

Asymmetric Behavior in Stem Cells

Bridget M. Deasy

Abstract Asymmetry in the stem cell niche refers to the notion that daughter cells are different from each other. There is significant evidence that many stem cell divisions result in one daughter cell that is similar to the parent cell and, hence, necessarily allows for self-renewal of the stem cell phenotype, whereas the other daughter cell is a differentiated or committed cell type. In this chapter we will discuss the role of asymmetry in stem cell divisions and the evidence that supports different asymmetric scenarios in different model systems. We first present the early asymmetric divisions that have been described in first divisions of the zygote and in gametogenesis. Next, we will discuss evidence of asymmetry in postnatal stem cells. Here we will describe two systems in particular – the hematopoietic system and muscle stem cells. Lastly, we will present a theory of the immortal strand hypothesis in which the role of DNA strand segregation is discussed as it relates to asymmetry in cell divisions and the protection of the self-renewing stem cell.

Keywords Polarized · Polarity · Niche · Microenvironment · Immortal strand · Cancer · Cell expansion · Cell therapy · Lineage · Division history

1 Stem Cell Asymmetry

Asymmetry in stem cell behavior refers to the notion that daughter cells are different from each other (Fig. 1A). It has been shown that some stem cell divisions result in one daughter cell that is similar to the parent cell and, hence, necessarily allows for self-renewal of the stem cell

phenotype, whereas the other daughter cell is a differentiated or committed cell type. Asymmetry in the phenotype of the daughter cells can occur in theory from two different mechanisms. First, there may be directed or random events occurring within the cytoplasm that result in asymmetric partitioning of cytoplasmic contents and, hence, distinct daughter cell phenotypes (Fig. 1B). Alternatively, the event of cell division may yield daughter cells that are equivalent at birth, and the cells then respond to extrinsic cues that prompt one cell to differentiate while the other does not. This scenario implies that the equivalent daughter cells are positioned in the microenvironment such that they receive different cues and, hence, the result is two different phenotypes (Fig. 1C).

Studies of asymmetry in stem cell biology must specifically define the aspect in which the resulting daughter cells differ. For example, to one investigator, asymmetric division may refer to a behavioral parameter such as cell division activity – one daughter cell is actively dividing and one daughter cell is nondividing (quiescent, terminally differentiated, or senescent). To another investigator, asymmetry may mean that one daughter cell maintains its location, while one daughter cell is physically moved to a new position. Asymmetry may also mean that one daughter cell expresses a specific transcription factor, and one daughter cell does not express that transcription factor. Therefore, the point in time at which an investigator can identify differences in the daughter cells, and hence recognize asymmetry, will vary with the parameter that is being investigated.

Here, we will discuss the role of asymmetry in stem cell divisions and the evidence that supports each of these scenarios in different model systems. We first present the early asymmetric divisions that have been described in first divisions of the zygote and in gametogenesis. Next, we will discuss evidence of asymmetry in postnatal stem cells. Here we will describe two systems in particular – the hematopoietic system and muscle stem cells. Lastly, we will present a theory of the immortal strand hypothesis in which the role of DNA strand segregation is discussed as it relates to asymmetry in cell divisions and the protection of the self-renewing stem cell.

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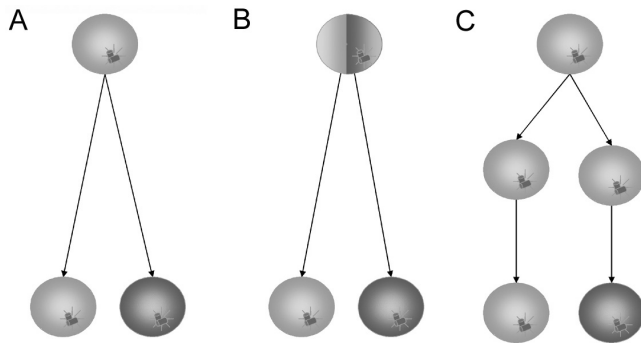


Fig. 1 Asymmetry in cell division gives rise to daughter cells with unique properties. **(A)** Asymmetry. General concept of asymmetric division with unique daughter cells. Observations of this general asymmetric scenario are often made without an understanding of underlying mechanism. Intrinsic or extrinsic factors play a role in whether the daughter cells are different due to internal cues or environmental cues. Asymmetry may occur in theory by two mechanisms. **(B)** Asymmetric Division. The parent cell may have cytoplasmic asymmetry, or the process of division that involves the centrosomes and mitotic spindle alignment may result in unequal partitioning of molecular determinants. The result is two unique daughter cells. **(C)** Asymmetric Fate. Asymmetry may arise from a parent cell that gives rise to two equal daughter cells at the time of division, but the daughter cells respond differentially to the microenvironment and adopt different phenotypes or undergo different developmental programs. The result is two unique daughter cells. The ability to distinguish which of these two patterns may be occurring in a given system depends on the spatial and temporal resolution of the experimental analysis, and the asymmetric parameter of interest. In addition, we show in this chapter, that some stem cell niches involve both mechanisms to maintain the stem cell phenotype and permit cell differentiation

2 Asymmetry in Embryonic and Germ Cells

2.1 Zygote First Division

Clearly, the most potent of stem cells is the zygote, having totipotent capability to give rise to all cell types of the organism and support development of extraembryonic tissues (e.g., the placenta). The notion that the first division of the zygotic cell establishes two cells with unique fates appears contradictory to the established finding that, in many systems, all cells of the 4-, 8-, or 16-cell stage have potential to give rise to all cell types [1–3]. Totipotency in the early cell stages was first shown by Hans Spemann in the newt salamander, and later, others showed that loss of totipotency in mammals spanned a range from the 2-cell stage up to nuclei totipotency of sheep embryos at the 64-cell stage [2]. Blastomeres that are separated at the two-cell stage show equal potential to become viable organisms, yet, more recent findings also support the occurrence of asymmetry in the first two blastomeres, or daughter cells, of the zygote.

Asymmetry has been examined comprehensively in embryonic development of the nematode *Caenorhabditis elegans*, and in the insect model *Drosophila melanogaster*.

