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# Developmental Cell Biology

*Frank J. Dye*
*Western Connecticut State University, Department of Biological & Environmental Sciences, 181 White Street, Danbury, CT 06810, USA*



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**Keywords**

**Cell cycle**
A series of phases, designated G1, S, G2 and M, that a cell sequentially passes through between the time it is formed by cell division and the time it itself divides.

**Chimera**
An organism consisting of parts derived from more than one pair of parents. The chimera of Greek mythology consisted of a lion's head, a goat's body, and a serpent's tail. Chimeras may be made in the laboratory by, in the case of mammals, removing the zona pellucida of each embryo and pushing the sticky embryos together, which merge together to form a single chimeric embryo.

**Embryonic stem cells (ESCs)**
Stem cells derived from the inner cell mass of the blastocyst stage embryo.

**Germ layers**
The three traditional germ layers of the nineteenth century – ectoderm, mesoderm, and endoderm – were expanded during the twentieth century to include a fourth germ layer, the neural crest. Recent inductions of induced pluripotent stem (iPS) cells have shown that differentiated cells developmentally derived from one germ layer (e.g., fibroblasts derived from mesoderm) may be induced to form differentiated cells from another germ layer (e.g., nerve cells normally derived from ectoderm).

**Growth factors**
Extracellular polypeptide signal molecules that can stimulate a cell to grow or proliferate.

**Induced pluripotent stem (iPS) cells**
Adult differentiated cells that have been converted to cells with the pluripotency of embryonic stem cells, generally accomplished by the activation of specific transcription factors.

***In- vitro* fertilization (IVF)**
Human/mammalian fertilization occurring under artificial conditions outside of the body.

**Intracellular signal transduction**
Cascades of chemical reactions used by cells to convey information from the cell surface to the interior of the cell (e.g., the nucleus) where the information is acted upon. The cell makes use of multiple and interacting intracellular signal transduction pathways.

**microRNAs (miRNAs)**
Post-transcriptional regulators that bind to complementary sequences of target messenger RNA (mRNA) transcripts, usually resulting in gene silencing.

**Organogenesis**
Organ formation.

**Pluripotent cell**
A cell that is capable of giving rise to many of the cell types of an organism.

**Receptors**
Proteins that bind other molecules or ions and, as a result of the binding, influence cellular activity.

**Stem cell**
A cell that undergoes mitotic cell division to give rise to the same type of cell. At some point, stem cells leave the pool of mitotically dividing cells to begin a process of cell differentiation.

**STAP cells**
Stimulus-triggered acquisition of pluripotency cells. During early 2014, STAP cells were reportedly created by simply immersing mouse cells briefly into a mild acid bath. About 7–9% of cells from newborn mice survived the acid treatment and took just a week to form STAP cells. Haruko Obokata and her colleagues, of the RIKEN Center for Developmental Biology in Kobe, Japan, and Harvard Medical School, reportedly transformed blood, skin, brain, muscle, fat, bone marrow, lung, and liver cells from newborn mice into STAP cells. When injected into mouse embryos, STAP cells reportedly not only incorporated into any body tissue but also formed parts of the placenta. According to news reports, later in 2014, Obokata, who led the researchers reporting that dipping adult cells in acid could change them into stem cells, agreed to retract one of the papers describing the result.

**Transcription factors**
Proteins that attach to DNA at a gene regulatory site, and by so doing influence the rate of transcription of a specific gene.

It is said that Thomas Hunt Morgan, the recipient of a Nobel Prize in Physiology or Medicine, in 1933, for his discoveries concerning the role played by the chromosome in heredity, started out as an embryologist but switched to genetics. At the time, the

awarded the first Nobel Prize in embryology, a Nobel Prize in Physiology or Medicine, in 1935, for his discovery of the organizer effect in embryonic development. Sixty years later, in 1995, a Nobel Prize in Physiology or Medicine was awarded to Edward B. Lewis, Christiane Nusslein-Volhard, and Eric F. Wieschaus for their discoveries concerning the genetic control of early embryonic development. Ironically, Edmund Beecher Wilson's book, *The Cell in Development and Heredity*, 3rd edition, published in 1925, is considered to be a classic in cytology (cell biology), genetics and embryology. The point being made is that the twentieth century witnessed the dissolution of the boundaries between embryology (developmental biology) and genetics. The following chapter makes it clear that this integration continues at an unabated pace.

## 1 Introduction

Developmental cell biology is concerned with the consequences of what cells do in the context of developing organisms. Developmental biologists are also concerned with events before fertilization (e.g., gametogenesis) and after birth – or its equivalent in nonmammalian species (e.g., metamorphosis and regeneration). The range of species in the purview of the developmental biologist covers, essentially, the entire living world – animals, plants, protists, fungi, and even prokaryotes. In the creation of a human being, cell proliferation is required to create the trillions of cells of which the body is composed. Cell differentiation provides the more than 200 different types of cell identified to date in the human body, while morphogenesis shapes the body form characteristic of the human species.

The present understanding of molecular and cellular mechanisms that underlie these phenomena has been progressing at an ever-increasing pace, a testimonial to the army of cell and developmental biologists at work across the planet. To understand the regulation of the cell cycle, apoptosis, cell differentiation, pattern formation and cell movement (to mention but a few such phenomena), to understand the integration of their underlying molecular and cellular mechanisms, and to understand the developmental consequences of their operation, makes the early twenty-first century an exciting time to be a developmental cell biologist. It is also a hopeful time for those people suffering the ravages of dreadful diseases or injuries, such as cancer or spinal cord injuries, and for those awaiting the promise of regenerative medicine.

## 2 Historical Perspective

Cell biology involves the investigation of the molecular basis of how cells work, for example, how cells move, how organelles are moved within cells, and how genes are regulated. Developmental biology is concerned with understanding how these processes are integrated to create the organism: for example, how cells move collectively to accomplish gastrulation; how cells are polarized as part of cell differentiation; and how cell communication affects the origin of different cells types within close proximity to each other to create an organ composed of functionally integrated tissues. Midway through the second decade of the twenty-first century, the boundaries between biochemistry, molecular biology, genetics, cell biology,

and developmental biology have all but vanished.

## 2.1 Origins of Cell Biology

The Cell Theory of Schwann and Schleiden (1838/1839) is a scientific milestone of the nineteenth century. Schwann and Muller (Johannes Muller, German physiologist and comparative anatomist) saw the implications of the cell theory for human pathology. Rudolph Virchow, who stated the famous aphorism, "omnis cellula e cellula," laid the foundation for cellular pathology during the middle of the nineteenth century, replacing the long-lived humoral pathology concept of disease causation. The realization that organisms are composed of cells, coupled to the development of improved microscopy, made the study of early development possible. During the last quarter of the nineteenth century, the fundamental details of mitosis and meiosis were elucidated at the level of the light microscope.

## 2.2 Origins of Developmental Biology

The nineteenth century – a century pregnant with advances in cytology (cell biology) and embryology (developmental biology) – saw the laying of the foundations of descriptive and comparative embryology, while the end of this same century provided the beginnings of experimental embryology. Karl Ernst von Baer (1792–1876), an Estonian naturalist and pioneer embryologist, is considered by some to be the father of modern embryology. He was the first to describe the mammalian egg (1827), and became the first to trace the development of the egg to produce the embryo; his *Uber Entwickelungsgeschichte der Tiere* (1828) became a standard text of embryology. Also regarded as the father of comparative embryology, Von Baer was the first to make embryology truly comparative. Together with Christian Pander (a Russian zoologist, regarded as a founder of the science of embryology), he formulated the germ layer concept as a structural fact for vertebrate embryology (see also Ref. [1]). Wilhelm Roux (1850–1924), a German embryologist, is considered to be a pioneer in (and by some, the founder of) experimental embryology. On the basis of his experimental results, Roux concluded that eggs are self-differentiating rather than being driven by external conditions.

## 2.3 Relationship between Cell and Developmental Biology

By the dawn of the twentieth century, cell biology and developmental biology – although not generally referred to as such at the time – were burgeoning disciplines of biology. Early in the twentieth century, Wilson [2] (1856–1939), an American zoologist specializing in cytology and embryology, wrote *The Cell in Development and Heredity* (3rd edition, 1925). This book is correctly considered to be a classic in, at least, the fields of cytology (cell biology) and embryology (developmental biology). According to Gilbert [3], the experiments of Hans Spemann (1869–1941; a German zoologist and 1935 recipient of the Nobel Prize in Physiology or Medicine for his discovery of the organizer effect in embryonic development) and his students framed the questions that experimental embryologists asked for most of the twentieth century (see also Ref. Saha [4]). In the editorial of the article, "Where Cell Biology and Developmental Biology Meet," in the inaugural issue of the journal, *Developmental*

*Cell*, **1** (1): 1 (2001), Siegel and Sweet proposed that it really does seem as though we are beginning to understand how cells function – both individually and as parts of multicellular organisms – and that there is a growing interaction and overlap between cell biology and developmental biology. During the second half of the twentieth century, the merging of cell and developmental biology was reflected in the titles of books and journals (e.g., *Annual Review of Cell Biology* became *Annual Review of Cell and Developmental Biology*, *In Vitro* became *In Vitro Cell and Developmental Biology*, and, in 2001, Cell Press established a new journal, *Developmental Cell*). Just as cell biologists endeavored to explain cellular phenomena at the molecular level, during the twentieth century it became increasingly clear that development needed to be explained at the cellular level. Attempts to explain the mechanisms and control of cell proliferation, cytodifferentiation, embryo patterning, and morphogenesis necessitated molecular understanding [5].

