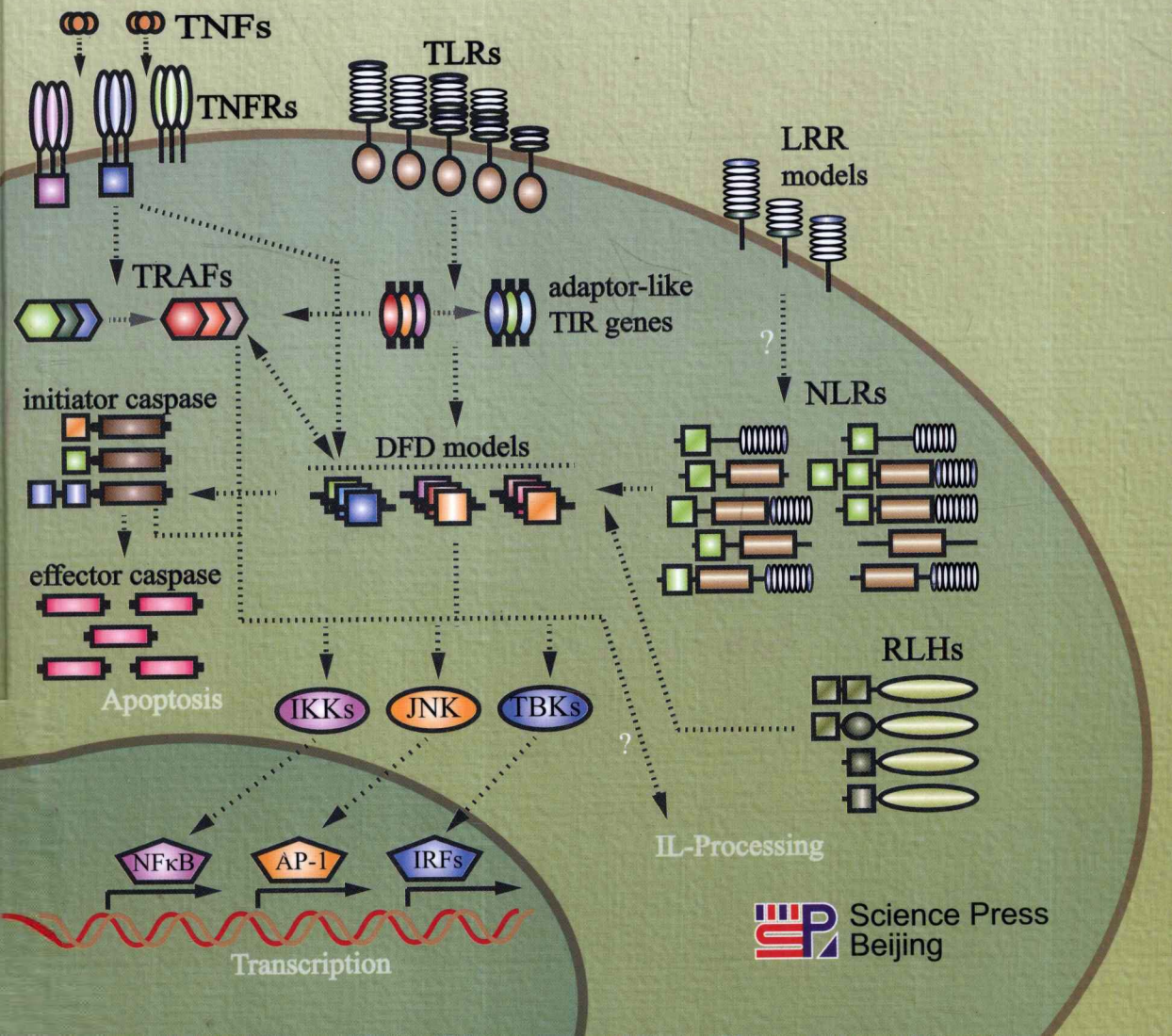
AMPHIOXUS IMMUNITY

– Tracing the Origin of Human Immunity

Anlong Xu



Science Press
Beijing

Amphioxus Immunity

- Tracing the Origin of Human Immunity

Anlong Xu

Science Press
Beijing

Anlong Xu Ph.D.
Sun Yat-sen University
Guangzhou, P. R. China
lssxal@mail.sysu.edu.cn

In the front cover, a picture of *Branchiostoma belcheri tsingtauense* is shown. Predicted TLR, NLR, RLH and TNF pathways and associated adaptors and transcription factors in *Branchiostoma floridae* are depicted in the below. In the back cover, the light-colored background is a masterpiece of world-famous architect Antoni Gaudi. The branched tree performs the mechanism of expanded protein variation produced by domain shuttling, recombination and duplication. The green auto-fluorescence is emitted from amphioxus oral cirri under ultraviolet rays, and the web site of our laboratory about amphioxus is listed.

