

Cell biology of stem cells: an enigma of asymmetry and self-renewal

Haifan Lin^{1,2}

¹Yale Stem Cell Center and ²Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06511

Stem cells present a vast, new terrain of cell biology. A central question in stem cell research is how stem cells achieve asymmetric divisions to replicate themselves while producing differentiated daughter cells. This hallmark of stem cells is manifested either strictly during each mitosis or loosely among several divisions. Current research has revealed the crucial roles of niche signaling, intrinsic cell polarity, subcellular localization mechanism, asymmetric centrosomes and spindles, as well as cell cycle regulators in establishing self-renewing asymmetry during stem cell division. Much of this progress has benefited from studies in model stem cell systems such as *Drosophila melanogaster* neuroblasts and germline stem cells and mammalian skin stem cells. Further investigations of these questions in diverse types of stem cells will significantly advance our knowledge of cell biology and allow us to effectively harness stem cells for therapeutic applications.

Introduction: cell biology of asymmetry for self-renewal

Stem cells exist in early embryos and individual tissues. Embryonic stem cells have the ability to ultimately differentiate into all types of cells in our bodies, whereas tissue stem cells (also known as adult stem cells) serve as immediate sources of cell supply to their resident tissues. Stem cell research has offered the promise of effective cell-based therapies in treating many debilitating diseases such as diabetes, neurodegenerative diseases, and cancer. The therapeutic potential of stem cells has inspired the imagination, intense interest, and targeted investment of scientists, clinicians, and the general public toward this fascinating area of biology. At present, human embryonic stem cell research is politically charged, with biologists engaging in ethical debates. Meanwhile, much of the research effort has been channeled to harnessing stem cells into desired cell types for clinical applications. Such translational research has yielded some exciting results in tissue therapy by transplantation. Excitement notwithstanding, there is still a long way to go in understanding the fundamental mechanisms of stem cells before new therapies will be effectively

established. However, this aspect of stem cell research has not garnered as much attention.

As evident from the three papers in this series of reviews, stem cell biology is, by and large, an integral part of cell biology and presents a vast new terrain of basic cell biology for exploration. The hallmark of a stem cell is its ability to self-renew while generating many daughter cells that are committed to differentiation. Intimately related to this ability are a host of fundamental questions that await investigation: How can we definitively identify a stem cell? What defines a stem cell in molecular terms? What signaling events control stem cell proliferation and differentiation? How does a stem cell behave in its biological context? What happens to a differentiated cell when it is reprogrammed into a stem cell or vice versa? Solutions to these wide-ranging and perplexing questions of cell biology are all related to understanding the single defining feature of stem cells—their self-renewing ability. The self-renewing ability of stem cells is tightly related to their ability to undergo self-renewing asymmetric divisions. The concept of self-renewing asymmetry should be applicable, either strictly during each mitosis or loosely among several mitoses, to all types of tissue stem cells and perhaps even to embryonic stem cells to account for their self-renewal. A stereotypical asymmetric division gives rise to both a daughter stem cell and a daughter cell that has acquired a more differentiated fate. This unique asymmetry allows a stem cell to self-replicate while producing numerous differentiated progeny. It is distinct from another form of asymmetric division that produces two daughter cells that are different from each other as well as from the mother, as often seen for progenitor cells. For those stem cells that undergo apparently symmetric divisions, the self-renewing asymmetry still exists among several divisions because, even stochastically, 50% of the daughter cells need to acquire a more differentiated fate after the divisions. Therefore, how the self-renewing asymmetry is achieved is a central question in stem cell biology.

The three reviews in this issue effectively summarize the latest progress in our understanding of mechanisms that underlie self-renewing asymmetric division of three of the best-characterized tissue stem cell systems—*Drosophila melanogaster* neuroblasts, *Drosophila* germline stem cells, and mammalian skin stem cells. Discoveries from these three model systems complement one another, each revealing a unique aspect of the asymmetric mechanism. Together, they present a comprehensive landscape of molecular mechanisms underlying the self-renewing asymmetric

Correspondence to Haifan Lin: haifan.lin@yale.edu

division of stem cells. It is a pleasure to comment on these exciting discoveries from a more general perspective.

