sheath produced by unique glial cells independent of the myelin sheath. These cells should be different from the Schwann cells for the myelin sheath. This question is discussed in Chap. 6.

Compactness of the Multilayered Sheath of the Penaeus Shrimp The fine structure of a myelinated fiber of *P. chinensis* and a high-magnification view of a part of its myelin sheath are shown in Fig. 5.3. The pictures show that the myelin sheath is composed of alternate repeats of the main dense lines and the intraperiod lines, which are similar to those observed in the fine structure of the vertebrate myelin sheath. Moreover, the compactness of the myelin sheath of *P. chinensis* nerve fibers reported by Huang et al. (1963), as well as that of the outer component of the myelin sheath of *P. japonicus* nerve fibers reported by Kusano (1966), can be compared with that of the myelin sheath of vertebrate nerve fibers. It is well known that the period of the compact layers of the vertebrate myelin sheath is about 10 nm, whereas that of the compact layers of the myelin sheath of *P. chinensis* and *P. japonicus* was reported to be 8 or 9 nm, respectively (Huang et al. 1963; Kusano 1966). Thus, the compactness of the myelin layers of both the *Penaeus* shrimp and the vertebrate nerve fibers is of the same order.

The Multilayered Sheath Is Concentrically Formed In the paper by Huang et al. (1963), it was reported that in the cross-sectional preparation of the myelinated fibers of *P. chinensis* there were radial narrow zones of coarser lamellae repeating at about 75 nm mixed in the compact multilayered sheath of the nerve fibers. In further studies it was clarified that the narrow coarser laminated zones are composed of tip-to-tip connected lateral loops with several microtubules in them arranged in a line radial to the axon axis (Fig. 5.4a). The electron micrograph was taken from the cross

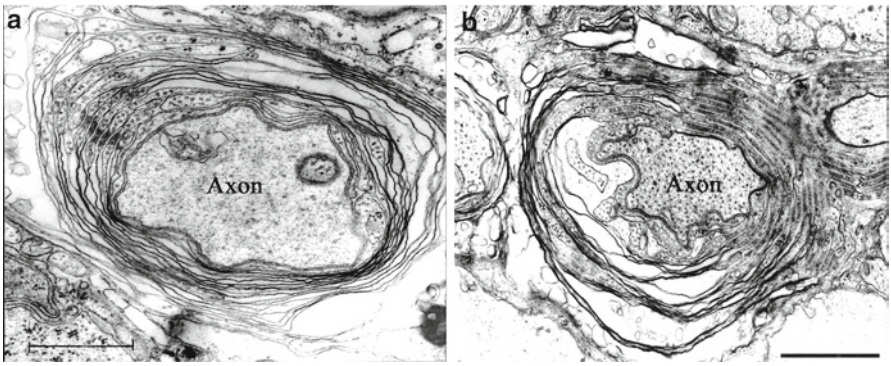

Fig. 5.4 Cross-sectional electron micrographs of developing nerve fibers of an immature shrimp, *P. chinensis* (body length ~5 mm). (**a**) Formation of myelin sheath with zone of loop-to-loop connection. Neither submyelinic space nor microtubular sheath was yet present. (**b**) Large circular-type myelin layers. Both submyelinic space and microtubular sheath began to appear. *Bars* 1 μm. (From Xu, unpublished data)

section of the ventral nerve cord of an immature *P. chinensis*. It is evident that the layers of the myelin sheath are embracing the axon in two hemispherical groups forming a circular shape from both sides. Each of the layers ends with a lateral loop, which is structurally analogous to those of the myelin layers ending at the paranodal region of the vertebrate nerve fibers (see Fig. 3.4). Each lateral loop is tightly tip-to-tip connected with its partner from the other side. The connected two lateral loops are arranged in a line radial to the axon axis. In this myelinated fiber there is one lateral loop line. Actually, the line number in the myelin sheath of a nerve fiber is dependent on its size. The larger the nerve fiber diameter the greater the number of lateral loop lines, indicating that the myelin sheath of the thicker fiber consists of more than one glial cell to surround an axon in a circle. The lateral loop line seems to bear a resemblance to the S-L incisures in the myelin sheath of the vertebrate nerve axon.

A circularly shaped, coarser multilayered region was rarely observed in the cross section of the developing myelinated fiber of *P. chinensis* (Fig. 5.4b). This type of coarser layer should be the image of the connections of the lateral cytoplasmic canals of the extended cell membrane of the two neighboring glial cells along the long axis of the fiber in cross section. It is interesting to note that these circularly shaped coarser layers were observed earlier in the sheath of the prawn (*Macrobrachium niponensis*) nerve fibers reported by Yeh and Huang (1962) (see Fig. 3.6b).

The genesis of the myelin sheath of the *Penaeus* shrimp nerve fibers from the extended cytoplasmic membrane of the glial cells was demonstrated by electron microscopy of the cross section of the ventral nerve cord of immature *P. chinensis* (Fig. 5.5a). The picture shows that an axon invades into the cytoplasm of a glial cell

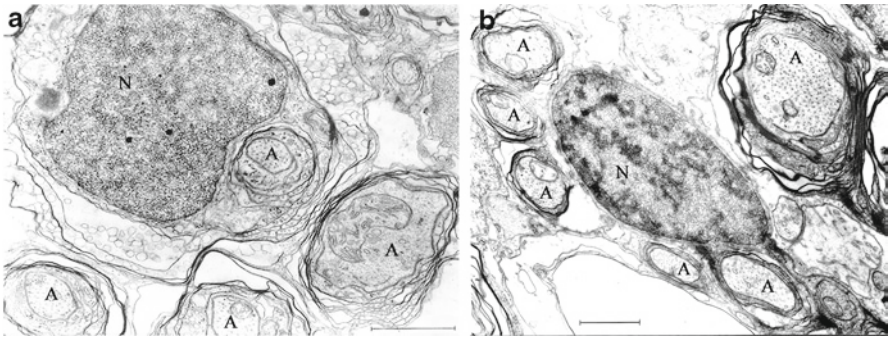

Fig. 5.5 Electron micrographs of cross-sectioned ganglion of ventral nerve cord of an immature shrimp, *P. chinensis* (body length ~5 mm). (**a**) Axon invading into myelin-forming glial cell. (**b**) A single glial cell forms myelin sheath for several axons. *A*, axon; *N*, nucleus of myelin-forming cell. *Bars* 1 μm. (From Xu, unpublished data)

that is forming the myelin sheath, as indicated by the presence of compact myelin layers between the nucleus and the axon. Another picture shows that a single myelin-forming glial cell forms the myelin sheath for several axons (Fig. 5.5b). In this connection the myelin-forming glial cell of the myelinated fibers of the *Penaeus* shrimp is analogous to the oligodendrocyte that forms the myelin sheath for several axons of the central nerve axons of vertebrates. This fact seems to indicate that the oligodendrocyte appears earlier than the Schwann cell in the phylogeny of vertebrate nervous systems.

These experimental results demonstrated that the myelin sheaths of both vertebrates and the *Penaeus* shrimp are formed by the extended plasma membrane of glial cells, which eliminated their cytoplasm except for a part called the cytoplasmic canal. However, there is an obvious difference in the mechanism for forming the structures of the two kinds of myelin sheath. Although each layer of *Penaeus* shrimp myelin is also made by the combined cell membranes framed with the cytoplasmic canal, it is not formed by a continuous double membrane of glial cell, as in the case of vertebrates. Instead, a layer of the *Penaeus* shrimp myelin is originated from two glial cells located in both sides of the axon. They meet each other to embrace the axon, leaving a wide gap space under the sheath formed by many layers. The layers observed in a cross section of the nerve fiber run in a shape of semicircle or less and end with lateral loops, connecting with their partners from the opposite side.

The diagrammatic drawing of the fine structure of the *Penaeus* shrimp myelinated fiber in cross section is shown together with that of vertebrates in Fig. 5.6 (upper panels). The pictures show that the intraperiodic lines and the major dense lines in the myelin sheaths of both vertebrates and *Penaeus* shrimp are formed by fusion of the outer and cytoplasmic faces of the glial cell membrane, respectively, and the compact layers are formed by fusion of the extended plasma membranes

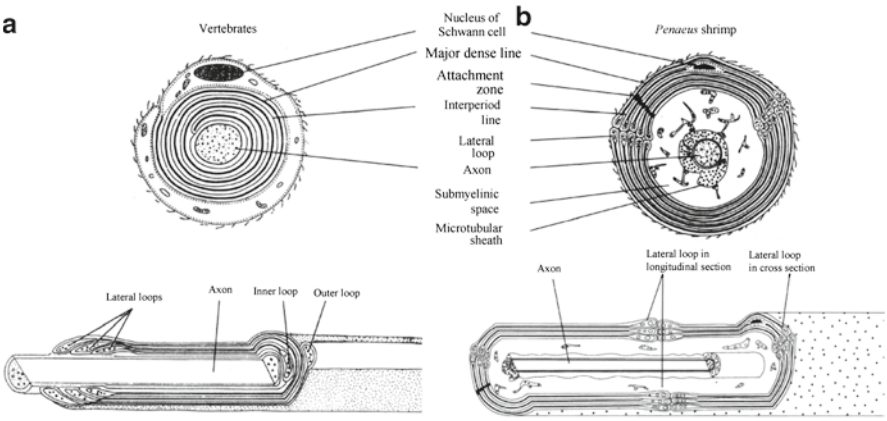

Fig. 5.6 Schematic diagrams of formation of fine structures of myelin sheath of vertebrate (**a**) and *Penaeus* shrimp (**b**) nerve fibers. *Upper panels*: Cross-sectional view. (From Xu (Hsu) and Terakawa 1999.) *Lower panels*: Longitudinal sectional view (*left:* from (Hirano and Dembitzer 1967) with permission by Rockefeller University Press); *right:* from Xu, unpublished data)

completely squeezing the cytoplasm out, except for a part in the region of the cytoplasmic canal. The lateral loop structures of the myelin sheath in the vertebrate and *Penaeus* shrimp nerve fibers are schematically shown in Fig. 5.6 (lower panels).

5.5 Electrical Properties of the Myelin Sheath Measured in the Giant Fiber Preparation of *Penaeus japonicus*

From the distance of electronic spreading of the applied potential along the myelinated giant fibers of *P. chinensis*, Fan et al. (1961) concluded that the myelin sheath of the nerve fibers should have a high electrical resistance and a low electrical capacitance. It was Kusano (1966) who accurately measured the electrical constants of the myelin sheath of *P. japonicus* nerve fibers for the first time. For comparison, some related values of the frog myelinated fiber are shown in Table 5.1.

As shown in Table 5.1, the two electrical constants of the myelin sheaths of frog and *P. japonicus* are nearly of the same order, showing that the electrical constants of *Penaeus* shrimp myelin are comparable to those of the vertebrate myelin. However, it is evident that the thickness of the myelin sheath of *P. japonicus* is about five times that of the frog. The reason for this is probably that the extended plasma membrane itself or the concentrically formed layers of the myelin sheath of the *Penaeus* shrimp is less effective than that of the frog to constitute the electrical resistance and capacitance.

The functional role of myelination to speed up the conduction velocity of nerve impulses is described and discussed in Chap. 8.

Table 5.1 Electrical resistance and capacitance of the myelin sheaths of frog and *Penaeus japonicus*

	Frog (Hodgkin 1964)	*P. japonicus* (Kusano 1966)
External diameter of fiber (μm)	14	120
Myelin sheath		
Thickness (μm)	2	10
Specific resistance (Ω/cm^2)	$1.0–1.6\times10^5$	$>3\times10^4$
Specific capacitance (F/cm^2)	$0.25–0.5\times10^{-8}$	$<1.35\times10^{-8}$

5.6 Overall View of the Myelin Sheaths of Vertebrate and Invertebrate (*Penaeus* Shrimp) Nerve Fibers

Nerve axons usually are coated with some kind of glial sheaths. Among them the most developed one is called the myelin sheath, which consists of compact multilayers. The nerve axons coated with the myelin sheath are called myelinated fibers, which had been concluded to be unique to the nervous system of vertebrates. However, in fact, the myelinated fiber has been found also in the nervous system of the *Penaeus* shrimp, which belongs to the invertebrates.

1. The myelin sheath of vertebrates is regularly interrupted by a node of Ranvier, which is a circularly unmyelinated gap about 1–1.5 μm in width in the peripheral nerve fiber and 1–10 μm or wider in the central nerve fibers. The myelin sheath of the *Penaeus* shrimp is continuous without the circular unmyelinated gap, such as the node of Ranvier, but possesses regularly distributed oval unmyelinated windows, which are called fenestration nodes. Note: The structure of the "fenestration node" in the myelinated fibers of the *Penaeus* shrimp, and the internodal distances between fenestration nodes, are described later.
2. In vertebrates, it is a Schwann cell that forms an internodal segment of the myelin sheath for a peripheral nerve fiber. A nucleus is located outside of each segment of the myelin sheath. However, it is an oligodendrocyte that forms the myelin sheath for numbers of central nerve fibers, and in each segment of the myelin sheath, no nucleus is located. In the *Penaeus* shrimp, it is a myelin-forming cell that forms the myelin sheath for several nerve fibers, and the nuclei of the myelin-forming glial cells are distributed randomly in the myelin, showing that the myelin is formed by overlapping the extended cell membrane of several glial cells.
3. The myelin sheath of both the peripheral and central nerve fibers of vertebrates is formed by the extended plasma membrane of a kind of glial cell (Schwann cell or oligodendrocyte), and the myelin sheath of nerve fibers of the *Penaeus* shrimp is also formed by the extended plasma membrane of a kind of glial cell (the myelin-forming glial cell).
4. The fine structures of the myelin sheaths of both vertebrates and the *Penaeus* shrimp consist of alternately repeating major dense layers and the intraperiod layers, which are made by fusion of the cytoplasmic membranes and outer faces

of the extended cell membranes of the glial cells, respectively. The periods of the major dense layers in both cases are about 10 nm.

5. The multilayers of the myelin sheath of vertebrates are formed by the spiral wrapping with the plasma membrane of a Schwann cell or oligodendrocyte around the axon, whereas those of *Penaeus* shrimp are formed by the extension of plasma membranes of glial cells to embrace the axon from both sides, so that it forms concentric multilayers. In both cases the cytoplasmic canal or tube is interposed in the extended cytoplasmic membrane of the glial cells.
6. Both the continuously and spirally formed multilayers in the vertebrate myelin sheath and the concentrically formed multilayers in the *Penaeus* myelin sheath are characterized by high electrical resistance and low capacitance. Therefore, the node of Ranvier and the fenestration node of the myelin sheaths together serve as the morphological basis for saltatory conduction of nerve impulses.

Chapter 6
Two Unique Structures Discovered in the Myelinated Fibers of the *Penaeus* Shrimp

An extraordinarily wide gap space under the myelin sheath and a unique thin wall were not only found in the fixed nerve fiber preparations, but were also observed in the fresh single nerve fiber preparations of all species of *Penaeus* shrimp examined. Here, two micrographs are cited from the corresponding papers to show the thin axon with its thin wall in the fresh myelinated single fiber preparations of *P. chinensis* and *P. japonicus*, respectively (Fig. 6.1).

As mentioned earlier, in the early phases of the research, it was not easy to identify with certainty the location of the axoplasmic membrane (axolemma) in the unusual structure of shrimp myelinated fibers, and the axon was temporarily described as a "fiber-like structure" in the papers of Huang et al. (1963) and Yeh et al. (1963). Also, the induced thinking that the excitable membrane should be directly under the myelin sheath was contradictory to the results of electrophysiological experiments, affecting the decision of authors to identify the location of the axon. The procedure of electrophysiological experiments was as follows:

When a micropipette electrode was longitudinally inserted into the gap space between the myelin sheath and the axon of the medial giant fiber preparation of *P. chinensi*s through the cut end, the action potential recorded was positive and monophasic, and gradually increased to its maximum with insertion (Fig. 6.2). The amplitude of the recorded action potential was still increased slightly to the maximum when a ligature was put on the cut end of the preparation. Figure 6.2 shows three overlapping action potentials taken during the process of insertion of the micropipette electrode into the gap space of the preparation.

The amplitude of the intragap potential was as high as 70 mV, which should be considered as almost equal to that of the normal-sized intra-axonal action potential. However, at the same time, the baseline of the recording did not shift at all during the micropipette insertion; i.e., no sign of DC potential was observed, indicating that the recording could be extra-axonal. Because of these contradictory facts, the authors wavered between the submyelinic structure and the central fiber-like structure as the location of the excitable membrane in the myelinated nerve fibers of *P. chinensis,* and the term "fiber-like structure" was temporarily used to designate the axon.

K. Xu and S. Terakawa, *Myelinated Fibers and Saltatory Conduction in the Shrimp: The Fastest Impulse Conduction in the Animal Kingdom*,
DOI 10.1007/978-4-431-53924-7_6, © Springer Japan 2013

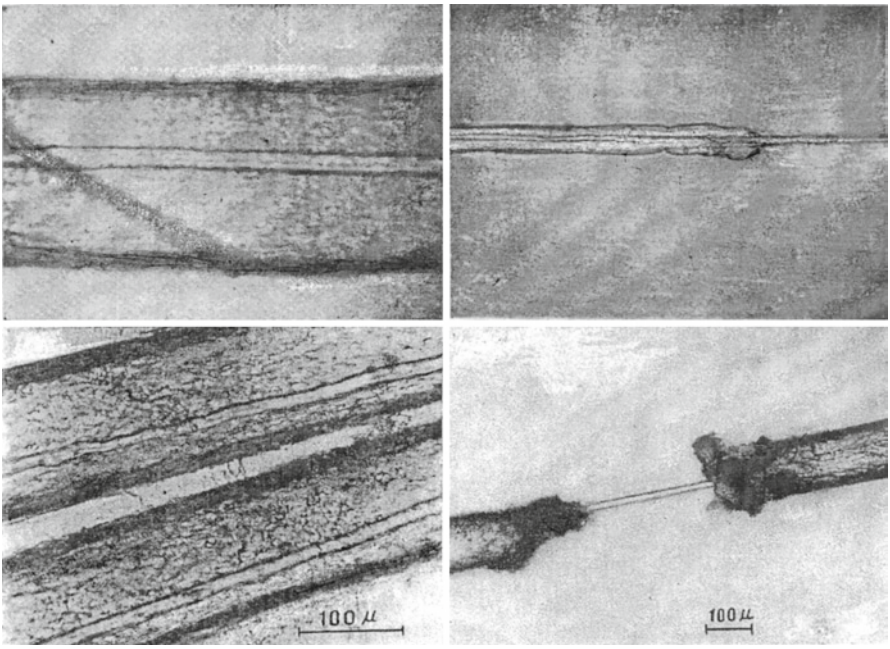

Fig. 6.1 Micrographs of living myelinated fiber preparations of *Penaeus* shrimp. *Left*: Giant fibers. *Right*: Small fibers. *Top*: *P. chinensis* (Yeh et al. 1963). *Bottom*: *P. japonicus* (Kusano 1966)

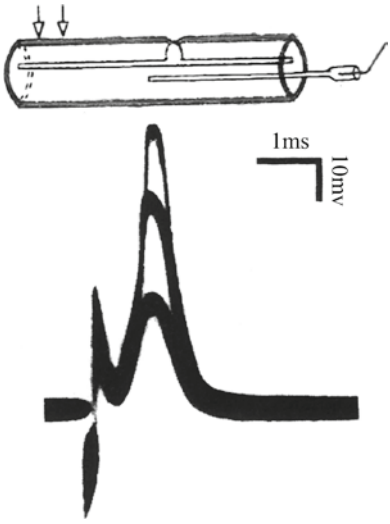

Fig. 6.2 Action potentials recorded during insertion of glass capillary electrode into submyelinic space of medial giant fiber preparation of *P. chinensis*. *Upper panel* shows scheme of experiment. (From Xu, unpublished data)

After identification of the "fiber-like structure" as the axon by histological methods, a line of functional evidence was built to affirm the conclusion. An intra-axonal recording was made to clarify the electrical characteristics of both the axon and the myelin sheath to analyze the electrical change in the wide gap space of the shrimp myelinated fibers during impulse conduction. Unfortunately, many attempts made in the early phase to insert a microelectrode through the myelin sheath and the wide gap space into the thin axon ended in failure. Then, a new approach to insert the microelectrode into the axon was taken; namely, the unique structure at the site of the giant synapse of the median giant fiber to the motor giant fiber was employed as a preparation. There, the synaptic membranes of the two axons are tightly connected together and thus are not covered by both the thin specific axon wall and the myelin sheath. A medial giant fiber preparation with a small segment of motor giant fiber attached at the giant synapse region was made from the ventral nerve cord of *P. chinensis.* The myelin sheath together with the axon of the motor giant fiber segment was longitudinally cut with fine needles to expose the postsynaptic site of the giant synapse. Then, through the synaptic membranes, the microelectrode was inserted into the axon of the medial giant fiber. Such recordings, repeated many times successfully, showed a positive and monophasic action potential. However, there was still no accompanying resting membrane potential.

