PREFACE

One critical difference between an embryo and a machine is that a machine is never required to function until after it is built, whereas every animal has to function as it builds itself.

Scott Gilbert, in "Developmental Biology," 6th ed., 2000

1. THE FOUNDATION OF MAMMALIAN DEVELOPMENT

Development of a fertilized human egg into an adult of 75 kg requires the production of 29 trillion cells or about 60 trillion meters of DNA. That's enough DNA to stretch from the earth to the sun and back 200 times! Include the approximately 1 trillion hematopoietic cells produced each day in adults, together with the turnover in epithelial cell populations, and the number of cell divisions in a human life span is truly astronomical!

Remarkably, the first 7–9 rounds of cell division that follow fertilization, the period termed preimplantation to peri-implantation development, establish the machinery that regulates cell proliferation and differentiation throughout these trillions of cell divisions. Each time a cell divides, it must duplicate its nuclear genome accurately once, but only once. Genes in the fertilized egg must soon be expressed to compensate for the loss of maternally inherited RNA and proteins. A developmental program must be established that determines which genes will become active, in which cells, and at what stage in development. The three primary cell lineages that are established in the blastocyst provide the progenitor cells for the fetus, placenta and adult. They also are the source of stem cells that can be derived, propagated, manipulated and differentiated *ex utero*. Thus, preimplantation development is the foundation on which the adult is built. Unfortunately, mistakes appear to occur frequently at the beginning of human development, because miscarriage during early pregnancy is as high as 31% (Regan & Rai, 2000; Wilcox et al., 1988). This book is dedicated to the proposition that understanding mammalian preimplantation development is critical to understanding fertility, embryonic development, and the birth of healthy offspring.

2. THE UNIQUE GOALS OF PREIMPLANTATION DEVELOPMENT

The mouse has been a popular experimental model, because it is cost-effective, amenable to the tools of genetics, biochemistry, and molecular

biology, and has a short gestation period. For example, implantation occurs 4 days postcoitum in mice, whereas humans average 9 days and cows require 30 days until implantation (Lee & DeMayo, 2004). These attributes permitted an extraordinary understanding of preimplantation development in mice that is now being extended to humans. Although the time elapsed between events can vary markedly, the sequence of events from fertilization to implantation is essentially the same in mice, rats, guinea pigs, rabbits, pigs, sheep, cows, and primates. What is striking is that the developmental goals of mammals are unique when compared with animals of other phyla.

In sexual reproduction, the onset of development is triggered by fertilization. Since nuclear DNA replication is dormant in the sperm, oocytes and eggs of mollusks, birds, fish, reptiles, insects, amphibians, and mammals, nuclear DNA replication begins in the *fertilized egg* (also termed *1-cell embryo* or *zygote*). In the well-characterized examples of insects, amphibians, and mammals, fertilization produces a zygote containing two *pronuclei*, one for the paternal chromosomes and one for the maternal chromosomes, and both the maternal and paternal pronuclei replicate their DNA concurrently. The pronuclei then fuse during the first mitosis and cytokinesis to form a *2-cell embryo* in which each cell contains a single diploid nucleus with one set of paternal and one set of maternal chromosomes (Fig. 1). Mitochondrial DNA replication occurs independently of nuclear DNA replication and is linked to the energy requirements of the cell.

The events that occur in a *1-cell embryo* are essentially the same in most, if not all, metazoa, but then they change dramatically following the first mitosis. In sexual reproduction, DNA transcription does not occur in sperm, and it stops in oocytes when they undergo meiotic maturation to form eggs (Zhang et al., 2013). Transcription of the zygote's genes follows DNA replication, although the time when it occurs varies widely among animals. For example, the zygotes of insects develop by repeated rounds of nuclear DNA replication, each separated by a mitosis, but without DNA transcription and cytokinesis. The result is a multinucleated cell termed a syncytial blastoderm that then undergoes cellularization and zygotic gene expression. Amphibian embryos undergo repeated cell cleavage events (cell division in the absence of cell growth), and nuclear DNA replication is followed by mitosis and cytokinesis in the absence of DNA transcription. The result is a multicellular blastula that then activates zygotic gene expression and continues cell proliferation using the mitotic cell cycle, which includes a period of cell growth termed the G1 phase. Mammalian zygotes, however, continue development by cell cleavage in the presence of zygotic gene expression. In the