### 2.4 Nuclear Equivalence

In a recent publication, Daley [6] traced the well-known history of the concept of nuclear equivalence; namely:

1) In 1885, August Weismann published the theory that development worked by "qualitative divisions" among daughter cells, which *segregated* subsets of heritable material to specify their unique traits.
2) In 1888, Wilhelm Roux pricked and ablated one cell of a two-cell frog embryo and observed the formation of a "half-embryo," and suggested that, even at the two-cell stage, the embryonic blastomeres were nonequivalent – an experiment consistent with the notion of *qualitative* division.
3) In 1892, Hans Driesch challenged that interpretation when he microdissected and separated sea urchin embryos at the two-cell stage and observed the formation of two equivalent sea urchins, thereby extending the notion of *nuclear equivalence* at least to the two-cell stage.
4) Later, Hans Spemann tied tiny hairs from his daughter's head around early-stage newt embryos, separating early blastomeres and observing the formation of two normal newts (albeit one smaller than the other), proving *developmental equivalence* up to the eight-cell stage.
5) Spemann famously envisioned, but never technically realized, a "fantastical experiment" whereby the nucleus of a highly differentiated cell might be transplanted back to the egg, to test whether it would remain specialized or would manifest embryonic potential.
6) Reporting precisely that experiment in 1952, Briggs and King suggested that cells *lose* the ability to support normal embryonic development as development and cell specialization progresses (*Rana pipiens*).
7) Gurdon established that *normal* development to adulthood could be achieved by the transfer of fully differentiated nuclei from the intestinal cells of feeding-stage larvae (*Xenopus*, 1962). Gurdon's profound contribution represents the foundation of the current assumptions about *nuclear equivalence* … the intellectual foundation of the excitement that has consumed the last 15 years of *stem cell biology* [6] (italics added for emphasis by the present author (see Sect. 7; Stem Cells).

## 3
## Cell Activities Underlying Development

### 3.1
### Intracellular Signal Transduction

Developmentally important cellular activities include cell division and differential gene expression. In a multicellular organism, these activities are under the control of the cell's environment, including signals from other cells. Although some of these signals (e.g., steroid hormones) are nonpolar and able to pass through the plasma membrane of the cell, other signals are polar (e.g., hormone epinephrine) and attach to receptors in the plasma membrane. In order to convey information carried by the signal from the cell surface to the interior of the cell (e.g., the nucleus), where the information is acted upon, the cell makes use of cascades of chemical reactions that make up intracellular signal transduction pathways.

#### 3.1.1 Receptors

Receptor proteins are proteins that bind other molecules or ions and, as a result of the binding, can influence cellular activity; examples include insulin receptors in the cell surface and progesterone receptors in the cytoplasm. A *morphogen* is a substance that can direct the differentiation of cells since, along a gradient of morphogenetic substances, the cells respond differently at different concentrations. A morphogen receptor gradient is a gradient of those molecules that recognize the morphogen; the expression of the *Brachyury* and *goosecoid* genes has been correlated with the number of activin receptors on each cell that binds activin.

#### 3.1.2 Intracellular Signal Transduction

Signal transduction involves the conversion of a signal from one form to another form; for example, the conversion of a cAMP signal to a kinase signal as an intracellular signal transduction pathway progresses into the interior of a cell. The receptor tyrosine kinase (RTK) signal transduction pathway is an intracellular signal transduction pathway that is important in development, and was one of the first pathways to unite various areas of developmental biology. In the case of migrating neural crest cells of humans and mice, the RTK pathway is important in activating the microphthalmia transcription factor (Mitf) to produce pigment cells. Homologous signal transduction pathways are composed of homologous proteins arranged in a homologous manner. Such pathways form the basic infrastructure of development, yet the targets of these pathways may differ among organisms; for example, the dorsal–cactus pathway used in *Drosophila* for specifying dorsal–ventral polarity is also used by the mammalian immune system to activate inflammatory proteins. When homologous developmental pathways are used for the same function in both protostomes and deuterostomes, they are said to have a "deep" homology. Homologous signal transduction pathways illustrate the conservation of molecular mechanisms across taxons.

#### 3.1.3 Transcription Factors

A transcription factor is a molecule that attaches to DNA at a gene regulatory site and by so doing influences the rate of transcription of a specific gene; a protein that regulates the transcription of genes, often, but not exclusively, by binding to *cis*-regulatory elements (promoters and enhancers). Transcription factors exist as two general types: (i) basal transcription factors (e.g., TFIID, TFIIA) which attach to gene promoters; and (ii) cell-specific transcription factors (e.g., Pax6, Mitf) which bind to gene enhancers. *Trans*-regulatory

factors are usually transcription factors. There are four major families of transcription factors based on DNA-binding motifs: homeodomain; basic helix-loop-helix (bHLH); basic leucine zipper (bZip); and zinc finger. The *trans*-activating domain of a transcription factor is concerned with activating or suppressing the gene's transcription.

A transcription initiation complex is, collectively, the RNA polymerase and associated basal transcription factors that attach to a gene promoter. A battery is a group of genes regulated by the same transcription factor. Zygotic genes are those genes expressed by the embryonic genome; the embryo's genes as opposed to maternal genes expressed in the oocyte by the maternal genome. In early *Drosophila* development, most of the zygotic genes, which first are activated along the anteroposterior and dorsoventral axes set up by maternal genes, encode transcription factors, which then activate more zygotic genes.

Combinatorial regulation is the control of gene transcription by two or more transcription factors; the spatial patterns of gene expression are often delimited by the combined action of transcription factors. Historically, the bicoid protein gradient provided the first reliable evidence for the existence of morphogen gradients that had been postulated to control pattern formation. The bicoid protein is a transcription factor that acts as a morphogen; it switches on certain zygotic genes at different threshold concentrations, thus initiating a new pattern of gene expression along the axis. The bicoid protein is a member of the homeodomain family of transcriptional activators and activates the *hunchback* gene by binding to regulatory sites within the promoter region.

## 3.2 Cell Signaling

Signaling between and among cells is necessary for order, that is the organism, to emerge from potential chaos, which is multicellularity. Signaling between cells of the anterior pituitary gland and the gonads orchestrates the development of gametes, and signaling between cells may result in the form of cell death known as apoptosis.

### 3.2.1 Juxtacrine Signaling

Juxtacrine signaling is a mode of cell–cell communication in which signaling molecules are retained on the surface of the signaling cell and interact with receptor proteins on adjacent cell surfaces. An example is the interaction between Bride of Sevenless protein and its receptor Sevenless, in fly eye photoreceptor specification (see Sections 3.2.2, 3.2.3 and 3.2.4).

### 3.2.2 Autocrine Signaling

Autocrine signaling is a mode of cell–cell communication in which signaling molecules (autocrine factors) attach to receptors on the same cell that produced them. An example is the explosive proliferation of placental cytotrophoblast cells in response to platelet-derived growth factor (PDGF), which these cells themselves produce.

### 3.2.3 Paracrine Signaling

Paracrine signaling is a mode of cell–cell communication in which signaling molecules (paracrine factors) act as local mediators and only affect cells in the immediate environment of the signaling cell. Paracrine factors are the protein molecules used in paracrine signaling; *these factors are the inducing factors of the classical experimental*

*embryologists.* Most paracrine factors fall into one of four major families: Hedgehog; Wnt; transforming growth factor-β (TGF-β); and fibroblast growth factor (FGF).

#### 3.2.4 Endocrine Signaling

Endocrine signaling is a mode of cell–cell communication in which signaling molecules (endocrine factors) are released into the circulatory system and may affect cells (target cells) that are some distance from the signaling cell. An example is the effect of anterior pituitary gland hormones on the gonads. Endocrine factors are molecules (hormones) that function in endocrine signaling, and include estrogens, testosterone, progesterone and polypeptide hormones such as the gonadotropins. Endocrine mimics (endocrine disruptors) are exogenous substances that act like hormones in the endocrine system and disrupt the functions of endogenous hormones; examples are DDT, polychlorobiphenyls (PCBs), bisphenol A, and phthalates.

#### 3.2.5 Growth Factors

Growth factors are extracellular polypeptide signaling molecules that can promote cell proliferation, and regulate cytodifferentiation, cell survival, and cell death. FGFs constitute a family of protein growth factors that were first identified as mitogens for fibroblasts in tissue culture, and which stimulate the proliferation of many cell types, inhibit the differentiation of various types of stem cells, and *act as inductive signals in embryonic development*. For example, FGF – which plays a key role in the induction of ventral mesoderm in *Xenopus* embryos – is secreted by vegetal cells. Apoptosis may be initiated by a withdrawal of growth factors from the cell, or by an active response to a signal.