Both undergo an extensive number of asymmetric divisions during development. In particular, all 959 cells of the worm have been traced (from 671 divisions) through the work of J. Sulston and colleagues [4, 5]. In particular, the first division of the *C. elegans* zygote involves *par* (partitioning) genes that lead to two cells of different developmental pathways; one cell develops to the ectodermal lineage and one cell to the endodermal and mesodermal lineages. The *par* genes are highly conserved. In many species, the membrane around the sperm entry position (SEP) is marked by a fertilization cone that consists of cytoplasmic elements including *par* proteins [6].

Studies with mammalian embryos, predominantly mouse embryos, show that the asymmetry of the first zygote division also may be established by environmental cues [7, 8]. First, the primary cleavage of the zygote that results in two cells with bilateral symmetry appears to be oriented with respect to the sperm entry position [9, 10]. It has also been demonstrated that the cleavage axis for the first division can be predicted with high probability by the SEP markers [9]. Further, the daughter cell that receives the SEP marker also has a tendency to divide before its sister cell. In *C. elegans*, the *par* proteins will accumulate near the SEP. Tracking the lineage of the cell membrane also showed that this earlier dividing, SEP-inheriting cell contributes preferentially to the embryonic part of the mouse blastocyst (Fig. 2). Another environmental cue that appears to play a role in polarity of the blastocyst is the polar body of the second meiotic division [11]. The final step in gametogenesis (also discussed below) yields a smaller haploid cell, or polar body, associated with the larger zygote. Gardner et al. [11] have shown that the location of the polar body has a tendency to be aligned with the boundary between the embryonic and extraembryonic regions. This axis relates to the animal-vegetal pole – the axis of bilateral symmetry is normally aligned with the animal-vegetal axis of the zygote and the embryonic-extraembryonic axis is orthogonal to it. Lineage analysis again shows that the cells that are adjacent to the polar body give rise to cells of the animal pole [12]. In sum, the SEP and polar body location appear to predict the first cleavage plane and these environmental cues may be the earliest signals that direct lineage fate of the blastocyst in the mouse blastocyst [7, 9] (Fig. 2).

2.2 Gametogenesis

Another clear pattern for asymmetric divisions is demonstrated in germ cell differentiation. Primordial germ cells (PGCs) are the embryonic precursors to the gametes. Primordial germ cells (PGCs) are the embryonic precursors to the gametes (also see Chapter 5). The point in embryogenesis at which germ lineage determination is made differs among species. In insects, nematodes, and some amphibians, for

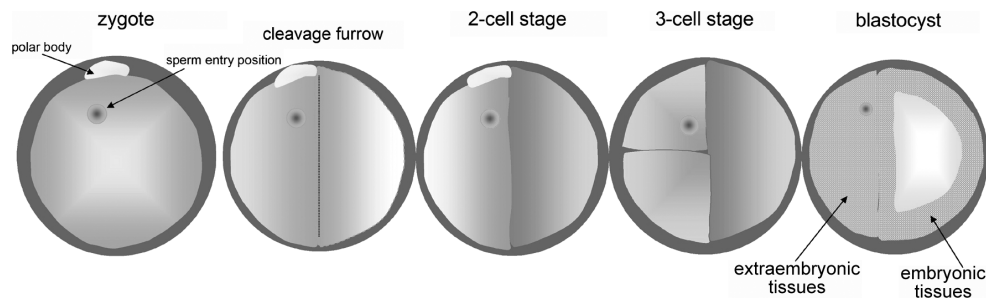


Fig. 2 Asymmetry in first cell cleavage or division of the mouse embryo. The sperm entry position (SEP) and the polar body appear to have a role in directing polarization of the early division of the zygote. In many species, the SEP is associated with positioning of the developing embryo. Later, the cell lineage shows partitioning between the embryonic and extraembryonic tissues. This figure is adapted from Zernicka-Goetz,

M., Development, 2002, 129:815 [7]. One of the challenges to understanding asymmetry at this early time point involves reconciling asymmetry with the demonstrated equal developmental plasticity of the early blastomeres

example, specific maternal cytoplasm of the zygote, called germ plasm, is responsible for signaling germline differentiation [13, 14]. Germ plasm is comprised of RNA, protein, and polar granules, or electron-dense structures that are associated with mitochondria [15]. The first division of the nematode *Ascaris* zygote, for example, results in two cells with different developmental potential; with one cell being committed entirely to somatic cells while the other may give rise to both germ cells and somatic cells [16, 17] (Fig. 3). In the *Drosophila* model, a number of maternal genes in the oocyte play a role in specifying germ cell fate. Here the nuclei destined to become germ cells are located at one pole of the developing syncytium. Nuclei associated with this cytoplasm are the first to form a unique cell membrane or cellularize to form cells of a distinct cell fate [18].

In mammals and other amphibians, germ cell differentiation appears to be signaled much later through cell-cell interactions of gastrulation [19]. In studies of the developing mouse embryo, it appears that cell interactions associated with gastrulation induce germ cell specification [20] and that the process is mediated by secreted factors of the bone morphogenetic protein (BMP) family [21]. Alkaline phosphatase expression has been classically used to identify PGCs. Cell divisions that give rise to cells of specific fates have not been identified here. Rather, cells appear to adopt distinct fates based on positional information. Whatever the mechanism of specification, once primordial germ cells are specified, the process of differentiation to mature gametes again involves asymmetry.

