李庆伟　刘 欣/著

中国七鳃鳗研究

Ⅰ 功能基因

Lampetra japonica
Research in China

科学出版社

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北京

内 容 简 介

在海洋生物中，七鳃鳗是现存脊椎动物门中最古老的物种，它印记了无脊椎动物的进化史，又为脊椎动物的起源与进化提供丰富的遗传信息基础。本书介绍了本课题组近几年在七鳃鳗功能基因和比较基因组研究方面的主要成果，包括七鳃鳗的形态解剖，肝脏和口腔腺组织的 cDNA 文库的构建及分析以及与抗炎、抗凝、抗肿瘤、抗心血管疾病相关的基因克隆，重组蛋白表达、纯化及生物学活性测定及作用机制研究，旨在为深入研究疾病的治疗和新药的研发提供理论基础。另外，本书所提供的研究方法也会为科研工作者及相关专业研究生提供有益的借鉴。

本书可供功能基因及比较基因组学研究相关的科研工作者、教师及相关专业研究生和本科生参考使用。

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序

随着国际人类基因组计划合作组织于2004年10月宣布成功绘制一张精度大于99%、误差小于十万分之一的人类基因组图谱，基因组学的研究热点开始由结构基因组学转向功能基因组学。目前在功能基因组学研究中，人们最关注的问题之一是发现和鉴定与人类重大疾病相关的基因，以及对一些具有重要生物学功能的基因进行克隆、功能鉴定、生物制药开发及临床应用。由此可见，功能基因是21世纪生物制药产业的基石、临床基因疗法技术开发的生长点和知识产权归属的制高点。因此，有效地挖掘和保护功能基因资源是极其重要的。

21世纪是海洋世纪，海洋生物资源的开发和利用已成为世界各海洋大国竞争的焦点之一，其中基因资源的研究、保护和利用更是各国争夺的焦点。美国、日本、英国、法国、俄罗斯等均投入巨资发展海洋药物研究及相关海洋生物技术，先后推出“海洋生物技术计划”、“海洋蓝宝石计划”、“海洋生物开发计划”等。世界上一些著名的大学也相继建立海洋药物研究机构。美国、日本和欧盟一些发达国家和组织近年来不断加强海洋药物研究的经费投入，逐渐走在了世界生物制药的前列。目前，世界各国已从海洋微生物及动植物中分离得到10 000多种新型化合物，其中有200余种化合物申请了世界专利保护。我国自“九五”以来设立了海洋“863”计划，海洋生物技术研发迅速发展、成绩斐然，克隆了一大批重要功能基因，其中包括潜在的药用基因、工业用酶基因、抗逆性基因、抗病基因、免疫相关基因，以及与动植物生长繁殖有关的基因。

李庆伟教授的研究团队在2004年率先启动对列入《中国濒危动物红皮书》的无颌类脊椎动物——日本七鳃鳗（*Lampetra japonica*）的全面研究，获得了国家、部委和省市多项基金的资助。七鳃鳗属于七鳃鳗目、七鳃鳗科，是现存脊椎动物亚门中最古老的物种。由于其在进化上的特殊地位，七鳃鳗成为研究脊椎动物起源与进化的关键物种，在脊椎动物胚胎发育、各个器官分化的比较研究中有大量的文献报道。但对该物种功能基因展开如此广泛和具有深度的研究，该书作者的研究无疑填补了国内外的空白。只有短短几年，即见著作面世，颇感欣慰。纵观全书，深感成绩斐然，作者利用基因组学、蛋白质组学、比较基因组学、生物信息学的技术与方法以及高效能克隆表达技术，开展了高通量七鳃鳗重要功能基因的筛选、鉴定、克隆、重组蛋白表达及生物学功能研究，筛选和鉴定了一批拥有自主知识产权的，与抗炎、抗凝、抗病毒、抗肿瘤、抗心血管疾病相关的药用蛋白基因以及免疫相关基因。该书为国内首部有关无颌类脊椎动物功能基因方面研究的专著，其研究方法和研究成果可为从事分子生物学、生物化学、生物信息学及生物制药研究领域的科研工作者及研究生所借鉴。相信该书的出版，确能给我

国未来海洋生物资源的保护与开发以及海洋生物功能基因的发现与应用带来极大的推动作用。愿借此序，为之一赞。

中国科学院院士

2010年10月28日

Foreword

In October 2004, as The Human Genome Organisation announced that a complete human genome map with precision greater than 99%, error less than 10 parts per million has been successfully drawn, Focus on genomics research began to shift from structural genomics to functional genomics research. At present, one of the most concerns of functional genomics is to discover and identify major disease-related human genes, and to clone and reveal their important biological functions for bio-pharmaceutical development and clinical application. Thus, functional genomics is the cornerstone of biological pharmaceutical industry in the 21st century, is the growing point of clinical gene therapy technology development and the commanding heights of the intellectual property ownership. Therefore, the effective protection and mining of genetic resources are extremely important.