Editor: Xin Tao

Cover Designer: Leiming You

Illustrator: Dongjuan Yuan

ISBN: 978-7-03-032316-3

16 Donghuangchenggen North Str.
Beijing, China

Dedicate to my Ph.D. mentor Dr. Harris Lewin and

Post doctor mentor Dr. Helen M Ranney

About the Author

Anlong Xu was awarded a government scholarship to study in the United States after his B.S. degree in biology from Zhongshan University in 1985. He went to the University of Illinois at Urbana-Champaign (UIUC) in Sept. 1986 to pursue his graduate study in immunogenetics under Dr. Harris Lewin's supervision and obtained his Ph.D. from UIUC in 1992. Dr. Xu then did his post-doctoral research for 2 years under the supervision by Dr. Helen M. Ranney, who was a professor in medicine at the University of California, San Diego, and member of US National Academy of Science and Institute of Medicine. Dr. Xu joined a San Diego, CA, based Alliance Pharmaceutical Corp from 1994-1996 and worked on new drug discovery. After 10 years of study and research work in the U.S., he joined the faculty of Department of Biochemistry at College of Life Sciences, Sun Yat-sen (Zhongshan) University, his alma mater, in 1996. Dr. Xu currently is a professor in molecular biology and immunology, Director of State Key Laboratory of Biocontrol, Director of National Engineering Center for Marine Biotechnology of South China Sea. Dr. Xu was appointed to Vice-President for research and development of Sun Yat-sen University in 2008 after serving as Dean of College of Life Sciences for 8 years. Dr. Xu has published more than 100 papers in peer-reviewed international journals, such as *N Engl J Med, Nature, Science Signaling, Genome Res, A J Hum Genet, PNAS, J Immunol, ISMEJ, J Biol Chem, E J Immunol, Mol Immunol, Dev Comp Immunol, Human Immunol, Immunogenetics, Fish & Shellfish Immunol*. Dr. Xu is currently a member of American Association of Immunologist, American Society for Biochemistry and Molecular Biology, American Association for the Advancement of Science, Chinese Society of Biochemistry and Molecular Biology and Chinese Society of Immunology,

and is currently President of Guangdong Society of Biochemistry and Molecular Biology and Vice-President of International Society for Developmental and Comparative Immunology. He currently serves on the Editorial Board for the following international journals: *Annual Review of Animal and Veterinary Biosciences, BMC Genomics, Animal Biotechnology*. His main research is the study on the origin and evolution of vertebrate immune system.

Foreword

In his book ***Amphioxus Immunity***, Anlong Xu covers a remarkable range of information about this small fish-like organism and its evolutionary context. This compilation of what is currently known about amphioxus, with a sharp focus on its immune system, is especially timely for many reasons. The recent availability of the amphioxus genomic sequence and its comparison with other metazoan genomes firmly establishes the phylogenetic position of amphioxus as the representative head of the chordates. The new phylogeny tree thus places amphioxus basal to the sister chordate lineages, tunicates and vertebrates.

Amphioxus has single copies of the ancestral genes that gave rise through two rounds of genome wide duplication to up to four identifiable paralogous genes in jawed vertebrates, including humans. There is still debate about whether the first round of genome wide duplication occurred before or after divergence of the jawless and jawed vertebrate lineages. Nevertheless, the resultant gene redundancy undoubtedly facilitated the evolutionary selection of new gene functions and more complicated biological systems, including those devoted to immune defense.

The competitive struggle for survival during the evolution of living forms on our planet inevitably led to the development of a wide variety of mechanisms for use in recognizing and repelling invasion by neighboring organisms. The diversity and complexity of the immune defense systems that have been recognized in bacteria, plants and animals defy simple classification, but they can be broadly categorized into innate and adaptive immune systems. An important distinction between the two types of immunity is that, for innate immunity, the genes encoding recognition elements are inherited in a ready to use form by each individual organism, whereas, for adaptive immunity, the genes for the recognition receptors are inherited in pieces that undergo combinatorial assembly during the differentiation of specialized lymphoid cells. This combinatorial assembly strategy results in the generation of a very large repertoire of clonally diverse lymphocytes, each of

which has its own unique receptor as the basis for recognition and response to a specific pathogen. Members of the lymphocyte population are thus available to recognize and respond to specific pathogens on first encounter and to give rise to long-lived progeny to provide specific immunological memory.

