Preface

Analyses of erythroid gene expression and regulation have historically been performed at the cutting edge of basic and clinical research, whether they be the first description of developmentally regulated changes in sites of hemoglobinogenesis (Jordan and Spiedel, 1923; McCutcheon, 1936), determination of sickle-cell anemia as an aberration of a single genetic locus (Pauling *et al.*, 1949), analysis of the quaternary, three-dimensional structure of hemoglobin (Perutz, 1987), the early successes of molecular cloning and gene structure determination (Lawn *et al.*, 1978), or the introduction of foreign genes into mice (Costantini and Lacy, 1981).

These analyses have also led to an extraordinary compendium of genetic aberrations that alter the proper regulation of red blood cell expression patterns (Stamatoyannopoulos *et al.*, 2001). Those related to globin regulation run the gamut of causes and provide clinical examples of molecular mutations that cover all aspects of transcriptional regulatory mechanisms, whether they be located within the enhancers and promoters, or whether they affect posttranscriptional controls such as splicing and processing/polyadenylation (Bunn, 2001; Weatherall, 1994). It is likely true that for every molecular mechanism that has been discovered since the advent of molecular biology, there is a clinically relevant example to be found within the globin regulatory cascade.

Such an expansion of the biological horizon has also been realized by studying the cellular aspects of red blood cell onset as it follows from hematopoietic differentiative mechanisms (Metcalf and Moore, 1971; Morrison *et al.*, 1995; Till and McCulloch, 1980). The recognition of a stem cell, and the conceptual details that define such a cell that are so pervasive today in many systems, has its origins within studies originally performed in circulating blood cells that aimed to identify how these varied cells were continuously established and propagated during the life of an individual (McCulloch and Till, 2005). Indeed, well into the fourth decade during which the self-renewal and multipotential concepts of hematopoiesis have been experimentally described and refined, the complexities of the biology have forced some of the initially posited questions to remain outstanding, for example whether multiple embryonic origins arise independently or not (Samokhvalov *et al.*, 2007).

Not surprisingly, unanticipated directions in cellular and molecular mechanisms demanded novel approaches to address these compelling issues of red gene regulation. For example, insights on the phenomena of distal

locus control elements and their role in erythroid-specific and developmentally appropriate expression (Behringer *et al.*, 1990; Enver *et al.*, 1990; Grosveld *et al.*, 1987) have been abetted by the ability to analyze large constructs that contain the complete genomic globin cluster in transgenic mice (Gaensler *et al.*, 1993; Peterson *et al.*, 1993; Strouboulis *et al.*, 1992).

One might have expected that, after so many decades of serious study, there would be few surprises in store for those who study red blood cell biology. As will be seen by perusal of this volume, this is clearly not the case, and it is in the spirit of these pioneering studies that this volume has been assembled. We begin with the embryological origins of the red blood cell, and McGrath and Palis describe the important role of primitive erythropoiesis in establishing the first population of red blood cells in the embryo. Intensive study of this has led to surprising observations that challenge the dogma of primitive red blood cell properties and their life in circulation. In addition, the close relationship between erythroid and megakaryocyte development has unexpectedly been found within these primitive cells.

Manwani and Bieker then focus in Chapter 2 on the immediate environment within which these red blood cells are first formed, known as the erythroblastic island. Even though these were first described almost 50 years ago, it is only recently that cellular and molecular details have begun to illuminate their importance for the normal process of erythroid development, and reinforces the important issue that studying cells in culture may not yield the complete picture on what actually occurs within the developing embryo. Such a concept has become more appreciated very recently, especially with the idea of the "niche" (itself first described almost 30 years ago within the context of hematopoietic development (Schofield, 1978)), a configuration of heterologous cells localized in the fetal liver or the bone marrow within which cell–cell interactions between its residents play a critical role in proper maturation of maturing hematopoietic cells that are eventually released into circulation (Scadden, 2006). In this context, the erythroblast island can be considered a specialized micro-niche.

