

# Preface

Embryology is a branch of biology that has an immediate bearing on the problem of “life.” Life cannot be fully accounted for without an understanding of its dynamic nature, which expresses itself in the incessant production of new organisms in the process of ontogenetic development. Therefore, embryology is defined as the science of the development of an embryo from the fertilization of the ovum to the fetus stage. Teaching of embryology has long been an established feature at universities throughout the world, both for students in biology and students in medical sciences. During the twentieth century most of this science has been overshadowed by experimental-based genetics and cell biology, which have turned classical embryology into “developmental biology.” Several universities are now teaching developmental biology instead of embryology as a course in biology programs. Significant contributions made in the twenty-first century in the fields of molecular biology, biochemistry, and genomics, integrated with embryology and developmental biology, provide an understanding of the molecular portrait of a “developmental cell.” This integrated approach to development is incorporated in the present book as “stem cell biology,” a new sister branch of embryology/developmental biology that emphasizes the study of self-renewal, differentiation, pluripotency, and nuclear programming, which are characteristics of stem cells. In a broad sense, *stem cell biology is nothing more than an understanding of embryology and development together at the molecular level using engineering, imaging, and cell culture principles*. With such a wide scope, this book can only be an introduction to stem cell biology.

*Stem Cells and Regenerative Medicine: From Embryology to Tissue Engineering* is mainly intended for readers in the biotechnology and molecular medicine fields. Although quite a number of books already exist covering stem cells, this book differs, in that it is the first text completely devoted to the basic developmental, cellular, and molecular biologic aspects of stem cells and their clinical applications in tissue engineering and regenerative medicine. We took serious consideration in choosing the chapters and sections in this book to maintain the theme of *Molecular Embryology to Tissue Engineering*.

This book focuses on the basic biology of embryonic and cancer cells and their key involvement in self-renewal, muscle repair, epigenetic processes, and therapeutic applications. Significant contributions, such as nuclear reprogramming–induced pluripotency, and stem cell culture techniques using novel biomaterials, are also

covered. This text consists of 36 chapters, grouped into six parts. Most of the chapters are written by experts in the field from academia and industry. The goal is to have this book serve as a reference for graduate students, post-docs, and teachers and as an explanatory analysis for executives and scientists in biotech and pharmaceutical companies. Our hope is that this volume will provide a prologue to the field for both newcomers and those already active in the field.

The term “stem cell” appeared in the scientific literature as early as 1868 in the work of the eminent German biologist Ernst Haeckel. Haeckel, a supporter of Darwinian evolution, developed a number of phylogenetic trees to represent the evolution of organisms from common ancestors and called these trees *Stammbaume* (“stem trees”). In this context, he used the term *Stammzelle* (“stem cell”) to describe the ancestor unicellular organism from which he presumed all multicellular organisms evolved. He referred to fertilized egg as the source that gives rise to all cells of the organism. Later, in 1887, Theodor Boveri and Valentin Hacker identified the earliest germ cells in animal embryos. In 1892, Valentin Hacker described stem cells as the cells that later in development produce oocytes in the gonads. Thus, in these early studies, the term stem cell referred to what we call the “germline lineage,” “primordial germ cells,” and “germline stem cells.” In 1896, Edmund Wilson, an embryologist, reviewed the finding of these German scientists in his book *The Cell in Development and Inheritance*, which was published in English and became an inspirational work for a generation of embryologists and geneticists, especially in United States. Given his wide readership, Wilson is generally credited as having coined the term “stem cell.”

Nuclear programming is the process that instructs specialized adult cells to form early pluripotent stem cells. Pluripotent stem cells possess the capacity to become any type of mature cell in the body and therefore have great potential for experimental and therapeutic purposes. Using the concept of “cellular reprogramming,” Briggs and King in 1952 produced normal tadpoles by transplanting nuclei from blastula cells to enucleated eggs in the frog *Rana pipens*. However, transplanting nuclei from differentiated cells was achieved by John Gurdon in 1962 in the African clawed toad, *Xenopus laevis*, which is now known as the classic nuclear transfer experiment. It took more than another decade (1975) for Gurdon to succeed in producing healthy and sexually mature fertile frogs with functional muscle, beating hearts, well-differentiated eyes, and all of the other organs. This experiment provided the first clear evidence that cell specialization does not involve irreversible inactivation in the genes required for development of an animal. This conceptual framework led to the start of the field of nuclear reprogramming, and Gurdon became known as the “father” of nuclear reprogramming (cloning). It took almost another 10 years to clone an adult sheep, Dolly (in 1996), by Kevin Campbell and Ian Wilmut of the Roslin Institute in Edinburgh, Scotland. This experiment dramatically extended Gurdon’s concept from frogs to mammals. The Dolly-related work of somatic cell nuclear transfer was further extended to produce monkeys, cows, dogs, mice, and other animals. These remarkable contributions stimulated other researchers to think about using nuclear transfer to generate pluripotent human embryonic stem cells for cell replacement therapy.