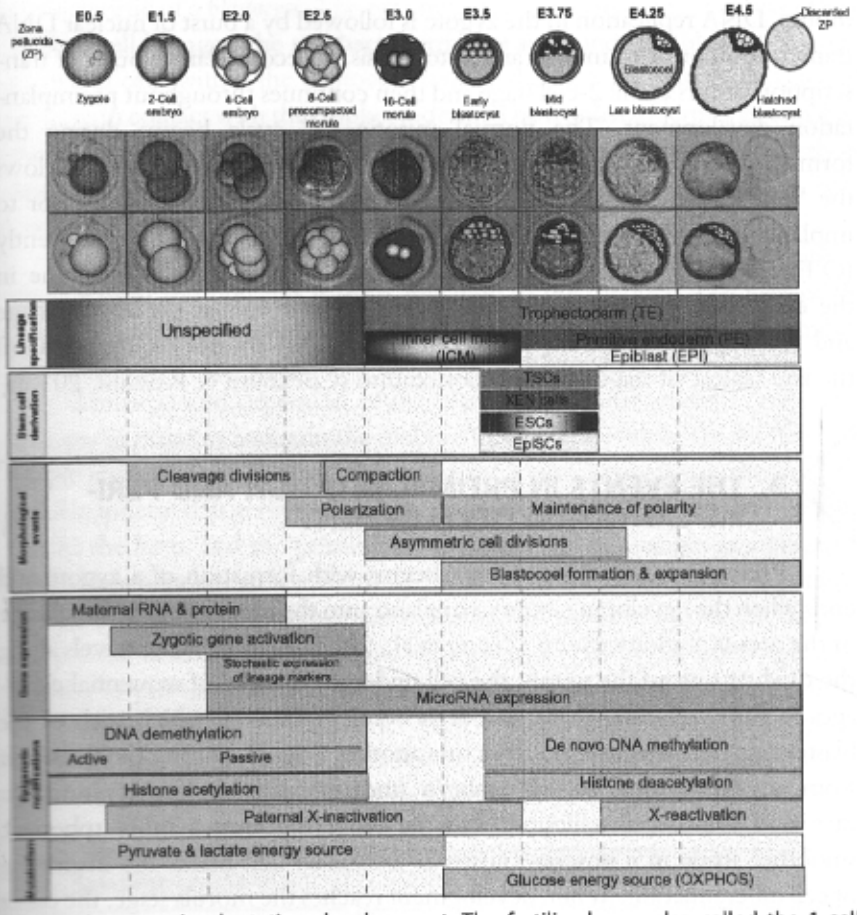

Fig. 1 Mouse preimplantation development. The fertilized egg, also called the 1-cell embryo or zygote, encapsulated by a glycoprotein layer termed the zona pellucida (ZP), undergoes three rounds of cell cleavage divisions to form the 8-cell embryo. At this stage, the totipotent blastomeres, yet unspecified for any defined cell lineage, become flattened, polar and compacted together. Over the next two divisions (8 → 16 and 16 → 32 cells), the first cell lineage decision generates two cell populations. The outer layer of the embryo consists of a monolayer of epithelial trophoblast cells that constitutes the trophectoderm (TE), which give rise to the placenta. The TE (*blue* (*gray* in the print version)) expresses the *Cdx2* gene, but not the *Pou5f1/Oct4* gene. The inner cell mass (ICM) enclosed by the trophectoderm consists of pluripotent blastomeres, which give rise to both the embryo and the extraembryonic endoderm. The ICM expresses the *Oct4* gene, but not the *Cdx2* gene. This step is followed by formation of the early blastocyst, which is characterized by a fluid-filled cavity (blastocoel) generated by water pumped into the blastocyst by the TE. Blastocoel formation requires a major increase in oxidative phosphorylation (OXPHOS) by the trophectoderm that is reflected