The niche induces asymmetric division

Self-renewing asymmetric division of a stem cell is controlled by both extrinsic signaling and intrinsic mechanisms. Much progress has been made in understanding intercellular mechanisms, especially the identification of niches for various types of tissue stem cells and elucidation of the role of the niche in regulating asymmetric stem cell division. Perhaps the best-illustrated role of the niche in regulating stem cell division comes from the study of germline stem cells in the *Drosophila* ovary and testis (see Yamashita and Fuller on p. 261 of this issue; for more detailed information, also see Lin, 2002). In female flies, somatic niche signaling requires the TGF β pathway and another signaling pathway defined by the YB and PIWI proteins, which are required in niche cells for germline stem cell maintenance. The TGF β and YB–PIWI pathways converge in germline stem cells to repress the expression of *bag of marbles* (*bam*), a gene that is necessary and sufficient for promoting stem cell differentiation (Chen and McKearin, 2005; Szakmary et al., 2005). The niche function is also assisted by Hedgehog signaling and requires niche cell–stem cell adhesion as mediated by epithelial cadherin (King et al., 2001; Song et al., 2002). The niche induces the attachment of one pole of the stem cell spindle to the niche cells (Deng and Lin, 1997). Such attachment is mediated by a spectrin-rich structure called the spectrosome and a cytoplasmic dynein-mediated mechanism (Deng and Lin, 1997; McGrail and Hays, 1997).

Similarly, the *Drosophila* male germline stem cell system contains somatic niche cells (hub cells) that secrete the unpaired ligand for the JAK–STAT (Janus kinase–signal transducer and activator of transcription) signaling pathway to maintain germline stem cells, as reviewed by Yamashita and Fuller (2008). As a stem cell divides, one pole of its mitotic spindle is anchored to the niche cells, ensuring the asymmetric division that allows only one of the two daughter cells to maintain contact with the niche cells and, as such, retains the stem cell fate. This attachment requires adherens complexes that contain cadherin, β -catenin, and adenomatous polyposis coli 2 (APC2) protein, which is similar to the anchorage of mitotic spindle in *Drosophila* embryonic epithelial cells (for review see Lin, 2003).

Although the role of niche in the asymmetric division of mammalian stem cells has not been as clearly illustrated, Fuchs and colleagues have shown that embryonic basal epidermal cells use their polarity to divide asymmetrically with respect to the underlying basal lamina, generating a committed suprabasal cell and a proliferative basal cell (Lechler and Fuchs, 2005; see Fuchs on p. 273 of this issue). Because skin stem cells are a subpopulation of mitotically active basal epidermal cells, it is conceivable that these stem cells divide in an asymmetric fashion to self-renew and to produce differentiated keratinocytes. Moreover, integrins and cadherins in the basal lamina are essential for the proper localization of apical complexes containing atypical PKC (aPKC), the Par3–LGN–Inscuteable protein, and NuMA (nuclear mitotic apparatus protein)–dynactin. This asymmetric localization may be functionally important because similar

complexes in *Drosophila* neuroblasts are essential for asymmetric division, as reviewed in this issue (see Chia et al. on p. 267 of this issue). The requirement of integrins and cadherins suggests that the extracellular matrix, such as basal lamina, can also serve as a stem cell niche or part of a niche. Such an acellular niche also contains signaling molecules such as laminin 5, which is a stable ligand for integrin in hemidesmosomes and focal adhesions. In addition, the basal lamina may serve as mechanical support to the stem cell system. Moreover, its resident proteoglycans and other proteins may function as molecular sinks for growth factors that either promote or restrict the proliferation of epidermal cells, thus serving as a signaling source for these molecules.