To our disappointment, the work had to be laid aside from 1964 to 1984 as a consequence of the historical events in China. Expecting no possibility to reopen research work on the study of the giant fibers of *P. chinensis* and to finalize the unfinished experiments, Hsu et al. (1975b) published a note reporting the data on the failure of recording the resting membrane potential from the axon of the giant fibers of *P. chinensis* when the recording microelectrode exactly penetrated into the presynaptic axon through the postsynaptic site of the giant synapse. At the same time in this note, it was mentioned that the data seemed to be discrepant with the resting membrane potential confirmed in the ganglion neurons of the *Penaeus* shrimp. Kusano (1966) had already measured the resting membrane potential of the ganglion neurons of *P. japonicus* to be about −60 mV.

Fortunately, from 1983 to 1985, Xu was invited to work for a few months every year in the laboratory of Prof. Watanabe, where Kusano had completed his experimental work on the *P. japonicus* giant fiber. Xu's first work completed together with S. Terakawa in the laboratory was to renew the experiment of inserting the microelectrode into the axon through the giant synaptic membranes at the medial giant fiber with a giant synapse preparation of *P. japonicus*. To examine the null value for the resting potential of the axon, the dye (Lucifer yellow)-containing microelectrode was used to confirm the location of the microelectrode tip in the giant fiber. At first, for a while, the positive monophasic action potential was recorded without the accompanying resting membrane potential. In many such cases, the injected dye merely diffused in the entire wide gap space. Later, it happened that a positive monophasic action potential of about 70 mV was recorded with the resting membrane potential of about −60 mV by inserting the microelectrode through the

synaptic site of the giant synapse. In those cases, the injected dye clearly diffused only in the presynaptic axon. Namely, it turned out that the preparation with an unusually simplified structure of the giant synapse was luckily used in those successful experiments (see Hsu and Terakawa 1984). The lack of success when inserting a microelectrode through the postsynaptic site into the presynaptic axon should be because of its extreme thinness in comparison with the postsynaptic axon (see Fig. 2.4).

The basic electrical characteristics of the wide extracellular gap space and the axon of the *Penaeus* shrimp myelinated fibers were finally established after detouring through some incorrect judgments. Fan et al. (1985) published a note in which they analyzed "the paradox of near zero resting potential" of the shrimp myelinated fiber using several unusual means, such as a treatment of the giant fiber with 50% glycerol–50% 500 mM KCl for 5–6 days or heating it to 80°C. It seems that the authors were also puzzled by incorrectly inserting the microelectrode into the gap space instead of the thin axon in the myelinated fiber.

After completely establishing the basic morphological and electrophysiological characteristics of the axon of the unusually structured myelinated fibers of the *Penaeus* shrimp, Xu and Terakawa (1991) easily measured the resting membrane potential at the medial giant fiber preparation of *P. japonicus* by inserting a microelectrode into its thin axon through a hole made in the myelin sheath by a needle. As expected, the conductive activity of the preparation was lost at once by making a hole in the myelin sheath of the preparation, but the resting membrane potential of the axon was not affected. The conclusion was that there is no difference in the level of the resting membrane potential recorded from the axon of the medial giant fiber determined through the myelin hole and that of the motor giant fiber recorded by directly inserting a microelectrode into the axon through the intact myelin. In both cases, the resting membrane potential was determined to be 64.3 ± 7.4 mV on average. It should be mentioned that the insertion of the microelectrode directly through the myelin sheath into the axon of the motor giant fiber was a success only by chance with much effort.

In the next step of the research, it was important to clarify the mechanism of the full-sized positive monophasic action potential recorded from both the extracellular gap space and the axon of the myelinated fibers of the *Penaeus* shrimp. It was evident that the subject was tightly connected to establishment of the saltatory conduction hypothesis instead of the tunnel hypothesis. This point is described and discussed in detail in Chap. 9.

6.1 Wide Extracellular Gap Space Filled with an Amorphous Gel Substance Under the Myelin Sheath of the Nerve Fibers of the *Penaeus* Shrimp

In the myelinated fibers of vertebrates, the gap between the myelin sheath and the axon is as narrow as 15–30 nm without exception. The narrow gap structure is especially beneficial to speed up impulse conduction in the saltatory manner along the

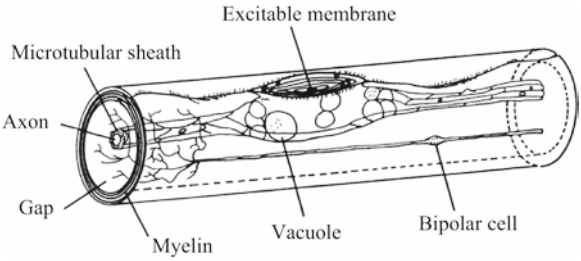

Fig. 6.3 Schematic diagram shows a kind of *bipolar cell* found in the submyelinic space of nerve fibers of *P. japonicus*. (From Terakawa and Xu 1987)

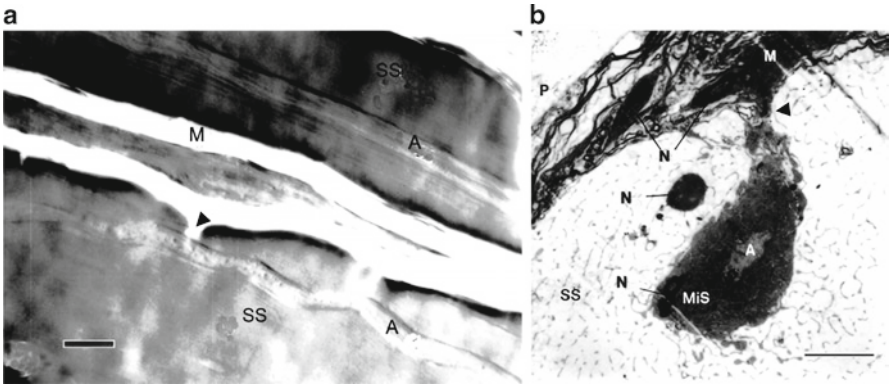

Fig. 6.4 Axon–myelin sheath relationship. (**a**) Differential interference contrast image of two living giant fiber preparations of *P. japonicus*. Axon attaches to a knob of myelin sheath (*arrowhead*). (**b**) Cross-sectional electron micrograph of myelinated fiber of *P. chinensis* shows attachment of axon to knob of myelin sheath (*arrowhead*). *A*, axon; *M*, myelin sheath; *SS*, submyelinic space; *MiS*, microtubular sheath; *N*, nucleus of myelin-forming cell; *P*, perineurium. *Bars* (**a**) 20 μm; (**b**) 5 μm. (From Hsu and Terakawa 1996)

myelinated fibers from node to node. An extremely wide gap space under the myelin sheath of the nerve fibers was found in all the aforementioned species of *Penaeus* shrimp. For example, the largest diameter of the gap space measured was surprisingly as wide as about 130 μm in the medial giant fibers of about 150 μm in diameter of *P. chinensis* (the axon diameter was only 15 μm) (Hao and Xu 1965). The extracellular wide gap space of the shrimp myelinated fibers was designated by the term submyelinic space.

Terakawa and Xu (1987) reported that the submyelinic space is filled with an amorphous gel substance that contains a few bipolar cells extending numerous processes in a dendritic shape. A portion of the myelinated fiber of the *Penaeus* shrimp with a node and a bipolar cell in submyelinic space is schematically shown in Fig. 6.3.

Although the axon of the shrimp myelinated fibers is basically separated from the myelin sheath, it is tightly connected with the myelin sheath at paranodal regions of the nerve fibers. Also, at the paranodal region, the axon is also loosely connected with the myelin sheath at a few points somewhere in the submyelinic space of the nerve fibers (Fig. 6.4a). An electron microscopic picture of an attachment point of

the axon to the myelin sheath is shown in Fig. 6.4b. The fine structure of the paranodal region of the *Penaeus* shrimp myelinated fibers is described in Chap. 8.

An important function of the submyelinic space is to speed up the conduction velocity of the myelinated fibers of the *Penaeus* shrimp. This subject is described and discussed later in Chap. 9. Another possible function of the submyelinic space is suggested to be a buffering of the myelinated fibers against the hydric pressure change during the vertical migration of the animal. It is known that the shrimp, at least *P. chinensis*, dives more than 100 m deep into the sea to pass the winter. However, regrettably no related experiment has been performed to date.

Nowadays, the functional relationship of the axon and the myelin sheath of the vertebrate nerve fibers is one of the highlights in the study of neuroscience. It is obvious that the myelinated fibers of the *Penaeus* shrimp with the wide submyelinic space should be a useful preparation for the comparative study on the functional relationship between the axon and the myelin sheath. Especially, the biochemical characteristics of the gel substance in the gap space should be an interesting subject.

6.2 Unique Thin Nucleated Wall Containing Microtubule Bundles and Tightly Enclosing the Axon of the Myelinated Fibers of the *Penaeus* Shrimp

The thin wall of the myelinated axons of the *Penaeus* shrimp is another unique structure, which tightly encloses the axon and is composed of the flattened processes of a microtubule-rich glial cell (Yeh et al. 1963; Kusano 1966; Hama 1966; Hsu et al. 1980; Terakawa and Xu 1987). In the processes of the glial cell, the microtubules are assembled in bundles and laid longitudinally along the axonal axis. The abundant microtubules in a cross section of a myelinated fiber of *P. chinensis* are shown in Fig. 6.5. The unique axon wall was designated by the term microtubular sheath according to its specific morphology.

Many facts showed that the microtubular sheath is clearly different from the myelin sheath, and it should be an independent sheath in the myelinated fibers of the *Penaeus* shrimp. These facts are as follows:

1. Morphologically, the microtubular sheath is composed of bundles of longitudinally directed microtubules, and it has no birefringence property, whereas the myelin sheath is composed of compact and concentric layers showing a clear birefringence.
2. Functionally, the microtubular sheath has a very low electrical resistance and serves the axon mainly as a supporting tissue, whereas the myelin sheath has a very high electrical resistance and a low capacitance and is able to speed up the conduction velocity of the nerve impulse.
3. The microtubular sheath ontogenetically appears later than the myelin sheath. While myelination is continuing, neither the microtubular sheath nor the submyelinic space of the nerve fibers appears in the developing nerve fiber of *P. chinensis* (see Fig. 5.4a, b).

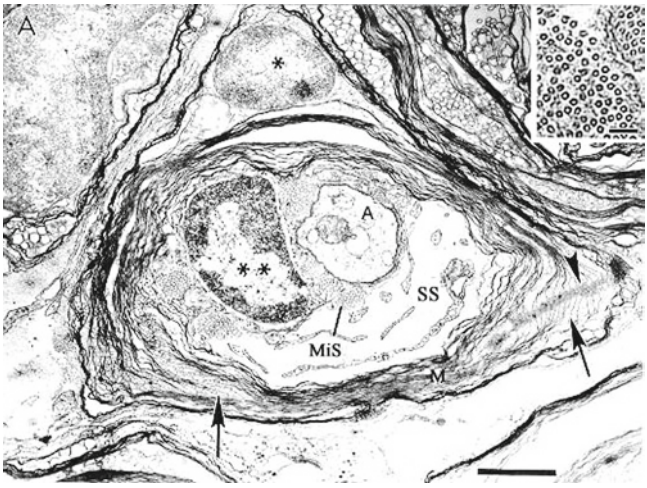

Fig. 6.5 Electron micrograph of middle-sized myelinated fiber of *P. chinensis*. Microtubular sheath (*MiS*) contains a nucleus. *A*, axon; *M*, myelin sheath; *SS*, submyelinic space; *one asterisk* (*), nucleus of myelin sheath-forming cell; *two asterisks* (**), nucleus of microtubular sheath-forming cell; *arrows*, zone of loop-to-loop connection of myelin sheath; *arrowhead*, attachment zone. *Inset:* High-magnification view of part of microtubular sheath. *Bars* 10 μm; *inset* 0.1 μm. (Modified from Xu (Hsu) and Terakawa 1999)

The microtubular sheath and myelin sheath are evidently formed by a different kind of glial cells, which are temporarily called the microtubule-rich glial cell and the myelin-forming glial cell, respectively. Moreover, according to the electrophysiological data, the submyelinic space is a fully sealed space and ontogenetically occurs between the myelin sheath and the microtubular sheath. Naturally, there is an open question about the origin of the large amount of the gel substance in the submyelinic space. A possibility is that the gel substance might be produced by some kind of glial cell(s). It seems that the aforementioned bipolar cell in the submyelinic space controls the metabolism of the gel substance, but the reality awaits experimental elucidation.

6.3 Overall View of Two Specific Structures of the Myelinated Fibers of the *Penaeus* Shrimp

In opposition to the vertebrate myelin sheath, which tightly encloses the axon with a gap of only 20 nm or so left between the myelin sheath and the axon, the myelinated nerve fiber of the *Penaeus* shrimp has two unique structures, called the submyelinic space and the microtubular sheath, located intermediately between the myelin sheath and the axon. The submyelinic space is obviously wide,

and the microtubular sheath is very thin. The diameter of the axon (together with the microtubular sheath) of the larger myelinated nerve fibers is commonly about one-tenth as thin as the host fiber. Even though the diameter of the axon of the giant motor fibers is thicker, it comprises about one-fourth of its host fiber.

1. The thin axon of the myelinated fibers of the *Penaeus* shrimp is tightly surrounded by the thin microtubular sheath, which is enclosed in the flat process of a kind of microtubule-rich glial cell. The function of the unique sheath seems to be as mechanical and metabolic support for the axon.
2. The submyelinic space between the microtubular sheath and the myelin sheath is filled with an amorphous gel substance. In the space, a few bipolar cells were found, which probably are the microtubule-rich glial cells to form the microtubular sheath. The axon with the microtubular sheath is freely located in the gap space; it is also tightly connected with the myelin sheath in the paranodal regions, as well as loosely attached to the myelin sheath somewhere in several points of the myelin sheath. The main function of the wide gap space as a parallel pathway with the axoplasm is to speed up the conduction velocity of nerve impulse. This subject is analyzed and discussed later.
3. The submyelinic space and the microtubular sheath of the *Penaeus* shrimp myelinated fibers develop after myelination of the nerve axon during ontogeny (Xu et al. 1994). The origin of the gel substance in the wide submyelinic space is uncertain at the moment and awaits further study.

Chapter 7
Studies on Impulse Conduction of Nerve Fibers

7.1 Some Significant Events of Studying the Electrical Impulse of Nerve Fibers

Excitation of nerve fibers is conducted as an electrical impulse, namely, the pulse of action current generated on the basis of the conformational change of ionic channels. As early as in the mid-19th century, by constructing a sensitive galvanometer, E. du Bois-Reymond succeeded in recording a steady negative current from the cut end to the intact portion of a frog nerve (injury current). When he excited the nerve with a current from the stimulating electrode, he observed that the injury current was neutralized. He called the current change evoked by nerve stimulation a "negative variation." The former phenomenon shows that the cut end of nerve fibers (to be equal to the inside of the nerve axons) is electrically negative to the intact region of nerve fibers, and the latter shows that in the excited point of the nerve fibers occurs a negative electrical change, which neutralized the negative injury current. The two phenomena were actually considered to be the discoveries of the resting membrane potential as well as the action potential that is generated during excitation of nerve fibers (see Tasaki 1982, pp. 9–10).

L. Hermann (1899–1906) called the "negative variation" of du Bois-Reymond the "action current" and further proposed a local circuit hypothesis of conduction of the action current along nerve fibers. Hermann recognized that the potential change associated with the excited region of an axon would send a small current in a circuit down the axonal cylinder through what we now call the axoplasmic membrane or axolemma, and back in the extracellular space to the excited region. The local circuit current evoked by stimulation flows to both sides of the excited region. Thus, the local circuit hypothesis suggested that the propagation of action current is an electrical self-stimulation process (see Tasaki 1953a, p. 38; Hodgkin 1964, p. 30).

Utilizing the method of "cutting down" the motor nerve to a muscle so that only two or three nerve fibers remained in functional continuity, Adrian and Bronk (1929) successfully recorded distinct signs of nerve impulses traveling along individual nerve fibers. It was

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established that the action potential of nerve fibers has a similar shape or waveform, indicating that the electric impulse of nerve fibers obeys the all-or-none principle.

In the beginning of the 20th century, adopting the local current flow hypothesis, J. Bernstein published the membrane theory of nerve conduction (see Hodgkin 1964, p. 30). Bernstein postulated that there is selective potassium permeability in the excitable cell membrane, and he recognized that excitable cells are surrounded by a membrane that is selectively permeable to potassium ions at the resting state. Actually, he correctly explained the resting membrane potential of nerve fibers as a diffusion potential built up by the tendency of positively charged potassium ions to diffuse from their high concentration in the cytoplasm to their low concentration in the extracellular solution. At the same time, he also suggested that during excitation the permeability of the nerve plasma membrane transiently increases to many species of ions in general, and consequently the resting membrane potential should be momentarily neutralized. From this point of view it could be deduced that the amplitude of action potential could not be higher than that of the resting membrane potential. Later experimental results showed that this was not the case.