The road to embryonic stem cells and beyond began in the 1960s with the work of Leroy Stevens from the Jackson Labs, Bar Harbor, Maine, who discovered embryonal carcinoma cells while studying testicular carcinomas. Later Stevens and colleagues demonstrated that these embryonal carcinoma cells are indeed pluripotent stem cells. In the mid-1970s, Gail Martin's postdoctoral work with Martin Evans at the University of Cambridge led her to develop *new in vitro* clonal culture methods of embryoid cells. In the early 1980s, Martin, then at the University of California at San Francisco, and Martin Evans and Matthew Kaufman of the University of Cambridge independently isolated stem cells from mouse embryos and coined the term "embryonic stem cells." It took almost 10 years for Jamie Thompson of the University of Wisconsin to culture monkey embryonic stem cells and subsequently human embryonic stem cells in 1999. Thompson's work propelled the activity of stem cell research and cell propagation technologies in general.

There are two routes to producing a living animal: (1) injection of a somatic cell nucleus into an enucleated egg (nuclear reprogramming) and (2) use of an embryo to produce embryonic stem cells. In a quite astonishing discovery, Kazutoshi Takahashi and Shinya Yamanaka of Kyoto University in Japan in 2006 for the first time turned adult mouse skin fibroblast cells into pluripotent cells. This breakthrough of inducing fibroblasts was achieved by stable transfection of only four transcription factors (Oct4, Sox2, Klf4, and c-Myc), and these are now referred to as induced pluripotent stem (iPS) cells. The discovery of iPS cells turned the field of nuclear reprogramming upside down. This work was extended and further confirmed by several groups that generated iPS cells from individuals with various neurodegenerative diseases, raising the hope of cell replacement therapy and making personalized medicine a reality. A section of this book with six chapters details the concepts behind nuclear reprogramming and induced pluripotent stem cells.

In 1868, Ernst Neumann suggested that hematopoiesis occurs in bone marrow. He used the term "stem cell" to refer to the common precursor of the blood system in 1912. The debate about the existence of a common hematopoietic stem cell continued for several decades until definitive evidence was provided in 1961 by two Canadian scientists, James Till and Ernest McCulloch. Blood and the system that forms it, known as the hematopoietic system, consists of many cell types with specialized functions (some of these include red blood cells, platelets, granulocytes, macrophages, B and T lymphocytes, etc). Generally, the hematopoietic system is destroyed by radiation and chemotherapeutic agents that kill dividing cancer cells. In order to quantitatively assess the radiation sensitivity of normal bone marrow cells, a colony-forming unit assay was developed by Till and McCulloch, who coined the term "pluripotent hematopoietic stem cells" (HSCs). Today, we know that the best locations for HSCs are bone marrow, umbilical cord blood, and embryonic stem cells. In 1959, for the first time, Edward Donnall Thomas of the University of Washington used HSCs for treating leukemia and lymphomas through bone marrow transplantation. The efficient expansion of HSCs in culture remains one of the major research themes of stem cell biology. Combined applications of genomics, proteomics, and gene therapy approaches will further help to widen the

horizon for clinical applications. According to Irving Weissman of Stanford University Medical School, the progeny produced from hematopoietic stem cells exhibits properties that include self-renewal, differentiation, migration, and apoptosis. A few chapters in the third part of this book highlight of the use of HSCs for bone marrow cell therapy, heart transplantation, and cell replacement therapy for neurologic disorders.

The term “tissue engineering” was first used by Eugene Bell of MIT in 1984, and later was also used extensively by Wolter and Meyer in 1984. Tissue engineering combines cells, engineering, and materials methods with suitable biochemical and physiochemical factors to improve or replace biologic functions. In other words, it deals with the repair or replacement of portions of or whole tissues such as bone, blood vessels, bladder, skin, and artificial organs. According to Robert Langer and Joseph Vacanti, it “applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ.” Powerful developments in the multidisciplinary field of tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices (“scaffolds”), cells, and biologically active molecules. A section of this book with five chapters highlights recent developments in biomaterials, three-dimensional culture systems, lab-on-a-chip concepts, and microtechnologies used in attempts to understand stem cell biology.

Regenerative medicine is a new branch of medicine that attempts to change the course of chronic disease, in many instances regenerating failing organ systems lost due to age, disease, damage, or congenital defects. The term “regenerative medicine” was first referred to in 1992 by Leland Kaiser and then popularly used by William Haselstine of Human Genome Sciences. The term regenerative medicine is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to treat diseases using cell therapies or transplantation methods. This field holds the promise of regenerating damaged tissues and organs in the body by stimulating previously irreparable organs to heal themselves. Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself. A section in this book is entirely devoted to describing the use of stem cells in muscle repair and treating cardiac and urologic diseases.

Gurdon has spent much of his career deciphering the molecules and mechanisms that an egg uses to “rejuvenate” nuclei. We know a lot about nuclear transfer, but the question remains of how to regulate and control the most efficient way to reprogram the nucleus. Although both Gurdon’s (nuclear reprogramming) and Yamanaka’s (iPS) technologies can generate living animals, we do not know the molecular mechanisms underlying these two strategies. The potential of iPS cell technology in biology and medicine is enormous; however, it is still in its infancy, and there are many challenges to overcome before various applications can be used successfully. We still need to understand the components of oocytes or eggs used to depress

somatic gene expression and discover the direct cell-type switches by over-expressed transcription factors. It is also important to identify the basis for the stability of the differentiated state of cells, which will help us to understand how egg-reprogramming factors operate. Finally, mapping of the “embryome” is a necessity, and it looks as though it will become available soon, which will help us to understand the intricacies and epigenetic imprints of embryos.

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This book is the first joint project of father and son. A portion of the royalties will be contributed to the Dr. Appasani Foundation, a nonprofit organization devoted to bringing social change through the education of youth in developing nations.

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