### 3.3 Cell–Cell Interaction

Cells may interact with each other in a variety of ways. In addition to cells interacting through cell signaling of the juxtacrine, autocrine, paracrine and endocrine varieties, cells may make physical contact with each other through a variety of cell junctions, termed tight junctions, gap junctions, and desmosomes.

#### 3.3.1 Cell Junctions

Cell junctions are specialized regions of contact between cells, and generally fall into three categories: tight junctions; gap junctions; and desmosomes.

- *Tight junctions* are specialized contacts formed between cells that establish partitions between isolated compartments of the body. During the compaction of early mammalian embryos, tight junctions form between the cells of the trophoblast; these cells with their tight junctions seal the blastocyst cavity off from the embryo's environment.
- *Gap junctions* are specialized contacts formed between cells that establish cytoplasmic continuity between the cells. Cells with these junctions rapidly communicate with each other. During the compaction of early mammalian embryos, gap junctions form between the cells of the inner cell mass (ICM).
- *Desmosomes* are a type of cell junction that join *one epithelial cell to another* and provide structural integrity to an epithelium; *hemidesmosomes* join *epithelial cells to the basal lamina*, a specialized extracellular matrix (ECM) on which epithelial cells reside.

#### 3.3.2 Cell Signals

A pathway in the context of cell–cell signaling consists of the components required

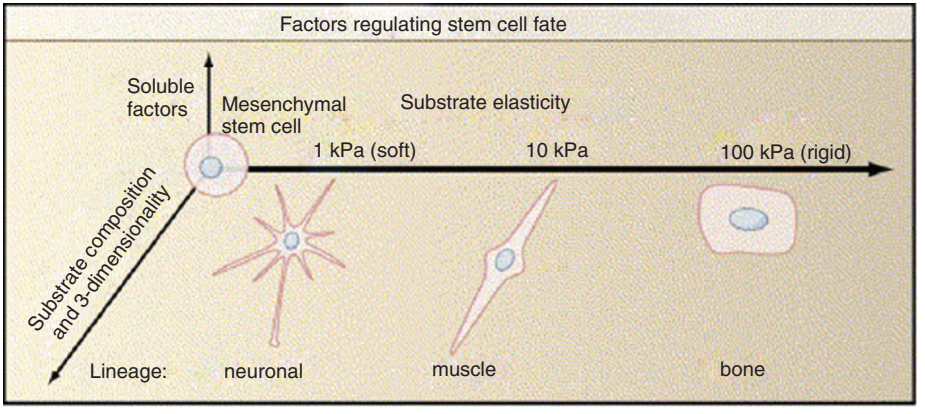


**Fig. 1** Effect of substrate elasticity on stem cell fate. Reproduced with permission from Ref. [8].

for the sending, receiving and transduction of a signal, including one or more ligands, membrane-associated receptors, intracellular signal transducers and, depending on the type of pathway, transcription factors.

### 3.4 Cell–Matrix Interaction

Growing, quiescent and dying cells may all be found within the same microenvironment during morphogenesis. Additionally, normal stem cell biology depends on the availability of the stem cell niche. The ECM forms one component of the cell's microenvironment. In fact, it has been shown that the tension exerted by a stem cell's ECM may determine the nature of that stem cell's differentiation. Engler *et al.* [7] examined the effect of matrix elasticity on the differentiation of human mesenchymal stem cells (MSCs), and showed that soft matrices favored the differentiation of mesenchymal stem cells into neuronal-like cells, a moderate elasticity promoted myogenic differentiation, and a rigid matrix stimulated osteogenic differentiation (see Fig. 1).

Recently, Eyckmans *et al.* [9] have reviewed the burgeoning field of mechanobiology, maintaining that mechanical forces are ubiquitous *in vivo* and that these forces directly impact cell function, and that such forces regulate morphogenesis, cell migration, cell adhesion to the ECM, as well as cell proliferation and differentiation. Although, Eyckmans and coworkers suggested that the beginnings of mechanobiology date back to 1892, and that mechanobiology received relatively little attention for much of the twentieth century, the recent renaissance in studies of mechanics – primarily in cell culture – has been based on tools that enable the measurement and manipulation of mechanical forces *in vitro*. The topics considered by Eyckmans *et al.* were: (i) the current understanding of the role of mechanical forces in cell biology; (ii) techniques that are being developed to enable such studies; and (iii) recent efforts to consider mechanical forces in development. Mechanobiology constitutes yet another example of the merging of cell and developmental biology.

#### 3.4.1 Integrin Signaling

Cells reside in a protein network, the ECM, which they secrete into the extracellular space. The ECM exerts a profound control

over cells, the effects being mediated primarily by integrins, a family of cell-surface receptors that attach cells to the matrix and mediate the mechanical and chemical signals from it. The integrins span the plasma membrane, bind the fibronectin of the ECM, and provide anchorage sites for the actin microfilaments of the cytoskeleton; that is, they integrate the extracellular and intracellular scaffolds. Most integrins recognize several ECM proteins, and individual ECM proteins (e.g., fibronectin, laminins, collagens, and vitronectin) bind to several integrins. Integrins can signal through the plasma membrane in either direction: the extracellular binding activity of integrins is regulated from inside the cell, while the binding of the ECM elicits signals that are transmitted into the cell.

Adherent cells must be anchored to an appropriate ECM to survive; depending partly on the signals from the matrix, they either proliferate or exit the cell cycle and differentiate. This anchorage requirement is lost in neoplastic cells. The cytoplasmic domains of integrins always lack enzymatic activity, and thus they transduce signals by associating with adaptor proteins that connect the integrin to the cytoskeleton, cytoplasmic kinases, and transmembrane growth factor receptors. Integrin signaling and assembly of the cytoskeleton are intimately linked. As integrins bind to the ECM, they become clustered in the plane of the plasma membrane and associate with a cytoskeletal and signaling complex that promotes the assembly of actin filaments. The reorganization of actin filaments into larger stress fibers, in turn, causes more integrin clustering, thus enhancing the matrix binding and organization by integrins in a positive feedback system. As a result, ECM proteins, integrins and cytoskeletal proteins assemble into aggregates on each side of the membrane. Well-developed aggregates detectable by immunofluorescence microscopy are known as focal adhesions and ECM contacts. Thus, integrins serve as integrators of the ECM and the cytoskeleton, the property for which integrins are named.

### 3.4.2 **Cell Movement**

Cell-shape changes and cell movements underlie the morphogenetic movements that create the form of the developing organism. Examples of the burgeoning literature on this topic include reviews by Keller [10], Adler [11], and Hall [12]. Polarized cell movements shape the major features of the vertebrate body plan during development. The head-to-tail body axis of vertebrates is elongated during the embryonic stages by "convergent extension" tissue movements during which cells intercalate between one another and transverse to the elongating body axis to form a narrower, longer, array. Recent studies have shown that these polarized cell movements are controlled by homologs of genes that control the polarity of epithelial cells in the developing wing and eye of *Drosophila*.

The actin cytoskeleton mediates a variety of essential biological functions in all eukaryotic cells. In addition to providing a structural framework around which cell shape and polarity are defined, its dynamic properties provide the driving force for cells to move and to divide. Understanding the biochemical mechanisms that control the organization of actin is therefore a major goal of current cell biology, with implications for both health and disease. Members of the Rho family of small guanosine triphosphatases have emerged as key regulators of the actin cytoskeleton, and, furthermore, through their interaction with multiple target proteins, they ensure a coordinated control of other cellular activities such as gene transcription and

adhesion. Observations have suggested that members of the Rho GTPase family are key regulatory molecules that link surface receptors to the organization of the actin cytoskeleton. The JAK/STAT pathway is a central component of the signal transduction network that controls cell proliferation, fate, and movement. Despite the present understanding of actomyosin function in individual migrating cells, little is known of the mechanisms by which actomyosin drives collective cell movement in vertebrate embryos. The collective movements of convergent extension drive both the global reorganization of the early embryo and local remodeling during organogenesis. Shindo and Wallingford [13] have reported that planar cell polarity (PCP) proteins control convergent extension by exploiting an evolutionarily ancient function of the septin cytoskeleton. By directing septin-mediated compartmentalization of cortical actomyosin, PCP proteins coordinate the specific shortening of mesenchymal cell–cell contacts, which in turn powers cell interdigitation. The data obtained by Shindo and Wallingford have illuminated the interface between developmental signaling systems and the fundamental machinery of cell behavior, and should provide insights into the etiology of human birth defects such as spina bifida and congenital kidney cysts [13].

## 4 Cell Differentiation

As the field of cell differentiation has generated vast literature, two models of cell differentiation will be considered here: (i) the role of retinoblastoma protein (pRB) in differentiation; and (ii) a model of how retinoic acid (RA) and FGF9 act antagonistically to determine germ cell fate.The active role of pRB in differentiation has been studied in a number of cell lines inducible for differentiation. In each instance, an early event in differentiation was the dephosphorylation of pRB, which correlated with cell cycle arrest in $G_0/G_1$, a prerequisite to enter the differentiation pathway. During the differentiation of muscle cells, pRB accumulates in the nucleus and forms complexes with muscle-forming transcription factors such as MyoD and myogenin, thus preventing pRB rephosphorylation and locking the cell in the differentiated state. However, the inactivation of pRB reverses the differentiated phenotype and allows cells to re-enter the cell cycle. Ajioka *et al.* [14] reported a new mouse model of retinoblastoma, which bears on the relationship between cell differentiation and the cell cycle. These authors showed that retinoblastoma is not driven by uncontrolled expansion of retinal progenitor cells, but rather is the result of cell cycle re-entry and expansion of differentiated horizontal interneurons in the retina (see Fig. 2).