Using an intracellular micropipette electrode, Hodgkin and Huxley (1939) exactly measured the resting membrane potentials of the *Loligo* giant axon to be negative at 50–70 mV. At the same time they found that the amplitude of action potential is as high as 100–120 mV, showing that at the peak of action potential there is an overshoot, during which the membrane potential transiently becomes inside positive. The overshoot phenomenon could not be explained by the "breakdown" of the selective permeability of the plasma membrane during excitation proposed by Bernstein, and it was necessary to search for a new ionic mechanism of action potential.

During the same period of time, Hodgkin (1939) experimentally proved Hermann's local circuit hypothesis of nerve impulse conduction in single nerve fiber preparations of sea crab and squid, respectively. In the experiments the conduction velocity of the nerve fiber preparations was recorded by placing the stretched nerve fiber in seawater between the stimulating and recording leads as a control. Then, a decrease in conduction velocity was observed by replacing the stretched nerve fiber into the oil. Otherwise, it was increased by connecting the strips of metallic conductors onto the stretch of the fiber by means of a mercury switch. The experimental results proved that conduction velocity of nerve fibers is dependent on the external electrical resistance in the circuit of action current or the local current.

In the mid-20th century, in the *Loligo* giant axon preparation, Hodgkin and Huxley further analyzed the ionic mechanism responsible for generation of the action potential using the voltage clamp technique, which provides ionic current at certain voltages. Those authors established that the ionic current consists of two independent components: the early inward sodium current, which is selectively blocked by tetrodotoxin (TTX), and the delayed steady-state outward potassium current, which is selectively blocked by tetraethylammonium (TEA). They proposed that there are two kinds of independent ion channels in the axoplasmic membrane, namely, the voltage-gated sodium channel and the voltage-gated potassium channel, which are responsible for generation of the corresponding two ionic components of action current, respectively. Now not only has the channel concept been fully established by experimental work, but also many ion channel molecules including

the sodium and potassium channels in the excitable membrane have been cloned and studied by genetic engineering.

In the excitable membrane of nerve axons, the voltage-gated sodium channel and potassium channel are present, allowing ions to permeate selectively from one side of the excitable membrane to the other according to their concentration gradient across the membrane. The nerve action current arises from the transient opening of the sodium channel, and terminates by the coordinated closing of the sodium channels and opening of the potassium channels. Thus, the sodium theory of nerve action current was substituted for the "breakdown" hypothesis of Bernstein.

7.2 Mechanism of Impulse Conduction in Unmyelinated Nerve Fibers

In living organisms, except for unusual circumstances, an action potential usually arises at one end of an axon and travels to the other. The mechanism of the impulse propagation along an axon can be schematically expressed as in Fig. 7.1. Suppose an action potential is conducted to a point on the axon. The spatial distribution of potential along the axon can be shown in the upper part of the figure. The distance occupied by the action potential depends on its duration and the conduction velocity of the axon. For example, if the duration of the potential change is 2 ms and it is conducted at 10 m/s, then the action potential will be spread over a length of 20 mm along the axon. The occupied distance is shown in the lower part of the figure.

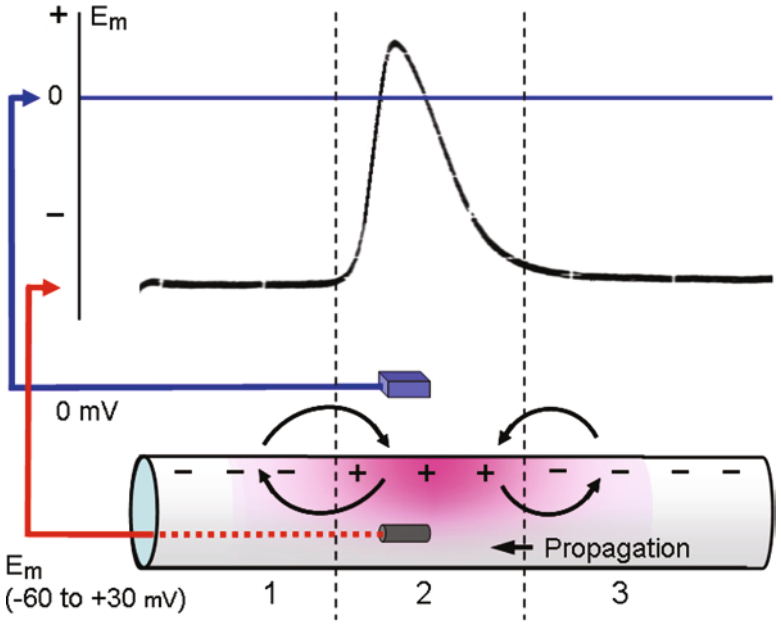

Fig. 7.1 Schematic diagram of mechanism of action current flow during action potential propagation from *right* to *left*

During the overshoot period (region 2 of Fig. 7.1), the sign of the membrane potential is reversed. The potential difference creates a local current flow to the inactive region ahead of the action potential (region 1 in Fig. 7.1). The local current induces there the opening of Na channels, which allows influx of Na ions according to their concentration gradient. As the depolarization caused by the influx of Na current reaches the threshold (indicated by an arrow), a rapid rising phase of a new action potential will be self-generated in the region near the leading edge of the active region. On the other hand, the potential difference creates a local current flow to the region behind the active one as well (region 3 in Fig. 7.1). However, it is unable to induce depolarization of that region because there is a refractory period of excitation during the slow falling phase of the action potential, which results from the inactivation of the Na channel and the activation of the K channel. Thus, an action potential once initiated and propagated cannot go backward by itself by reversing its direction of propagation. If an action potential is experimentally evoked in the middle of an axon, the potential peak is split into two parts, which travel away from each other to both sides of the axon. That is, to say, there is no inherent directionality in propagation, because in this case the rapid rising phase of action potential is capable of depolarizing equally the regions both ahead and behind the active region.

7.3 Experiments for Establishing the Saltatory Conduction Hypothesis with the Myelinated Fiber Preparations of Toad and Frog

As pointed out previously, as early as in 1871, Ranvier discovered the nodal structure in peripheral myelinated fibers of vertebrates. Since then. for a long period of time the function of Ranvier's node had not been clarified. In 1925, R.S. Lillie clearly proposed the possible role of Ranvier's node in impulse conduction in the myelinated fibers. He suggested that nerve impulse conducted in the myelinated fibers could be from one node of Ranvier to the other in a manner similar to the saltatory conduction of chemical activity in his iron wire nerve model (see Tasaki 1982, p. 33). By the way, this was a good example of the contribution of an artificial model experiment to neurobiology.

In the beginning of the 1930s (in the past century), the single myelinated fiber preparation of toad was successfully prepared in the laboratory of Kato in Japan. The living nerve fiber preparation provided a good possibility of study on the specific characteristics of impulse conduction in the myelinated fibers of vertebrates. In 1939–1942, Tasaki et al. in Kato's laboratory accomplished the basic experiments in the single fiber preparation to show that the nerve impulse is conducted in the myelinated fibers in a saltatory manner through the nodes of Ranvier (see Tasaki 1953a, 1982, p. 2). Tasaki's classical experiments, which are usually cited in texts and reference books of physiology and neurobiology, were as follows:

1. The threshold to electrical stimulation was tested by moving a unipolar micropipette electrode along a single myelinated fiber preparation of toad. It was

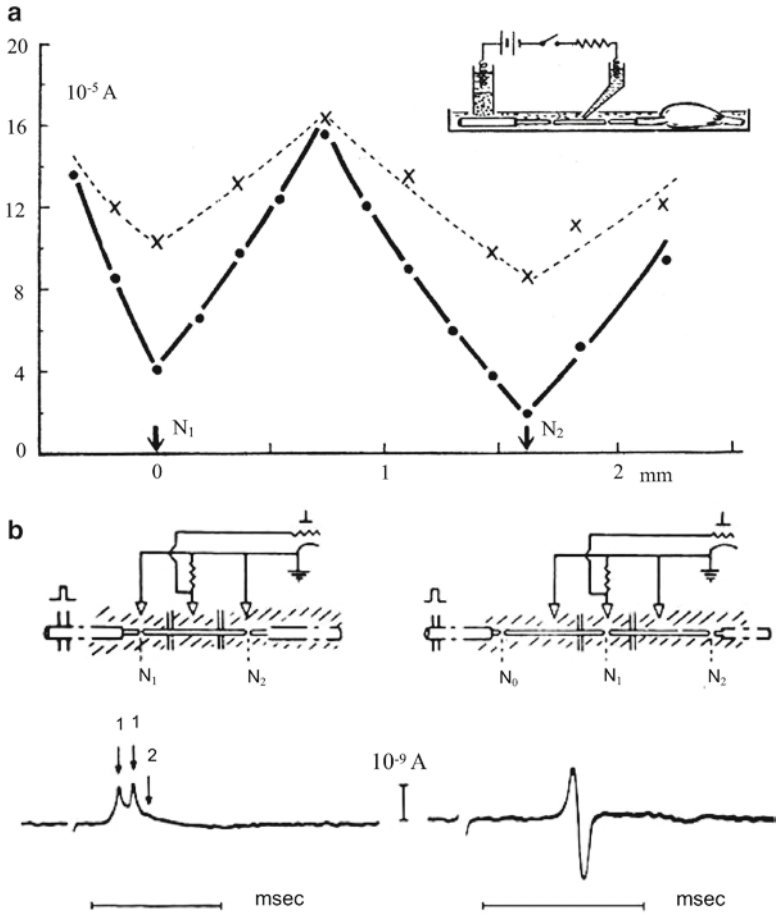

Fig. 7.2 Threshold of myelinated nerve fiber. (**a**) Thresholds to electrical stimulation along a single myelinated fiber preparation of toad determined with unipolar micropipette electrode. *Right upper diagram*, scheme of experimental arrangement. *Filled circles and crosses* show the thresholds obtained with cathode and anode current stimulation, respectively; N_1 and N_2 indicate nodes of Ranvier. (**b**) Action current was recorded from nodal region (*right*) but not from internodal region (*left*) of single myelinated toad fiber preparation. *Upper panels*, schemes of experimental arrangements. N_0, N_1, and N_2 indicate nodes of Ranvier. (From Tasaki 1982; the book is in public domain)

shown that the threshold was regularly lowered at the nodal regions (Fig. 7.2a). The authors explained the experimental result by an assumption that the myelin sheath is an electrical isolator and consequently the stimulating electrical current enters and leaves the nerve fiber only through the nodes of Ranvier.

2. To test whether the action current also leaves and enters the nerve fiber only through the nodes of Ranvier during impulse conduction, the authors placed a single myelinated fiber preparation in a chamber separated into three pools containing Ringer's solution by narrow air gaps. The myelin sheath on the gap was dried by the air to increase its electrical resistance at the gap, and the action current

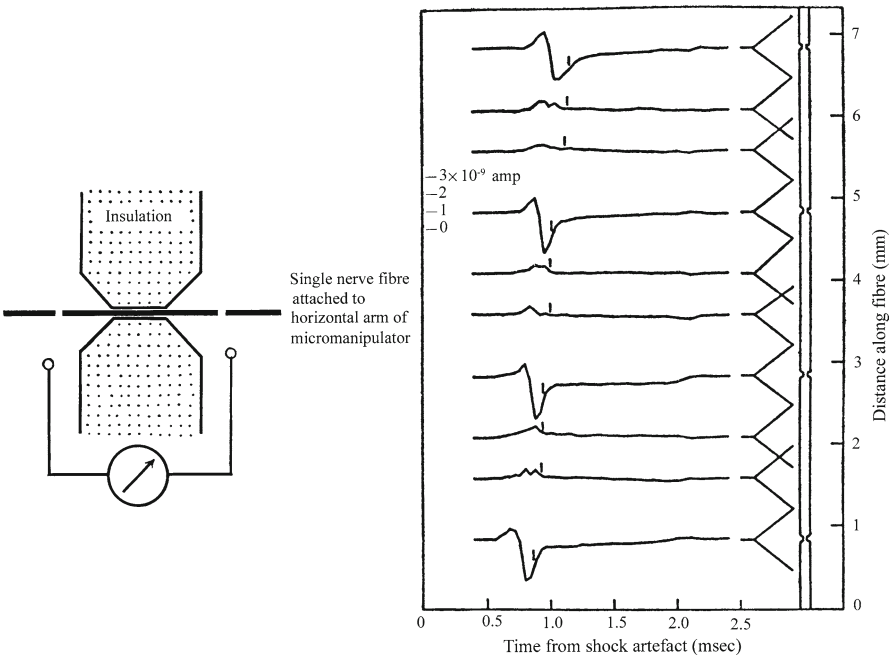

Fig. 7.3 Action current recorded only from nodal regions of single myelinated fiber preparation of frog. *Left panel* shows experimental scheme. (From Huxley and Stämpfli 1949, with permission by John Weiley and Sons)

was recorded. The experimental results showed that as the myelinated segment of the single fiber preparation placed in the central (recording) pool, no inward current was recorded under the stimulation of the nerve fiber (Fig. 7.2b, left). The two small peaks are the components of the inward action currents, which are generated in the nodes of Ranvier on both sides of the pools. When the segment of the single fiber preparation with a node of Ranvier was placed in the recording pool, an inward action current could be evoked (Fig. 7.2b, right).

The observations of Tasaki were confirmed by Huxley and Stämpfli (1949) using a newly constructed device to record the nodal action current along the single myelinated fiber preparation of the frog. In their method the distribution of action current was measured by placing a single myelinated fiber inside a short length of a fine capillary made of an insulator. The action current across the capillary was recorded. By sliding the fiber preparation through the capillary, the recorded action current was found to be a function of time and space. The inward currents are confined to the nodes, and the myelinated segments show only a smaller outward component (Fig. 7.3).

The relationship of the conduction velocity (V) in meters per second or the internodal distance (L) in millimeters with the fiber diameter (D) in micrometers was determined, respectively, at the single small myelinated fiber preparations of bull

frog by Tasaki et al. (1943). The statistic relationships were expressed by the following formulae:

Conduction velocity (V) m/s = 2.05 D (μm)
Internodal distance (L) = 0.146×10^3 D (μm)

The average internodal conduction time (T) was obtained:

Internodal conduction time (T) = L/V = 0.071 (ms)

The result means that a node of Ranvier is brought into action by the current developed by the neighboring node during saltatory conduction with a latency of only 0.071 ms, and the result undoubtedly shows that the conduction of the node is time consuming and that of the internodal segment is extremely short and independent of the fiber diameter.

The saltatory conduction hypothesis built up on the basis of the aforementioned experiments was soon accepted and extended to the myelinated fibers of all vertebrates by most physiologists and neurobiologists. Then, in a review of Stämpfli (1954) entitled "Saltatory conduction in nerve," three essential points were considered to be criteria or experimental proofs for the saltatory conduction hypothesis:

1. The excitable axoplasmic membrane is confined only to the nodal region of myelinated fibers.
2. The internodal axoplasmic membrane is a passive core conductor.
3. Activity is conducted from node to node through local electrical currents.

However, some authors (e.g., Lorente de Nó and Honrubia 1964, 1965) proposed a so-called tunnel hypothesis, which claimed that the nerve impulse propagating along the axoplasmic membrane of the myelinated nerve fibers should be also continuous by local action currents which could not be recorded from the outside of the myelin sheath during impulse conduction. In any event, at that time the saltatory conduction hypothesis had to be proven further.

Tasaki (1953b) had tried to test directly whether the reversal of the resting membrane potential takes place in the internodal axoplasmic membrane during impulse conduction by inserting a microelectrode deeply into the myelin sheath itself or into the axon at a single myelinated fiber preparation of the toad, respectively. However, in the experiment, no clear result was obtained, because neither the intra-axonal action potential (less than 40 mV) nor the resting membrane potentials (from 0 to −30 mV) reached satisfactory values.

In an intact myelinated fiber of desheathed sciatic nerve trunk of the frog, Xu (1985) repeated the aforementioned experiment by Tasaki. A positive monophasic action potential was recorded (Fig. 7.4) by inserting a microelectrode into the axon (A) or into the deep layer of the myelin sheath (B) of an intact single myelinated fiber, respectively. The microelectrode tip inserted into the axon or the deep layer of the myelin sheath was identified by the ionophoretic injection of 5% horseradish peroxidase (HRP). The results showed that both recorded action potentials were positive and monophasic. The difference of the two potentials was mainly in the amplitudes: the intra-axonal action potential was about 70 mV, whereas the potential

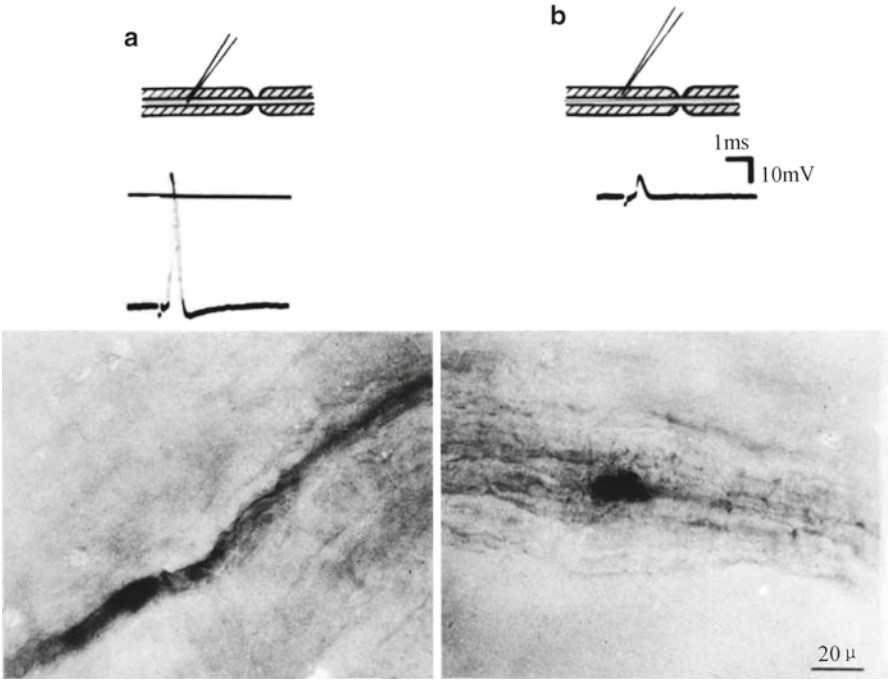

Fig. 7.4 Resting and action potentials recorded with microelectrode inserted into axon (**a**) or deep layer of myelin sheath (**b**) of myelinated fiber in desheathed sciatic nerve trunk preparation of frog. *Upper panels*, experimental schemes; *lower panels*, traces of tip of microelectrode location with 5% horseradish peroxidase (HRP). (From Xu 1985)

recorded from the deep layer of the myelin sheath was about 10 mV. The experiment proved that there is no reversal of the resting membrane potential, *i.e.*, no excitation in the internodal axoplasmic membrane occurs during impulse conduction.