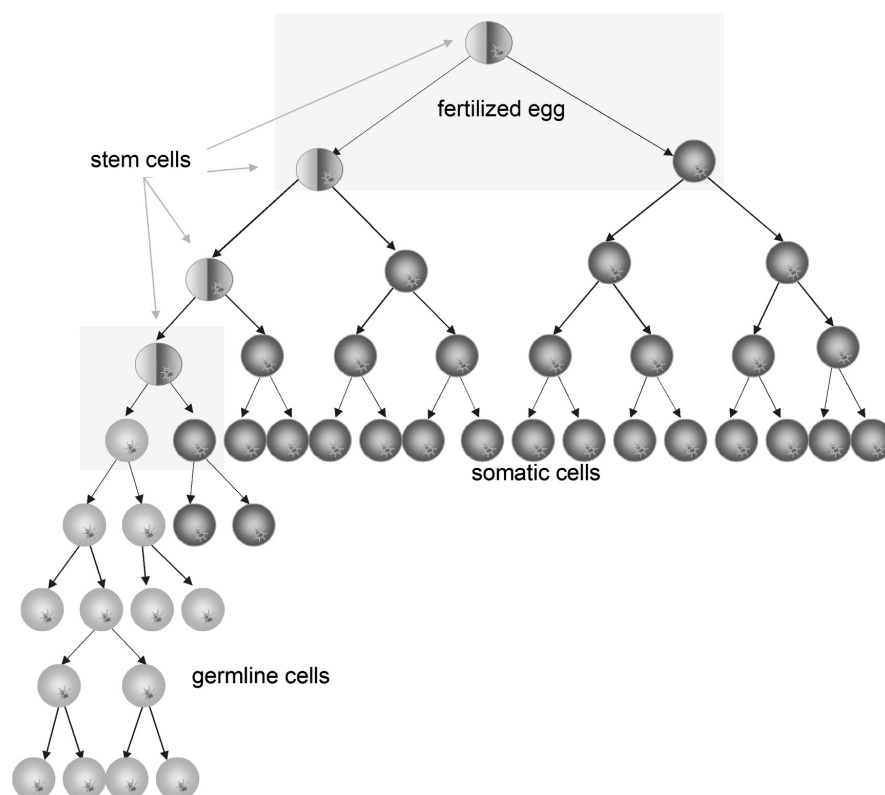
The well-described differentiation of the *Drosophila* male primordial germ cells to sperm provides a clear example of stem cell asymmetry and the role of the stem cell niche. Like other species, the *Drosophila* testes contain compartments of cells at the various stages of spermatogenesis. A cluster of post-mitotic somatic cells, termed hub cells, resides at the apical tip of the fly testis and this hub is surrounded by the germline stem cells [22] (Fig. 4A). Upon cell divi-

sion, the male germ cell gives rise to 1 cell which will remain adjacent to the hub, and retain the stem cell phenotype, and 1 cell which is physically displaced from the hub and is no longer in direct physical contact with the hub. The displaced cell, termed the gonialblast, gives rise to transiently amplifying cells and spermatogonia. The apical hub cells express the ligand Unpaired (Upd), which activates the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway in adjacent germ cells [23, 24]. This pathway is required for self-renewal of the germ cells [24, 25]. Further, this local acting ligand appears to have limited diffusion [26] and may therefore act on the adjacent germ cells to signal self-renewal, while cells further from the hub initiate differentiation [27]. In addition to the hub cells, signals from the cyst progenitor cells also regulate germ cell differentiation; the epidermal growth factor receptor pathway acting within the cyst cell plays a role in inducing differentiation and regulating amplification in the germ cells [28, 29].

It has been proposed that the male germ cell niche of *Drosophila* also requires the function of adherens junctions and specific orientation of mitotic spindles to ensure that one daughter cell self-renews and remains within the niche and the other daughter cell is displaced [27, 30]. Yamashita et al. [30] showed that dividing germline stem cells use mechanisms involving centrosome activity and a cortically localized protein to orient the mitotic spindles perpendicular to the hub cells of the niche. The high concentration of the E-cadherin homolog (Shg) at the interface of hub cells and germ cells, and the architectural association of the adherens with intracellular APC of germ cells, would facilitate an asymmetric division in which one daughter cell remains in the niche and self-renews and the other is displaced and initiates differentiation [30].

An asymmetric pattern also is observed in many species of female germ cell development. The differentiation of female primordial germ cells to oocytes involves two steps of asymmetric meiosis (Fig. 4B). The daughter cells differ from

Fig. 3 Asymmetry in cell cleavage of *Ascaris*. A unique mode of asymmetric lineage development is observed in the invertebrate *Ascaris* nematode. Asymmetry results from portions of the genome being lost in some daughter cells. The somatic cells have reduced chromatin content, while cells of the germ line retain a full chromosome complement. Adapted from Ham, *Mechanisms of Development*, 1980, Mosby Publishers, St. Louis, MO [16]



each other mainly in cytoplasmic volume. In the process, the germ cell gives rise to the diploid oogonium, which may undergo symmetric divisions to give rise to more oogonia or may mature to an oocyte. In the first asymmetric division of meiosis, the primary oocyte gives rise to a secondary oocyte and a polar body. Both daughters receive a second complement of chromosomes; however, one of the daughters, termed a polar body, randomly receives a much smaller portion of the cytoplasm. In the second meiotic event, the secondary oocyte gives rise to the haploid ootid, which will mature to the oovum, and another smaller polar body.

Further asymmetry is observed in the epigenetic characteristics of parental genomes of the fertilized egg. Imprinting during gametogenesis gives rise to differential developmental roles for the maternal and paternal genomes in embryonic and extraembryonic tissues [31, 32]. In mammals and a number of other species, the higher degree of methylation of the maternal DNA and histones, as compared to the paternal DNA and histones methylation, is responsible for epigenetic asymmetry. A rapid loss of methylation occurs in the hours following zygote formation and some regions are resistant to demethylation [33–35]. The mechanism responsible for the methylation differences is not clearly understood. It may be that the high level of methylation at the maternal zygote protects against the demethylase activity of the zygote. Or the differential may be due to increased targeting of the paternal genome by the demethylases. The results of Nakamora et al. [35] suggest that the maternal factor called PGC7/Stella protects the maternal genome

from demethylation after it localizes to the nucleus, where it maintains the methylation of several imprinted genes. Additional epigenetic asymmetry is observed during development. The DNA and histone methylation and polycomb gene silencing are asymmetric in the embryonic (deriving from the inner cell mass) versus extraembryonic tissues (mainly deriving from the trophoectoderm) [36, 37]. X chromosome inactivation is random generally in the embryonic and somatic tissue but imprinted in the extraembryonic placental and umbilical tissues.

In relation to the current interest in stem cells for therapeutics, the potential of ESCs and PGCs has been examined. The potential of ESCs has been widely discussed. In vitro and in vivo studies have shown that PGCs may give rise to pluripotent stem cells that are capable of giving rise to cells of multiple lineages. However, transplantation of PGCs to the mouse blastocyst showed that the cells did not contribute to either germ cells or somatic cells [38]. Regulatory molecular mechanisms that control development of the mammalian EC and germline cells are the focus of ongoing studies. This will contribute to both the potential use of these cells and an understanding of the role of division patterns in basic biology of early ECs and PGCs.