Recent studies conducted by Bowles *et al.* [16] have provided details about how the sex determination of mammalian germ cells occurs during fetal development and depends on signals from gonadal somatic cells. It had been established previously that RA triggers ovarian germ cells to enter meiosis and thereby commit to oogenesis whereas, in the developing testis, the enzyme CYP26B1 degrades RA and the germ cells are not induced to enter meiosis. Bowles *et al.* [16], using *in-vitro* and *in-vivo* models, showed that FGF9 produced in the fetal testis acts directly on germ cells to inhibit meiosis; in addition, FGF9 maintains the expression of pluripotency-related genes and upregulates markers associated with male germ cell fate. Based on these data, it was concluded that two independent and mutually antagonistic

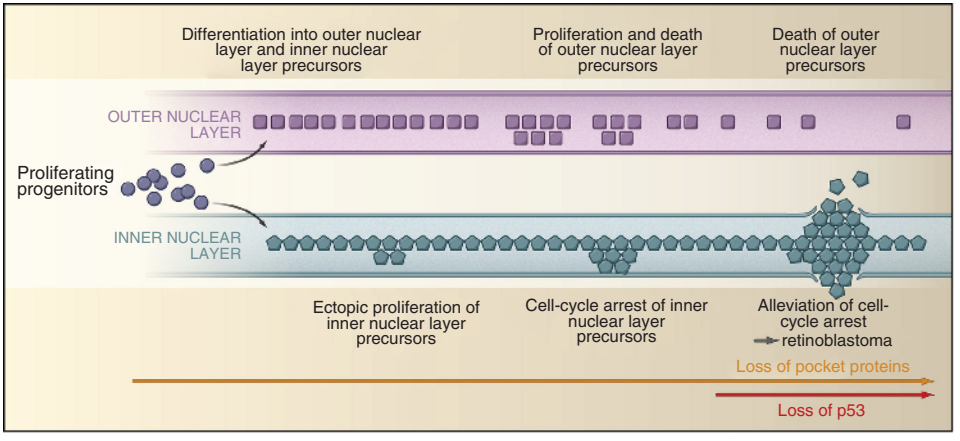


**Fig. 2** Retinoblastoma development in mice. The loss of pocket proteins allows proliferating progenitor cells in the retina to differentiate into outer and inner nuclear layer precursor cells. These cells continue to proliferate at least for a while, but then certain retinal cell types (rods, cones, ganglion, and bipolar cells) die, whereas other retinal cells (amacrine, horizontal, and Muller glia cells) survive but become arrested in the cell cycle. A second event that suppresses the p53 pathway may alleviate cell-cycle arrest, leading to proliferation of differentiated cells. Reproduced with permission from Ref. [15].

pathways involving RA and FGF9 act in concert to determine mammalian germ cell sexual fate commitment, and support a model in which the mitosis/meiosis switch is robustly controlled by both positive and negative regulatory factors (see Fig. 3).

D'Angelo *et al.* [17] showed that a specific change in nuclear pore complex (NPC) composition is required for both myogenic and neuronal differentiation. The transmembrane nucleoporin Nup210 is absent from proliferating myoblasts and embryonic stem cells (ESCs), but becomes expressed and incorporated into NPCs during cell differentiation. Furthermore, the prevention of Nup210 production by RNAi blocks myogenesis and the differentiation of ESCs into neuroprogenitors. D'Angelo and colleagues found that the addition of Nup210 to NPCs did not affect nuclear transport, but was required for the induction of genes that are essential for cell differentiation. These results identified a single change in NPC composition as an essential step in cell differentiation, and established a role for Nup210 in gene expression regulation and cell fate determination (see Fig. 4).

## 5 The Cell Cycle and Development

The relationship between the cell cycle and development is a topic that is of great interest to developmental cell biologists. Since its elucidation during the 1950s, the canonical cell cycle, $G_1$, S, $G_2$, M, has been studied intensively by cell biologists. It has long been known that the cell cycle of embryos undergoing cleavage is not classical, but rather is an abbreviated cycle consisting of S alternating with M. This embryonic (before gastrulation) cell cycle, using maternal gene products and not providing time ($G_1$) for cell growth,

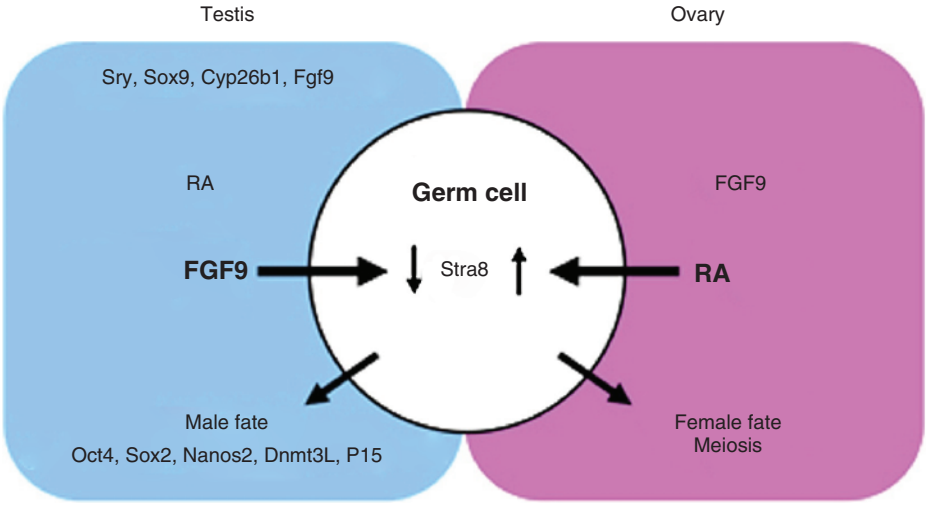


**Fig. 3** Retinoic acid (RA) and fibroblast growth factor 9 (FGF9) act antagonistically to determine germ cell fate. The germ cell sexual fate is determined by two signaling molecules produced by the somatic cells of the gonad, FGF9, and RA. Cyp26b1 and Fgf9 are highly expressed in the testis, but are downregulated in the ovary. Since CYP26B1 degrades endogenous RA, levels of RA are low in the testis while FGF9 levels are high. In the ovary, RA is not degraded and FGF9 levels are low (one white cell shown) to upregulate Stra8 (RA) or to prevent its upregulation (FGF9). Stra8 expression in gonadal germ cells is essential for entry into meiosis, by an unknown mechanism. FGF9 acts directly on germ cells to antagonize Stra8 expression, maintain expression of pluripotency markers, Oct4 and Sox2, and to induce male germ cell fate markers, Nanos2, Dnmt3L, andP15. Reproduced with permission from Ref. [16].

results in cleavage of the zygote into an ever-increasing number of blastomeres of progressively decreasing size; that is, multicellularity is achieved at the expense of cell size. The midblastula transition of early *Xenopus* development has been used to study the conversion of S/M to $G_1$/S/$G_2$/M. Cell cycle alteration and regulation during development is a fundamental topic in considering the relationship between the cell cycle and development (also see Ref. [18]).

*Xenopus* oocytes and early embryos provide excellent systems for studying the relationship between the cell cycle and development. *Xenopus* embryos bracketing the midblastula transition provide a developing system for the study of the sequential establishment of cell cycle checkpoints and the connection of signaling pathways to regulation of cell cycle progression.

The extent to which the cell cycle can be altered, to serve the changing needs of the developing organism, is provided by studying *Drosophila* development. *Drosophila* embryonic cells undergo minimal growth between divisions, and the key event during this developmental phase is patterning. As development progresses to the larval phase, the developmental objective changes, and so too does the nature of the cell cycle. The major objective of *Drosophila* larval tissues is cell growth rather than cell proliferation: the larval cells undergo repeated rounds of endoreplication, an effort that may be designed to increase the total gene dosage in a given cell in an effort to support drastically increased cell size.