7.4 Difference in the Distribution of Ion Channels in the Axoplasmic Membranes Between Unmyelinated and Myelinated Fibers of Vertebrates

The action current flows continuously in the unmyelinated nerve fibers, indicating that both the voltage-gated sodium channels and the voltage-gated potassium channels should be uniformly distributed in the axoplasmic membrane. The numbers of the sodium channels in the axoplasmic membrane of various unmyelinated fibers were determined, and they were shown to be rather low. For example, using the voltage clamp technique and tritiated TTX, the values given for the squid giant axon vary from 100/μm^2 (Chandler and Meves 1965) to 500/μm^2 (Levinson and Meves 1975). The density of the sodium channels in the axoplasmic membrane of the

rabbit unmyelinated C-fibers was determined to be about 110/μm^2 (Ritchie et al. 1976). The potassium channel is also shown to be distributed throughout the axoplasmic membrane of squid and unmyelinated fibers in general (Tasaki 1959).

The nerve impulse is conducted along the myelinated fibers in a saltatory manner from node to node. Experiments showed that the reversal of the resting membrane potential during impulse conduction does not take place in the internodal axoplasmic membrane, indicating that excitation limitedly occurs only in the nodal regions of the myelinated fibers of frog (Tasaki 1953b; Xu 1985). According to these experimental results, the sodium channels responsible for the generation of action current should be concentrated in the nodal axoplasmic membrane, and should be absent or, if there are any, should not be sufficient for generating action potential in the internodal axoplasmic membrane. Experiments further showed that in the nodal region of frog nerve fibers two types of ionic current are generated: the early inward current carried by sodium ions and the delayed steady-state outward current carried by potassium ions (Dodge and Frankenhaeuser 1958). Later, in the myelinated nerve fiber of rabbit, the early inward current carried by sodium ions was shown to be present, but the delayed steady-state outward current carried by potassium ions was absent (Rogart and Stämpfli 1981).

Using a saxitoxin-binding assay, Ritchie and Rogart (1977) determined the densities of sodium channels in the nodal region and the internodal region of rabbit sciatic nerve. Values of about 10,000/μm^2 and less than 25/μm^2 were obtained for the nodal and the internodal regions, respectively. The lack of contribution of voltage-dependent potassium currents to the action potential in rat and rabbit nodes of Ranvier, as well as in the nodes of a variety of other mammals, was well established (Chiu et al. 1979). Thus, the potassium channels are concluded to be absent in the mammalian node of Ranvier, at least in the peripheral nervous system (Brismar 1980).

These experimental results showed that following myelination of the nerve fibers of vertebrates the distribution of both the sodium and potassium channels in their axoplasmic membrane is rearranged. Consequently, in a review by Roots (1984, p. 24), it was concluded that:

It might be suggested that the next step in evolution was the concentration of sodium channels at the functional nodes and the exclusion of potassium channels from the nodal area.

As to the distribution of the potassium channels in the axoplasmic membrane of paranodal and internodal regions of the mammalian myelinated fibers, some electrophysiological experiments indicated that chronic destruction of the myelin sheath somehow led to the appearance of potassium channels in membrane areas where they did not exist previously. The acute treatments to disrupt the myelin sheath were applications of lysolethicin to dissolve the myelin, collagenase to loosen the connective tissue in the nodal region, and hypo- or hypertonic solutions to induce axonal volume changes. It was suggested that the appearance of potassium channels in the whole of the internodal axoplasmic membrane, not just the paranodal region, brings about generation of the outward potassium current on depolarization (Chiu and Ritchie 1982). Referring to the complementary distribution of channels in the

axoplasmic membrane, Ritchie (1986, p. 111) concluded that the nodal membrane is rich in sodium channels but poor in potassium channels, whereas the internodal membrane is rich in potassium channels and has relatively few sodium channels.

However, the appearance of potassium channels in the internodal membrane upon disruption of the myelin sheath does not prove the existence of the channels in normal myelinated fibers. In a later chapter of this book, we present evidence for the absence of the voltage-gated potassium channels in the internodal axoplasmic membrane of the normal myelinated fibers of an invertebrate, the *Penaeus* shrimp. Such a complementary distribution of the voltage-gated potassium channels in the internodal axoplasmic membrane is not the case in invertebrate myelinated nerve fibers.

7.5 Saltatory Conduction Was Concluded to Have Evolved Before Vertebrates

Node-like structures were observed under the light microscope in the nerve fibers of crustaceans (see Holmes 1942) and under the electron microscope in the crab *Cancer irroratus* (McAlear et al. 1958), in the prawn *Palaemonetes vulgaris* (Heuser and Doggenweiler 1966, and the prawn *Macrobrachium niponensis* (Yeh and Huang 1962). Yet no saltatory conduction has been established in these nerve fibers in spite of the fact that, in the nervous system of some species, giant fibers conduct the nerve impulse at a rather high speed. Recently, Davis et al. (1999) and Weatherby et al. (2000) reported a node-like structure on the myelin-like sheath of the nerve fibers of copepods and showed that the small planktonic crustaceans have an escape response that accelerates them to 200 body lengths per second within milliseconds. To be sure, no saltatory conduction was yet observed in the nerve fibers of these sea animals.

As a matter of fact, the saltatory conduction phenomenon based on functional nodes was reported only in a few nerve fibers of invertebrates such as a pair of the medial giant fiber of *P. chinensis* (Hsu et al. 1964, 1975a) and *P. japonicus* (Kusano and LaVail 1971), and in the median giant fiber of an earthworm (*L. terrestris*) (Günther 1973, 1976). Summarizing these related reports on the saltatory conduction based on functional nodes of invertebrate nerve fibers in their book, Bullock et al. (1977, p. 61) concluded that:

True nodes with a similar functional meaning have not been demonstrated in invertebrates, but functional spots acting as nodes are strongly suggested in earthworms and shrimps.

It is evident that the functional nodes observed in the nerve fibers of the earthworm and the shrimps were called “functional spots,” which were considered to merely have a function similar to that of the node, such as the node of Ranvier, in vertebrates. In other words, up to now, it has been generally concluded in the neurobiological literature that neither a true node, such as the node of Ranvier, nor saltatory conduction based on the true node, is present in the nervous system of invertebrates.

Chapter 8
Saltatory Conduction Found in the Nervous System of Two Model Invertebrates, the Shrimp (*Penaeus*) and the Earthworm (*Lumbricus terrestris*)

As already described, the finding of the saltatory conduction phenomenon in the nervous system of a few invertebrate animals was mentioned in some neurobiological literature, but a conclusion on the lack of saltatory conduction in the nervous system of invertebrates had not yet been generally revised. The reason for this seemed to be that a saltatory conduction in invertebrates is based on the functional spots, which could not be accepted as a true node. Therefore, before presenting the experimental findings that demonstrate the phenomenon of saltatory conduction in the nervous system of invertebrates, it is necessary to discuss the common understanding of the true nodal structure of the myelinated fibers.

8.1 Necessary Conditions for Identifying the Nodal Structure of Myelinated Nerve Fibers

The node of Ranvier in the myelinated nerve fibers is undoubtedly considered as the standard of a true node. Here, let us cite some explanations of the term "node of Ranvier" from the glossaries of some texts and reference books of neurobiology, as follows:

Nodes of Ranvier: "Interruptions in the myelin sheath at regular intervals (typically 1–2 mm)" along the axon. . . The voltage-gated Na^+ channels in the exposed axon membrane at each node provide a source of current that helps propagate the action potential." (Kandel et al. 1995, p. 710, Glossary)

More recent books of neurobiology describe the nodes of Ranvier as periodic gaps in the myelination of axons (Purves et al. 1997), or as localized areas devoid of myelin occurring at intervals along a myelinated axon (Nicholls et al. 2001).

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Commonly, the true nodal structure, as the node of Ranvier, is considered to be:

1. Interruptions in the myelin sheath at regular intervals
2. Capable of conducting the action potential in a saltatory manner along the nerve fibers
3. Abundant in the voltage-gated sodium channels and scarce in the voltage-gated potassium channels in its axoplasmic membrane

8.2 Saltatory Conduction Based on the Functional Nodes Found in the Giant Nerve Fibers of the *Penaeus* Shrimp

Saltatory conduction as a phenomenon in the nervous system of invertebrates was first found in a pair of the medial giant fibers of *P. chinensis* by Hsu et al. (1964, 1975a). By experiments similar to the classical one performed by Tasaki (1953a), saltatory conduction in the medial giant fibers was demonstrated as follows.

As a pair of the medial giant fibers superficially and dorsally lie in the whole length of the ventral nerve cord of the shrimp *P. chinensis*, a strip of the connective tissues above it was removed to expose it directly to the medium. The threshold to electrical stimulation was measured along an intact medial giant fiber by using a moving unipolar stimulating micropipette electrode. The results showed that the threshold along the abdominal portion of the medial giant fiber is regularly lowered at the ganglionic regions of the ventral nerve cord and the sites of the third nerve root leaving the ventral nerve cord (Fig. 8.1a). However, the threshold along the thoracic portion of the medial giant fiber is regularly lowered at the ganglionic regions only, because that portion is lacking in the third nerve root branching (Fig. 8.1b).

As shown in Fig. 2.2a, one of the medial and one of the lateral giant fibers, respectively, have an unmyelinated branch passing through an abdominal ganglion of the ventral nerve cord of *Penaeus* shrimp. The two unmyelinated branches cross to the opposite side of the ganglion respectively and are found in each abdominal ganglion. In Fig. 2.2b, it is shown that a pair of the medial and a pair of the lateral giant fibers with a Y-typed motor giant fiber form four giant synapses, which are located in each connective of the abdominal segment of the ventral nerve cord. The experimental results showed that the stimulating current enters and leaves the axon only through regions of the unmyelinated branch in each ganglion and the synaptic membrane of the giant fiber in each connective of the abdominal portion of the ventral nerve cord.

Another experiment was carried out using a single medial giant fiber preparation of *P. chinensis* in a chamber with three pools (Fig. 8.2, upper panel). When the myelinated segment of the preparation was placed in the recording pool, no inward action current was recorded (Fig. 8.2a). The action current was recorded only when the ganglionic or the synaptic region of the preparation was placed in the recording pool of the chamber (Fig. 8.2b). According to these results, the local action current is supposed to enter and leave the axoplasmic membrane also through regions of the unmyelinated branch in each ganglion and the synaptic membrane of the giant fiber in each connective segment of the ventral nerve cord.

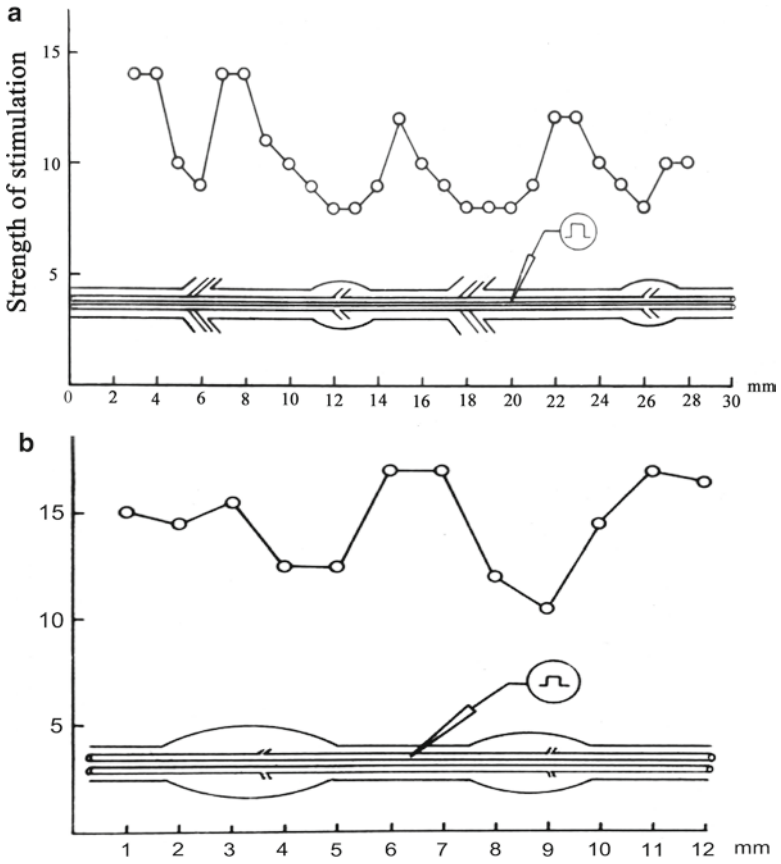

Fig. 8.1 Thresholds to electrical stimulation along abdominal part (**a**) and thoracic part (**b**) of medial giant fiber in ventral nerve cord preparation of *P. chinensis* determined with unipolar microelectrode. (From Hsu et al. 1975a)

It is evident that the unmyelinated branch in each ganglion and the synaptic membrane in each connection between two neighboring abdominal ganglia of the ventral nerve cord of the shrimp take the role of so-called functional nodes for the saltatory conduction of impulses. The same kind of functional nodes was found also in a pair of the lateral giant fibers (see Fig. 2.2a, b), and supposedly their impulse is also conducted in a saltatory manner, although experiments were carried out mainly in the medial giant fiber preparations.

Subsequently, saltatory conduction was reported in the same named single giant fiber preparation of *P. japonicus* using the electrophysiological method by Kusano and LaVail (1971). The functional nodes for this saltatory conduction are also the unmyelinated branches of the giant fiber in ganglia and the synaptic membrane regularly distributed in the abdominal segment of the ventral nerve cord of the shrimp.

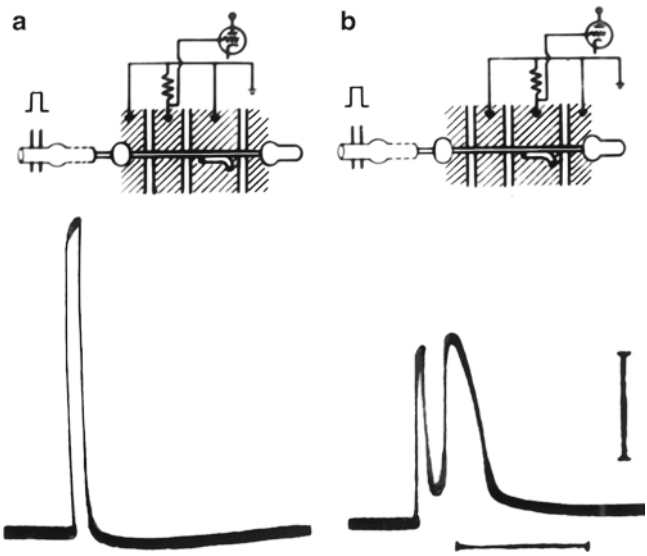

Fig. 8.2 Action current absent in internodal part (**a**), but recorded from nodal region (**b**), of medial giant fiber preparation of *P. chinensis*. *Upper panels*, experimental arrangement. (From Hsu et al. 1975a)

8.3 Saltatory Conduction Based on the "Spot Openings" in the Median Giant Fiber of the Earthworm (*Lumbricus terrestris*)

Following the finding of saltatory conduction in a pair of medial giant fibers of *P. chinensis* and *P. japonicus* among invertebrates, Günther (1973, 1976) reported saltatory conduction in the median giant fiber of the earthworm (*L. terrestris*), in spite of the fact that the sheath of the giant fiber is loosely multilayered. The nerve impulse was shown to be conducted through unsheathed branches in the ganglion of the nerve cord and spot openings along the median giant fiber (Fig. 8.3).

8.4 Experiments Analyzing the Ion Currents of the Functional Nodal Membrane in the Medial Giant Fiber Preparation of *Penaeus japonicus*

Terakawa and Hsu (1991) analyzed the ionic action currents of a functional nodal membrane (the giant synaptic membrane) in the medial giant fiber preparation of *P. japonicus* using the sucrose-gap voltage-clamp technique. A special acrylic

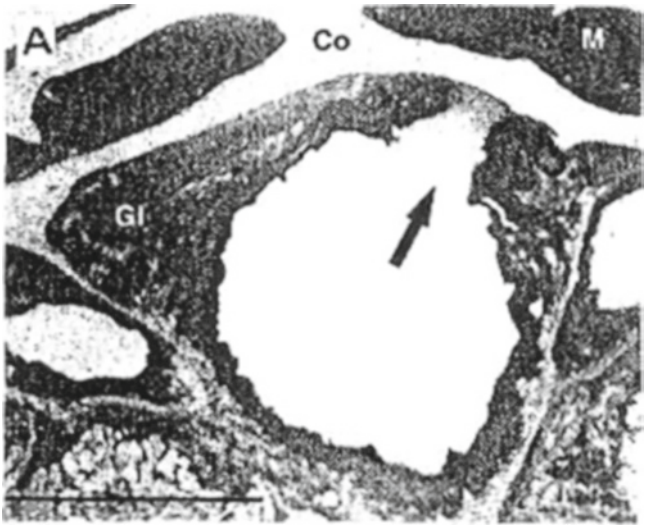

Fig. 8.3 Electron micrograph of "spot opening" in median giant fiber of earthworm (*Lumbricus terrestris*). *Arrow*, location of nodal membrane; *Co*, collagenous capsule of nerve cord; *Gl*, glial tissue; *M*, muscle of cord envelope. *Bar* 40 μm. (From Günther 1976, with permission under Springer Link)

chamber was made for the electrical recording, with three parallel vertical grooves that were 1 mm wide and separated from each other by walls 0.3 mm thick. A notch was made in the middle of each wall. A single fiber preparation about 3 cm long containing a nodal membrane was dissected from the ventral nerve cord of *P. japonicus*. An incision about 1 mm in length was made in the myelin sheath above the nodal region to expose the axon to the medium for better isolation with the sucrose solution. The nerve fiber preparation was mounted in the chamber perpendicularly to the grooves so as to cross all three grooves at the notch. First, the functional node was placed in the central groove of the acrylic chamber. The portions of the fiber in each groove were separated by petroleum jelly. Natural seawater was passed continuously through the central groove, while an isotonic sucrose solution was passed through both side grooves. Both ends of the fiber were immersed in 0.6 M KCl solution in pools prepared beside the grooves. By this means, the nodal membrane in the central groove was electrically isolated from both ends of the fiber. One end of the fiber in a pool was used for supplying a voltage-clamping current to the nodal membrane in the central groove, and the other for recording the potential difference across the same membrane (Fig. 8.4, upper panel).