21st century is the century of ocean, the marine resources development and utilization have become a rivalry focus of the great oceanic countries in the world, in which the genetic resources research, protection and utilization are the focal point of all countries. The United States, Japan, Britain, France, Russia and other countries have invested heavily in drug research and development of marine technology related to marine life, have launched the "marine biotechnology plan", "Ocean Sapphire plans", "Marine Biological Development Scheme" separately. Some of the world renowned universities have also established marine drug research institutions. The United States, Japan and some developed countries of the European Union continue to strengthen the expense of marine drug research, and gradually walk in the forefront of the world's biopharmaceutics in recent years. Currently, in the world more than 10 000 kinds of new compounds were isolated from the marine microbes, plants and animals, of which more than 200 compounds have been applied for international patent protection. In China, marine "863" plan has been established since the "Ninth Five-Year", marine biotechnology research has been developing rapidly with great successes, a large number of important functional genes were cloned, including the potential pharmaceutical genes, industrial enzyme gene, adversity resistance gene , disease resistance gene, immune-related genes as well as genes involved in growth and reproduction of plants and animals.

The research team of Professor Li Qingwei first started full researches on a jawless vertebrate-Japanese lamprey (*Lethenteron japonicum*) in 2004, which is in the inclusion of "China Red Data Book of Endangered Animals". The researches have been supported

by a number of funds from nation, ministries and provinces. Lampreys which has belonged to order Petromyzontiformes and family Petromyzontidae, are the most ancient species of extant vertebrate subphylum. Because of its special position in evolution, lampreys become the key species for studying the origin and evolution of vertebrates; a large number of comparative studies were reported in vertebrate embryonic development and various organs differentiations. However, as for such a broad and in-depth research of the functional genes of the species, authors' studies will undoubtedly fill the gaps at home and abroad. Although only a few years, the works are published, I am quite pleased. Throughout the book, one might be deeply impressed that authors carried out high-throughput screening, cloning, recombinant protein expression and biological function identification of important lampreys functional genes by using of genomics, proteomics, comparative genomics, bioinformatics technology and methods, and efficient cloning and expression technologies. They have been screening and identifying of a group of anti-inflammatory, anticoagulant, anti-virus, anti-tumor, anti-cardiovascular disease-related genes and immune medicinal related genes with independent intellectual property rights. This is the first monograph about jawless vertebrates on aspects of functional gene research, and its research methods and results in molecular biology, biochemistry, bioinformatics and biopharmaceutics can be used by researchers and postgraduate students. I believe that the publication of this book do give a tremendous boost on protection and development of our future marine living resources and marine life as well as the discovery and application of functional genes. I would like to write this preface to pay a compliment to authors.

Zhang Yaping

Academician of Chinese Academy of Sciences

October 28, 2010

前　言

随着社会、经济的发展和人类活动的干预，海洋环境正在不断地恶化，海洋生物多样性正遭到破坏，海洋生物基因资源的保护和利用，显得更加紧迫。研究海洋生物基因组及功能基因，能深层次地探究海洋生命的奥秘；发掘海洋生物基因，有利于保护海洋生物资源；从海洋生物的功能基因入手，有助于开发具有我国自主知识产权的海洋基因工程新药，部分解决海洋药源问题。

目前，“模式生物与人类疾病”研究在国际上刚刚起步，但在比较基因组学领域已有大量研究成果。通过比较小鼠的基因组发现了人类的肥胖基因。果蝇与人类基因组的比较，识别了548个与人类疾病同源的基因，并成功地建立了亨廷顿舞蹈病的果蝇模型。此外，斑马鱼、河豚、秀丽新小杆线虫也被用于人类疾病的研究。当前，科学家们正在进行“模式生物果蝇与心脏病”、“利用条件基因剔除技术研制人类疾病小鼠模型”、“人类自身免疫疾病的小鼠模型”、“斑马鱼模式生物体在人类基因功能研究中的应用”以及“模式生物乙型肝炎模型”等领域的研究。可见，利用模式生物研究人类疾病的发病机制、发现人类疾病相关基因以及建立人类疾病的动物遗传模型，具有重大的理论和实践意义。