2.3 Neurogenesis

Neurogenesis during embryonic development has been well characterized using the *Drosophila* model system. Key in

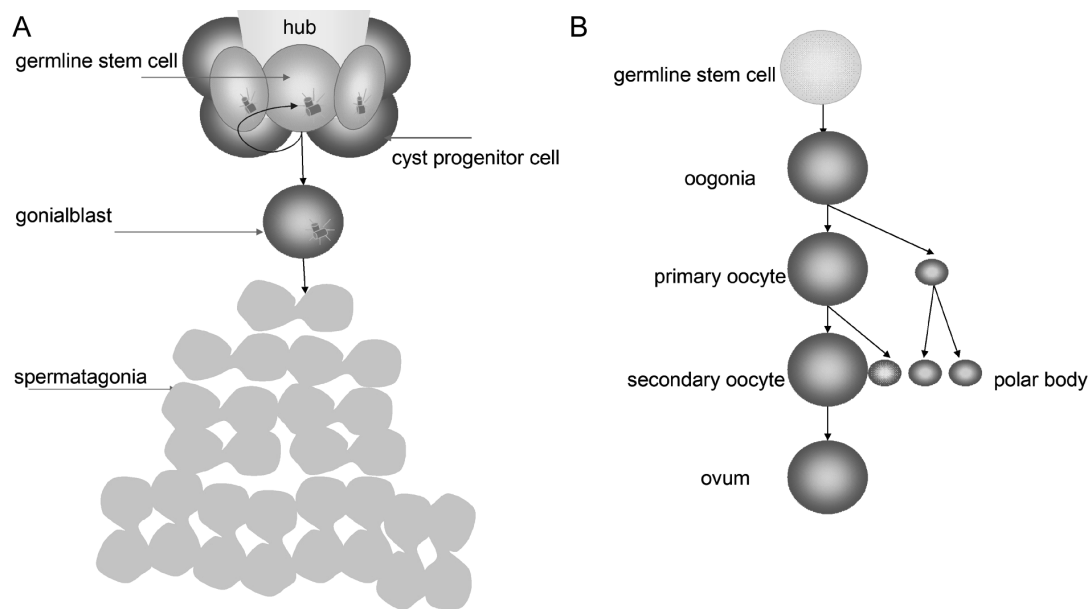


Fig. 4 Gametogenesis. **(A)** A cluster of post-mitotic somatic cells resides at the apical tip of the *Drosophila* testis and this hub is surrounded by the germline stem cells. The male germ cell gives rise to one cell that will remain adjacent to the hub, and retain the stem cell phenotype, and one cell, the gonialblast, which is physically displaced from the hub and is no longer in direct physical contact with the hub. The gonialblast gives rise to transiently amplifying cells, through four divisions, and spermatagonia. The hub cells express the ligand Unpaired, which activates the JAK-STAT pathway in adjacent germ cells, and is required for self-renewal the germ cells. Signals from the cyst progenitor cells also regulate germ cell differentiation. Finally, dividing germline stem cells use mechanisms involving the centrosomes and a cortically localized protein to orient the mitotic spindles perpendicular to the hub and facilitate an asymmetric division in which one daughter

cell remains in the niche and self-renews and the other is displaced and initiates differentiation. **(B)** The coordinated process of oogenesis in several mammalian species creates one ovum and three smaller polar bodies. The daughter cells differ from each other mainly in cytoplasmic volume. The primordial germ cell gives rise to the diploid oogonium, the oogonia, and then the oocyte. In the first asymmetric division of meiosis, the primary oocyte gives rise to a secondary oocyte and a polar body. While both daughters receive a second complement of chromosomes, one of the daughters, termed a polar body, will randomly receive a much smaller portion of the cytoplasm. The second meiotic event, whose timing varies among species, will give rise to the mature oovum, and another smaller polar body. In humans, the second meiotic division occurs after fertilization

this process is Numb, a membrane-bound intracellular protein that directs fate specification of neuron and sheath cells (cells that form a sheath around the dendrite of the neuron) and other cells associated with the external sensory organ. Asymmetry related to Numb and neurogenesis has been extensively described elsewhere, for *Drosophila* and mammals [39–43], and will only be highlighted here.

The lineage of the sensory organ precursor (SOP) cell eventually gives rise to five cells of the *Drosophila* external sensory organ. Rhyu et al. [44] first showed that Numb protein segregates asymmetrically and this event is required for the fate specification of the daughter cells. Spindle orientation also plays a role in the asymmetric divisions as it orients the plane of cell cleavage. The crescent-shaped surface localization of Numb on the cell correlates with the mitotic spindle arrangement. The first SOP division, occurring along the anterior-posterior axis, results in pIIa and pIIb; pIIb subsequently divides along the apical-basal axis to give rise to a glial cell and pIIIb, which again divides apical-basal to yield a sheath cell and a neuron. All divisions in the lineage are asymmetric (Fig. 5).

A number of other cytoplasmic factors interact with or inhibit Numb and affect cell fate specification [45–47]. Notable among these factors is Notch. Notch signaling was shown to inhibit neuronal differentiation in *Drosophila* and other species [48]. Numb is an inhibitor of Notch signaling [49]; it prevents nuclear translocation of Notch and antagonizes its activity. Morrison et al showed that a transient activation of Notch was sufficient to cause an irreversible loss of neurogenic differentiation potential; accelerated glial differentiation was also observed following Notch activation [50]. In sum, interactions between Numb, Notch [and Numblike (d-Numb homolog)] play important roles in controlling asymmetry and directing neuronal cell fate specification.