The experimental results confirmed that the ionic currents could be recorded only when the nodal region of the fiber was placed in the central seawater pool. The inward currents induced by depolarizing voltage pulses were completely suppressed by TTX and greatly prolonged by scorpion toxin, suggesting that there are sodium currents mediated by the voltage-gated Na channels. The late outward currents were

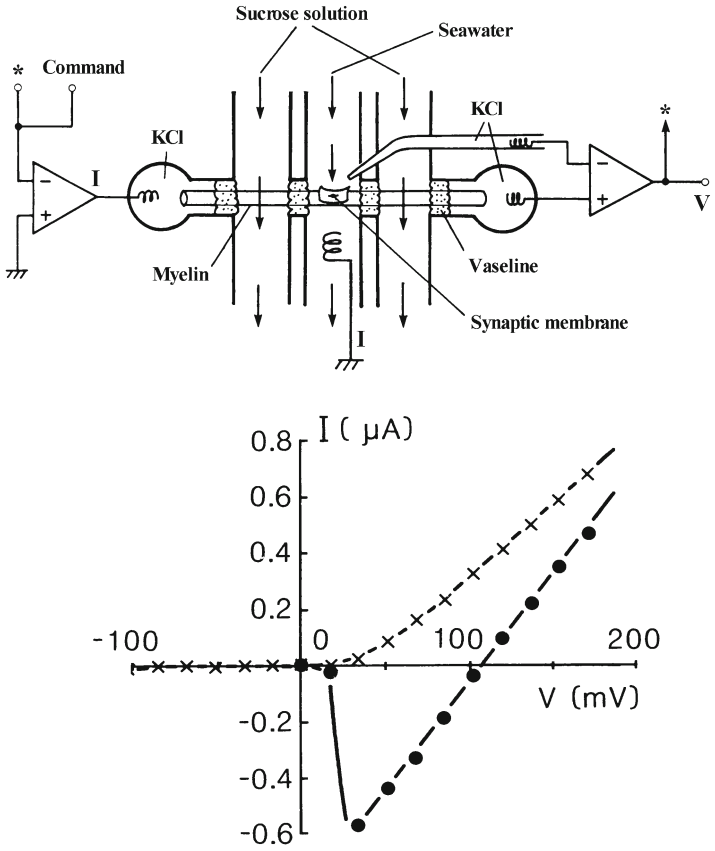

Fig. 8.4 I-V curve of ionic currents measured by sucrose-gap voltage-clamp method in functional (synaptic) nodal membrane of medial giant fiber preparation of *P. japonicas*. *Upper panel*: Experimental scheme of sucrose-gap voltage-clamp method. (Modified from Terakawa and Hsu 1991)

induced only by depolarizing pulses larger than 50 mV in amplitude. These currents could be suppressed by TEA, suggesting that they are potassium currents, mediated by the voltage-gated potassium channels. The potassium current was negligibly small when measured at the voltage that induced the maximum inward current, suggesting a low density of the K channels in the functional nodal membrane. A typical example of the current–voltage (I-V) curve showed a small K current compared with the Na current (Fig. 8.4).

The experimental results showed that the phenomena of both the concentration of sodium channels in the nodal membrane and the exclusion of potassium channels from it occur in the giant synaptic membrane of the medial giant fibers of the shrimp. Thus, at least a "functional spot," as it was called in the literature (see Bullock et al. 1977, p. 61), of the invertebrate myelinated fiber possesses all the aforementioned

essential functional conditions for identification of the nodal structure. Further, at the same time, this membrane plays the role in synaptic transmission. Thus, it is better to call "the functional spots" found in invertebrates as "functional nodes."

It is evident that the functional nodes were observed only in a few giant myelinated fibers of invertebrates, in contrast to the node of Ranvier, which is widely found in all myelinated fibers of vertebrates. From this point of view, the common presence in myelinated fibers should be an additional necessary condition for the true node.

8.5 Saltatory Conduction Based on Fenestration Node, a Novel Node of Common Type in the Myelinated Fibers of the *Penaeus* Shrimp

Following the discovery of the saltatory conduction phenomenon in the medial giant fiber of *P. chinensis* (Hsu et al. 1964, 1975a) and *P. japonicus* (Kusano and LaVail 1971), an intensive search began for the nodal structure in the medium- and small-sized myelinated fibers in the shrimp. Usually, neither a regularly distributed synaptic membrane nor an unmyelinated branch could be found. At the beginning of the work, although many slice preparations of the nerve fibers in the ventral nerve cord of *P. chinensis* were made and different methods for staining the nerve tissue were used, a nodal structure similar to the node of Ranvier could not be found in the nerve fibers.

Then, it came to mind that the medial and the lateral giant fibers in a pair of the circumesophageal connectives, *i.e*., the connectives between the head and subesophageal ganglia, as well as in the caudal half of the last abdominal connective, are as long as 3 cm or even longer in adult *P. chinensis* and *P. japonicus*, and that neither a synapse nor an unmyelinated branch is present. Luckily, by using a differential interference contrast microscope (DIC microscope), we found a kind of nodal structure on these portions of a single living giant fiber preparation of *P. japonicus*. Generally speaking, the thin axon in the giant nerve fiber is slightly swollen in the nodal region (Fig. 8.5a). Especially when viewed from the side of the nodal structure, a short natural interruption in one side of the myelin sheath is noted. On this site, the axon is attached to an unmyelinated opening (Fig. 8.5b). In a top view at a higher magnification, the nodal structure looks like an ellipsoid or circles arranged concentrically (Fig. 8.5c). Indeed, the novel nodal structure is an unmyelinated window for the myelinated nerve fiber, and we refer to this window structure as the fenestration node (FN) (Xu (Hsu) and Terakawa 1993; Hsu and Terakawa 1996).

Taking the swollen portion of the axon or the short natural interruption in one side of the myelin sheath as an indication, the FN in the myelinated fibers of the *Penaeus* shrimp can be easily identified even under the conventional bright-field microscope. Thus, the FN was easily established to be present in all observed myelinated fibers of all species of the *Penaeus* shrimp examined, which are listed in Chap. 2.

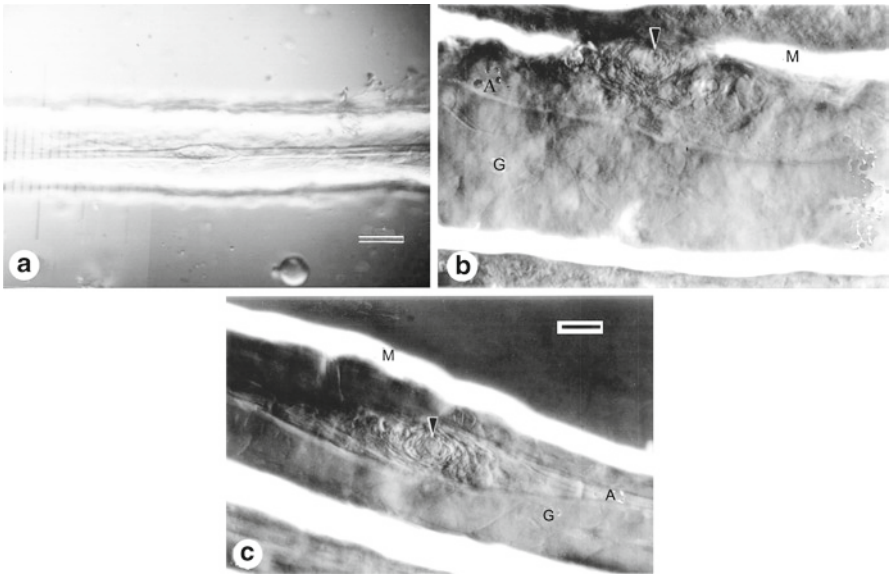

Fig. 8.5 Differential interference contrast images of living myelinated fiber preparation of *Penaeus* shrimp show fenestration node in these nerve fibers. (**a**) *Top view* of fenestration node in giant fiber preparation of *Penaeus semisulcatus*. (**b**) *Side view* of fenestration node in giant fiber preparation of *P. japonicus* (note natural gap of myelin sheath in its upper side). (**c**) *Top view* of fenestration node in the giant fiber of *P. japonicus* (*arrowhead*). *A*, axon; *M*, myelin sheath; *G*, gap space or submyelinic space. *Bars* (**a**) 50 μm; (**b** and **c**) 20 μm. (Modified from Hsu and Terakawa 1996)

The FN is regularly spaced along the smooth continuity of the myelin sheath. The internodal distance of the FNs is roughly proportional to the diameter of the fiber. For example, it is about 3 mm in myelinated nerve fibers with a diameter of approximately 40 μm, but is as long as 15 mm in the circumesophageal segment of a medial giant fiber of 150 μm in diameter. The long axis of the elliptical FN also depends on the diameter of the host myelinated nerve fibers. For example, the largest outmost ring of the myelin layers around the fenestration is about 50 μm in a medial giant fiber of 150 μm in diameter, and approximately 5 μm in fibers 30–40 μm in diameter. In the region of the FN, the swollen part of the axon contains many vacuoles of different shapes and sizes. This appearance does not appear to be a sign of morphological damage, because the vacuoles were observed in fresh nerve fiber preparations showing normal impulse conduction (Hsu and Terakawa 1996).

The electron microscopic studies revealed that the opening in the myelin sheath is formed just over the swollen part of the axon, and the myelin layers are seamed and fused together at the edge around the fenestration node (Fig. 8.6a, b). Both the myelin sheath and the microtubular sheath are absent in the fenestrated region, and instead a few perineurial cells loosely cover the axoplasmic membrane with digitated infoldings of various lengths (Fig. 8.6b). At this edge, the myelin sheath is tightly attached to the axoplasmic membrane, forming a close junction between them. The edge of the microtubular sheath is also tightly attached to the myelin

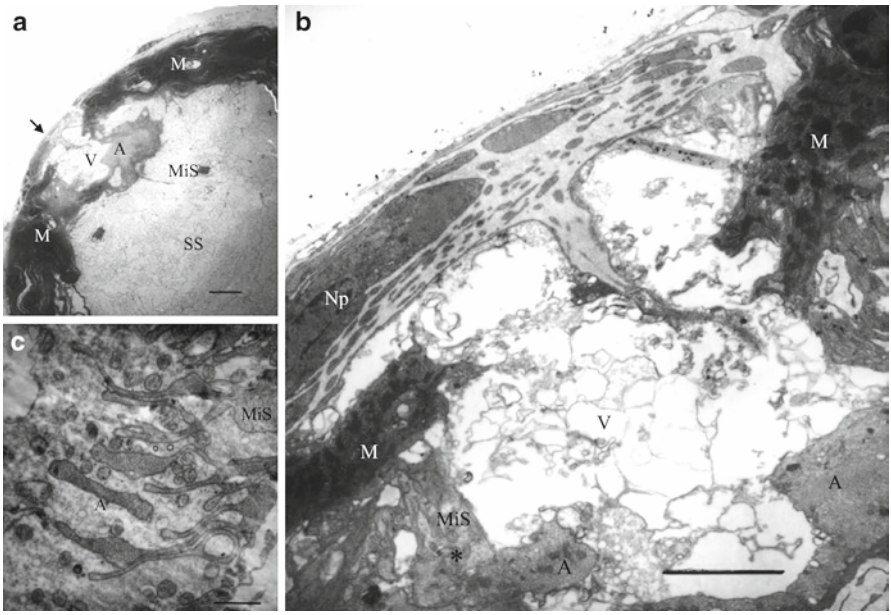

Fig. 8.6 Electron micrographs of fenestration node of myelinated fibers of *P. chinensis*. (**a**) Cross-sectional view of fiber with a fenestration node marked by an *arrow*. (**b**) Enlarged view of fenestration node region shows edge of myelin sheath bridged by perineurial cell and microtubular sheath processes. (**c**) Enlarged view of infoldings (interdigitation) formed by axolemma with processes of microtubular sheath (these structures were found in regions indicated by *asterisk* in (**b**)). *A*, axon; *M*, myelin sheath; *MiS*, microtubular sheath; *SS*, submyelinic space; *P*, perineurium; *Np*, nucleus of perineurial cell; *V*, vacuole. *Bars* (**a**) 10 μm; (**b**) 5 μm; (**c**) 1 μm. (From Xu and Terakawa 1999)

sheath so that the submyelinic space is electrically insulated from the external space. The axoplasmic membrane, *i.e.*, the excitable nodal membrane, is attached to the window and located slightly above the narrowest part of the FN in the myelin. Many infoldings are formed by the axoplasmic membrane at the nodal region, partly by perineurial cell processes but mainly by multiple processes of the microtubular sheath (Fig. 8.6b, c). These infoldings contribute to the total area of the excitable membrane in the node, which would help increase the density of local loop currents and the electrical conductance there for promoting saltatory conduction.

Both the FN of shrimp and the spot opening in the median giant fiber of the earthworm (*L. terrestris*) reported by Günther (1973, 1976) are unmyelinated windows, but differences in the fine structure between them are evident. In the spot opening of the earthworm, the nodal membrane is extended toward the upper level of the myelin sheath and is connected directly to the collagenous capsule of the nerve cord. Neither infolding of the axoplasmic membrane nor vacuole is present in the spot opening of the earthworm. Moreover, the spot openings are sporadically distributed in the giant fiber of the earthworm, whereas the FNs are definitely present in all myelinated fibers of the *Penaeus* shrimp.

8.6 Ionic Currents of the Nodal Fenestration Membrane in the Myelinated Fiber Preparation of *Penaeus japonicus*

The ionic action currents of the nodal fenestration membrane in the giant fiber and the large myelinated fiber preparations of *P. japonicus* were analyzed using the sucrose-gap voltage-clamp method that had been used for studying the functional nodal (synaptic) membrane (Hsu and Terakawa 1996). An incision about 1.0 mm in length was also made in the myelin sheath of the preparation to expose a part of the axon with a fenestration node to the medium of seawater for better isolation with sucrose solution. A large ionic current was evoked by applying a clamping pulse of +80 mV when a portion of nerve fiber bearing a single FN was placed in the seawater-containing central groove of the chamber (Fig. 8.7a). Then, the same voltage pulse applied after placing the FN in the adjacent sucrose-containing groove did not evoke any ionic current change. Finally, the ionic current was evoked again by placing the node back in the seawater-containing groove. The experimental results showed that the excitable axoplasmic membrane is localized in the region of the fenestration node.

The inward currents induced by depolarizing voltage pulses were also completely suppressed by tetrodotoxin (TTX) and greatly prolonged by a scorpion toxin, suggesting that they are the Na current, mediated by the voltage-gated Na channels. The small outward currents at the late phase of the clamping pulse were suppressed by TEA, suggesting that they are the K current, mediated by the voltage-gated K channels. The current–voltage (I-V) relationship obtained from a FN of a myelinated fiber of *P. japonicus* (Fig. 8.7b) indicates that the nodal action current largely arises from the activity of Na channels located in the nodal membrane. Although there is a small outward K current at the late phase of the clamping pulse, this current would not be enough to eliminate the excitation during the action potential.

The density of Na channels in the nodal fenestration membrane of the *P. japonicus* myelinated giant fiber was roughly calculated to be 530 channels/μm^2. This value is smaller than that estimated for the functional (synaptic) nodal membrane of the medial giant fiber (Terakawa and Hsu 1991). As has already been described, in the nodal membrane of amphibians are found a high density of sodium channels and a small number of potassium channels, whereas in that of mammals, such as the rabbit, there is a concentration of only sodium channels, fully excluding the potassium channels. Now we know that in the nodal fenestration membrane of the *Penaeus* shrimp there is also a concentrated number of sodium channels, although their density is slightly smaller than that in the functional nodal membrane of the shrimp. Also, the density of potassium channels in both the fenestration and functional nodal membranes is estimated to be quite small. So, from the evolutionary point of view, the nodal membranes of *Penaeus* shrimp seem to be ranked in the middle position between those of the amphibians and the mammals. There was also no detectable range of voltage-gated Na and K channels in the internodal axoplasmic membrane of *Penaeus* shrimp myelinated nerve fibers.

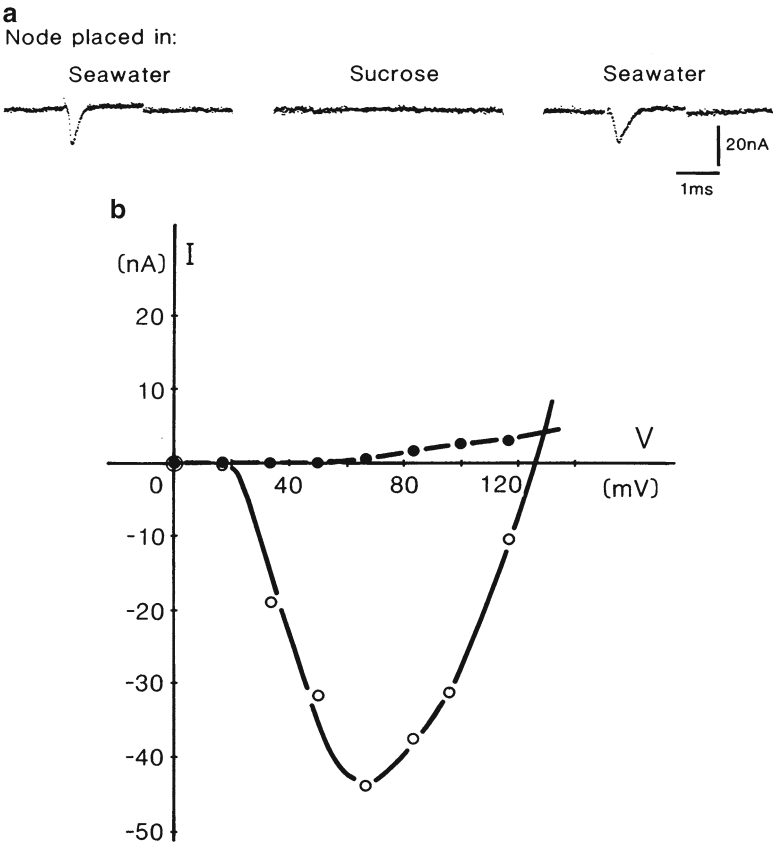

Fig. 8.7 Ionic current recorded by sucrose-gap voltage-clamp method in myelinated fiber preparation of *P. japonicus* possessing a fenestration node. (**a**) Ionic current recorded with node placed in seawater-containing groove (*left*), with node in adjacent sucrose-containing groove (*middle*), and with node returned to seawater-containing groove (*right*). (**b**) I-V relationship of fenestration nodal membrane measured by sucrose-gap voltage-clamp method. Peak value of initial inward current, *open circles*; late steady value of outward current, *filled circles*. Sucrose-gap voltage-clamp method as illustrated in Fig. 8.4 (*top*) was used. (From Hsu and Terakawa 1996)

8.7 Overall View of Saltatory Conduction in the Myelinated Nerve Fibers of Vertebrates and *Penaeus* Shrimp

Saltatory conduction is the mechanism of action current flow along myelinated nerve axons, so named because that the local action current was supposed to flow from one node to the next based on self-generation of the current in the nodes.