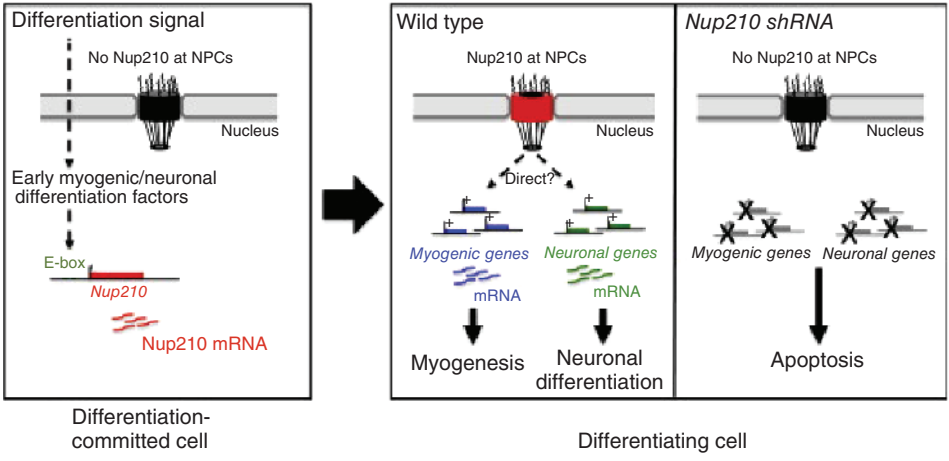


**Fig. 4** Schematic model of Nup210 regulation of cell differentiation. In undifferentiated myoblasts the expression of Nup210 is repressed. Early differentiation signals activate Nup210 gene expression. In myoblasts, Nup210 induction is likely carried out by myogenin/MyoD binding to its promoter E boxes. Nup210 protein is then recruited to the NPC where it regulates the expression of genes required for myogenic and neuronal differentiation. Prevention of Nup210 addition to the NPC by shRNAs prevents the activation of Nup210-regulated genes and leads to the death of the differentiation-committed cell by apoptosis. Reproduced with permission from Ref. [17].

Imaginal disc cells in the developing larva must undergo both growth and patterning, and exhibit a "classical" $G_1$/S/$G_2$/M cell cycle, with a growth-sensitive cell cycle checkpoint at the $G_1$/S boundary and a patterning checkpoint at the $G_2$/M boundary. The developmental program thereby neatly adjusts the cell cycle to promote patterning without growth, growth without patterning, or growth and patterning simultaneously, as each is required for proper development.

The polycomb group (PcG) proteins control development and cell proliferation through chromatin-mediated transcriptional repression. Mohd-Sarip *et al.* [19] described a transcription-independent function for the PcG proteins Posterior sex combs (PSCs) in regulating the destruction of cyclin B (CYC-B). A substantial portion of PSC was found outside canonical PcG complexes, instead of being associated with CYC-B and the anaphase-promoting complex (APC). Cell-based experiments and reconstituted reactions have established that PSC and Lemming (LMG; also called APC11) associate and ubiquitylate CYC-B cooperatively, marking it for proteosomal degradation. Thus, PSC appears to mediate both developmental gene silencing and the post-translational control of mitosis. Direct regulation of cell cycle progression might be a crucial part of the PcG system's function in development and cancer [19].

## 6 Organogenesis

Tissues and organs are composed of differentiated groups of cells. Each organ has a characteristic structure and function, which emerges during development of the embryo, and thus needs to be studied on an individual basis. The practical applications

of a general understanding of organogenesis will include the repair and/or replacement of a patient's compromised organs, most likely starting with the patient's own stem cells [20].

The process of organogenesis has a prerequisite step, namely the specification of spatially defined regulatory domains that promote the differentiation programs. This commonplace mechanism enables the recognition and definition of regulatory fields as discrete territories of specific gene activities. Selector genes control the formation and identity of the various fields, while field-specific genes are a special class of selector genes that have the unique property of directing the formation of complex, specialized structures such as organs. Examples of such organ-identity genes include: *Pax6/eyeless*, which is required for eye formation in *Drosophila* imaginal discs, and *Pit 1* which, together with *Gata2*, controls pituitary differentiation. Fang *et al.* [21] carried out a genome-wide expression analysis of embryonic development and reported transcriptome profiles of human early embryos covering development during the first third of organogenesis. In this case, two major categories of genes were identified that displayed gradually reduced or gradually increased expression patterns across this developmental window. The decreasing group appeared to include stemness-specific and differentiation-specific genes that are important for the initiation of organogenesis, whereas the increasing group appeared to be largely differentiation-related and indicative of diverse organ formation (see Fig. 5). Based on these findings, a putative molecular network was devised that may provide

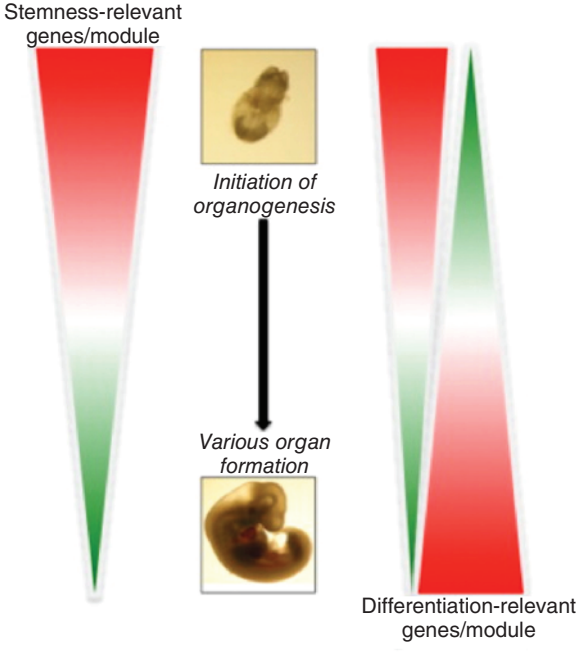


**Fig. 5** Transcriptome analysis of early organogenesis in human embryos. Reproduced with permission from Ref. [21].

a framework for the regulation of early human organogenesis.

The complexity of the human brain has made it difficult to study many brain disorders in model organisms, and has highlighted the need for an *in-vitro* model of human brain development. Lancaster *et al.* [22] have developed a human pluripotent

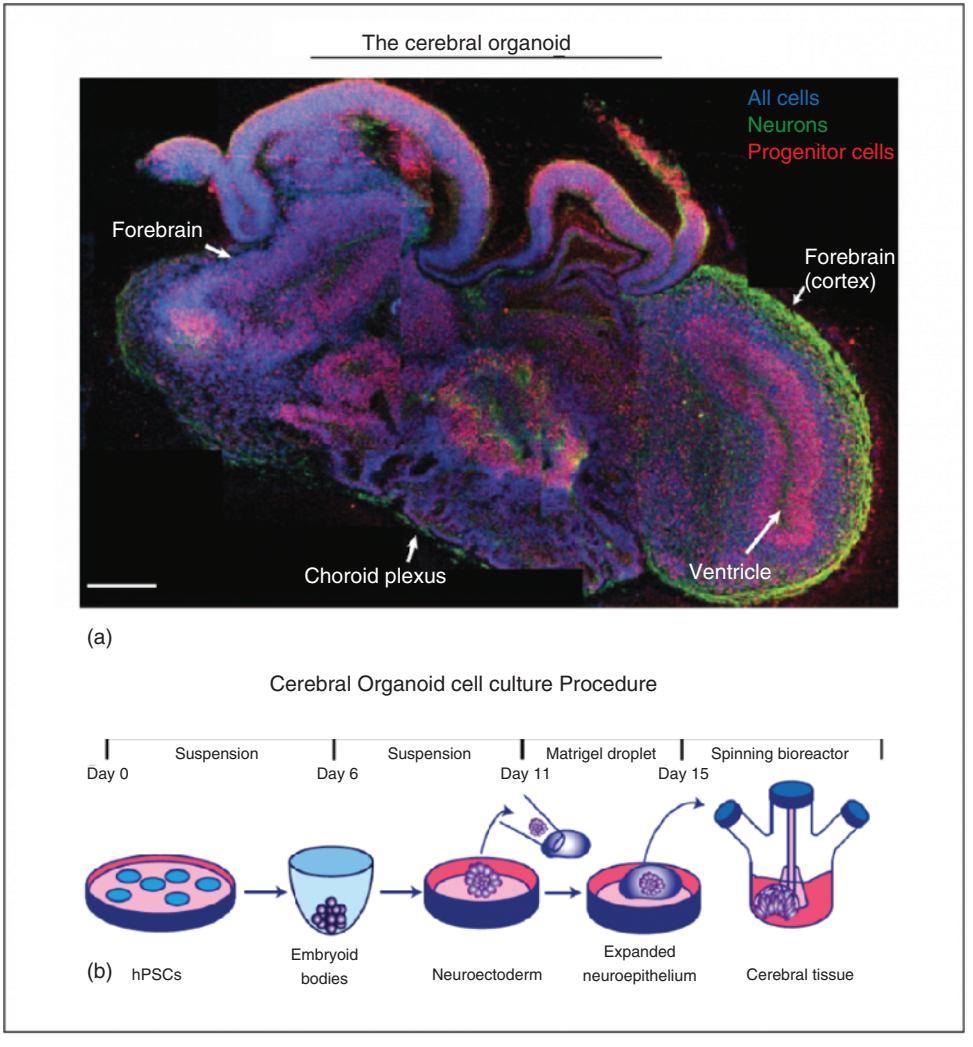


**Fig. 6** Cerebral organoids introduce new methods of modeling defects in neural development, particularly those of the cerebral cortex. (a) Organoids are composed of neural progenitors (red) and neurons (green), as well as other cell types (blue marks all cells). In addition, organoids develop tissues such as neocortex, choroid plexus, and ventricle; (b) The cerebral organoid culture system begins with dissociated pluripotent stem cells which are first directed to a neural fate. Neuroectoderm (early neural stem cells) are then embedded in Matrigel, after which they are cultured in a spinning bioreactor and direct their own development in the absence of most exogenous growth factors. Modified from Ref. [22] by Mark Springel, SITN, Harvard University.

stem cell-derived three-dimensional (3D) organoid culture system, termed cerebral organoids, which develop various discrete, although interdependent, brain regions (see Fig. 6). These include a cerebral cortex containing progenitor populations that organize and produce mature cortical neuron subtypes. Cerebral organoids have also been shown to recapitulate features of human cortical development, namely characteristic progenitor zone organization with abundant outer radial glial stem cells. Finally, RNA interference (RNAi) and patient-specific induced pluripotent stem (iPS) cells were used to model microcephaly, a disorder that has been difficult to recapitulate in mice, and also demonstrated premature neuronal differentiation in patient organoids, a defect that could help to explain the disease phenotype. Together, these data show that 3D organoids can recapitulate development and disease, even in this most complex human tissue [22].