在海洋生物中，七鳃鳗是迄今所知道的最原始的无颌脊椎动物，其最早的化石记录可以追溯到奥陶纪，与寒武纪晚期底栖的甲胄鱼类有共同的祖先；与人类、其他具有高度发达中枢神经系统的灵长类共同隶属脊椎动物亚门（Vertebrata）。七鳃鳗与盲鳗同属无颌纲（Agnatha），是约22种原始鱼形无颌脊椎动物的统称，均隶属于七鳃鳗科（Petromyzonidae）。2006年，南非金山大学和美国芝加哥大学的科学家们在南非东开普省格雷厄姆斯敦附近的沃特山岩石群中发现了一块有3.6亿年历史的七鳃鳗化石。这块迄今发现的最古老的鱼类化石显示，自七鳃鳗在3.6亿多年以前出现以来它的外观几乎没有发生变化，证明现代七鳃鳗是意义不同寻常的活化石，也是目前所知仅存的最原始的无颌脊椎动物。七鳃鳗是颌口类脊椎动物的近亲，与颌口类脊椎动物不同的是，它的体内缺乏活动的上下颌，终生保留脊索，无成形的脊椎骨和成对的附肢等。但是，七鳃鳗与颌口类脊椎动物又存在着一些共同的特征，如开始出现复杂的脑的分化，出现心脏及肝脏等。比较解剖学和胚胎学研究揭示，所有的脊索动物在其个体发育的一定阶段或终生均具有共同的三大主要特征——背神经管、脊索和咽鳃裂，这些清楚地表明该门动物起源于一个共同的祖先，七鳃鳗是现存脊椎动物亚门中最古老的物种。因此，七鳃鳗不仅是研究脊椎动物起源与进化的关键物种，同时也是研究脊椎动物胚胎发育、器官分化的最佳模型。

由于七鳃鳗在进化上所处的重要地位，2004年8月4日，美国国家人类基因组研究所在英国 *Nature* 杂志网站上宣布将绘制七鳃鳗全基因组图谱，用于与人类基因组进

行比较研究，进一步探索生物进化史。特别是2004年*Nature*报道，科学家对七鳃鳗所含有的脊椎动物免疫系统的原始元素所做的新的搜索工作，在与脊椎动物淋巴细胞类似的细胞上发现了新型可变淋巴细胞受体，发现七鳃鳗是利用一种不寻常的基因重排过程来产生受体多样性。这一发现对了解人类免疫系统起源提供了关键线索。七鳃鳗是联系无脊椎动物与脊椎动物之间的重要阶元，从遗传信息基础来说，它必定印记了无脊椎动物的进化历史，同时作为脊椎动物最直接的祖先，又为脊椎动物的起源与进化提供丰富的遗传信息基础。总之，深入开展以七鳃鳗为基础的遗传发育研究，对于理解和揭示脊椎动物的起源和进化具有重要的意义。另外，以七鳃鳗的基因组研究为基础，通过与人以及其他脊椎动物基因组的比较研究，有可能发现重要的人类疾病相关基因，揭示人类疾病的发病机制，从而建立人类疾病的七鳃鳗动物遗传模型，为深入研究疾病发病机制以及为疾病的治疗和新药的研发提供理论基础。