In addition to basal epidermal cells, mouse neuroepithelial stem cells and hematopoietic precursor cells undergo both asymmetric and symmetric divisions. In the mammalian central nervous system, embryonic neuroepithelial cells first undergo symmetric division to expand their population and then switch to asymmetric divisions for neurogenesis. This switch involves a change in cleavage plane orientation from perpendicular to parallel to the plane of the apical lamina, leading to an asymmetric distribution to the daughter cells of the apical plasma membrane, which constitutes only a minute fraction (1–2%) of the entire neuroepithelial cell plasma membrane (Kosodo et al., 2004). Somewhat similarly, mouse hematopoietic progenitor cells are capable of both symmetric and asymmetric divisions in cultures supported by stromal cells (Wu et al., 2007). A pro-differentiation stromal cell line increased the frequency of asymmetric division, whereas a pro-proliferation stromal cell line promoted symmetric division. These observations indicate that niche signaling can also control the asymmetry of stem cell division at a populational level.

Inherited cell polarity determines asymmetric division

Although niche induction accounts for asymmetric division in some types of stem cells, it may not play a role in all types of stem cells. For *Drosophila* neuroblasts, the initial cue for symmetry seems to depend solely on the cell itself, as reviewed by Chia et al. (2008). The neuroblasts are derived from embryonic epithelial cells and inherit their polarity, with one end being apical and the other being basal. This allows molecules that determine cell fate to be segregated along the apical-basal axis. The mitotic spindle is also oriented along this axis such that the plane of division is perpendicular to the axis. This means that one daughter cell inherits the apical molecules and remains a neuroblast; the other inherits the basal components and becomes a ganglion mother cell.

Studies on *Drosophila* neuroblasts in the past 15 years have identified a group of proteins localized to the apical cortex that determine the asymmetry of stem cell division. These proteins are organized into two complexes linked by the Inscuteable protein. The first complex includes Bazooka–Par3, aPKC, and Par6, which regulates the tumor suppressor Lethal (2) giant larva (LGL). Such regulation is likely via phosphorylation, which, in turn, affects the activity of LGL in the localization of basal complexes. Conversely, LGL inhibits the basal localization

of aPKC, thereby restricting aPKC to the apical cortex (Lee et al., 2006). Therefore, the regulation of LGL and aPKC is likely to be mutual inhibition. The second apical complex contains heterotrimeric G protein signaling mechanism components: G α i, Partner of Inscuteable (Pins), and Locomotion defect (Loco). These two complexes work in parallel to control the asymmetric localization of cell fate regulators, the apicobasal orientation of the mitotic spindle, and the asymmetric structure of the spindle itself. The coordination of all of these aspects of asymmetry is essential for the asymmetric fates of the two daughter cells, as reviewed by Chia et al. (2008), and will be discussed in further detail in the following sections.

Interestingly, key components of the Par3 complex have also been found in the apical cortex of mammalian skin stem cells, as reviewed by Fuchs (2008). The mouse Numb homologue is localized asymmetrically during hematopoietic precursor cell division, similar to the asymmetric behavior of Numb in *Drosophila* neuroblasts (Wu et al., 2007). These observations raise the possibility that the asymmetric mechanism discovered in the *Drosophila* neuroblast is conserved during evolution.

Centrosomal asymmetry contributes to asymmetric division

A fundamental aspect of the asymmetric division mechanism is the asymmetric property of centrosomes during stem cell division. The mother and daughter centrosomes are known to differ in size, molecular composition, the ability to organize microtubules, and even the ability to localize mRNAs or possibly other cell fate determinants, as systematically discussed by Yamashita and Fuller (2008) and by Chia et al. (2008). In both *Drosophila* male germline stem cells and neuroblasts, the large mother centrosome organizes a more extensive population of astral microtubules and is selectively retained in the daughter stem cell after stem cell division. This feature has also been found in mammalian cultured cells. Additionally, the two centrosomes may differentially associate with cell fate determinants, which would be an effective mechanism for the asymmetric segregation of cell fate determinants. Finally, anchoring of the mother centrosome to the niche is also important for the oriented asymmetric division.

The multifaceted difference between the mother and daughter centrosomes may be a consequence of the structural difference of their resident old and young centrioles, as discussed by Yamashita and Fuller (2008). The exploration of asymmetric features in centrosomal biogenesis and function represents a new area of stem cell research with general implications in cell and cancer biology.

