PREFACE

Hox genes were discovered almost 30 years ago as one of the very first unifying principles in development of Bilaterian species. These genes code for a family of conserved transcription factors which, in most species, are organized in clusters along chromosomal territories. Their action and distribution along the anteroposterior axis of the embryo, as well as their deployment in time, exhibit a striking order which reflects their linear organization on the chromatin. This peculiar arrangement, termed colinearity, was first recognized by Ed Lewis in the fly embryo. Despite very active research during the past three decades, *Hox* gene regulation and function remain extremely mysterious. Particularly, the molecular mechanism underlying the fundamental property of colinearity found in organisms ranging from flies to humans remains unknown.

Hox mutations exhibit spectacular homeotic properties, whereby the identity of a body segment can be changed in that of a different segment. Whereas these effects are now well characterized in vertebrates and invertebrates at the phenotypic level, the molecular details of the targets and functions of Hox proteins underlying these identity changes are poorly understood. Thus, despite the wealth of research focused on *Hox* genes, major questions are still to be answered.

A large body of literature on *Hox* genes has been published since the seminal paper from Ed Lewis in 1978, but strikingly, very few monographs have been devoted to this fascinating topic. Thus, the goal of this book is to provide a comprehensive and up-to-date summary of recent developments in the field of Hox biology. This is a large field and due to space limitations, some areas might be covered more extensively than others. In this book, we cover some history of the characterization of the Hox complexes in the fly, as well as discussions of the organization, regulation, and function of *Hox* genes in patterning the body axis in invertebrates (essentially *Drosophila*) and in vertebrates.

The book begins with a chapter by Robert K. Maeda and François Karch who recapitulates the history of the discovery of the BX-C complex in the fly and describes the striking colinear organization of the *cis*-regulatory elements controlling expression of the *Ubx*, *AbdA*, and *AbdB* genes initially recognized by Ed Lewis. This organization is strikingly different from that of vertebrate *Hox* clusters where no such colinear distribution of the *cis*-regulatory sequences is observed. Genes involved in the early patterning of the embryo, such as the gap and pair rule genes, control the initiation of *Hox*

gene expression, whereas their maintenance requires the genes of the Polycomb and Trithorax complexes that act on chromatin to respectively maintain the repressed or activated configurations of *Hox* genes. The chapter also describes our current understanding of the role of specific chromatin domains and regulators in the colinear regulation of the BX-D *cis*-regulatory elements. The next chapter by Walter Gehring, Urs Kloter, and Hiroshi Suga presents genetic and phylogenetic arguments, supporting the notion that the second thoracic segment in the fly (T2), which is specified by Antenapedia, corresponds to a developmental and evolutionary ground state in Bilaterians. In vertebrates, this ground state would correspond to the thoracic level patterned by the *Hox6* group. Based on these arguments, the authors further argue about the origin of the *Hox* clusters by duplication and unequal crossing-over, leading to the progressive addition of genes in between the two extremities of the cluster. In various circumstances in flies and vertebrates, the posterior *Hox* genes have been shown to be functionally dominant over the anterior ones, a property called posterior prevalence or phenotypic suppression. The arguments developed and the model proposed by Gehring and colleagues in this chapter clearly challenge this notion for the genes expressed anterior to T2 in flies.

Strikingly, very little is known about the mode of action and the targets regulated by Hox proteins. A longstanding paradox in the field is the relative lack of specificity of the Hox binding sequences compared to the exquisite developmental functions assumed by individual Hox proteins. In the third chapter, Richard Mann, Katherine Lelli, and Rohit Joshi discuss this question and argue in favor of different modes of Hox regulation. They survey the different kinds of Hox targets characterized, and distinguish very specific targets recognized by a single paralog, from targets showing less specificity and, hence, recognized by several Hox factors. Hox proteins can act with cofactors, such as the TALE proteins, that help cooperative binding to DNA. This cooperative binding induces conformational changes revealing novel, specific binding properties of the complexes to their DNA targets. Finally, they discuss how Hox proteins also interact with collaborators, forming what they call a Hoxasome, which influences the transcriptional outcome of the Hox-based regulation.