The diversity and constraints of presently known mechanisms for innate immunity is described by Xu, who also traces the evolution of the central genetic elements used for these heritable defense systems. The unifying theme for innate immunity is the use of germ-line encoded pattern recognition receptors that can recognize molecular patterns shared by many potential pathogens. The expression of a limited spectrum of cell surface and intracellular pattern recognition receptors can thus be used to sense a wide variety of potential pathogens and trigger cell signaling cascades leading to the activation of genes responsible for effective defense responses.

Whereas innate immune systems are universal, adaptive immune systems based upon clonally diverse lymphocytes have been defined only in vertebrates. Surprisingly, jawed and jawless vertebrates employ very different genes to encode their antigen specific receptors, although the lymphocytes that express them are very similar. In jawed vertebrates, combinatorial assembly of different immunoglobulin variable (V), diversity (D) and joining (J) gene segments during lymphocyte differentiation in the thymus or hematopoietic tissues results in the generation of highly diverse receptor repertoires for T and B lymphocytes. In jawless vertebrates, the combinatorial assembly of different leucine-rich-repeat (*LRR*) gene segments to complete variable lymphocyte receptor (*VLR*) genes during lymphocyte differentiation in thymus-equivalent or hematopoietic tissues results in the generation of clonally diverse T- and B-like lymphocytes. The similarity between these lymphocyte differentiation pathways in both vertebrate lineages suggests that bifurcated lymphocyte differentiation evolved in a common vertebrate ancestor before different primordial genes were co-opted for modification to serve antigen recognition purposes in the alternative adaptive immune systems of jawless and jawed vertebrates.

In this context, amphioxus is not only the best available model to gain insight into the beginning of vertebrate evolution, it also provides a pivotal representative for the study of how an adaptive immune system might have

gradually emerged. Obsessed with the daunting challenge of gaining insight into the evolutionary puzzle of how an adaptive immune system evolved to augment pre-existing mechanisms for innate immunity, Xu and his colleagues have sifted through the genomic sequences of two amphioxus species, *Brachiostoma floridae* and *Brachiostoma belcheri* (which they sequenced) to find an abundance of genes employed in innate immunity. While this exhaustive search does not reveal orthologous genes for those used in the combinatorial generation of antigen specific receptors in vertebrates, ancestral gene candidates were found for immunoglobulin receptors and many of the signaling elements used for activation of vertebrate lymphocytes. Most tantalizing is their identification of lymphocyte-like cells in the gill and intestinal regions. These lymphocyte-like cells may be immobile and, indeed, blood in the amphioxus circulatory system is acellular. Nevertheless, the tissue based lymphocytes of amphioxus respond to bacterial pathogens with an increase in size and the up-regulation of several genes characteristically used in vertebrate lymphocyte differentiation.

There are many interesting evolutionary principles yet to be learned from studies in the amphioxus model, not least among them being the unfinished story about how lymphocytes and their functions evolved. **Amphioxus Immunity** is loaded with information that will be useful for anyone who wishes to learn more about the origin of vertebrates and adaptive immunity.

Max D. Cooper, M.D.
Georgia Research Alliance Eminent Scholar
Professor of Pathology and Laboratory Medicine
Emory University School of Medicine
1462 Clifton Road, NE
DSB, Room 403C
Atlanta, GA 30322
email: max.cooper@emory.edu

Preface

I came to know the word of "Immunity" in Chinese for the first time when I was given a shot of vaccine during my childhood years in my hometown, a small town called Poyang Town and named after the largest freshwater lake of China, Poyang Lake, near the middle of Yangtze River, in Jiangxi province, China. After elementary school, middle school and high school in Poyang, I was admitted to Sun Yat-sen University (SYSU), my first alma mater, after National College Entrance Exam. At university, I had a chance to learn the basic terms of immunology, such as antibody and antigen, with major in biology. After graduating from SYSU, I was awarded a government scholarship to study in the United States in 1985, and was eventually admitted to the Ph.D. program of Immunogenetics at the University of Illinois at Urbana-Champaign (UIUC), in 1986. Under Dr. Harris Lewin's supervision, I was fascinated by the complexity and diversity of animal and human immunity, which prompted me to quest from where this immunity came and how it was formed during the evolution. In addition to my scientific curiosity, my way to think about scientific questions and to conduct scientific experiments was completely shaped up by my Ph.D. mentor's hard training, which has ever since influenced my scientific career until today. After graduating from UIUC, my second alma mater, with both M.S. and Ph.D. degrees, I was fortunate to work with a world famous female physician scientist, Dr. Helen M. Ranney, Professor in the Department of Medicine, University of California San Diego (UCSD), in 1992 as her last post-doc, and then went with Dr. Ranney to work at a San Diego-based pharmaceutical company, Alliance Pharmaceutical Corp after post-doc research. Dr. Ranney has also made great influence on my scientific career like Dr. Lewin. In consultation with the two important persons in my scientific career, I decided to come back to my home country China to joint faculty of the Department of Biochemistry, College of Life Sciences, SYSU, my first alma mater, in 1996.

