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Invited Review

Asymmetric stem cell division and pathology: insights from *Drosophila* stem cell systems

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Abstract

Adult stem cells maintain many tissues and organs throughout the life of an organism by serving as renewable sources of differentiated cells. While stem cells remain in a relatively undifferentiated state, their daughters can commit to differentiation to acquire distinct cell fates. Therefore, a stem cell's choice between self-renewal and commitment to differentiation is of critical importance to the maintenance of functional tissues and organs. Many adult stem cells can divide asymmetrically to produce one self-renewed stem cell and one differentiated daughter, preserving the critical balance between stem cell and differentiated cell populations. Stem cell dysfunction and/or malfunction have been proposed to lead to several human pathologies, including tumourigenesis and tissue degeneration, yet whether a failure of asymmetric division is a primary cause of stem cell-related pathologies remains largely uninvestigated. Here, I discuss the implications of asymmetric stem cell division in pathology.

Keywords: **stem cell; *Drosophila*; cell fate; asymmetric division; pathology**

Introduction

Asymmetric stem cell division is the most fundamental requirement for the development of multicellular organisms. A multicellular organism is not simply a mass of cells but rather is a conglomeration of distinct cell types, which have essential roles at specific times and locations to contribute to the overall function of the individual. The cells of an individual can be as different from each other as an electric cable, a piece of glass and a towel; neurons transmit information with electricity, lens cells in the eye become transparent to transmit images and gut cells absorb nutritious liquid. More strikingly, the cells that compose a multicellular organism's body originate from a single cell, the zygote. The zygote develops into a multicellular organism through a series of asymmetric cell divisions. Not surprisingly, the mechanism for asymmetric cell division appears to have been well conserved through evolution.

Recently, the asymmetric cell divisions of adult stem cells have become the focus of many researchers' attentions. Stem cells continue to divide throughout the life of an organism, self-renewing their own identity as well as replenishing the supply of differentiated cells. Many adult stem cells have the potential to divide asymmetrically, giving rise to one stem cell and one differentiating cell, preserving both populations in the correct ratio. Asymmetric cell divisions also occur during development, where a single cell may divide to form two cell types, A and B, one of which will in turn divide to give rise to cell types C and D, and the other E and F. Compared to the complexity of embryonic development, the asymmetric divisions of adult stem cells may not seem that exciting because every division produces the same pair (one stem cell and one differentiating cell). Asymmetric stem cell division is an important issue in stem cell biology because imbalances in the stem cell and differentiated cell populations can lead to pathology, including tumourigenesis and tissue degeneration. The dysfunction and/or malfunction of stem cells have been speculated to cause the progression of many diseases because of the fundamental contributions stem cells make to tissue homeostasis. An excess of stem cell self-renewal may lead to tumourigenesis, whereas an excess of differentiation may lead to tissue degeneration, due to the depletion of the stem cell population. Indeed, compelling evidence suggests that a failure in the stem cell specification and/or differentiation programme leads to tumourigenesis and tissue ageing [1]. However, there is little direct evidence that a failure in asymmetric stem cell division leads to disease. In this review, I summarize the recent findings concerning asymmetric stem cell division in the scope of pathology, with a particular focus on cancer and tissue ageing.

Mechanisms of asymmetric stem cell division

There are two main strategies for asymmetric cell division; one controlled by the asymmetric segregation of fate determinants into two daughter cells ('cell-intrinsic'), the other controlled by the asymmetric placement of the two daughter cells into different microenvironments ('cell-extrinsic'). These two strategies can be combined or function independently.