## 7 Stem Cells

A stem cell is a cell which undergoes mitotic cell division to give rise to the same type of cell and, at some point, leaves the pool of mitotically dividing cells to begin the process of cell differentiation. Adult stem cells are, in effect, an embryonic population of cells, continually producing cells that can undergo further development within an adult organism. The path of differentiation that a stem cell descendant enters depends on the molecular milieu in which it resides. It is noteworthy that most stem cells are relatively quiescent.

The term pluripotent describes a cell that is capable of giving rise to several of the cell types of an organism; examples include pluripotent epidermal stem cells and pluripotent hematopoietic stem cells. A lineage-restricted stem cell is a cell that can produce only one type of cell in addition to renewing itself; an example is the burst-forming unit, erythroid (BFU-E).

Embryonic germ (EG) cells are derived from primordial germ cells (PGCs); these pluripotent stem cells may be produced in culture by treating PGCs with stem cell factor, leukemia inhibition factor, and basic FGF2.

Embryonic stem cells (ESCs) are derived from the embryo; for example, those derived from normal mouse inner mass cells cultured *in vitro*.

The meristem is a part of a plant characterized by dividing cells; these regions of plants contain stem cell populations that produce cells, some of which go on to differentiate into plant tissues and some of which constantly renew the stem cell population.

iPS cells are a type of pluripotent stem cell artificially derived from a non-pluripotent cell (typically an adult somatic cell) by inducing a "forced" expression of certain genes. The recent creation of iPS cells has raised the question of whether the genomes of these cells and those of human ESCs have similar pluripotent states. Guenther *et al.* [23] suggested that a comparison of the global chromatin structure and the gene expression programs of these two cell types would provide a robust means of assessing whether the genomes of the cells have similar pluripotent states. The results obtained showed that: (i) genome-wide maps of nucleosomes with certain histone modifications indicate that there is little difference between ESCs and iPS cells with respect to this criterion; and (ii) gene expression profiles confirmed that the transcriptional programs of ESCs and iPS cells showed very few consistent differences.

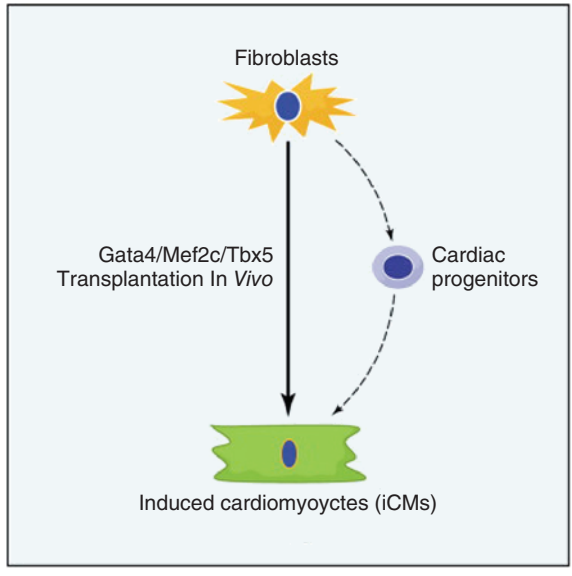


**Fig. 7** Direct reprogramming of fibroblasts into functional cardiomyocytes. Reproduced with permission from Ref. [24].

This led to the conclusion that these variations did not serve to distinguish ESCs from iPS cells. The reprogramming of fibroblasts to iPS cells raises the possibility that a somatic cell could be reprogrammed to an alternative differentiated fate, without first becoming a stem/progenitor cell. A large pool of fibroblasts exists in the postnatal heart, yet no single "master regulator" of direct cardiac reprogramming has been identified. Ieda *et al.* [24] reported that a combination of three developmental transcription factors (i.e., Gata4, Mef2c, and Tbx5) rapidly and efficiently reprogrammed postnatal cardiac or dermal fibroblasts directly into differentiated cardiomyocyte-like cells. The induced cardiomyocytes expressed cardiac-specific markers, had a global gene expression profile similar to cardiomyocytes, and contracted spontaneously. Fibroblasts transplanted into mouse hearts at one day after transduction of the three factors also differentiated into cardiomyocyte-like cells. Ieda *et al.* [24] suggested that these findings showed that functional cardiomyocytes can be directly reprogrammed from differentiated somatic cells by defined factors (see Fig. 7). The same authors also speculated that the reprogramming of endogenous or explanted fibroblasts might provide a source of cardiomyocytes for regenerative approaches.

As noted by Daley [6], the legacy of Gurdon had established the principle of conservation of the genome during cellular differentiation. Gurdon's success with nuclear transfer had established that a molecular machinery within the egg cytoplasm was sufficient to reprogram a somatic genome to a pluripotent state. Ultimately, in 2004, in a review entitled *The First Half-Century of Nuclear Transplantation*, Gurdon surmised that "*… a second half-century* of nuclear transplantation should identify the molecules and mechanisms that achieve nuclear reprogramming" (italics added by present author). In just over a

year after the landmark report on reprogramming from Takahashi and Yamanaka [25] several groups described the successful reprogramming of human cells (in 2007). With this accomplishment, the field turned its attention away from somatic cell nuclear transfer (SCNT) and converged on this new factor-based reprogramming technology, to exploit its practical implications. Applied reprogramming was quickly applied to patient-derived fibroblasts and a large collection of disease-specific iPS cell lines for conditions as diverse as Parkinson's, diabetes and primary immune deficiency were established. As Daly [6] points out, "… the Yamanaka experiments have ushered in an era of *cellular alchemy*…. this technology heralds an era during which any patient's cells represent the ingredients for tissue repair and regeneration" (italics added by present author).

## 8 Cloning

In 1996, Campbell and Wilmut succeeded in deriving two cloned sheep from a differentiated cell line established from a nine-day-old embryo, and a year later achieved worldwide acclaim by successfully deriving a single sheep, "Dolly," from the mammary cells of an adult ewe. In their case, success necessitated adaptations of the cell cycle of the donor nucleus to better match that of the recipient oocyte. These data extended Gurdon's principle of nuclear equivalence (see above) for all somatic cells

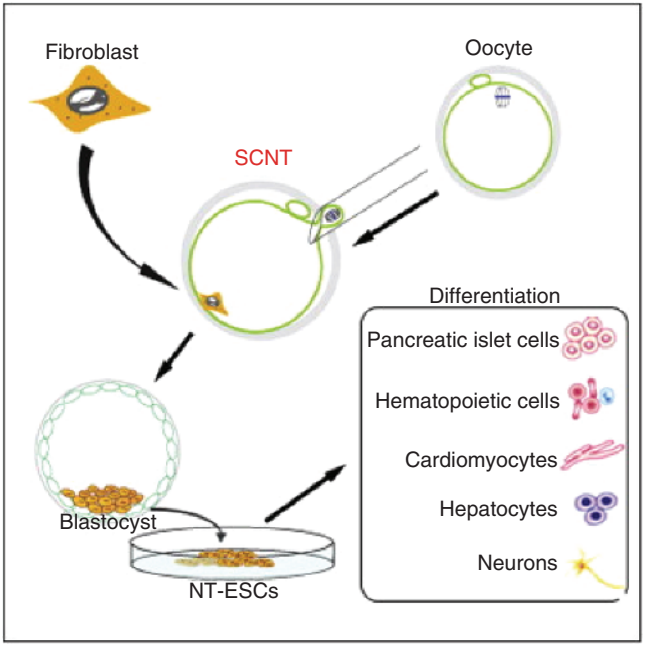


**Fig. 8** Cytoplasm of human oocytes reprograms transplanted somatic cell nuclei to pluripotency. NT-ESCs can be efficiently derived from high-quality human oocytes. Human NT-ESCs are similar to ESCs derived from fertilized embryos. Reproduced with permission from Ref. [26].