3 Asymmetry in Postnatal Cells

As the germ layers are formed from the ESCs, and cell determination and organogenesis evolve during embryonic development, it is believed that asymmetry may generate stem cells that maintain the stem cell pool specific for

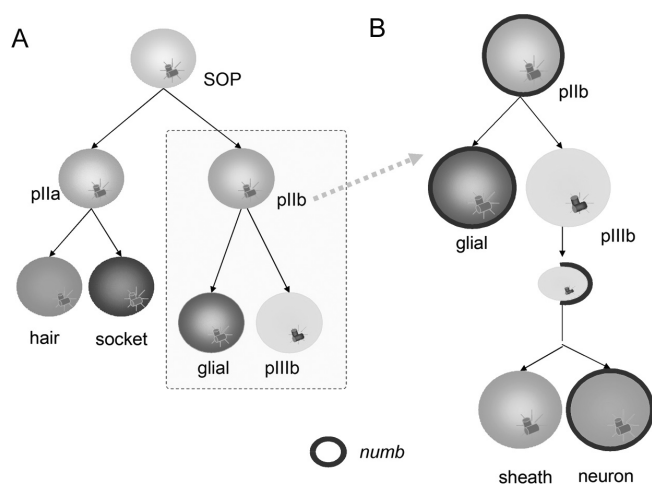


Fig. 5 Asymmetry in neurogenesis of *Drosophila* external sensory organ. The SOP (sensory organ progenitor) cell lineage gives rise to five cells of the external sensory organ – a hair cell, a socket cell, a glial cell, a sheath cell and a neuron. (A) The SOP cell divides to give rise to pIIa and pIIb. The progenitor pIIa yields the hair and socket cells, while the progenitor pIIb yields a glial cell and a pIIIb cell. The progenitor pIIIb undergoes an additional division to yield the sheath cell and neuron. (B) Numb expression regulates cell fate determination. Other important factors (not shown here) include the niche, the mitotic alignment, and the presence or absence of notch, inscuteable, and delta. All divisions are asymmetric

different organs and tissues, and other cells that initiate the process of differentiation through transiently amplifying stages and become the progenitors of somatic cells. Postnatal or adult stem cells are resident tissue-specific stem cells that are responsible for tissue homeostasis and tissue repair.

3.1 Hematopoietic Stem Cells

Stem cells of the blood tissue have been the model system for studying adult-derived stem cells. Hematopoietic stem cells (HSCs) give rise to all blood cell types, which fall into two general categories: myeloid lineages – monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes, platelets, dendritic cells; and lymphoid lineages – T-cells, B-cells, NK-cells, and dendritic cells. In addition, postnatal HSCs from cord blood, peripheral blood, and bone marrow have been used successfully in therapy to treat blood disorders and some types of cancer. (See Chapters 9, 10, 15 and 30 for additional discussions of HSCs.)

Early clonal assays revealed that single HSCs and progenitors are capable of giving rise to colonies of mixed progenies that include, for example, macrophages eosinophils, neutrophils, basophils, erythrocytes, and megakaryocytes [50–52]. These studies provided evidence for a single cell origin with multilineage potential, and also showed that a structurally intact, or physical, microenvironment was not necessary for

multilineage differentiation. The findings opened the door to questions regarding the cellular mechanisms that lead to the mixed colonies. In subsequent studies, the question was asked whether asymmetry may occur in the originating cell division. Indeed, studies of paired daughter cells that result from a HSC division suggested that there is asymmetry in daughter cell developmental potential [53–56]. Using single-cell micromanipulation, daughter cells were physically separated and the differentiation fates of the cell progeny were examined. Suda et al. [54] found that there were differences in the differentiation directions of the progeny that derived from the sister cells, and these were termed nonhomologous pairs. Because the cells were in similar environmental conditions, yet they produced different progeny, this suggested that there was a stochastic element in cell fate determination. In these studies, some daughter cell pairs also revealed significant differences in colony size, and therefore proliferation rates [53, 54, 56]. The results of Leary et al. [55, 56] supported the findings as they reported that sister multipotent progenitors had differences in colony-forming potential. Asymmetric cell phenotypes, here determined by asymmetry in differentiation fate, were observed in up to 17% of the paired progenitor cells of human umbilical cord blood [57]; the fate did not appear to be affected by cytokines, which again supports the idea of a stochastic component. Other results performed on clones and subclones, rather than sister cells, provided further support for the notion of asymmetry in that they demonstrate heterogeneity and intrinsic control in cell fate [58].

Studies using defined phenotypes have also demonstrated asymmetry in HSCs' fate. HSCs isolated on the basis of CD34 expression, a surface glycoprotein that functions in hematopoiesis and hematopoietic cell adhesion [59, 60], were examined by time-lapsed microscopy and it was observed that HSC divisions resulted in some daughter cells remaining as quiescent cells while other daughter cells underwent extensive proliferation [61]. Other groups subsequently showed that the CD34 cells that gave rise to myeloid-lymphoid initiating cells had slower division times and were associated with asymmetry more so than CD34 cells that gave rise to colony-forming units [62]. They also showed that contact with supporting cells, which may mimic the microenvironment, caused an increase in daughter cell asymmetry [63]. In these studies, approximately 30% of the defined CD34 cells gave rise to daughter cells with mixed proliferation rates [61, 62]. Geibel et al. [64] also showed that both primitive and more committed cells gave rise to differentially specified daughters. However, it was within the primitive compartment that the majority of cells appeared to diverge asymmetrically, while the majority of divisions of the committed cells led to symmetric cell expansions.

It has been shown clearly that stem and progenitors of the hematopoietic compartment can give rise to daughter cells

whose progeny have different cell fates. However, it has not been shown that there is asymmetry in intracellular determinants at the time of HSC cell division. There is still a focus to identify segregation of molecular determinants within the cell such that unique phenotypes between the daughter cells is apparent at the time of cell division. A recent study indeed suggests that an interaction between CD34 cells affects the cleavage plane of cell division, and may subsequently result in unequal distribution of Notch-1 to the daughter cells [65]. Certainly, although one type of asymmetry has been shown in an in vitro culture setting, it is not known whether HSCs divide symmetrically or asymmetrically in vivo.

3.2 Skeletal Muscle Stem Cells

The skeletal muscle cell compartment of adult tissues, like the blood cells, includes a variety of cell types. The stem cell that is described classically in skeletal muscle is the satellite cell, which fuses to form the mature multinucleated muscle fiber. Satellite cells, which appear to be committed precursor cells, were first described based on their location and morphology [66]. Satellite cells surround the mature functional cell of skeletal muscle; the specific niche for satellite cells is in between the sarcolemma and the basal lamina of the muscle fiber. In adult muscle, satellite cells remain quiescent until external stimuli trigger re-entry into the cell cycle. Their progeny, myoblasts, fuse to form new multinucleated myofibers [67–71]. Cell surface markers associated with the in situ satellite stem cell phenotype, either in the quiescent or activated state, include M-cadherin, c-met, CD34, Pax7, and CD56 [70, 72–77]. These cells have been described as having multilineage differentiation potential [78] and have been examined as candidates in cell therapy for muscle repair [79–83].