日本七鳃鳗所具有的特殊生活方式，又赋予它诸多特有的功能蛋白基因。虽然国内外对七鳃鳗的营养价值进行了研究，而利用生物技术对其基因及蛋白质进行药物开发几乎没有报道。为此，近几年我们在国家“863”计划项目“七鳃鳗药用相关基因的筛选、鉴定与功能研究”（2007AA09Z428）、“973”计划项目“原始免疫重排机制和形成机制及比较免疫学研究”（2007CB815802）、国家自然科学基金项目“七鳃鳗口腔腺分泌PR-1蛋白-L251中性粒细胞抑制活性鉴定”（No. 30470936）以及辽宁省科技厅科研基金、辽宁省教育厅创新团队计划项目和大连市科技局科技攻关项目的共同资助下，已完成了日本七鳃鳗肝脏、类淋巴细胞和口腔腺组织的cDNA文库的构建，共得到肝脏组织的10 077条EST序列，类淋巴细胞的10 500条EST序列和口腔腺组织1281条EST序列，并对其进行了初步的生物信息学分析，发现了一些与抗炎、抗凝、抗病毒、抗肿瘤、抗心血管疾病相关的目标蛋白基因以及免疫相关的基因。并对3个含RGD/KGD模体的类去整合素、视黄酸与干扰素诱导的致死蛋白-19（类GRINM-19蛋白，GRIMIN）、翻译控制肿瘤蛋白（translationally controlled tumor protein，TCTP）、类gelsolin蛋白、类sorcin蛋白、具SCP结构域的类神经毒素以及L251蛋白等的蛋白质表达、纯化及重组蛋白的生物学活性测定、作用机制及功能展开了深入的研究。

本书是我们近几年在功能基因组比较研究以及有效地挖掘、保护和开发海洋生物重要功能基因资源，开发功能蛋白生物制药领域的研究成果总结。相信本书的出版会对从事该领域研究工作的同行有所帮助，对我国海洋生物制药的发展具有一定推动作用。在本书的撰写过程中，得到同事王继红、吴毓，以及博士、硕士研究生白洁、高琪、韩晓曦、刘岑洁、麻晓庆、孙晶、孙婴宁、薛壮、于水艳、张楠楠、张丕桥、周丽伟、朱丽娜等的大力协助，他们在各自的研究中做了大量深入的工作，谨致以深切谢意！对科技部、自然科学基金委多年的大力支持表示衷心的感谢！最后感谢科学出版社在本书出版方面给予的热情支持！

李庆伟

2010年春于辽宁师范大学

Preface

With the social and economic development and intervention of human activities, the marine environment is continually deteriorating, marine biological diversity is being destroyed, marine genetic resources conservation and use is even more urgent. Studying marine genomics and functional genomics could help to explore deeply in the mysteries of marine life, exploring marine life genes could help to protect marine biological resources, investigating functional gene of the marine life could help to develop Chinese own intellectual property rights of new marine genetic engineering drugs, a partial solution to the problem of marine drug resource.

Currently, the "Model Organisms and Human Disease" study, in recent years has just started in the international arena, but in the field of comparative genomics there have been a lot of research results. By comparing the mouse genome, human obesity gene was found. The comparison of *Drosophila* and human genome helped to identify 548 genes homologous to human disease, and successfully establish a *Drosophila* model of Huntington's disease. In addition, zebra fish, puffer fish, *C. elegans* have also been used to study human diseases. Currently, scientists are carrying out "Model Organism *Drosophila melanogaster* and Heart Disease", "Use of Conditional Gene Knockout Technique to Develop Mouse Models of Human Disease", "Human Autoimmune Disease Mouse Model", "Zebra Fish Model Organism in the Human Genome Function of Application " and " Model Organisms Hepatitis B Model " and other areas. It shows that the use of model organisms of human disease pathogenesis, disease-related genes in humans and the creation of animal genetic model of human disease, are of great theoretical and practical significance.

In the marine species, lampreys (*Petromyzon*) is by far the most primitive known jawless vertebrates, their fossil record dates back to the earliest Ordovician and late Cambrian, sharing common ancestors with ostracoderms. Lampreys are classified in *Vertebrata* together with highly developed human and other primate with central nervous system. Lamprey and hagfish belong to Agnatha, 22 species lamprey are collectively referred to primitive fish shaped jawless vertebrates, and all are Petromyzonidae. In 2006, scientists of University of the Witwatersrand (South Africa) and the University of Chicago found a 3. 6 billion-year history of lamprey fossil in South Africa Eastern Cape near Grahamstown Walter Hill rock group. This piece of the oldest fish fossils ever found shows its appearance has almost not changed since lampreys occur in 360 million years ago. It proved that modern lampreys are remarkable living fossils, and is cur-

rently the only remaining known the most primitive vertebrates without jaws. Lamprey is a close relative of jawed vertebrates, it is different from jaw vertebrates in its lack of activity of the upper and lower body, a lifetime notochord, without forming the spine and paired appendage. However, lamprey and jawed vertebrates have some common characteristics: the emergence of complexity of brain and the differentiation of heart and liver. Comparative anatomy and embryology studies reveal that all vertebrate deve-lopment in a certain stage of their individual or common life exist the three main features—dorsal neural tube, notochord and pharyngeal gill slits, a clear indication that these animal origin a common ancestor. Lampreys are the oldest species in extant vertebrate Subphylum, therefore, lampreys are not only the key vertebrate species in studying the origin and evolution of vertebrates, but also the best model in studying vertebrate embryonic development and organ differentiation.