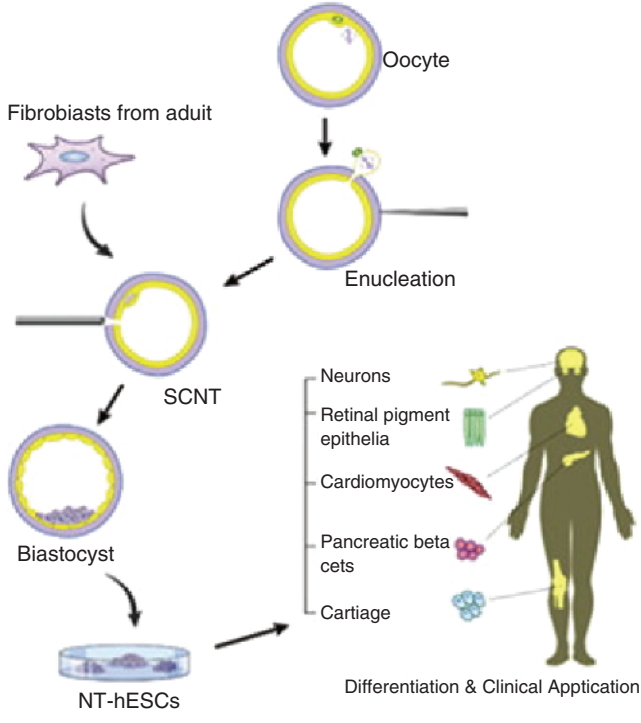


**Fig. 9** Human ESCs were derived via SCNT from 35- and 75-year-old males. Reproduced with permission from Ref. [27].

to fully differentiated cells of an adult mammal, an experiment that had implications far beyond the scientific community [6]. In 2013, a cloning technique was described for reprogramming human cells to create stem cells; the technique – SCNT – fused mature cells from an infant to egg cells that had their DNA-containing nuclei removed (Fig. 8) [26].

During 2014, it was reported that human cloning to produce stem cells can function even with cells from middle-aged or elderly people. In this study, Chung *et al.* [27] employed a recently developed technique to generate human ESCs via SCNT, using dermal fibroblasts from 35- and 75-year-old males. The results demonstrated the applicability of SCNT for adult human cells, and supported the further investigation of SCNT as a strategy for regenerative medicine (Fig. 9).

## 9 Chimeras

A chimera is an organism consisting of parts derived from more than one pair of parents. The *chimera* of Greek mythology consisted of a lion's head, a goat's body, and a serpent's tail. Chimeras may be made in the laboratory by (in the case of mammals) removing the zona pellucida of each embryo and pushing the sticky embryos together, so that they merge to form a single chimeric embryo. This embryo, if transferred into the uterus of a maternal host, may develop into a chimeric organism.

Such constructs have been created with, for example, different strains of mice. ESCs are stem cells derived from the embryo; for example, derived from normal mouse inner mass cells cultured *in vitro*. The use of these cells to create chimeras allows the introduction into ESCs of an engineered gene with a known mutation, such that mice can be bred which have the same mutation in all of their cells.

Beatrice Mintz, an American developmental biologist, is especially well known for developing mouse chimeras. In one of her experiments, early embryos consisting of only a few cells were removed from pregnant mice and placed in close contact with similar cells of genetically unrelated embryos to form a composite that was then implanted in a mouse uterus to create a cellular mosaic. This technique enabled Mintz to trace the tissue site of specific genetic diseases. A recent report by Kobayashi *et al*. [28] described the creation of viable rat–mouse chimeras that developed to term and became fully functional adults. In order to show that xenogeneic organ complementation could be achieved, these investigators injected fluorescently labeled mouse or rat iPS cells into rat or mouse blastocysts and returned them to blastocyst-compatible (to avoid rejection) pseudopregnant females. The donor cells (i.e., the injected iPS cells) not only made major contributions to the tissues of the host, but it was also found that the host blastocyst could control the size of the chimera, as well as imposing additional morphogenetic regulations (as exemplified by the presence or absence of a gallbladder). The study by Kobayashi and coworkers also showed that cells derived from rat iPS cells were able to completely rescue a genetic deficiency of the host mouse blastocyst, resulting in a normal functional pancreas, and confirming that xenogeneic organ complementation is achievable. In reviewing these studies, Solter [29] suggested that, although xenogeneic organ complementation is unlikely to be a viable strategy for regenerative medicine, the elegant studies of Kobayashi *et al.* should provide a wealth of information for research groups seeking to better understand the biology of stem cells and mammalian development (see Fig. 10).

Totipotent cells in early embryos are progenitors of all stem cells and are capable of developing into a whole organism, including extraembryonic tissues such as placenta. Pluripotent cells in the ICM are the descendants of totipotent cells, and can differentiate into any cell type of a body except extraembryonic tissues. The ability to contribute to chimeric animals upon reintroduction into host embryos is the key feature of murine totipotent and pluripotent cells. Rhesus monkey ESCs and isolated inner cell mass cells failed to incorporate into host embryos and develop into chimeras, although chimeric offspring were produced following the aggregation of totipotent cells of the four-cell embryos (Fig. 11). These results provide insights into the species-specific nature of primate embryos, and suggest that a chimera assay using pluripotent cells may not be feasible [30] (Fig. 11).

## 10 MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are post-transcriptional regulators that bind to complementary sequences of target messenger RNA transcripts (mRNAs), which usually results in gene silencing. miRNAs are short RNA molecules (on average only 22 nucleotides long), and the human genome may encode over 1000 miRNAs, that can target about

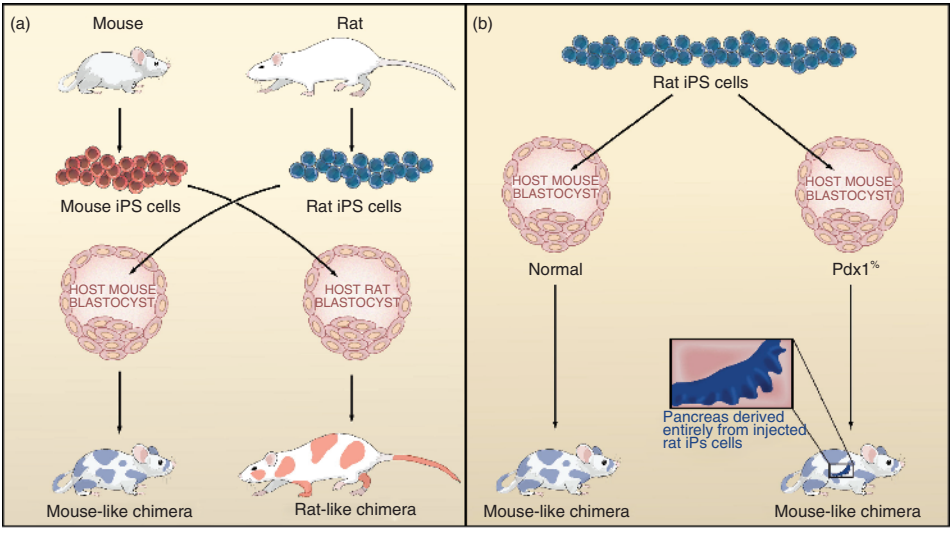


**Fig. 10** The generation of rat–mouse chimeras. (a) Induced pluripotent stem (iPS) cells were derived from adult mouse and rat cells and labeled with different fluorescent proteins. Rat (blue) and mouse (red) iPS cells were injected into reciprocal blastocysts (mouse into rat, and vice versa) to produce intergeneric chimeras. From these blastocysts, several chimeras were born and some survived to adulthood. The contribution of injected donor stem cells was observed throughout the body of the host. The size and morphology of the newborn and adult chimeras was determined by the host blastocyst; (b) Fluorescently labeled rat iPS cells (blue) were injected into normal mouse blastocysts (left) or blastocysts lacking the *Pdx1* gene (right), which encodes the transcription factor pancreatic and duodenal homeobox 1 that is required for pancreas development. Chimeras derived from normal or *Pdx1*-deficient mouse blastocysts showed an extensive contribution of rat cells to all tissues. However, in the *Pdx1*-deficient chimeras, the entire pancreas was derived from donor rat cells (inset, blue) and was fully functional, including the production of insulin by β islet cells. Reproduced with permission from Ref. [29].

60% of mammalian genes and are abundant in many human cell types. miRNAs may each repress hundreds of mRNAs, and are well conserved in eukaryotic organisms; they are thought to be a vital and evolutionarily ancient component of genetic regulation.