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mouse, DNA replication in the zygote is followed by a burst of nuclear DNA transcription prior to mitosis and cytokinesis. A second, larger burst of transcription occurs at the 2-cell stage and then continues throughout preimplantation development. The normal mitotic cell cycle begins during the formation of a compacted morula (Fig. 1). This change in strategy allows the "placental mammals" (eutherians) to begin cell differentiation prior to implantation, which ensures that placenta and fetus develop concurrently (O'Farrell, Stumpff, & Su, 2004). Development is essentially the same in the early human embryo, although compaction occurs at the 16-cell stage and the trophectoderm and inner cell mass (ICM) are not established until the late blastocyst stage at 6 days postcoitum (Cockburn & Rossant, 2010).

3. THE EVENTS IN PREIMPLANTATION AND PERI-IMPLANTATION DEVELOPMENT

Preimplantation development begins with formation of a zygote and ends when the developing embryo implants into the inner mucous membrane of the uterus (endometrium) (Zhang et al., 2013). As the zygote travels along the oviduct toward the uterus, the cell undergoes a series of sequential cleavages to form a 2-cell, 4-cell, and then 8-cell embryo in which each of the blastomeres remains separate from one another (Fig. 1). During the transition from an 8-cell to a 16-cell embryo (morula), the blastomeres undergo increased cell-to-cell adhesion, and the embryo adopts a more spherical, smoother shape in a process known as *compaction* that marks the beginning of cell differentiation. When development reaches the morula stage, the outer blastomeres differentiate into a monolayer of epithelial cells (trophectoderm) that is essential for the formation of the fluid-filled cavity (blastocoel)

Fig. 1—Cont'd in a switch in energy sources from pyruvate and lactate to glucose. Concomitant with blastocoel formation, the second lineage decision results in the segregation of the ICM into primitive endoderm (PE) cells that express *Gata6* (*red* (*dark gray* in the print version)) and epiblast cells (Epi) that express *Nanog* (*yellow* (*light gray* in the print version)). *Nanog*- and *Gata6*-expressing cells are arranged in a "salt and pepper" pattern in the early blastocyst, but by the late blastocyst stage, are sorted into a layer of primitive endoderm that separates the epiblast from the blastocoel. Prior to implantation, the late blastocyst hatches out of the zona pellucida. Different stem cell populations are typically isolated in vitro from blastocyst-stage outgrowths. These include trophoblast stem cells (TSCs), embryonic stem cells (ESCs), epiblast stem cells (EpiSCs), and extraembryonic endoderm stem cells (XEN cells). Numbers above the phase contrast images indicate the number of days postcoitum at the indicated embryonic stage. *Images and figures were provided by Alyson A. Lokken and Amy Ralston.*

characteristic of a *blastocyst*. The preimplantation embryo now consists of two distinct cell lineages: the trophectoderm that surrounds the blastocoel, and a cluster of cells within the blastocoel termed the *inner cell mass* (ICM), which remains in contact with the trophectoderm. The trophectoderm is essential for the subsequent implantation of the embryo, and it contributes exclusively to formation of the placenta, whereas the fetus is derived from the ICM, with minor contributions from the endoderm (Sills et al., 2016).