More recently, a number of other stem-cell-like populations have been identified from the adult skeletal muscle tissue. These phenotypes include side population or SP cells [81, 84–89], mesoangioblasts [90–92], pericytes [93–95], and endothelium-related cells such as AC133 cells [96, 97], preplate muscle-derived cells [98–100], and myo-endothelial cells [101]. The developmental origins and relationships among these cells are still being investigated (for review, see [102]). However it is generally believed that the satellite cell is downstream of the other cell types, which often do not express the Pax7 transcription factor that appears to induce satellite cell specification [76]. The mix of cell types present in an adult muscle biopsy have led to similar questions regarding the role of asymmetry in muscle cell population heterogeneity and hierarchy.

The general notion of muscle stem cell self-renewal implies that the cell division results in one daughter cell that

maintains the stem cell phenotype and one daughter cell that is committed to the myogenic lineage. While it has not been demonstrated conclusively that asymmetry of this sort occurs with adult muscle stem cells, there is growing evidence that supports this idea. Olguin and Olwin [103] examined clonal cultures, initiated with 500 cells, and found heterogeneity within individual clones – both differentiated progeny and cells that regained quiescent phenotype markers (Pax-7+ and MyoD[−] or myogenin[−]). Clonal and subclonal cultures of muscle-derived stem cells have also demonstrated mixed phenotypes in terms of both marker expression (CD34, Sca-1, and myogenic markers) [99, 104] and proliferative behavior [105]. Although it was not reported that these clonal cultures were explicitly initiated with single cells [99, 104, 105], a separate study observed proliferative heterogeneity in a single cell colony, from a population of muscle stem cells, which was tracked using time-lapsed imaging [106]. Zammit et al. [107] also proposed a model for asymmetry in cell fates of daughters of satellite cells based on their observations of cell clusters on muscle fibers. Cell clusters were heterogeneous in the expression of Pax7 and MyoD [107]; these results were also supported by findings of Pax7+/MyoD[−] cells in chicken muscle cell cultures, initiated with 10 cells/plate, which showed both Pax7+/MyoD[−] and Pax7+/MyoD⁺ progeny in the cultures [108]. Actively dividing satellite cells (BrdU or PCNA+) also showed asymmetric cellular localization of Numb [109], an inhibitor of Notch signaling, and a determinant of asymmetry in *Drosophila* neurogenesis [44]. Numb was asymmetrically localized to one pole of the cell, and Numb+ and Numb[−] progenitors showed different patterns of expression of myogenic genes. Adjacent cells that may represent daughter cells showed Numb+/Pax3[−] and Numb[−]/Pax3+ cell pairs, Numb+/Myf5⁺ and Numb[−]/Myf5[−] pairs, and Numb+/desmin+ and Numb[−]/desmin[−] pairs [109].

In vivo experiments for muscle regeneration have shown that there may be only a subset of satellite cells or myoblasts that contribute to new myofibers; these studies support the notion of self-renewal [110–113]. A specific subpopulation of slowly dividing cells (refractory to [³H]thymidine uptake in culture) appeared to survive intramuscular transplantation and proliferate well in vivo [110]. In studies in which intact myofibers, with associated satellite cells, were transplanted to muscles of immunocompromised, dystrophic (*mdx*-nude) mice, investigators observed the generation of large numbers of donor-derived functional Pax7 satellite cells, which supports the concept of stem cell self-renewal in satellite cells [112]. These reports, however, were not designed to identify asymmetric events that yielded these potentially self-renewing subpopulations.

Kuang et al. [114] performed in situ examination of adjacent satellite cells that appear as the daughter cells of a satellite cell division. The orientation of the mitotic spindle within

the stem cell niche appears to influence divisional symmetry. Asymmetric division may occur when the mitotic spindle is oriented perpendicular to the fiber axis and cytokinesis gives rise to 1 Myf5⁻ self-renewing cell that remains in contact with the basal lamina and 1 Myf5⁺ committed cell that is adjacent to the plasma membrane but does not contact the basal lamina. They propose that symmetric divisions may occur parallel to the axis of the myofiber, and give rise to either two self-renewing cells or two committed myogenic cells – both daughter cells contact the basal lamina and the plasma membrane [114, 115] (Fig. 5). The proposed asymmetry will be strengthened by additional studies that include temporal analysis to determine whether the cells are different at the time of cell division or if the cells adopt these different fates.

Broad heterogeneity has been described for satellite cells and other muscle stem cell populations [112, 116–121]. As illustrated for other examples of asymmetry and shown in Fig. 6, clonally derived mixed populations could arise from unequal partitioning of cytoplasmic components or the cells may stochastically adopt unique cell fates. While some data suggest that population heterogeneity derives from asymmetric divisions, additional clonal studies utilizing single cells will strengthen the understanding of this stem cell activity in adult muscle stem cells.

4 Immortal Strand Hypothesis

As stem cells are responsible for the long-term health and maintenance of tissue throughout the adult life of the organism, it is necessary for these cells to have a mechanism to resist the accumulation of replication errors that would occur during normal tissue repair. A proposed mechanism by which cells protect themselves from DNA damage could also give rise to asymmetric divisions in stem cell self-renewal and differentiation. In 1975, John Cairns hypothesized that stem cell division may involve segregation of new and old DNA strands [122] (Fig. 7). If most spontaneous mutations arise during DNA replication, and since DNA is replicated semi-conservatively, Cairns hypothesized that the strand that acquires the mutation would be the strand passed to the progeny of stem cells. The nonmutated strand, in theory, could be retained by the self-renewed stem cell, that is, the immortal daughter cell. In this way, an immortal strand would be maintained through successive divisions while the mutation(s) would accumulate in the mortal daughter that would become the differentiated tissue cells or senescence in time. Further, this analysis can be extended to show how heterogeneity could result in the expanding population. Distinct phenotypes can be categorized based on the DNA template and