The heart, more than any other organ, requires precise functionality on a second-to-second basis throughout the lifespan of the organism. Even subtle perturbations in cardiac structure or function have catastrophic consequences, resulting in lethal forms of congenital and adult heart disease. Such intolerance of the heart to variability necessitates especially robust regulatory mechanisms to govern cardiac gene expression. Recent studies have revealed central roles for miRNAs as governors of gene expression during cardiovascular development and disease. The integration of miRNAs into the genetic circuitry of the heart provides a rich and robust array of regulatory interactions to control cardiac gene expression. miRNA regulatory networks also offer opportunities for therapeutically modulating cardiac function through the manipulation of pathogenic and protective miRNAs. The roles of miRNAs as regulators of cardiac

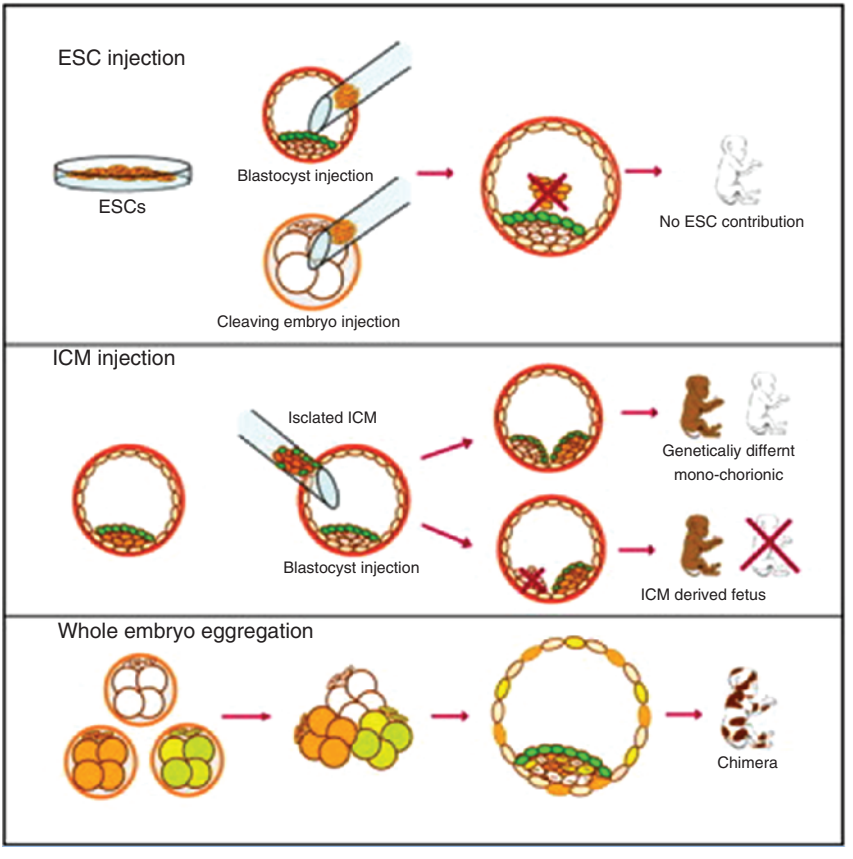


**Fig. 11** Summary of chimera studies with monkey embryos and embryonic cells. Rhesus monkey ESCs as well as isolated inner cell mass cells (ICMs), blastomeres, or whole embryos were tested for their ability to incorporate into host embryos and generate chimeric offspring. Established ESCs and freshly isolated ICMs failed to produce chimeras when injected into host blastocysts. However, ICMs developed into separate fetuses with placental support from the host embryo. Aggregating several four-cell embryos efficiently produced live chimeric offspring. Reproduced with permission from Ref. [30].

form and function, unresolved questions in the field, and issues for the future are discussed by Liu and Nelson [31].

## 11
## *In-Vitro* Fertilization/Nuclear Equivalence

The technique of *in-vitro* fertilization (IVF) provides an excellent example of the merging of cell biology and developmental biology. Since its introduction, IVF is estimated to have resulted in the birth of four million babies worldwide, beginning with the birth of Louise Brown in 1978. A pioneer in this field was Robert Edwards (1925–2013), who was the 2010 recipient of the Nobel Prize in Physiology and Medicine, for the development of human IVF therapy. In order to successfully carry

out human IVF it was first necessary to understand the cell and developmental biology of human gametes, zygotes, and early embryos *in vivo*, and then to apply this knowledge to the *in-vitro* conditions that allowed for success of the technique.

Another, more recent, example is the award of the 2012 Nobel Prize in Medicine or Physiology to Sir John Gurdon of the United Kingdom and Shinya Yamanaka of Japan, "…for the discovery that mature cells can be reprogrammed to become pluripotent." This discovery rested on a foundation of experiments regarding the question of nuclear equivalence, which began during the late nineteenth century, and which will impact not only on the manipulation of cells for medical purposes, but also on understanding the molecular basis of development, the trajectory of which will impact human health well into the twenty-first century.

## References


1. Churchill, F.B. (1991) The Rise of Classical Descriptive Embryology, in: Gilbert, S.F. (Ed.) *A Conceptual History of Modern Embryology*. The Johns Hopkins University Press, Baltimore, MD.
2. Wilson, E.B. (1925) *The Cell in Development and Heredity*, 3rd edn, Macmillan Publishing, New York.
3. Gilbert, S.F. (2010) *Developmental Biology*, 9th edn, Sinauer Associates, Sunderland, MA.
4. Saha, M. (1991) Spemann Seen Through a Lens, in: Gilbert, S.F. (Ed.) *A Conceptual History of Modern Embryology*, The Johns Hopkins University Press, Baltimore, MD.
5. Dye, F.J. (2012) Developmental Cell Biology, in: *Systems Biology*. Wiley-Blackwell, New York.
6. Daley, G.Q. (2012) Cellular alchemy and the golden age of reprogramming. *Cell*, **151** (6), 1151–1154.
7. Engler, A.J., Sen, S., Sweeney, H.L. (2006) Matrix elasticity directs stem cell lineage specification. *Cell*, **126** (4), 677–689.
8. Even-Ram, S., Artym, V., Yamada, K.M. (2006) Matrix control of stem cell fate. *Cell*, **126** (4), 645–647.
9. Eyckmans, J., Boudou, T., Yu, X., Chen, C.S. (2011) A hitchhiker's guide to mechanobiology. *Dev. Cell*, **21** (7), 35–47.
10. Keller, R. (2002) Shaping the vertebrate body plan by polarized embryonic cell movements. *Science*, **298**, 1950–1954.
11. Adler, P.N. (2002) Planar signaling and morphogenesis in *Drosophila*. *Dev. Cell*, **2** (5), 525–535.
12. Hall, A. (1998) Rho GTPases and the actin cytoskeleton. *Science*, **279**, 509–514.
13. Shindo, A., Wallingford, J.B. (2014) PCP and septins compartmentalize cortical actomyosin to direct collective cell movement. *Science*, **343** (6171), 649–652.
14. Ajioka, I., Martins, R.A.P., Bayazitov, I.T., Donovan, S. *et al*. (2007) Differentiated horizontal interneurons clonally expand to form metastatic retinoblastoma in mice. *Cell*, **131**(2), 378–390.
15. H. te Riele (2007) Retinoblastoma teaches a new lesson. *Cell*, **131** (2), 227–229.
16. Bowles, J., Feng, C.-W., Spiller, C., Davidson, T.-L. *et al*. (2010) FGF9 suppresses meiosis and promotes male germ cell fate in mice. *Dev. Cell*, **19** (3), 440–449.
17. D'Angelo, M.A., Gomez-Cavazos, J.S., Mei, A., Lackner, D.H., Hetzer, M.W. (2012) A change in nuclear pore complex composition regulates cell differentiation. *Dev. Cell*, **22** (2), 446–458.
18. Bock, G., Cardew, G., Goode, J.A. (Eds) (2001) *The Cell Cycle and Development*. Novartis Foundation Symposium 237, John Wiley & Sons, Ltd, West Sussex.
19. Mohd-Sarip, A., Lagarou, A., Doyen, C.M., van der Knaap, J.A., Aslan, Ü., Bezstarosti, K., Yassin, Y., Brock, H.W., Demmers, J.A., Verrijzer, C.P. (2012) Transcription-independent function of polycomb group PSC in cell cycle control. *Science*, **336** (6082), 744–747.
20. Arnone, M.I. (2002) Bringing order to organogenesis. *Nat. Genet.*, **30** (4), 348–350.
21. Fang, H., Yang, Y., Li, C., Fu, S. *et al*. (2010) Transcriptome analysis of early organogenesis in human embryos. *Dev. Cell*, **19** (1), 174–184.
22. Lancaster, M.A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L.S., Hurles, M.E. (2013) Cerebral organoids model human

brain development and microcephaly. *Nature*, **501**, 373–379.

23. Guenther, M.G., Frampton, G.M., Soldner, F., Hockemeyer, D., Mitalipova, M., Jaenisch, R., Young, R.A. (2010) Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. *Cell Stem Cell*, **7** (2), 249–257.
24. Ieda, M., Fu, J.-D., Delgado-Olguin, P., Vedantham, V. *et al*. (2010) Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*, **142** (3), 375–386.
25. Takahashi, K., Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, **126** (4), 663–676.
26. Tachibana, M., Amato, P., Sparman, M., Gutierrez, N.M. *et al*. (2013) Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell*, **153** (6), 1228–1238.
27. Chung, Y.G., Eum, J.H., Lee, J.E., Shim, S.H. *et al*. (2014) Human somatic cell nuclear transfer using adult cells. *Cell Stem Cell*, **14** (6), 777–780.
28. Kobayashi, T., Yamaguchi, T., Hamanaka, S., Kato-Itoh, M. *et al*. (2010) Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. *Cell*, **142** (5), 787–799.
29. Solter, D. (2010) Viable rat-mouse chimeras: where do we go from here? *Cell*, **142** (5), 676–678.
30. Tachibana, M., Sparman, M., Ramsey, C., Ma, H., Lee, H.-S., Penedo, M.C.T., Mitalipov, S. (2012) Generation of chimeric rhesus monkeys. *Cell*, **148** (1-2), 285–295.
31. Liu, N., Olson, E.N. (2010) MicroRNA regulatory networks in cardiovascular development. *Dev. Cell*, **18** (4), 510–525.