A protein shell termed the *zona pellucida* surrounds the zygote prior to implantation and prevents it from attaching to the oviduct. The cells of the blastocyst continue to proliferate until the pressure produced by the expanding blastocoel allows the embryo to hatch out of its zona pellucida and implant. The formation and expansion of the blastocoel requires dramatic metabolic changes in order to maintain the embryo's *energy homeostasis*. By the late blastocyst stage (100–200 cells), three distinct tissue lineages are present: the trophectoderm that gives rise exclusively to the placenta, the epiblast that gives rise to the fetus, and the primitive endoderm, which emerges as a polarized epithelium adjacent to the blastocoel with a basement membrane separating it from the epiblast. From the time the blastocyst is free in the uterus, through the process of attachment, until the beginning of trophoblast differentiation is known as *peri-implantation*. A peri-implantation blastocyst results from the ICM segregating into two discrete layers: the epiblast, which gives rise to the fetus, and the primitive endoderm that, along with the trophectoderm, produces extraembryonic tissues, such as the placenta (Fig. 2).

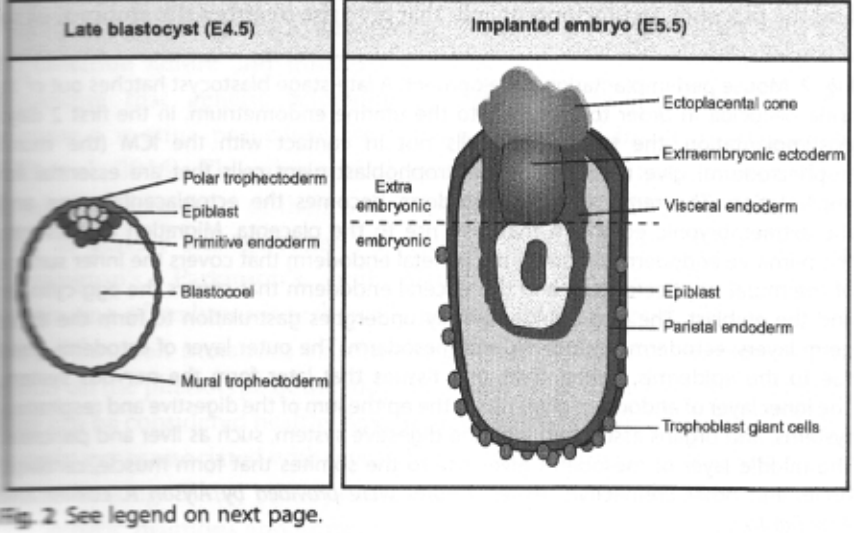

Fig. 2 See legend on next page.

4. RECENT ADVANCES

The purpose of this book is to highlight the fundamental importance of preimplantation development, and the recent breakthroughs in our understanding of the processes that govern it. Taken together, the 12 chapters provide a comprehensive picture of mammalian development from the 1-cell embryo to the period encompassing implantation of the blastocyst. The chapters are grouped into three sections.

Chapters 1–4 address cell proliferation. They address the problems and solutions encountered in triggering DNA replication in the zygote, ensuring that the entire genome is accurately duplicated only once each time a cell divides, but allowing special developmentally programmed cells to overcome this restriction and produce multiple copies of the genome in response to environmental signals. Fertilization also results in transcription and translation of genes in preimplantation embryos. Which genes are activated, and when are they activated? Are they activated in specialized cells at different times during preimplantation development? How are oocytes and eggs that are programmed to undergo meiosis reprogrammed into cells that undergo mitosis? How do cells transit from cell cleavage cycles, when DNA replication and mitosis occur in the absence of cell growth, to mitotic cell cycles when cell growth precedes DNA replication and mitosis?

Chapters 5–10 address cell differentiation. They address the transition from totipotent cells that produce lineages that will form both the embryo and the placenta, to pluripotent cells that give rise to either the embryo, or to