Spindle asymmetry determines the size difference of the two daughter cells

An intriguing feature of asymmetry as revealed by the study of *Drosophila* neuroblast division is the asymmetric geometry of the mitotic spindle. Particularly, the distance between the apical pole and equator of the spindle is greater than that between the basal pole and the equator. This results in an apically located larger daughter neuroblast and a basally located smaller differentiated cell (i.e., the ganglion mother cell). The spindle asymmetry

is controlled by both apical complex I (Bazooka–Par3 and aPKC–Par6) and apical complex II (G α i–Pins–Loco), with either complex alone being sufficient to maintain the geometric asymmetry of the spindle (Cai et al., 2003). In addition, the apical localization of these complexes leads to displacement of the spindle toward the basal cortex, which also contributes to the size difference between the two daughter cells. Given that the components of these complexes are evolutionally conserved, this mechanism may be involved in the asymmetric division of other types of stem cells that generates two daughter cells of unequal size.

Cell cycle regulators have novel roles in asymmetric division

A particularly exciting development in basic stem cell research in the past few years is the discovery of novel functions of cell cycle regulators in controlling the asymmetry of stem cell division, as timely reviewed by Chia et al. (2008). For example, the *cdc2/cdk1* level controls whether a neural or muscle progenitor undergoes symmetric or asymmetric division. In neuroblasts, high levels of CDK1 during mitosis are required for the asymmetric localization of apical and basal protein complexes. In addition, Aurora and Polo kinases act as tumor suppressors in neuroblasts by preventing excess self-renewal, implicating the function of asymmetric division in restricting overproliferation. The mutations of these two kinase genes affect the asymmetric localization of aPKC, Numb, Partner of Numb, and Notch, causing symmetric division to generate two daughter neuroblasts. In addition, anaphase-promoting complex/cyclosome is also required for the localization of Miranda and its cargo proteins (Prospero, Brain Tumor, and Staufen). More surprisingly, even cyclin E, a G1 cyclin, is involved in asymmetric neuroblast division.

Multiple lines of evidence suggest that the asymmetric function of these cell cycle regulators is not via their conventional function in cell cycle control but rather by directly impinging on the asymmetric localization and segregation machineries in neuroblasts. Mutants of some of these cell cycle genes exhibit tumor phenotypes, which is similar to the phenotype of genes required for apicobasal polarity in *Drosophila* epithelia and neuroblasts. These observations highlight the importance of asymmetry in preventing overproliferation (Chia et al., 2008).

Asymmetric division and tissue maintenance: skin as an example

Just as it is important to understand the asymmetric mechanism of stem cells, it is imperative to comprehend the biological impact of asymmetric stem cell division on the development and maintenance of tissues. In this regard, mammalian epidermal stem cells provide an unparalleled opportunity. As described in the review by Fuchs (2008), stem cells for the epidermis and its appendages (hair follicles and sebaceous glands) have been relatively well identified. In the epidermis, different types of differentiating keratinocytes are organized in an orderly fashion along the baso-apical axis. This reflects a gradient of differentiation from basally located stem cells to the most differentiated cells, the stratum corneum on the apical surface. This organization pattern is readily accessible for investigating how the asymmetric division of epidermal stem cells with a defined orientation

serves the need to replenish this tissue. Moreover, epidermal stem cells together with hair follicle and sebaceous gland stem cells contribute to the skin. The three types of stem cells behave similarly in their corresponding lineage, yet they can transiently contribute to another lineage during the injury repair process. This provides excellent opportunities for studying the coordinated control of different stem cell lineages within a tissue to ensure its development and homeostasis. For example, Wnt signaling plays a key role in promoting hair follicle versus epidermal development. After formation of the hair follicle primordium (placodes), Shh further promotes the growth and maturation of hair buds by turning on specific transcription factors. Fuchs (2008) elegantly addresses these issues.