We then zero in on the genetic control mechanisms for erythropoiesis. However, rather than describing the transcription factors and *cis*-acting control elements important for red blood cell gene expression, we describe novel observations related to epigenetic mechanisms of gene control. Wozniak and Bresnick begin in Chapter 3 by covering the important regulatory role of histone and their modifications, particularly as they relate to the regulation of the β-like globin locus and the GATA2 gene. Of particular interest has been the role of the histone code to this process, and how small differences in histone acetylation and methylation lead to significant changes in developmental regulation of these genes. The details of how tissue-restricted transcription factors play critical roles in helping to properly coordinate these histone marks in the proper way, and the issues that remain unresolved, are discussed.

The concept of epigenesis is further amplified by Ginder, Gnanapragasam, and Mian, who describe DNA methylation and its effects on red blood cell gene control in a range of species. Although the inverse correlation of DNA methylation and gene expression of the β-like globin regulatory domain was first noted almost 30 years ago, it is only recently that the molecular details have become apparent. Globin regulation continues to provide a fertile ground in which to address the mechanistic effects of DNA methylation, and Chapter 4 highlights the recent observations that link histone modifications with DNA methylation, and the potential role of specific proteins that recognize DNA modifications in developmental regulation of the globin and other red blood cell genes.

Attempts to obtain a three-dimensional idea of these molecular observations required a novel technology, and de Laat, Klous, Kooren, Noordermeer, Palstra, Simonis, Splinter, and Grosveld describe the detailed conformations and molecular dynamics that can be deduced by 3C and 4C approaches. These have helped to distinguish between models of long-range erythroid gene expression control mechanisms, and have moved the focus away from the one-dimensional regulatory observations of the past. The 3C technology can address local mechanisms one at a time, and indeed has also been used to demonstrate the importance of the EKLF and GATA1 transcription factors for establishing the proper structure at the β-like globin locus. But the expansion of this technology to the 4C approach described in Chapter 5 can lead to a more global understanding of the spatial relationships between loci at a given time within a particular cell type. In combination with high-resolution fluorescent microscopy, the nuclear architecture that follows from such long-range interactions can be ascertained to a resolution never previously seen.

One of the major end results of these genetic control mechanisms is to produce a red blood cell that expresses the proper level of hemoglobin to ensure adequate oxygen transport throughout the body. Iron is a critical component for this process, and Wrighting and Andrews describe the cellular and organismal control mechanisms that come into play to ensure that adequate levels of iron are properly incorporated and metabolized by the red blood cell. Of particular importance are the roles of hepcidin and ferroportin in this process, and Chapter 6 integrates the biochemical properties of these molecules and their interacting partners with disease states that result from their mutation or altered regulation.

Although oxygen transport is the most well-described property of red blood cells, nitric oxide is another colorless gas that interacts with hemoglobin and becomes chemically altered as a result. Čokić and Schechter provide a comprehensive review of the synthesis and regulation of nitric oxide and place this in the context of red blood cell differentiation. The role of accessory cells, such as endothelial and stromal cells, in this process is part of the wider framework in which nitric oxide exerts its effects of

erythroid cells at a variety of points. As detailed in Chapter 7, these impinge on both extracellular (e.g., vasculogenesis) and intracellular (e.g., hemoglobin function and expression) control mechanisms, leading to an unanticipated intersection with therapies used to increase fetal hemoglobin.

We then end with a detailed description by Ellis and Lipton of Diamond-Blackfan anemia, a red blood cell disorder that results from a specific type of genetic aberration, as a particularly cogent example of how the combination of basic and clinical observations has helped to unravel its molecular basis. Of particular surprise has been the determination that Diamond-Blackfan anemia is a disorder of ribosome synthesis or function, and the potential mechanisms by which this might lead to compromised red blood cell function are evaluated in Chapter 8. Interestingly, this situation is no longer unique, as there are now other red blood cell (and non-red blood cell) disorders that are also linked to ribosomal dysfunction. Given the pediatric nature of this disease, the authors discuss how established treatment options are being considered and reevaluated in the light of these novel genetic causes and the molecular mechanisms that are altered as a result.

It is hoped that the reader will appreciate why the authors of these chapters are still excited about the study of red blood cell development, even after all of the years of intensive efforts. The synergistic merge between basic research and clinical observations that characterize this field has no peer in science, and the "bench to bedside" road has been well traversed in both directions for decades. Its continuous history of novel observations illuminated by innovative approaches that exert a wide range of influence on other fields of study progresses unabated, and indeed will continue to lead to a deeper understanding and appreciation of the multilayered complexities of eukaryotic biology.

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