Fig. 2 Mouse peri-implantation development. A late-stage blastocyst hatches out of its zona pellucida in order to implant into the uterine endometrium. In the first 2 days postimplantation, the trophoblast cells not in contact with the ICM (the mural trophectoderm) give rise to polyploid trophoblast giant cells that are essential for implantation. The remaining trophectoderm becomes the ectoplacental cone and the extraembryonic ectoderm that give rise to the placenta. Migration of cells into the primitive endoderm produces the parietal endoderm that covers the inner surface of the mural trophectoderm, and the visceral endoderm that covers the egg cylinder and the epiblast. The egg cylinder quickly undergoes gastrulation to form the three germ layers: ectoderm, endoderm, and mesoderm. The outer layer of ectoderm gives rise to the epidermis, neural crest, and tissues that later form the nervous system. The inner layer of endoderm gives rise to the epithelium of the digestive and respiratory systems, and organs associated with the digestive system, such as liver and pancreas. The middle layer of mesoderm gives rise to the somites that form muscle, cartilage, bone, and other connective tissues. *Figures were provided by Alyson A. Lokken and Amy Ralston.*

multipotent stem cells that produce placenta and other extraembryonic tissues. Obvious signs of differentiation include compaction, wherein a loose collection of totipotent cells suddenly form a compact ball of tightly adhering cells. Embryo compaction is accompanied by a major transition in metabolic energy pathways concomitant with the first example of cell differentiation, specification of the trophectoderm lineage that drives blastocoel formation. Changes in gene expression correlate with epigenetic changes such as histone acetylation and methylation, DNA methylation, and inactivation of the paternal X chromosome. Cell fate acquisition involves changes in gene expression, cell polarity, and cell signaling. Cell fate acquisition not only includes separation of the trophectoderm from ICM, but it also involves the specification of progenitors of embryonic, trophoblast, and extraembryonic endoderm stem cells, all three of which can self-renew and are capable of differentiating into multiple lineage-specific cell types.

Chapters 11 and 12 address the differences that exist in preimplantation development among various mammalian species, and how genetic analysis of preimplantation embryos can overcome human infertility and facilitate the development of healthy offspring.

5. THE FUTURE OF PREIMPLANTATION DEVELOPMENT

5.1 Genetic Diagnosis

Preimplantation genetic screening has the power to improve successful pregnancy rates, minimize miscarriage risk, and limit multiple gestations. Nevertheless, background aneuploidy remains a major factor driving implantation failure and miscarriage for patients experiencing infertility, suggesting that genetic screening of preimplantation embryos should become a routine procedure for patients requesting in vitro fertilization (Sills et al., 2016). This raises ethical questions for parents using technologies to select genetic traits of their offspring. Should we select for genetic traits such as gender and giftedness, as well as against genetic traits such as Huntington's, Parkinson's, and Alzheimer's disease? Whole-genome sequencing of embryos is technically possible (Winand et al., 2014). Many loss-of-function mutations exist in the general population without serious effects on the phenotype of the individual, but more than 40% of individuals who can be considered healthy have mutations that are predicted to be damaging in genes associated with severe Mendelian disorders or are annotated as disease causing. Thus, preimplantation development is already a window into genetic diagnosis and embryo selection.

5.2 Genetic Intervention

How long will it be before preimplantation development is also a window into genetic modification of mammals? Embryonic stem cells, as well as induced pluripotent stem cells, are widely used to model early embryonic events, provide a vehicle for introducing new or altered genes into specific mammalian tissues, and form the basis of cell replacement therapies. Embryonic stem cells have been reverted to a totipotent state that can differentiate in the direction of trophectoderm as well as ICM. Stem cells have been derived from trophectoderm, endoderm, and epiblast tissues. Such stem cells could soon be used to correct genetic traits that are harmful to development, as well as to enhance those traits we deem helpful. It is all a question of ethics (Ethics Committee of the American Society for Reproductive Medicine, 2015), a discussion in which the entire society should be taking part. Just because genetic modifications in the germ line are possible, it does not mean that they are desirable.

6. CANCER STEM CELLS

Preimplantation development not only provides the foundation for construction of a healthy adult, but it might also sow the seeds for its demise. Consider the possibility that some pluripotent embryonic cells do not differentiate during postimplantation development, but enter a quiescent state that allows them to survive into the adult where they eventually resurface as the progenitors of cancers. If this hypothesis is correct, then great caution must be exercised in the application of pluripotent cells to gene therapy.

MELVIN L. DEPAMPHILIS

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