1. Saltatory conduction based on the node of Ranvier was concluded to be true in the myelinated nerve fibers of most vertebrates only. Then, the phenomenon

based on the functional nodes was first established in two pairs of the giant myelinated fibers of the *Penaeus* shrimp and in a giant fiber with the loosely multilayered sheath of an earthworm. More recently, a saltatory conduction based on a common type of novel nodal structure, called the fenestration node, was found in all myelinated fibers of the *Penaeus* shrimp (see Xu and Terakawa 1999). Thus, it has been proven that the saltatory manner of impulse conduction evolved in the myelinated fibers of both vertebrates and invertebrates.

2. The manner of impulse conduction along the myelinated fiber is slightly different between vertebrates and the *Penaeus* shrimp as a consequence of the difference in structures of the two kind of myelinated fibers. The question is discussed later.
3. As to the morphological bases of saltatory conduction, both the node of Ranvier and the fenestration node are narrow periodic openings in the myelin sheath of the nerve axons, but the former is a complete cylindrical interruption of the myelin sheath, whereas the latter is a hole-like opening through the myelin sheath. The functional nodes are also observed in a few myelinated giant fibers of the *Penaeus* shrimp and the earthworm.
4. The internodal distance between two Ranvier nodes is commonly about 2 mm in peripheral nerve fibers and a little longer in the central nervous system of vertebrates, whereas that between two fenestration nodes or functional nodes is roughly proportional to the diameter of the nerve fibers. For example, the internodal distance of the fenestration nodes varied from 3 mm in nerve fibers with a diameter of approximately 40 μm to about 12 mm in the circumesophageal segment of a medial giant fiber 170 μm in diameter.
5. The high concentration of sodium channels in the nodal axoplasmic membrane and the exclusion of potassium channels from it were established both in Ranvier's node of vertebrate myelinated fibers and in the fenestration node, as well as the functional (synaptic) node of invertebrate myelinated fibers. However, the voltage-gated potassium channels were determined to be absent in the internodal axoplasmic membrane of the myelinated fibers of *Penaeus* shrimp, whereas they were found abundantly in the internodal axoplasmic membrane of the mammalian myelinated fibers when the myelination was removed by acute treatment with some agents.

Chapter 9
Experimental Confirmation of the Saltatory Conduction Hypothesis

In Chap. 7, we learned that the hypothesis of saltatory conduction was based on the experiments by Tasaki et al. in the toad single myelinated fiber preparation. It was immediately supported by new experiments in the frog single myelinated fiber preparation by Huxley and Stämpfli (1949). Then, Stämpfli (1954), in a review, proposed three conditions for the experimental proof of the hypothesis of saltatory conduction. There were a few investigators who objected to the saltatory conduction hypothesis and insisted that the nerve impulse was also continuously conducted under the myelin sheath as a pass-through tunnel (Lorente de Nó and Honrubia 1964, 1965). At the present time, saltatory conduction in the myelinated fibers of vertebrates has been adopted by most neurobiologists and is included as a subject in most texts and reference books of neurobiology. Nevertheless, saltatory conduction as reported in the nervous system of the *Penaeus* shrimp in invertebrates is not yet commonly introduced in the neurobiological literature. In view of these circumstances, the saltatory conduction hypothesis needs to be thoroughly confirmed by experiments. Fortunately, the myelinated giant fiber of the *Penaeus* shrimp can be used as a suitable preparation for this purpose. Namely, the wide submyelinic space of the giant fiber can be considered as a "wide natural tunnel" between the myelin sheath and axon in the myelinated fibers. Moreover, its long internodal distance is advantageous for experimental operation. Making the best use of these properties in the giant fiber preparation of the *Penaeus* shrimp, the three conditions for the proof of the saltatory conduction hypothesis proposed by Stämpfli (1954) were all fulfilled.

9.1 Inexcitability of the Internodal Axoplasmic Membrane of Myelinated Nerve Fibers

A preparation of a single motor giant fiber, which has a relatively thick axon, and a portion of a giant synapse with the medial giant fiber, was made from the ventral nerve cord of *P. japonicus* (as schematically shown in Fig. 9.1). A glass

K. Xu and S. Terakawa, *Myelinated Fibers and Saltatory Conduction in the Shrimp: The Fastest Impulse Conduction in the Animal Kingdom*,
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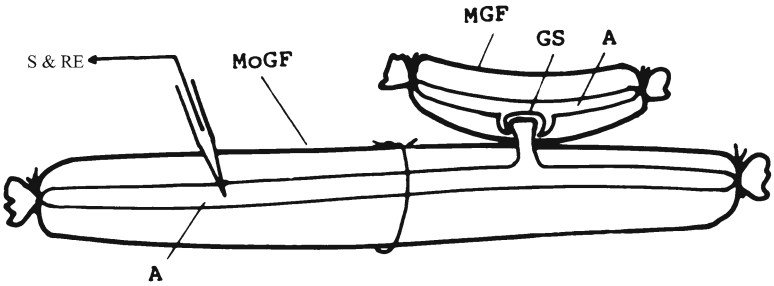

Fig. 9.1 Scheme of experimental arrangement shows intra-axonal recording of resting and action potentials from isolated piece of motor giant fiber preparation of *P. japonicus* with nodal (synaptic) region attached or removed by a single silk thread ligature. *A*, axon; *GS*, giant synapse; *MGF*, medial giant fiber; *MoGF*, motor giant fiber; *S & RE*, stimulation and potential recording. (Modified from Xu and Terakawa 1991)

microelectrode was successfully inserted through the myelin sheath into the axon of the motor giant fiber for both stimulation and recording. The normal resting membrane potential and action potential were recorded from the preparation to show that it was in good functional condition. Then, the synapse-containing portion of the motor giant fiber of the preparation was functionally excluded by placing a ligature of a single silk thread between the giant synapse and the tip of the microelectrode. In the internodal motor giant fiber preparation, the action potential could no longer be evoked by using both positive and negative stimulating currents. However, the value of the resting membrane potential of the preparation was not changed at all by placing the ligature. The experimental results showed that the internodal axoplasmic membrane of the myelinated fibers of the *Penaeus* shrimp is completely inexcitable, but it generates a resting membrane potential as equally as does the nodal axoplasmic membrane (Xu and Terakawa 1991).

Moreover, by the voltage clamp technique with a variable amplitude of the clamping pulse, it was established that no action current is evoked at the internodal axoplasmic membrane of the myelinated nerve fiber of *P. japonicus* (Hsu and Terakawa 1996). This finding means that there is not any kind of voltage-gated sodium channel.

By another experiment, the ion channels responsible for generating the resting membrane potential in the internodal axoplasmic membrane of the myelinated fibers of the *Penaeus* shrimp were tested (Xu and Terakawa 1991). The arrangement of the experiment was as follows. An internodal segment of the medial giant fiber preparation was made from the ventral nerve cord of *P. japonicus*. An incision about 2 mm in length was made in the myelin sheath to expose a part of the axon to the artificial medium. The preparation was placed in an experimental chamber, in which the concentration of K ions ($[K^+]$) in the medium (artificial seawater) could be changed automatically with a mini-pump. The change in resting membrane potential of the preparation was continuously recorded, and the effect of changing the $[K^+]$ in the medium on the resting membrane potential was examined. The experimental result is shown in Fig. 9.2. When the $[K^+]$ in the medium was raised stepwise, from a

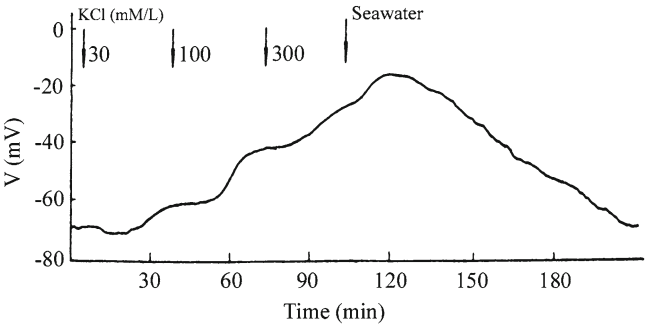

Fig. 9.2 Effects of changes of external [K^+] in seawater by replacing equivalent amount of Na^+ on the membrane potential at internodal preparation of myelinated giant fiber of *P. japonicus*. (From Xu and Terakawa 1991)

normal level of 10 to 30, 100, and 300 mM, the resting membrane potential of the internodal fiber preparation shifted in depolarizing direction from the control level of −69 to −59, −41, and −18 mV, correspondingly. When the K^+-rich medium was replaced with the normal one, the depolarized membrane potential gradually returned to its original level. In the experiment, the change in [K^+] in the medium from 30 to 300 mM induced a depolarization of the resting membrane by about 41 mV. The preliminary result indicated that it should be the non-gated potassium channel which is mainly responsible for the generation of the resting membrane potential in the internodal axoplasmic membrane. The resting membrane potential of the internodal preparation of the shrimp was not affected by an extracellular application of TEA, showing that the property of the non-gated potassium channel is different from that of the voltage-dependent one that is involved in the generation of action potential.

As mentioned previously, in the frog single myelinated nerve fiber preparation it was established that the reversal of the resting membrane potential does not occur during impulse conduction, showing the lack of excitability of the internodal axoplasmic membrane of the myelinated fibers of vertebrates (Tasaki 1953b; Xu 1985).

9.2 Excitability of Axoplasmic Membrane Confined to the Nodal Region of Myelinated Nerve Fibers

In contrast to the internodal axoplasmic membrane, only the nodal axoplasmic membrane is experimentally proven to be excitable in the myelinated giant fiber preparation of *P. japonicus*. A medial giant fiber preparation of about 1 cm in length, including a giant synapse, was dissected out at the level of the last thoracic and the first abdominal ganglia of the ventral nerve cord of *P. japonicus*. To keep the giant

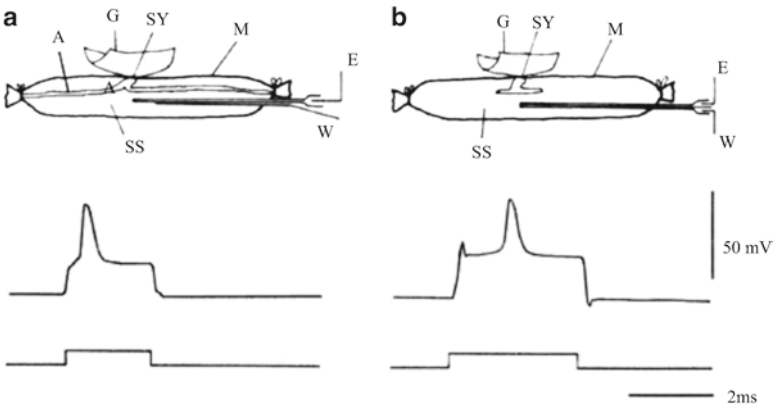

Fig. 9.3 Action potential recorded from submyelinic space at isolated piece of the medial giant fiber preparation of *P. japonicus*. *Upper panels*: Schemes of medial giant fiber preparation. *SS*, submyelinic space; *SY*, synaptic (nodal) membrane; *A*, axon; *M*, myelin sheath; *G*, remaining part of motor giant fiber; *E*, potential recording electrode; *W*, current supplying electrode. (**a**) Outward current of 0.2 μA was applied through '*W*' and action potential was recorded through '*E*.' (**b**) Action potential recorded from preparation shown in (**a**) after removing most of its axon. Current was applied during the period indicated by *bottom traces*. (From Hsu and Terakawa 1984)

synapse in a normal functional condition, a small portion of the motor giant axon was left attached to the synaptic site of the medial giant fiber preparation. A glass pipette electrode with a "piggyback" wire electrode attached was inserted longitudinally to the submyelinic space of the giant fiber preparation. A monophasic action potential about 50 mV in amplitude was evoked using the stimulating wire electrode as a control (Fig. 9.3a). Then, the electrodes were taken out, and the main parts of the axon under the myelin sheath were removed from both sides of the preparation without damaging the myelin sheath, by needle manipulation. After the operation the myelin sheath was stretched again and the same electrode was inserted into the gap. Both ends of the preparation were tied again. The stretch of the axon left to the synaptic part was about 2 mm in length. In this preparation, a monophasic action potential of about 50 mV in amplitude was recorded again from the submyelinic space (Fig. 9.3b). The experiment directly proved that the excitable membrane is limited in the axoplasmic membrane of the functional nodal (synaptic) region of the myelinated fibers (Hsu and Terakawa 1984).

The voltage clamp experiments further showed that the voltage-gated sodium channels alone, or with the voltage-gated potassium channels, are responsible for the generation and termination of action potential in the nodal axoplasmic membrane of the myelinated nerve fibers of both vertebrates (Dodge and Frankenhaeuser 1958) and the *Penaeus* shrimp (Terakawa and Hsu 1991; Hsu and Terakawa 1996). The aforementioned experiments showed that the excitable membrane of the myelinated fibers is confined to the nodal region in both vertebrates and the *Penaeus* shrimp.

9.3 The Local Action Current Is Conducted from Node to Node in Myelinated Nerve Fibers

In frog myelinated fiber preparations, it was established that about one-fifth the nodal action current is enough to excite the next node (Tasaki 1953a). In other words, a nodal action current is large enough to excite more than three neighboring nodes. In the myelinated fiber preparation of *P. chinensis*, it was usually observed that two or three notches appeared on the rising phase of the action potential in spite of being recorded from inside the axon or from the submyelinic space. The notches became more obvious when the intensity of the stimulus was reduced to a level near the threshold (Hsu et al. 1975). The appearance of the notches is probably related to the consecutive spread of the local action currents to three or four nodes separated by long internodal segments as passive conductors. To analyze the origin of the notches, a double-barreled capillary electrode was longitudinally inserted into the submyelinic space from the cut end of a medial giant fiber preparation of *P. chinensis*. The tip of the electrode was adjusted in place to just meet the first functional node of the preparation. One barrel of the electrode was used for stimulating and the other for recording (Fig. 9.4). After making a ligature at the cut end of the preparation, an action potential was taken as the control. Then, several ligatures were made in turn on the preparation from the distant end for testing the changes in the amplitude of the action potential. For example, in an experiment, the amplitude of the control action potential was 47 mV (which was evidently not

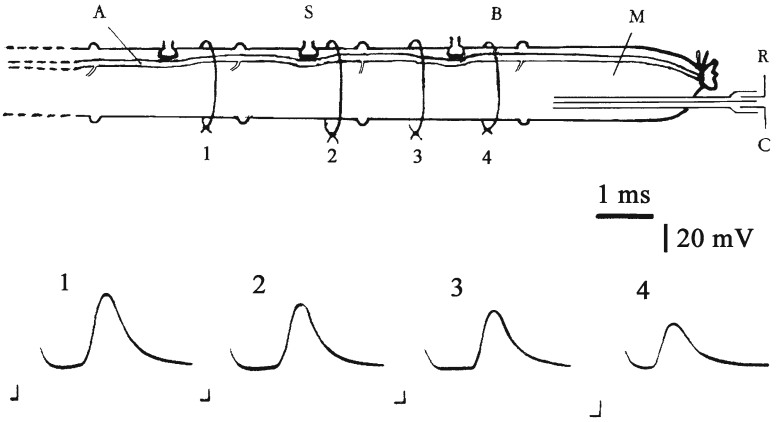

Fig. 9.4 Determination of changes in amplitude of action potential in medial giant fiber preparation of *P. chinensis* after placing ligatures with a single silk thread to remove the functional nodes in turn. After making ligatures *1*, *2*, *3*, and *4*, the amplitude of the action potential was 47, 40, 37, and 28 mV, respectively. *Upper panel*: Scheme of medial giant fiber preparation in ventral nerve cord. *A*, axon; *B*, unmyelinated branch of axon in ganglion of ventral nerve cord; *S*, synaptic region; *M*, submyelinic space; *R*, recording electrode; *C*, current supplying electrode. (Modified from Xu (Hsu) and Terakawa 1993)

large enough yet, because the tip of the electrode was not inserted deeply enough). The amplitude of the action potential remained at 47 mV after completing ligature 1, showing that the distal functional nodes do not contribute to the action potential. As ligatures 2, 3, and 4 were consecutively placed, the amplitude of the action potential was decreased stepwise to 40, 37, and 28 mV. By placing ligatures, the number of the functional nodes between the sites of the ligature and the tip of the electrode remained three, two, and one, respectively, and the amplitude of the action potential was accordingly decreased by 7, 3, and 9 mV, respectively. The stepwise change in the amplitude of the action potential proved that the action current flows from node to node by a local circuit during impulse conduction. If a nodal action current is large enough, it is able to elicit the excitation in more than three functional nodes. The contribution of a synaptic nodal area to the amplitude of action potential obviously is greater than that of a branching nodal area (Xu (Hsu) and Terakawa 1993).

9.4 Overall View of Saltatory Conduction as the Common Principle of Impulse Conduction in Myelinated Nerve Fibers

The hypothesis of saltatory conduction was constructed by Tasaki. Then, it was objected to a so-called tunnel hypothesis, which insisted that the nerve impulse conducted in the myelinated fibers should be also continuous, as in the case of the unmyelinated fibers. In more recent time, the phenomenon of saltatory conduction has been found in the myelinated fibers of the *Penaeus* shrimp and the median giant fiber of the earthworm. Now the saltatory conduction hypothesis is systematically proven in the specifically structured myelinated giant fiber preparation of *P. japonicus* and *P. chinensis*. Thus, saltatory conduction can be considered as a common principle of impulse conduction in the myelinated fibers of both vertebrates and invertebrates.

Chapter 10
Three Mechanisms or Strategies for Increasing Conduction Velocity of Nerve Fibers

10.1 Some Significant Events of Studying the Mechanism of Impulse Conduction in Nerve Fibers

In the early stage of development of physiology, the leading physiologists, including J. Muller, the teacher of H.L.F. Helmholtz, had visualized the mysterious process that travels along the nerve at such an immense velocity, which could not even be measured. Then in 1850, Helmholtz first determined the conduction velocity of the frog nerve fibers by two series of experiments. He unexpectedly found that the conduction velocity of frog nerve fibers is only about 27 m/s (see Tasaki 1982, pp. 10–13). His historic work opened the way in physiology to study the impulse conduction of nerve fibers.

More than half a century later, Erlanger and Gasser (1937) completed a famous work to record the compound action potentials of the nerve trunk of frog using a cathode-ray oscillograph and established that there is a simple relationship between the conduction velocity and fiber diameter. The authors classified the nerve fibers in the trunk into A, B, and C groups according to their conduction velocities of action potential:

Group A (the thick myelinated fibers): 15–42 m/s
Group B (the thin myelinated and the unmyelinated fibers): 1–6 m/s
Group C (the unmyelinated fibers): 0.2–0.5 m/s

Classification of nerve fibers into groups according to their conduction velocities and fiber diameters was taken as a principle applied in the study of nerve fibers of vertebrates. For example, there is a classification developed in a common reference book as follows:

Group A refers to myelinated fibers in the peripheral nervous system of mammals. The conduction velocities range from 5 to 120 m/s.
Group B consists of myelinated fibers in the autonomic nervous system, which have conduction velocities in the lower part of the A-fiber range.
Group C contains only the unmyelinated fibers conducting nerve impulses at less than 2 m/s.