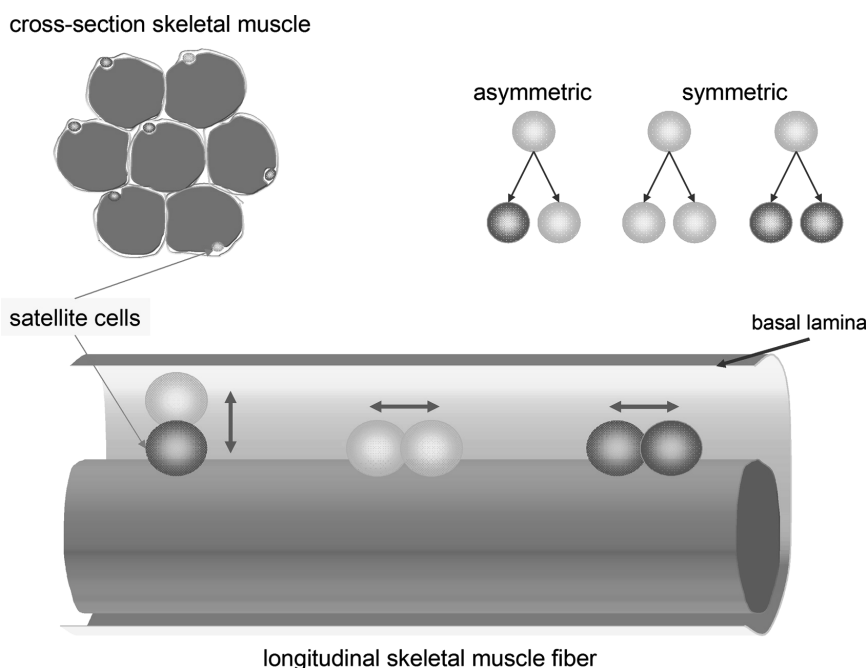


Fig. 6 Asymmetric division of skeletal muscle satellite cells. The orientation of the mitotic spindle within the stem cell niche may influence divisional (a)symmetry. Satellite cells reside in between the basal lamina and the plasma membrane of the muscle fiber. Asymmetric division may occur when the mitotic spindle is oriented perpendicular to the fiber axis and cytokinesis gives rise to one self-renewing cell (Pax7⁺/Myf5⁻) that remains in contact with the basal lamina and one committed cell

(Pax7⁺/Myf5⁺) that is adjacent to the plasma membrane but does not contact the basal lamina. Symmetric divisions may occur parallel to the axis of the myofiber, and appear to give rise to either two self-renewing cells or two committed myogenic cells – both daughter cells contact the basal lamina and the plasma membrane. Image based on *Cossu and Tajbakhsh Cell, 2007, 129:859* [115] and the work of *Kuang S et al, Cell, 2007,129: 999* [114]