Asymmetric stem cell division controlled by intrinsic fate determinants

The most intensively studied example of asymmetric stem cell division by cell-intrinsic mechanisms is the *Drosophila* neuroblast, where two sets of fate determinants are differentially segregated into the two daughter cells. *Drosophila* neuroblasts divide asymmetrically along the apical-basal axis [2]. Major fate determinants, such as Numb, Miranda and Prospero, form the 'basal crescent' (or basal complex) and are segregated into a daughter cell called the ganglion mother cell, which divides only once more and then commits to terminal differentiation. The 'apical crescent' (or apical complex) forms the opposite side of the basal complex in dividing neuroblasts and remains with the neuroblast. The apical complex contains an evolutionarily conserved polarity complex, including aPKC, Par6 and Baz (Par3) and other proteins, such as Insecutable and Pins. The apical complex controls the formation and position of the basal complex and also functions to orientate the mitotic spindle along the apical–basal axis, ensuring that the fate determinants are partitioned asymmetrically into the two daughter cells.

The cell polarity complex, aPKC–Par6–Par3, appears to be evolutionarily conserved and plays an essential role in establishing cell polarity, not just in asymmetrically dividing cells but also in symmetrically dividing polarized cells, such as epithelial cells. Thus, it is likely that the primary role of the aPKC–Par6–Par3 complex is to establish cell polarity, and this polarity is somehow interpreted by a downstream pathway to specify asymmetric and symmetric divisions. The examples of aPKC–Par6–Par3 in cell polarity include *C. elegans* early embryos, *Drosophila* oocyte formation, and *Drosophila* and mammalian epithelial polarity (reviewed in [3]; see references therein).

Asymmetric stem cell division controlled by extrinsic fate determinants

The *Drosophila* male and female germ line stem cells (GSCs) provide examples of asymmetric stem cell division governed by extrinsic fate determinants. The GSCs reside in microenvironments that specify stem cell identity, called the stem cell niche. The niche provides essential signals to maintain stem cell identity and thus can impose a limitation on the expansion of the stem cell population [4].

Somatic cells, called cap cells and terminal filament cells, constitute the niche of female GSCs and secrete the signalling ligand, dpp, which in turn activates TGFβ signalling in the GSCs to maintain stem cell identity [5] (Figure 1A). The major function of TGFβ signalling in GSC maintenance is to repress Bam, a master regulator of differentiation. In addition to cap cells and terminal filament cells, female GSCs are surrounded by escort stem cells (ESCs), which provide important signals to control GSC behaviour.

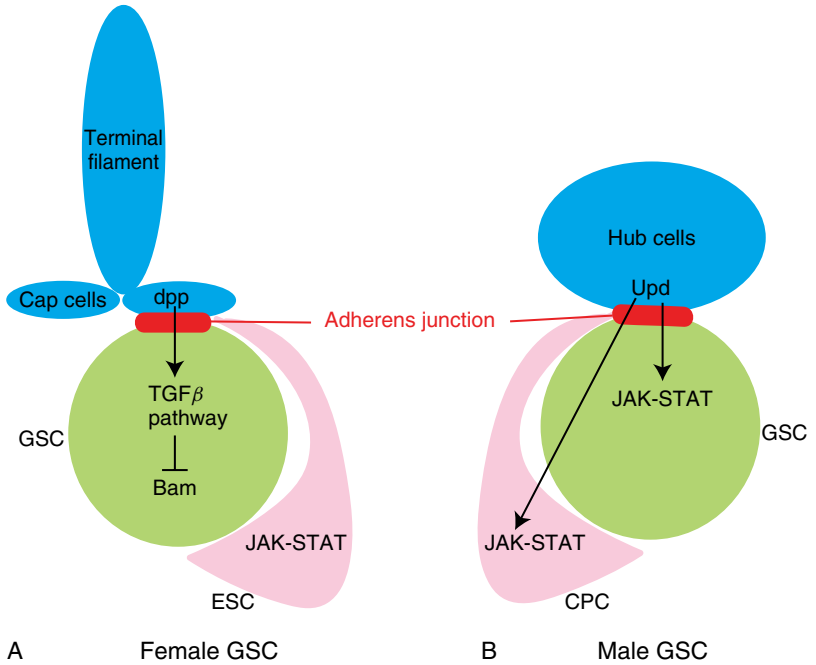

Figure 1. Signalling in the stem cell niche. *Drosophila* female (A) and male (B) germ line stem cells and their niche. Terminal filament/cap cells (female) and hub cells (male) send signals essential for GSC identity. Escort stem cells (ESCs, female) and cyst progenitor cells (CPCs, male) also provide environments that maintain GSC identity

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The ESCs are controlled by the JAK–STAT signalling pathway, hyperactivation of which leads to ESC and GSC over-proliferation [6]. ESCs are stem cells that generate escort cells, which encapsulate differentiating germ cells, presumably providing essential signals that promote differentiation.