Because lampreys are important in evolution, in August 4, 2004, the U. S. National Human Genome Research Institute Web site announced that they will draw the lamprey genome-map in the *Nature*, for a comparative study of the human genome and further explore the history of biological evolution. Particularly, in 2004, *Nature* reported that, scientists have explored lamprey immune system, which contains the original elements of vertebrate and the new investigation have found the new variable lymphocyte receptor on similar cells with vertebrate lymphocyte cells, proved that lamprey is to use an unusual process of gene rearrangement to generate receptor diversity. This finding provides a key clue for understanding the origin of the human immune system. Lamprey is a critical link stages between invertebrates and vertebrates, from the basis of genetic information, it will mark the evolutionary history of invertebrates and at the same time be the most direct ancestor of vertebrates, but also provide a rich foundation of genetic information for the vertebrate origin and evolution. In short, the deep study of lamprey-based genetic development has important significance for understanding and revealing the origin and evolution of vertebrates. In addition, based on lamprey genome research, and comparison with human and other vertebrate genomes, we may find important human disease-related genes, reveal human disease pathogenesis, and thus establish genetic model of lamprey on human disease for the study of disease pathogenesis and treatment of disease and provide a theoretical basis for research and development of new drugs.

Japanese lamprey possesses the special way of life, but also many unique protein features. Though lampreys were studied on the nutritional value worldwide, there were almost no reports on the use of biotechnology on their gene and protein for drug development. For this reason, in recent years, supported by national "863" Project, "Identification of Lamprey Medicinal Related Genes and Function Study" (2007AA09Z428); "973" project, "The Mechanism of Original Immune Rearrangement and Formation and

Comparative Immunology" (2007CB815802); National Natural Science Foundation project , " Identification of Lamprey Oral Gland Secretion of PR-1 Protein-L251 Inhibition of Neutrophil" (No. 30470936) and the Research Fund for Science and Technology, Department of Liaoning Province; Innovation Team Programme projects, Liaoning Provincial Department of Education; scientific and technological project, Dalian Municipal Science and Technology Agency, we have completed the Japanese lamprey liver, type lymphocytes and oral gland cDNA library construction, and sequenced a total of 10, 077 liver EST sequences, 10, 500 lymphocytes EST sequences and 1281oral glands EST sequences. By preliminary bioinformatics analysis, we found a number of anti-inflammatory, anticoagulant, anti-virus, anti-tumor, anti-cardiovascular disease-related target gene and immune-related genes. And 3 integrins with RGD / KGD motif, retinoic acid and interferon-induced death protein -19 (Class GRINM-19 protein, GRIMIN), translationally controlled tumor protein (TCTP), type gelsolin protein, type sorcin protein, a SCP domain of neural protein toxins and L251 protein were studied by expression, purification and biological activity analysis of recombinant proteins, and the mechanisms and functions were carried out in-depth study.

This book is the our research summary recent years of functional genomics, effective mining, protection and exploration of the important functions of marine genetic resources, development of functional protein in the field of biopharmaceutical. I believe this book published will be help to peer research in the field, and has a greater impetus in the development of our marine bio-pharmaceutical. In the book writing process, colleagnes Wang Jihong, Wu Yu and postgraduates Bai Jie, Gao Qi, Han Xiaoxi, Cen Liujie, Ma Xiaoqing, Sun Jing, Sun Yingning, Xue Zhuang, Yan Shuiyan, Zhang Nannan, Zhang Piqiao, Zhou Liwei, Zhu Lina and others offered great assistance, they have done a lot in their research work in depth, I would like to extend my deepest gratitude! I would also express my sincere thanks to State Science and Technology, National Natural Science Foundation support for many years! Finally thanks to Science Press for the enthusiastic support in book publishing!

Li Qingwei

Liaoning Normal University, in the Spring of 2010

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