In the fly and vertebrates, *Hox* genes are not expressed in the anteriormost part of the brain (telencephalon, diencephalon, and mesencephalon), which is patterned by other Homeobox-containing genes, such as *Otx* in vertebrates or *orthodenticle* in the fly. In the vertebrate central nervous system, *Hox* genes are involved in the patterning of the hindbrain and the spinal cord. The hindbrain or rhombencephalon corresponds to the posterior part of the brain, which is transiently segmented into seven rhombomeres. These segments define compartments that acquire distinct functional identities during development, and which control a variety of physiological functions such as respiration. In the hindbrain, *Hox* genes are expressed

segmentally, with their expression boundaries respecting the rhombomeric frontiers. Mutations in the mouse demonstrated that *Hox* genes play a key role in the control of the identity of the rhombomeres. The regulation and role of *Hox* genes in patterning the vertebrate nervous system has been extensively studied in the hindbrain and more recently in motoneurons. The fourth chapter by Stefan Tumpel, Leanne Wiedemann, and Robb Krumlauf summarizes our current understanding of the role of anterior *Hox* genes in early patterning of the hindbrain in vertebrates. It describes the *cis*-regulatory codes and regulatory networks established during hindbrain differentiation by the *Hox1* to *Hox4* paralog genes which are involved in initiating and maintaining *Hox* gene expression at the appropriate segmental level. Chapter 5, by Yuichi Narita and Filippo Rijli, focuses on the later functions of *Hox* genes in hindbrain development and, more specifically, on their role in the establishment of the complex neuronal connectivity that underlies various important physiological functions and behaviors.

In chapter 6, Jeremy Dasen and Tom Jessell discuss our understanding of the role of *Hox* transcription factors in the patterning of the spinal cord motoneurons. Recent elucidation of the role of these factors in the establishment of the various levels of motoneuron organization such as columns and pools is detailed, as well as the recognition of key cofactors such as FoxP1 in this process.

In vertebrates, aside from the nervous system, the role of *Hox* genes in axial patterning has been examined in great detail at the level of the vertebral column. The spine is progressively formed in a head-to-tail direction during embryogenesis by the rhythmic addition of vertebral precursors, termed somites. The somitic columns formed during embryogenesis become subsequently patterned into different anatomical regions when somitic derivatives differentiate to form the vertebrae and associated muscles. Mouse knock-out experiments have shown that somite regional identity is largely controlled by *Hox* genes. In chapter 7, Tadahiro Iimura, Nicolas Denans, and Olivier Pourquié describe the early colinear activation of *Hox* gene expression in the precursors of the vertebrate spine (the paraxial mesoderm), which roughly positions the Hox expression domains at the appropriate axial level. They discuss how this temporal expression sequence is translated into the characteristic colinear expression domains along the forming axial skeleton. A second repositioning phase involving the segmentation machinery is also required for the definitive positioning and subsequent maintenance of the Hox expression domains in the somites. Interestingly, such a two-step regulation of Hox expression in the developing embryo is also described for fly embryos in chapter 1 and for the hindbrain in chapter 4. Strikingly, in these different systems, it largely relies on different mechanisms.

Related issues are also discussed in chapter 8 by Teddy Young and Jacqueline Deschamps, which is furthermore concerned with the role of

the *Cdx* genes in the regulation of *Hox* genes in the embryo. *Cdx* genes belong to the ParaHox cluster, which was proposed to share a common evolutionary origin with the Hox cluster. In chapter 9, Deneen Wellik details the later function of *Hox* genes in patterning the vertebrate axis once the complex nested expression patterns are established in the embryo. The regional patterning of vertebrae was originally proposed to be dependent on the combinatorial action of all Hox proteins in the vertebral precursors—the Hox code. However, this idea was subsequently challenged by the concept of posterior prevalence, which assumed that only the posterior-most genes expressed in a given segment are involved in patterning this segment. The knockout of entire paralog groups in the mouse somehow reconciles these two ideas, demonstrating that whereas posterior genes are clearly dominant over anterior ones, some level of combinatorial functions of adjacent paralog groups are required for the appropriate patterning of vertebrae.

While this book is expected to meet the expectations of Hox aficionados, it is also intended to provide a survey of the field to newcomers. We hope that this book will take its place as a useful tool for those working in the ever growing field of Hox biology.

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