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In the 1940s, Tasaki et al. as well as Huxley and Stämpfli clarified the roles of the myelin sheath and node of Ranvier in the impulse conduction of vertebrate nerve fibers. The authors experimentally established that an electrical impulse is conducted in myelinated nerve fibers in a saltatory manner, and its conduction velocity is greatly accelerated. The action potential propagates along the nerve axon, whether it is coated with a myelin sheath or not, by the mechanism of self-generated local ionic current. It is evident that the axon as a conductor plays the role of both the resistor and the capacitor for the flow of ionic current.

Considering that a cylindrical nerve fiber and an undersea cable have the same formulated components, such as a core conductor and an insulating sheath around a conducting medium, Hodgkin and Rushton (1946) measured the characteristic change of the passive spread of applied current along a lobster axon with extracellular electrodes, and analyzed the data from the axon by using the differential equation derived for the electrical properties of the undersea cable. As a result of their work, the authors introduced several electrical constants for characterizing the passive electrical properties of a nerve fiber:

1. Membrane resistance (r_m) of an axon represents the resistive nature of the axon wall to the ionic current flowing through it. It is measured in a unit of Ω/cm.
2. Membrane capacitance (c_m) of an axon represents the isolating ability of the axon membrane to store electrical charges, *i.e.*, during the passage of the ionic current through the axonal double membrane, which works as a capacitor. It is measured in a unit of μF/cm.
3. Resistance of the axoplasm (r_i) represents the resisitive nature of the axoplasm to the ionic current flow passing through it. It is measured in a unit of Ω/cm.
4. Time constant (τ_m) of an axon is a measure of the rate of buildup or decay of a localized graded potential of axon. Actually, it is the time for the membrane potential to vary 63% (1–1/e) of the way toward its final value in response to a stepwise change in current. Numerically, $\tau_m = r_i c_m$.
5. Length constant (λ) of the axon is a measure of the distance over which a localized graded potential of an axon decreases to 1/e of its original size in response to a stepwise change in current. Numerically, $\lambda = (r_m/r_i)^{1/2}$.

These electrical constants of the nerve fiber are experimentally measurable and are applied to characterize the electrical properties of not only a nerve axon but also a muscle fiber.

10.2 Conduction Velocity and the Electrical Properties of Nerve Fibers

The conduction velocity of the action potential of a nerve fiber is tightly dependent upon its electrical characteristics. Generally speaking, the conduction velocity of an action potential depends on how quickly the action potential is generated in a nerve fiber, *i.e.*, a property related to the electrical threshold or the rate of rise. It also

depends on how far the action potential is spread along the nerve fiber, *i.e.*, a property related to the electrical constants, such as τ_m and λ of a nerve fiber.

Electrical Threshold of a Nerve Fiber This threshold is the level of membrane potential at which an action potential is generated. The level is called the threshold membrane potential. To drive a nerve fiber to its threshold, the current must pass from the axoplasmic side to the extracellular side through the axoplasmic membrane. Suppose that a bipolar stimulating electrode is used to measure the threshold of a nerve fiber. The stimulating current flows across the axoplasmic membrane into the axon in the vicinity of the positive electrode, then flows along the axoplasmic core, eventually flowing out through the axoplasmic membrane near the negative electrode in the extracellular fluid. The axons into which a current can enter easily are the most excitable ones, *i.e.*, they have the lower threshold. In general, the larger the diameter of the axon, the lower the axial resistance to the flow of longitudinal current because of the greater number of the intracellular charge carriers (ions) per unit length of the axon. Therefore, a greater fraction of total current enters the larger axon, so it is depolarized more efficiently than a smaller axon. For these reasons, larger axons are recruited at lower densities of current, *i.e.*, they have a lower threshold for stimulating current and conduct the action current faster than smaller ones.

Time Constant (τ_m) and Length Constant (λ) of a Nerve Fiber The myelin sheath of the vertebrates nerve axons consists of compactly fused cytoplasmic membranes of a glial cell tightly surrounding the axon with a gap about 20 nm in width. The number of myelin membranes layered concentrically in a period of about 10 nm ranges between a small number of 10 or 20 to a maximum of about 160, which means there can be as many as about 320 membranes piled in a series between the axoplasmic membrane and the extracellular fluid. Thus, the membrane resistance (r_m) of a nerve axon is increased by a corresponding factor, and the axonal membrane capacitance (c_m) is reduced to the same extent. Both parameters contribute to the increase in conduction velocity of nerve fibers.

It was established that if the time constant ($\tau_m = r_m c_m$) of a membrane is smaller, the excitable membrane of the nerve fiber will be depolarized to the threshold more quickly, *i.e.*, its threshold will be lower, and the conduction velocity will be higher. If the length constant [$\lambda = (r_m/r_i)^{1/2}$] of a nerve fiber is larger, the depolarizing current will spread for a longer distance ahead of the active region, and the conduction velocity will be higher.

Further, the myelin sheath of the nerve fiber of vertebrates is periodically interrupted by nodes of Ranvier, which cause the nerve impulse to be conducted in a saltatory manner, and the conduction velocity of the myelinated fibers is greatly increased.

10.3 Mechanisms for Increasing the Conduction Velocity of Nerve Fibers

From the aforementioned factors, two rules of nature for increasing the conduction velocity of nerve fibers were formulated in neurobiology.

Enlargement of Axon Diameter According to the experimental results, the electrical resistance of the axoplasm (r_i) decreases in proportion to the square of the axon diameter, and the electrical capacitance (c_m) of axon increases in direct proportion to the fiber diameter. The net effect of an increase in fiber diameter is a decrease in membrane time constant and is an increase in conduction velocity. For these reasons, larger axons are recruited at low values of current, whereas smaller axons are recruited at relatively greater current densities. This mechanism has been especially observed in the nerve axons of invertebrates, and reached an extreme in the giant axon of the squid, which realized a diameter of 1 mm.

Myelination of Axon Because the myelin sheath greatly increases the effective thickness of the axonal wall, the external electrical resistance of the local action current totally increases. At the same time the myelin sheath reduces the total electrical capacitance of the axonal membrane. Thus, myelination of the axon results in a great decrease in $r_i c_m$ and an increase in range of the local current spread.

The conduction velocity in the myelinated fibers of vertebrates is also greatly increased by the jump of impulse from node to node in the saltatory manner. For these reasons, a nerve impulse conducted in the myelinated axons is much faster than that in the unmyelinated axons of the same diameter. Exactly, the conduction velocity in the myelinated fibers is proportional to the fiber diameter, while in the unmyelinated fibers it is proportional to the root of the fiber diameter. Thus, differences in energy and space requirements between the two types of mechanism are exaggerated at higher conduction velocities. It is obvious that the myelination of the nerve axon facilitates impulse conduction while conserving both energy and space.

10.4 Two Strategies for Increasing the Conduction Velocity of Nerve Fibers Have Evolved in the Nervous System of Living Organisms

The two mechanisms for increasing the conduction velocity of nerve fibers are fully confirmed by experimental data on the conduction velocity of different kind of nerve fibers of both vertebrates and invertebrates. Some experimental data are cited from a common book of comparative physiology (Table 10.1).

Data on the velocities of nerve conduction in animals from lower to higher species not only are coincident with the aforementioned two mechanisms for increasing conduction velocity, but also give us occasion to suppose that conduction velocity of nerve fibers, whether in unmyelinated or in myelinated fibers, progressed with the development of animals during evolution. Thus, the two mechanisms are also described as strategies of increasing the conduction velocity in living organisms in texts and reference books on neurobiology.

Table 10.1 Relationship between conduction velocity and the diameter of nerve fibers

	Nerve fibers	Diameter (μm)	Conduction velocity (m/s)
Crab	U	4	0.1–0.5
	U	10–20	2–4
Squid	U	10–20	3–6
	U	400–600	15–20
Earthworm	(loose sheath)	60–80	17–25
Frog	U (C fiber)	0.2–0.3	0.5–0.7
	U (B fiber)	1.9–2.6	4–5.4
	M (A fiber)	20	42
Dog	U (C fiber)	0.3	1.5
	M (B fiber)	3.8	20.0
	M (A fiber)	13.9	73.2

U, unmyelinated fiber; *M*, myelinated fiber

10.5 New Mechanism or Strategy for Increasing the Conduction Velocity of Nerve Fibers Found in the Myelinated Fibers of the *Penaeus* Shrimp

In the nervous system of the *Penaeus* shrimp, evolution occurred not only for myelinated fibers among invertebrates in particular, but also for a new mechanism of nerve activity in general. As a result of analysis on the full-sized positive action potential recorded from both the axon and the submyelinic space, it was concluded that the main function of the submyelinic space is to increase the conduction velocity of nerve impulses by greatly decreasing the electrical resistance (r_i) of the axoplasmic equivalent to the local action current flow. The novel mechanism of the nerve fibers for increasing conduction velocity was probably evolved in response to the marine environment.

Tightly Sealed Wide Extracellular Space in the Nerve Fibers The wide gap space between the myelin sheath and the microtubular sheath of the axon extends along the whole length of the nerve fibers of the *Penaeus* shrimp. It is a tightly sealed cylindrical space. The axon runs freely throughout the space except for each paranodal region, where the myelinated and the microtubular sheaths fuse together and form a window to expose the nodal axoplasmic membrane, and in a few places it is slightly attached to the myelin sheath. Thus, the gap-gel in the submyelinic space and axoplasm in the axon are two concentric cylindrical spaces separated by a cylindrical axoplasmic membrane with a thin microtubular sheath.

Low Resistance and High Capacitance of the Axoplasmic Membrane It is evident that the resistance of the cylindrical axonal membrane of the *Penaeus* nerve fibers, even together with the microtubular sheath, is very low, and the capacitance of that, quite oppositely, is very high in comparison with those of the myelin sheath, respec-

Table 10.2 Comparison of specific resistance of the gap-gel (r_g) with that of axoplasm (r_i) of some marine invertebrate animals

Specific resistance (Ω/cm)			
Penaeus japonicus	r_g	23	Kusano (1966)
Squid	r_i	30	Cole and Hodgkin (1939)
Lobster	r_i	60	Hodgkin and Rushton (1946)
Frog	r_i	110	Hodgkin (1964)

tively. Actually, the myelin sheath is several hundred times as thick as that of the axoplasmic membrane. Consequently, the differences in resistance and capacitance between the axoplasmic membrane and the myelin sheath should fall in the same order, respectively.

As it was already mentioned, the internodal portion of the axoplasmic membrane of the myelinated fibers of the *Penaeus* shrimp is not excitable, but it generates the resting membrane potential equally to the nodal axoplasmic membrane. Furthermore, the non-gated K channel, which is freely permeable to K ions, was shown to be responsible for the generation of the resting membrane potential across the internodal portion of the axoplasmic membrane (Xu and Terakawa 1991).

Low Resistances of Both the Gap-Gel and Axoplasm Measurement of the specific resistance of the gap-gel was carried out in the giant fiber preparation of *P. japonicus* by Kusano (1966). However, the axoplasmic resistance of the myelinated nerve fibers of the *Penaeus* shrimp has not yet been determined. The electrical parameters of some marine invertebrate animals are cited from the literature in Table 10.2 as reference.

It could be surely supposed that the resistances of both the gap-gel and axoplasm of the nerve fibers of the *Penaeus* shrimp are of the same order. Thus, the gap-gel and axoplasm separated by the double-layered axon membrane are two coaxial low-resistance pathways of the action current in the nerve fibers of the *Penaeus* shrimp.

Similarity Between Ionic Composition of the Gap-Gel and Seawater No significant potential was recorded in a DC mode from the submyelinic space of the giant fiber of both *P. chinensis* and *P. japonicus* when the reference electrode was immersed in the external medium. Moreover, the resting membrane potential of the axon was not affected by making a hole in the myelin sheath (Xu and Terakawa 1991). These facts show that the ionic compositions in the gap-gel and in the external medium (artificial seawater) are similar, and at least the $[K^+]$ in the submyelinic space should be equal to that in seawater.

Mechanism of the Positive Monophasic Action Potentials Recorded from Both the Axon and the Submyelinic Space A resting membrane potential of about −65 mV was recorded from the axon of the giant fibers of *P. japonicus*. Normal-sized and similarly shaped positive monophasic potentials were recorded not only from the axon but also from the submyelinic space of the myelinated giant fibers of the shrimp. The difference in amplitude of the two action potentials was about 10 mV (Fig. 10.1).

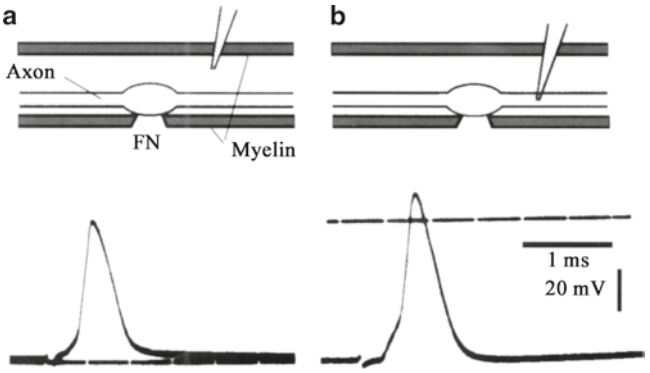

Fig. 10.1 Resting and action potentials recorded from submyelinic space (**a**) and axon (**b**) of motor giant fiber preparation of *P. japonicus*. *FN*, fenestration node. *Dashed lines* in (**b**) indicate potential level of 0. (From Xu (Hsu) and Terakawa 1993)

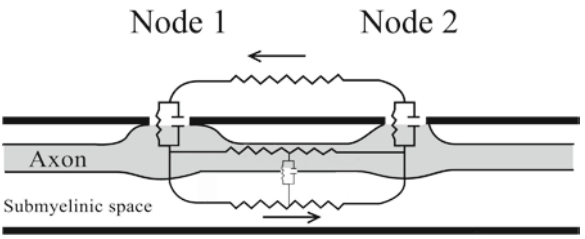

Fig. 10.2 Equivalent circuit of loop current between two fenestration nodes of myelinated nerve fiber of *Penaeus* shrimp. (Modified from Hsu and Terakawa 1996)

This fact showed that reversal of the resting membrane potential does not occur at the internodal portion of the axoplasmic membrane during impulse conduction. Otherwise, if excitation continuously occurs through the submyelinic space under the myelin sheath as a tunnel, the recorded potential would be biphasic.

From these experimental data, an equivalent circuit of the action current flow along the myelinated nerve fibers of the *Penaeus* shrimp was made (Fig. 10.2). Suppose the action current, which is generated at fenestration node 1, enters the axon. It flows through two parallel pathways, the axon and the submyelinic space, both leading to fenestration node 2. There the external current flows back to fenestration node 1 to close the local circuit through the external medium. According to the circuit, the positive monophasic action potential is conducted along the axon and spread into the submyelinic space, while at the same time it is passively influenced by the activity of the non-gated K channel in the axoplasmic membrane. Thus, the positive monophasic potential change of the axoplasmic membrane at the fenestration node is coupled to the potential in the submyelinic space with only a small difference in the amplitudes of the intra-axonal and intragap potentials caused by the electrical resistance of the non-gated K channels in the axoplasmic membrane.

A simulation test on the equivalent circuit showed that the rising phases of the spike of the two potentials fully coincided, but the their initial parts were obviously distorted (note: the simulation curve was kindly made by Prof. P.J. Liang). The results indicated that the capacitance of the axoplasmic membrane is large enough for inductive coupling of the quick potential change to the submyelinic space, but the slowly changing local potential should be distorted by the large capacitance of the axoplasmic membrane.

It could be concluded that the wide gap between the myelin sheath and the axon of the myelinated nerve fiber of the *Penaeus* shrimp was developed to increase conduction velocity, instead of the enlargement of the axon diameter, as is the case especially in other invertebrates (Terakawa and Hsu 1991; Hsu and Terakawa 1996).

10.6 Overall View of Three Mechanisms or Strategies for Increasing the Conduction Velocity of Nerve Fibers

Two strategies for increasing the conduction velocity of nerve fibers were established: the first is to increase the diameter of the axon core, and the second is the myelination of the axon. Examples of the former are observed in vertebrates, and quite commonly in invertebrates. The latter was concluded to be a result of development in the vertebrate branch of the evolutionary tree. Compared with the first strategy, the second greatly saves both energy and space. An example of the advantage of myelination is obtained by comparing two different kinds of nerve fibers, both of which conduct at 25 m/s at 20°C. A 500-μm-diameter unmyelinated giant axon of the squid requires 5,000 times as much as energy and occupies about 1,500 times as much space as a 12-μm-diameter myelinated fiber in the frog (see Siegel et al. 1994, p. 118).

Now, the third strategy for increasing conduction velocity is to widen the gap space between the myelin sheath and axon in the nerve fibers of the *Penaeus* shrimp. The submyelinic gap space is filled with a gel substance of low electrical resistance. Emergence of the gap space is functionally equivalent to an increase in diameter of axon core and therefore contributes to the speeding up of the nerve impulse. From an evolutionary point of view, the third strategy is ranked in the middle of two other strategies; that is, the advantage of this strategy is that energy is saved, but space is not.

In a word, a total of three strategies for increasing the conduction velocity of nerve fibers have evolved in living organisms.

Chapter 11
Ultrahigh Conduction Velocity Found in the Medial Giant Fibers of *Penaeus chinensis* and *Penaeus japonicus*

Erlanger and Gasser (1937) established that thicker myelinated fibers have higher conduction velocity. Among these, the highest conduction velocity was usually described to be 120 m/s of A-type mammalian myelinated fibers in texts and common reference books of neurobiology.

11.1 Conduction Velocities of 80–210 m/s in Giant Fibers of *P. chinensis* and *P. japonicus*

It was surprisingly observed that a pair of the giant nerve fibers in the ventral nerve cord of *P. chinensis* conducts excitation at an extremely high speed. Fan et al. (1961) accurately measured the conduction velocity of medial giant fiber preparations made from the ventral nerve cord of *P. chinensis*. They evoked an action potential in the nerve fiber preparation and recorded it by changing the distance between stimulating and recording electrodes three times. Then, the conduction velocity was obtained by the distance between two pairs of electrodes divided by the corresponding time difference in the recorded action potentials. The result showed that conduction velocity varied in the range of 80–200 m/s. The upper limit of the conduction velocity of the shrimp is unbelievably higher than 120 m/s, the highest conduction velocity of nerve fibers described in common texts of neurobiology.

To confirm the ultrahigh conduction velocity, the experimental result was further checked by a very simple method. After removing all fibers at the point of the root, the ventral nerve cord of the shrimp and a sciatic nerve trunk preparation of toad were placed side by side on the same stimulating and recording electrodes in an experimental chamber. The conduction velocities of the two preparations were measured at the same time (Fig. 11.1).