Concluding remarks

Current progress in studying the self-renewing mechanisms of stem cells demonstrates how basic stem cell questions are characteristically cell biological questions and how these questions can be effectively approached by cell biological approaches. Current findings also reveal that stem cells use evolutionally conserved molecular pathways and machineries for their asymmetric division and self-renewal. Thus, the unique properties of stem cells are more a result of the unique combination of cell-general mechanisms than the existence and effect of stem cell-specific molecules. The three reviews in this issue each in its own unique way cover this exciting progress as well as present challenging questions that await exploration. These challenges and progress invite cell biologists to the fascinating world of basic stem cell research.

I thank Valentina Greco, Travis Thomson, Li Liu, Xiao Huang, and Jianquan Wang for critical reading of this review.

The stem cell work in my laboratory is supported by the National Institutes of Health (grants HD33760, HD42042, and HD37760S1), the Connecticut Stem Cell Research Fund, the G. Harold and Leila Mathers Award, and the Stem Cell Research Foundation.

Submitted: 27 December 2007

Accepted: 7 January 2008

References

- Cai, Y., F. Yu, S. Lin, W. Chia, and X. Yang. 2003. Apical complex genes control mitotic spindle geometry and relative size of daughter cells in *Drosophila* neuroblast and pI asymmetric divisions. *Cell*. 112:51–62.
- Chen, D., and D. McKearin. 2005. Gene circuitry controlling a stem cell niche. *Curr. Biol.* 15:179–184.
- Chia, W., W.G. Somers, and H. Wang. 2008. *Drosophila* neuroblast asymmetric divisions: cell cycle regulators, asymmetric protein localization, and tumorigenesis. *J. Cell Biol.* 180:267–272.
- Deng, W., and H. Lin. 1997. Spectrosomes and fusomes are essential for anchoring mitotic spindles during asymmetric germ cell divisions and for the microtubule-based RNA transport during oocyte specification in *Drosophila*. *Dev. Biol.* 189:79–94.
- Fuchs, E. 2008. Skin stem cells: rising to the surface. *J. Cell Biol.* 180:273–284.
- King, F.J., A. Szakmary, D.N. Cox, and H. Lin. 2001. *Yb* modulates the divisions of both germline and somatic stem cells through *piwi*- and *hh*-mediated mechanisms in the *Drosophila* ovary. *Mol. Cell.* 7:497–508.
- Kosodo, Y., K. Roper, W. Haubensak, A.M. Marzesco, D. Corbeil, and W.B. Huttner. 2004. Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. *EMBO J.* 23:2314–2324.
- Lechler, T., and E. Fuchs. 2005. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature*. 437:275–280.
- Lee, C.Y., K.J. Robinson, and C.Q. Doe. 2006. Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. *Nature*. 439:594–598.
- Lin, H. 2002. The stem-cell niche theory: lessons from flies. *Nat. Rev. Genet.* 3:931–940.
- Lin, H. 2003. To be and not to be. *Nature*. 425:353–355.
- McGrail, M., and T.S. Hays. 1997. The microtubule motor cytoplasmic dynein is required for spindle orientation during germline cell divisions and oocyte differentiation in *Drosophila*. *Development*. 124:2409–2419.
- Song, X., C.H. Zhu, C. Doan, and T. Xie. 2002. Germline stem cells anchored by adherens junctions in the *Drosophila* ovary niches. *Science*. 296:1855–1857.
- Szakmary, A., D.N. Cox, Z. Wang, and H. Lin. 2005. Regulatory relationship among *piwi*, *pumilio*, and *bag-of-marbles* in *Drosophila* germline stem cell self-renewal and differentiation. *Curr. Biol.* 15:171–178.
- Wu, M., H.Y. Kwon, F. Rattis, J. Blum, C. Zhao, R. Ashkenazi, T.L. Jackson, N. Gaiano, T. Oliver, and T. Reya. 2007. Imaging hematopoietic precursor division in real time. *Cell Stem Cell*. 1:541–554.
- Yamashita, Y.M., and M.T. Fuller. 2008. Asymmetric centrosome behavior and the mechanisms of stem cell division. *J. Cell Biol.* 180:261–266.