PREFACE

The development of various tissues and cell types each with a specific structure and function is a highly complex process that requires precisely regulated gene expression. Among the various transcription factors that regulate developmental processes, arguably, the basic helix-loop-helix (bHLH) superfamily plays a prominent role in the establishment of tissue-specific gene expression programs. Moreover, these factors also play a role in tissue maintenance and homeostasis.

The bHLH transcription factor superfamily is highly conserved over evolution and has been identified in organisms ranging from yeast to humans. The bHLH motif was first recognized in the E-proteins E12 and E47, and now over 200 factors all of which share a bHLH domain have been identified in this superfamily. In addition to the conserved bHLH domain, some family members contain a Per-Arnt-Sim [PAS] domain or a leucine zipper [LZ] domain, whereas the Id proteins lack a basic region. Elegant studies over the years in various species and using different model systems have identified these factors as key regulators of lineage specification, sex determination, and cellular differentiation along multiple lineages. In addition, bHLH factors regulate immune cell function, circadian rhythms, hypoxia responses, toxin metabolism, cell proliferation, apoptosis, and many other biological processes. Given their requirement for growth, development, and differentiation of several cell types, it is not surprising that deregulated expression or activity of numerous bHLH factors is linked to human pathologies. This volume brings together our current knowledge on the structure, function, and involvement of bHLH transcription factors in development and in human disease.

In Chapter 1, Tajbakhsh and colleagues provide an in-depth review on the myogenic regulatory factors MyoD, Myf5, Myogenin, and Mrf4 that coordinate developmental myogenesis. They provide a perspective on regulation and function of these factors, as well their function in regulating chromatin structure through recruitment of chromatin-modifying and remodeling complexes.

Proneural genes that confer neural identity have been well studied both in *Drosophila* and in vertebrates by regulating Notch signaling. Chapter 2 by Huang et al. reviews proneural gene function and discusses the deregulated expression of these genes in various human disease including cancer and psychiatric disorders.

In Chapter 3, Tamura et al. review *Hand* genes that have been genetically proven to play a critical role in heart, craniofacial, and limb development. The human *Hand2* gene resides on 4q34.1 and its expression is reduced in 4q− syndrome suggesting it may be causally involved. The impact of *Hand2* gene dosage in developmental disorders and in the human chromosomal disorder partial trisomy distal 4q is discussed.

E-proteins that include E2A, HEB, and E2-2 have been widely studied as central regulators of T and B lymphocyte development. In addition, E-proteins heterodimerize with various tissue-restricted bHLH factors such as MyoD to regulate cellular differentiation. In Chapter 4, Belle and Zhuang provide an overview of the role of E-protein function in development of the immune system. They also discuss how deregulation of these factors leads to autoimmune disease, as well as T and B cell leukemias and lymphomas.

Id proteins function predominantly, but not exclusively, as dominant-negative inhibitors of E-proteins. Unsurprisingly, gain of Id proteins mimic loss of E-proteins and also block differentiation along multiple lineages. In Chapter 5, Ling et al. discuss the role of Id proteins as antagonists of E-proteins in the immune system, as well as in blocking differentiation and promoting tumorigenesis.

The *Enhancer of Split [E(Spl)]* locus which consists of seven bHLH genes was identified as spontaneous loss-of-function mutant resulting in a neurogenic phenotype in *Drosophila*. These genes are perhaps among the best-characterized targets of the Notch signaling pathway. In Chapter 6, Delidakis and colleagues provide a comprehensive review on the evolution, structure, and function of these genes in lateral inhibition that serves to restrain neuroblast commitment.

The vertebrate counterparts of E(Spl) are the *Hes* and *Hey* genes. In Chapter 7, Kobayashi and Kageyama provide a comprehensive review on the role of *Hes* genes as downstream effectors of Notch signaling. They discuss their function in the nervous system, in the maintenance of stem cells, as well as the oscillatory expression of *Hes* genes, which is important in somite segmentation.

In Chapter 8, Weber et al. describe the striking defects in heart and vascular development as evidenced by genetic disruption of *Hey1*, *Hey 2*, and *HeyL* in mice. They also discuss the role of Hey2 in cardiac function and its association with Brugada syndrome.

Stra13/Dec1 and Sharp-1/Dec2 bHLH factors exhibit domain structure similarity with the Hes and Hey proteins. However, these factors exhibit several distinctive transcriptional properties and functions in various tissues.

In Chapter 9, we review the function of these proteins in tissue repair and regeneration especially in the musculoskeletal system, as well as their function in homeostasis in the immune system. Growing evidence documenting deregulated expression of both genes in various pathologies is reviewed. In Chapter 10, Kato and colleagues discuss the role of these factors in circadian rhythms and adaptive responses.

Given the very large number of bHLH factors, it would be impossible to cover the entire superfamily in a single volume. Nonetheless, this volume attempts to bring together for the first time a compilation of in-depth reviews on several subfamilies that positively and negatively regulate development and differentiation of many cell types. These reviews reflect a continuum of progress that has been made toward understanding the biological functions of this superfamily.

Significant advances have been made in our understanding of bHLH factor function in developmental processes through loss-of-function and gain-of-function studies in mice and other model systems. Numerous cascades involved in cell fate determination during development and governing cell proliferation, differentiation, apoptosis, and migration are regulated by bHLH family members that are highly conserved from invertebrates to humans. Moreover, deregulated expression or activity of several factors is seen in human pathologies. In some instances, there is strong evidence of causal involvement in various disease conditions, whereas in others, direct evidence of mutations in human pathologies awaits further investigation. In spite of these rapid strides in linking bHLH factors to many human diseases, much remains to be understood with regard to the molecular mechanisms underlying their function. For instance, while most bHLH proteins bind to highly similar DNA-binding sites (E-box or N-box motifs), they interact with distinct genomic regions *in vivo* and regulate different sets of target genes. The mechanisms by which specificity and selectivity of DNA binding is achieved in the genome are not completely understood. The advent of ChIP-seq technology along with transcriptome analysis has started to shed light on genome-wide binding sites and identification of direct target genes. Posttranslational modifications that can alter stability, subcellular localization, DNA binding, dimerization, and association with cofactors remain to be deciphered in many cases. Epigenetic reprogramming is a hallmark of terminal differentiation during which cells acquire specialized functions and undergo dramatic alterations in gene expression. The identity of chromatin-modifying and remodeling complexes that influence tissue-specific differentiation programs by distinct bHLH transcription

factors is unclear in most tissues. Association with cofactors can influence transcriptional activity and function of bHLH proteins and may be relevant in pathologies where genetic mutations have not been identified in disease. Such studies aimed at understanding of the molecular and cellular basis of function will undoubtedly clarify and put into perspective how bHLH factors function in normal developmental programs and how their deregulation results in disease pathogenesis and progression.

This book is dedicated to the loving memory of my father who passed away as I was working on this book in December 2013. He led a life of honesty, hard work, perseverance, and goodwill to all that will be an everlasting source of inspiration.

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