In Fig. 11.1, the first spike is the action potential of the single medial giant fiber of *P. chinensis* and the second spike is the compound action potential of the sciatic nerve trunk of toad. It is undoubtedly shown that the conduction velocity of the

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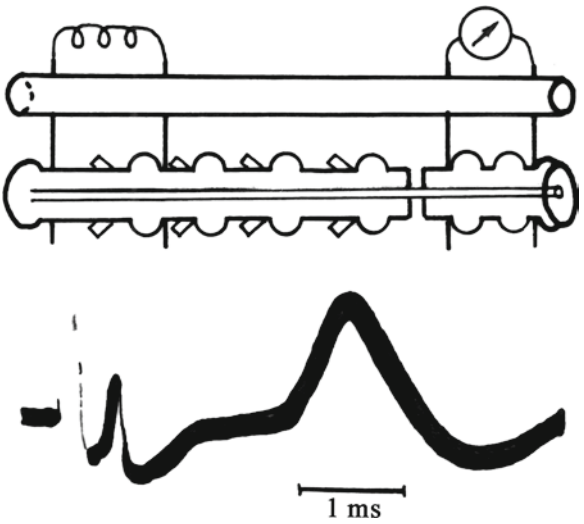

Fig. 11.1 Simultaneous recording of action potential of medial giant fiber in ventral nerve cord preparation of *P. chinensis* and compound action potential of sciatic nerve trunk preparation of toad. *Top*: Experimental scheme. *Bottom*: Two action potentials as recorded (see text). (From Xu, unpublished data)

shrimp giant fiber was notably higher than that of the compound action potential of the toad sciatic nerve trunk. In this experiment the conduction velocities of the two kinds of nerve fiber preparations were 130 and 25 m/s, respectively.

Later on, it coincidentally happened that the conduction velocity of the same named giant fiber of *P. japonicus* was reported to be 90–210 m/s by Kusano (1966). Thus, the wide dispersion of data obtained on the conduction velocity of the medial giant fiber of the shrimps was considered to be real experimental fact.

Since then, reports on the strangely high conduction velocity of the shrimp giant fibers had attracted the attention of many neuroscientists, but at the same time, some uncertainties naturally remained in the literature. For example, the conduction velocity of the giant fiber of *P. japonicus* was cited as more than 90 m/s (see Roots 1984, p. 21). The reason for the wide variation of conduction velocity measured and the factors determining the upper limit, *i.e.*, the conduction velocity above 200 m/s of the medial giant fibers of *P. chinensis* and *P. japonicus*, awaited clarification.

11.2 Analysis of the Wide Dispersion in Conduction Velocity of the Medial Giant Fiber of *P. chinensis* and *P. japonicus*

It has been mentioned that the body length of females is much greater than that of males in both *P. chinensis* and *P. japonicus.* On the other hand, the body lengths of both females and males of *P. chinensis* are slightly less than those of *P. japonicus*,

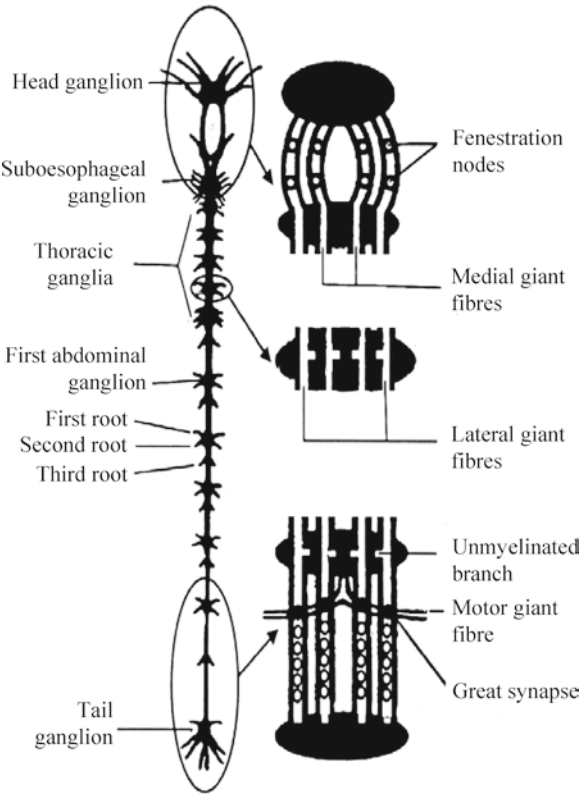

Fig. 11.2 Anatomical diagram shows nodal structures distributed in medial and lateral giant fibers of *P. japonicus*. Three *enlarged drawings* show arrangement of fenestration nodes (*open circles*), giant synapses (*filled circles*), and unmyelinated intraganglionic branches of giant fibers. (From Xu (Hsu) and Terakawa 1999)

respectively. The average body lengths of the two species of *Penaeus* shrimp were measured during the experiments as follows:

	Body length (cm)	
	Male	Female
P. chinensis	13	23
P. japonicus	15	25

To analyze whether the wide dispersion in the obtained data on the conduction velocity of medial giant fibers is related to shrimp body length, the number of the nodal structures distributed at the whole length of medial and lateral giant fibers of both *P. chinensis* and *P. japonicus* were counted. The distribution of the nodal structures, including the FN (fenestration node) and two kinds of functional node (unmyelinated branch in ganglion and giant synapse), of the medial and lateral giant fibers is shown in Fig. 11.2.

The nodes in the entire length of the giant fibers of both female and male from the two species of shrimp were counted, and the number was shown to be constant

in all cases except for the circumesophageal segment, which had two or three nodes. The number of nodes at each segment of the ventral nerve cord is as follows:

	Number of nodes
Circumesophageal	2 (3 in a few preparations)
Thoracic	5
Abdominal	9
Caudal	7
Total number	23 (or 24)

The total number of nodal structures is independent of shrimp body length; consequently, the ventral nerve cord preparation taken from shrimp of greater body length has a longer internodal distance. The lengths of the ventral nerve cord of female and male *P. chinensis* were measured to be about 20 and 10 cm, respectively, and their internodal distances were calculated to be about 8.7 and 4.3 mm on average. By assuming that the internodal conduction time for all internodal myelinated segments is similar, the wide dispersion of the conduction velocity of medial giant fibers are mainly ascribed to the difference in the internodal distance of the myelinated fibers.

11.3 Factors Determining the Ultrahigh Conduction Velocity of the Medial Giant Fibers of *P. chinensis* and *P. japonicus*

Several factors are involved in the mechanism of the ultrahigh conduction velocity of the medial giant fibers of *P. chinensis* and *P. japonicus*. Besides the heavy myelination of the axon, the giant fibers also possess several factors to accelerate their conduction velocity. The factors could be enumerated as follows.

The Thickest Myelin Sheath The thickness of the myelin sheath is usually dependent on the diameter of the shrimp nerve fibers. The diameter of a pair of the medial giant fibers is the largest among the myelinated fibers of the shrimp. Consequently, it has the thickest myelin sheath. For example, the myelin sheath of the giant fiber is as thick as about 15 μm when the giant fiber has a diameter of 150 μm. In contrast, the myelin sheath of the thicker myelinated fibers of vertebrates is only about 2 μm.

There is an optimal ratio between the axon and fiber diameters for maximizing impulse conduction. In vertebrate myelinated fibers, the optimal ratio was calculated to be 0.7 (see Hodgkin 1964). The diameter ratio of the submyelinic space cylinder to the host fiber in the shrimp was measured to be 0.69 ± 0.10 on average (mean ± SEM) ($n = 110$) (Hao and Xu 1965). Thus, the thickness of the myelin sheath could be considered as optimal for maximizing the conduction velocity of nerve fibers.

The Longest Internodal Distance The internodal distance of the medial giant fibers of female *P. chinensis* and *P. japonicus* is about 10 mm on average, whereas that of the peripheral myelinated fiber of vertebrates is about 2 mm.

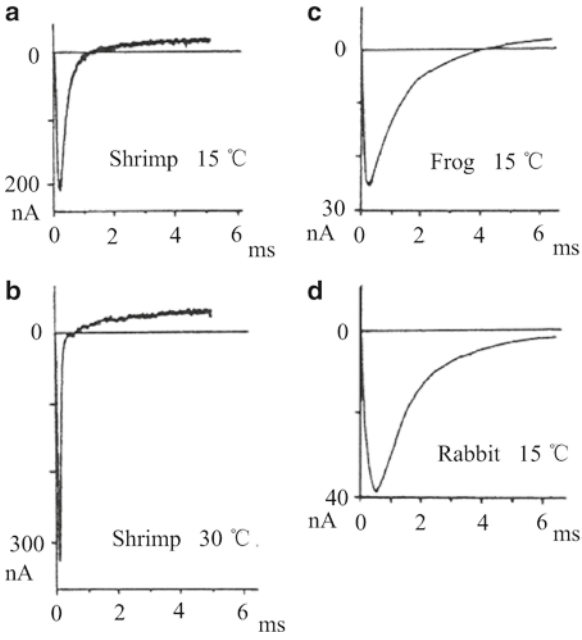

Fig. 11.3 Comparison of characteristics of Na current recorded from nodal region of medial giant fiber preparation of *P. japonicus* (**a**, **b**) with those of myelinated fibers of frog (**c**) and rabbit (**d**). The sucrose-gap voltage clamp method illustrated in Fig. 8.4 was used; temperature of solution varied as indicated. (From Terakawa and Xu 1987)

The Largest Submyelinic Space The medial giant fiber has the largest submyelinic space. For example, the greater diameter of the space (with the axon inside it) of the giant fiber reaches as much as 105 μm, which makes the fiber electrically equivalent to the same size of axon. It was described previously that the specific electrical resistances of the gap-gel and axoplasm of the giant fiber of the *Penaeus* shrimp are equally low.

Fast Action Current The time from onset to the peak of Na current recorded from the FN of the medial giant fibers of *P. japonicus* determined by voltage clamp technique is about 100 μs in the fastest case, whereas those of nodes of Ranvier of the myelinated nerve fibers of frog and rabbit were 150 and 180 μs, respectively (Terakawa and Hsu 1991). The dynamics of the Na current of the myelinated nerve fibers of frog and rabbit, as well as shrimp (*P. japonicus*), are shown in Fig. 11.3.

All these factors are favorable for fast nerve impulse conduction in the medial giant fiber of *P. japonicus* and *P. chinensis.*

11.4 Overall View of the Factors Contributing to the Highest Conduction Velocity of Nerve Fibers

1. The highest conduction velocity of nerve fibers had been commonly considered to be about 120 m/s, as reported in reference books of neurobiology, but recently it was found that a pair of the medial giant fibers of *P. chinensis* and *P. japonicus* conducts nerve impulses at velocities of 80–200 and 90–210 m/s, respectively.

2. The wide dispersion in the conduction velocity of the nerve fibers of the *Penaeus* shrimp was clarified to be mainly the result of the difference in the internodal distances of its medial giant fiber. The number of nodal structures in the giant fibers of *P. chinensis* and *P. japonicus* is nearly fixed. Thus, the longer medial giant fiber preparation has the longer internodal distance and the higher conduction velocity.
3. The thickest myelin sheath, the largest internodal distance, and the widest submyelinic space of the myelinated giant fiber are the main factors for the ultrahigh conduction velocity of the largest medial giant fibers of *P. chinensis* and *P. japonicus*. Moreover, the most rapid action current of the myelinated fiber of the shrimps is also a factor to increase conduction velocity.
4. The highest conduction velocity in the medial giant fiber of the *Penaeus* shrimp does not imply that the efficiency of movement in the shrimp is as high as that of the warm-blooded vertebrates. Theoretically, the velocity–diameter relationship for myelinated fibers established by Hursh (1939) predicts that a mammalian myelinated fiber would have a conduction velocity of 600 m/s at 37°C, if the diameter of the fiber had reached 100 μm (Hsu and Terakawa 1996).

Chapter 12
Remarks on the Evolution of Myelin Sheath, Glial Cells, Nodal Structure, and Conduction Function of Nerve Fibers

The findings of specific structures of the nerve fibers in the nervous system of the *Penaeus* shrimp in invertebrates give us occasion to make some suggestions about the phylogenetic development of the myelin sheath, nodal structure, and impulse conduction function of nerve fibers in neurobiology.

12.1 Evolution of the Myelin Sheath

The naked axon is common in the nerve tissues of the coelenterates, but the axons of all other phyla are mostly surrounded by some kind of glial sheath. The complexity of the glial sheath varies from a simple layer to the loose overlapping laminar sheath and the compact concentric multilayered sheath. It was considered that the loose myelin sheaths evolved independently in annelids, crustaceans, and chordates, and the most developed compact myelin sheath was concluded to have evolved only in vertebrates (Roots 1995).

Nerve axons coated with the compact myelin sheath, which is comparable to that of vertebrates in both structural and functional aspects, were recently found in the nervous system of the *Penaeus* shrimp. The compact myelin sheaths of both vertebrates and invertebrates should have evolved in parallel in their nervous systems, judging by the different types of formation of the fine structures in the two kinds of myelin sheath (Xu (Hsu) and Terakawa 1999) and the different chemical compositions of the vertebrate and invertebrate myelin.

12.2 Evolution of Glial Cells in Nerve Fibers

In vertebrates, two kinds of glial cells, such as the Schwann cell and oligodendrocyte, form the myelin sheath for the peripheral and central nerve axons, respectively. A Schwann cell supplies an internodal segment of myelin sheath, whereas an

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oligodendrocyte supplies that for several axons. Similar to the oligodendrocyte, a myelin-forming glial cell of the *Penaeus* shrimp supplies the myelin sheath for many axons. This fact seems to indicate that the oligodendrocyte-type glial cells occur earlier in phylogeny than does the Schwann cell, which could be considered as a more-developed glial cell.

In vertebrates, besides the myelin-forming cell such as the Schwann cell and oligodendrocyte, there is also a non-sheath-forming glial cell that may wrap several small axons generally lying in separate surface troughs (as shown in Fig. 3.8b). In the *Penaeus* shrimp, besides this kind of non-sheath-forming glial cell, there is a unique microtubule-rich glial cell that forms a thin microtubular sheath tightly surrounding the axon for its structural and metabolic support.

12.3 Evolution of Nodal Structures

Since 1875, the Ranvier node had been considered as a solitary structure for more than 100 years until its counterpart was found in the nervous system of the *Penaeus* shrimp of the invertebrates (Hsu and Terakawa 1996). The two kinds of node are different in their fine structure formation, indicating that they should also have independently evolved in the nervous system of vertebrates and invertebrates.

A few unmyelinated spot openings were found in an individual giant fiber with the loose myelin sheath of the earthworm (Günther 1973). This rare case obviously has a structural similarity with the more complicated and widely distributed fenestration node, an unmyelinated window, which was found in the compact myelin sheath of nerve fibers of the *Penaeus* shrimp. The resemblance of the structures seems to indicate that the two kinds of nodal structure are homologously generated in phylogeny; that is, in evolution, the spot opening found in the earthworm could be a prototype of the fenestration node found in the *Penaeus* shrimp.

It was first established in the myelinated nerve fibers of vertebrates that the Na channels are concentrated in the nodal axoplasmic membrane and the K channels are excluded from it. Recently, the same fact was established in both kinds of functional node and fenestration node of the myelinated fibers of the *Penaeus* shrimp (Terakawa and Hsu 1991; Hsu and Terakawa 1996). These facts showed that rearrangement of the ion channels in nodal and internodal axoplasmic membranes proceeded independently in evolution of the myelinated fibers of both vertebrates and invertebrates. Indeed, the rearrangement of the ion channels is a common step of evolution to reach the nodal structures.

Fixed numbers of nodal structures are distributed at the medial and the lateral giant fiber of the *Penaeus* shrimp. Consequently, the internodal distances of the giant fibers are proportional to the length of the ventral nerve cord of the animal. The number of fenestration nodes in the middle- and small-sized myelinated fibers could also be constant, but in fact, the larger the total number of nodes, the larger the fiber diameter. Thus, the growth of the fiber in length during body development of the animals should be mainly dependent upon the lengthening of the internodal distance.

In the literature, it was reported that the internodal distance of the myelinated nerve fibers of some fishes obviously increases with the growth of the body. For example, in an eel (*Conger*) the maximum internodal distance increases from 1.5 to 6.0 mm as the body length of the animal increases from 24 to 110 cm. In some other fishes, such as the adult torpedo, the internodal distance of the nerve fibers of the electric organ is within 1.0–1.5 mm (see Koshtoyants 1957). These facts showed that there should be at least two patterns to establish the node during myelination of nerve fibers in ontogeny: one is, first, to establish the numbers of the nodes during myelination, and the other is to form new nodes as myelination continues.

12.4 Evolution of the Conduction Function of Nerve Fibers

Continuous conduction of nerve impulses is found in the unmyelinated fibers of both vertebrates and invertebrates, and saltatory conduction is also found in the nerve fibers of vertebrates and invertebrates. The hypothesis of saltatory conduction in invertebrates was experimentally proven by using the myelinated giant fiber preparation of the *Penaeus* shrimp. Thus, saltatory conduction should be considered neurobiologically as a common principle of impulse conduction in all myelinated nerve fibers.

The compact multilayered sheath of nerve fibers had been considered as a prior condition of the occurrence of saltatory conduction. However, the median giant fiber of the earthworm conducts nerve impulses in a saltatory manner, despite that the fact that its sheath is loosely multilayered (Günther 1973). This observation shows that saltatory conduction as a function of nerve fibers seems to have occurred earlier than the appearance of the compact myelin sheath in phylogeny.

Enlargement of axon diameter and myelination of the axon were concluded to be two strategies for increasing the conduction velocity of nerve fibers. Now, a third strategy for increasing the conduction velocity of nerve fibers was found in the myelinated fibers of the *Penaeus* shrimp. The strategy is to widen the gap space between the axon and the myelin sheath instead of enlarging the axon itself. This strategy seems to have evolved particularly in marine invertebrate animals.

The nerve fibers of the *Penaeus* shrimp utilize the best of the strategies of both myelination and enlargement of the gap space between the axon and the myelin sheath for increasing conduction velocity. Moreover, the node of Ranvier in the myelinated nerve fibers of vertebrates and the fenestration node in those of the *Penaeus* shrimp are different in morphological structure. Consequently, the impulse conduction of nerve fibers should be accordingly divided into three types (see Fig. 1.3):

1. Continuous conduction of the unmyelinated nerve fibers in both invertebrates and vertebrates
2. Saltatory conduction of the myelinated fibers in vertebrates
3. Saltatory conduction of the myelinated fibers of the *Penaeus* shrimp in invertebrates

The medial giant fibers of large *P. chinensis* and *P. japonicus* are to conduct nerve impulses at a velocity as high as more than 200 m/s, which is the highest conduction velocity among all nerve fibers. However, the efficiency of the myelinated fibers of invertebrates may not be as high as the efficiency of those of warm-blooded vertebrates (Xu (Hsu) and Terakawa 1999).

Finally, it can be predicted that the record of conduction velocity of 200 m/s of a pair of medial giant fibers of *P. chinensis* and *P. japonicus* could be surpassed if the medial giant fiber preparation were taken from a *Penaeus* shrimp with a body length longer than about 30 cm.

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