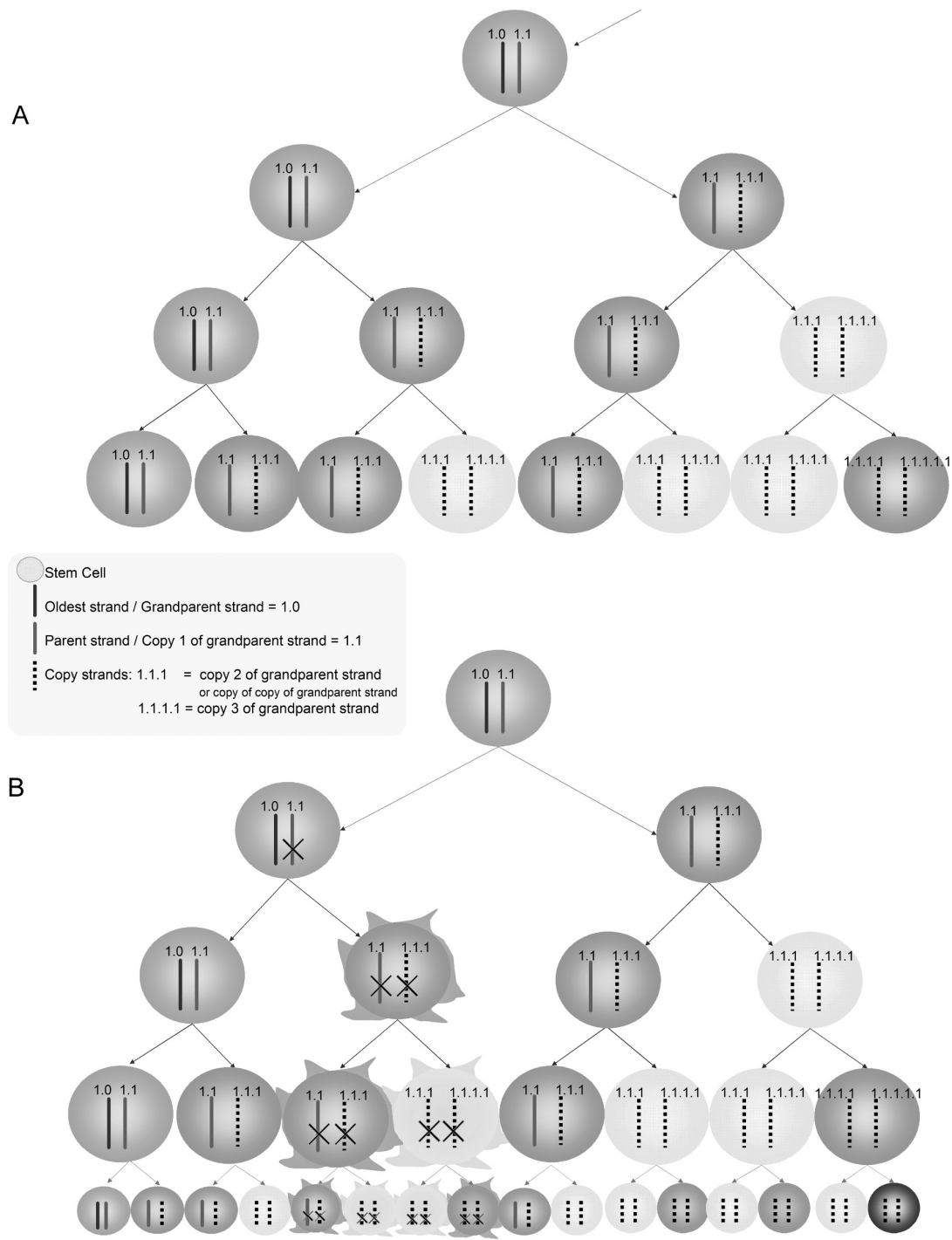


Fig. 7 Immortal strand hypothesis. **(A)** Asymmetry in strand segregation would allow for stem cell self-renewal. The (blue) stem cell would retain the oldest DNA strands. This cartoon shows segregation of one chromosome: the oldest/grandparent strand is blue and designated 1.0, and the parent strand (a copy of the grandparent strand) is red and designated 1.1. All other copies are dashed lines and designated copy numbers are 1.1.1, 1.1.1.1, 1.1.1.1.1 etc. If nonrandom strand segregation occurs among all chromatids in the cell, the result is asymmetric divisions and self-renewal of the stem cell. We extend Cairns analysis to show here how heterogeneity would result in the expanding population. If distinct phenotypes occur based on the DNA strand copy numbers, then these phenotypes can be categorized based on the template and

copy number. For example, after the three divisions shown in the lineage tree above, there would be one stem cell (1.0 and 1.1 strands, p0 phenotype), three pI cells would have (1.1/1.1.1 strands), three pII cells (1.1.1/1.1.1.1), and one pIII cell (1.1.1.1/1.1.1.1.1). **(B)** Further, as Cairns hypothesis showed, a mutation, X, which is heterozygous in the chromosome strands, would segregate and stem cells would be protected against duplication errors. Nonrandom segregation would be required to maintain the immortal strand. This figure also shows how heterogeneity could occur in cancer cells that may develop from the mutation. Some of the cells that derive from the original mutation would also have higher strand-copy numbers that increase the probability of errors in the DNA code (e.g., the green cells with X)

copy number (Fig. 6A). For example, after the three divisions shown in the lineage tree in Fig. 6A, there would be one stem cell that contains a grandparent strand (1.0) and a parent (1.1) strand, there would be three cells that have a parent strand (1.1) and a copy of the parent strand (1.1.1), there would be three cells that have copy of the parent strand (1.1.1) and a copy of a copy of the parent (1.1.1.1) and there would be one cell that has a copy of a copy of the parent (1.1.1.1) and a copy of a copy of a copy of the parent strand (1.1.1.1.1). As the lineage tree or colony grows, the phenotypic difference between the categories would become more distinct.

The immortal strand hypothesis assumes that there is minimal sister chromatid exchange. If this were not the case, the stem cell would not be able to retain a strain that did not have replication errors in the code. Further, cells that preserve immortal strands could avoid the accumulation of errors if they inhibit pathways for DNA repair [123]. Such pathways could potentially cause error-prone resynthesis of damaged strands. Finally, the immortal strands would need to be marked in some way in order for nonrandom segregation to occur. Cairns recognized that the centromeres need to be able to distinguish the sister chromatids and the centromeres would need to behave in a co-coordinated fashion [122].

Some evidence has been presented to support this hypothesis, although several questions regarding its plausibility remain [124, 125]. Several years prior to Cairns hypothesis, nonrandom segregation of sister chromatids was reported for mouse embryonic cells [126]. The investigators examined the incorporation of a pulse of tritiated thymidine in the grand-daughter cells and quantitatively observed unequal label distribution. There also are some early studies using lower organisms that may support the possibility of DNA strand co-segregation [124]. More recently, Potten et al. [127] examined the mouse epithelial in the crypts of the small intestinal mucosa for nonrandom strand segregation. The template DNA strands (or regenerating cells) were first pulse-labeled with tritiated thymidine, and subsequently received a bromodeoxyuridine (BrdU) pulse. Co-expression of these two DNA markers provided evidence that the cells were actively dividing. Long-term retention of the tritiated thymidine label, and concomitant loss of the BrdU label, illustrated that an immortal (label-retaining) strand was actively dividing and the label was segregated nonrandomly. A similar study of mouse neural stem cells used a bromodeoxyuridine label alone to show the long-term retention of (BrdU) in the actively dividing cells [128].

Two studies in skeletal muscle also support the idea of asymmetry in DNA strand segregation. Conboy et al. [129] also showed that mouse myogenic progenitors behaved similarly. In the *in vivo* studies, cells were first labeled with 5-chloro-2-deoxyuridine (CldU) and then a short pulse of 5-iodo-2-deoxyuridine (IdU). Asymmetric inheritance of CldU was evident by all of the detected label being

identified in only one daughter. Lastly, Shinin observed selective template-DNA strand segregation during satellite cell mitosis *in vivo*, and in culture; this provides strong indication that genomic DNA strands are nonequivalent [130]. Interestingly, this study also showed that Numb, previously described for its role in asymmetry, undergoes selective partitioning to one daughter cell. They also found that template DNA and Numb co-segregated in long-term label-retaining cells that express Pax7 [130].

There are some reports that appear to counter the immortal strand hypothesis. Studies that used mouse HSCs showed that co-labeling of BrdU (pulse 1) and halogenated 2-deoxyuridines (CldU or IdU, pulse 2) indicated that all HSCs segregate their chromosomes randomly; both *in vivo* and *in vitro* results supported this idea [131].

Overall, there is increasing support for the immortal strand hypothesis; there is also further development of the theory of the function of nonrandom strand segregation. For example, the silent sister hypothesis distinguishes that the purpose of nonrandom strand segregation is to direct gene expression and cell fate in stem and progenitor cells [125]. This idea is in line with the immortal strand hypothesis and highlights important players involved in cell determination – the epigenetic factors. This is likely to be the exciting future context in which the nonrandom strand segregation is investigated in stem cells.

5 Conclusions

Stem cells function to balance self-renewal with differentiation during embryonic development, and in adult tissue, to maintain tissue homeostasis. One mechanism to maintain stem cell self-renewal is asymmetric cell division, in which one cell self-renews while one cell initiates differentiation. The stem cell niche, or microenvironment, provides both biochemical and biophysical components for these regulated stem cell activities. An increased understanding of the extrinsic cues and intrinsic cues, including nonrandom strand segregation, will allow for the development of methods to control stem cell fate and perhaps increase the use of stem cells in cell therapeutics.

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