The first thing I decided to do for my scientific research after I came

back to China was to start something new which could utilize my scientific training in US and explore new avenue on immune research. I had many thoughts on my new research directions, and one of my top listed directions was to understand the origin and evolution of human immunity. However, I had no idea about which model species to pursue this very important quest. It took me one year to figure out that amphioxus was THE model organism to best address my question, and another two years to establish lab-based aquaculture system of amphioxus and the infection model for understanding host immune response to infections, which made my lab one of the pioneer labs in the world to study amphioxus immunity, although this species had been an iconic model for the evolutionary biology for more than 200 years. Instead of using conventional immunological methods only, my lab combined traditional immune methods with cellular, biochemical and molecular approaches, particularly genomic approach, to conduct a comprehensive survey on the immune response of amphioxus to bacterial infection from the beginning, which gave us a quick open-up for this brand new field. For last 10 years, my lab has contributed more than 30 papers related to amphioxus immunity. In summary, our contribution to the knowledge of amphioxus immunity, aiming to the understanding of origin and evolution of vertebrates immunity, especially human immunity, can be briefly described as followed:

1) Genomic analysis of the immune gene repertoire of amphioxus reveals extraordinary innate complexity and diversity, suggesting that our chordate ancestors had a remarkably elaborate innate immune system, but this system was somehow reduced in the vertebrate lineage. This finding provides an obvious evidence for the so-called "immunological big bang" for the explanation of the origin of vertebrate immunity.

2) Functional analyses of important innate immune genes involved in two basic innate immune signaling, TLR and TNF signaling, in amphioxus, suggest that the basic frameworks for these two signaling pathways have been established at the basal chordate which have laid foundation for the eventual formation of the two pathways in vertebrates.

3) Identification of lymphocyte-like cells along with related transcription factors and signaling molecules for lymphoid proliferation and differentiation indicates the emergence of adaptive immunity for vertebrates with the

presence of some basic components for adaptive immunity. Finding of extrinsic apoptosis pathway in amphioxus further substantiates the claim for the beginning of adaptive immunity in the basal chordate since extrinsic apoptosis pathway is generally believed to be co-evolved with adaptive immunity.

4) In addition to tracing the origin of existing system for immune response and regulation in vertebrates including humans, we may be in the position to reveal a novel mechanism involved in immune regulation which has never been described in other organisms by studying this model animal, such as findings of novel molecules for immune recognition and novel mechanism for immune regulation epigenetically by alternative 3'UTRs.

5) In immune signaling system, most proteins have characteristic and conserved multiple domains to exert specific functions to the proteins so as to interact with specific molecular partners. Our study on amphioxus immune signaling molecules indicates that different combinations of these domains (e.g. CARD, TIR, DFD, DEATH and etc) are the sources of evolution for the generation of new signaling molecules that can result in the interaction specificity. Understanding the evolution mechanism of these domains and their shuffling for the generation of new proteins with new functions should provide a novel vista to synthetic biology and insights that may help the treatment of diseases associated with mutated protein activity.

Finally, I would like to thank all of my students who have made various significant contributions to our understanding of amphioxus immunity, and I would not come to this far without their diligent and intelligent work on this research. I would also like to thank Professors XU Xun of Third Institute of Oceanography, State Oceanic Administration of China, ZHANG Shicui of Ocean University of China, ZHANG Hongwei of Shandong University, ZHANG Peijun of Institute of Oceanology, Chinese Academy of Sciences (CAS), WANG Yiquan of Xiamen University, CHEN Junyuan of Nanjing Institute of Geology and Palaeontology, CAS, GAO Fu of Institute of Microbiology, CAS, PENG Xuanxian of Sun Yatsen University and LIU Xiaolong of Shanghai Institutes for Biological Sciences, CAS for their scientific comments and academic discussions on my research. I would like to thank Professors Linda and Nick Holland of Institute of Oceanography, University of California San Diego and Hector Escriva of CNRS UMR,

UPMC Univ Paris 06, Banyuls, France for their academic communications and exchanges on my research. Particularly, I would like to thank Professor Max Cooper of Emory University for his longtime enthusiastic support to my research and his nice foreword to my book is deeply appreciated.

Anlong Xu
Sun Yat-sen University
Guangzhou, P.R. China, 510275
E-mail: lssxal@mail.sysu.edu.cn

Contents