The somatic hub cells in testes secrete a signalling ligand, called Unpaired (Upd), to activate the JAK–STAT pathway in the male GSCs, and thus function as a major component of the stem cell niche [7,8] (Figure 1B). The loss of the JAK–STAT pathway in the germ line leads to a failure of stem cell maintenance and over-expression of the ligand, Upd, leads to stem cell tumours. However, Leatherman and DiNardo recently reported that the activation of the JAK–STAT pathway only within the germ line is not sufficient in specifying GSC identity (although the JAK–STAT pathway is required within GSCs for stem cell identity) [9]. Instead, the pathway activation in somatic cells is enough to induce cyst progenitor cell (CPC) identity and, in turn, GSC tumours as well. CPCs are the somatic stem cells that encapsulate GSCs and are supposed to provide essential signal(s) that specify GSC identity and/or prevent differentiation. The results indicate that the activation of the JAK–STAT pathway in CPCs dominantly instructs CPC identity and GSC identity in neighbouring germ cells, while the pathway activation within germ cells are not enough (although required) to drive stem cell identity. This finding may place male GSCs closer to female GSCs than had been previously appreciated, and suggests the existence of closely related signalling pathways in male and female GSCs.

This newly revealed nature of the JAK–STAT pathway in specifying CPC and GSC identity are highly reminiscent of that seen in ESCs and GSCs in female gonads, raising the possibility that ESCs and CPCs are the counterparts that share many aspects in niche signalling. In addition, although it has been emphasized that the hub cells are the major components of the male GSC niche, other somatic cells (CPCs and cyst cells) clearly play important roles in stem cell identity and the differentiation of germ cells [10–12]. Furthermore, germ cells also appears to signal back to somatic cells to control their proliferation, since somatic cells start proliferating in the absence of germ cells [13]. Therefore, the interactions of many cell components promote the mutual maintenance of cell type identity, creating microenvironments for stem cells (and other cells as well). Here, I would like to remind readers that the concept 'niche' does not mean the component cells themselves sending the signal(s) to the stem cells. Instead, 'niche' is a term to describe the microenvironment, in which stem cells maintain their identity. In the example of male GSCs, the hub is not the niche but rather the place where GSCs can receive signals from the hub is the niche.

In these two cases (female and male GSCs), the fates of the daughters produced by stem cell divisions appear to be determined solely by the locations in which they are placed. In this regard, the two daughter cells of a stem cell division are intrinsically equivalent, but different microenvironments instruct these cells to become different cell types; the niche instructing germ cells to become stem cells. Indeed, partially differentiated germ cells that are displaced away from the stem cell niche can re-assume stem cell identity once they are placed back in the niche [14,15]. In such a microenvironment, GSCs divide asymmetrically by orientating their mitotic spindles perpendicular to the niche component cells. In this manner, one of the resultant daughter cells will maintain a close association with the niche, while the other will be pushed out of the niche.

The importance of spindle orientation

As easily deduced from the above examples (neuroblasts and male/female GSCs), spindle orientation is critical during asymmetric division, no matter how the asymmetry is established (intrinsic or extrinsic). In *Drosophila* male GSCs, the spindle orientation is prepared by an elaborate cellular mechanism, precisely controlling centrosome placement throughout the cell cycle. Before duplication, the single centrosome is always located close to the interface between the GSC and the hub cell [16]. After centrosome duplication, the mother centrosome stays close to the hub–GSC interface, while the daughter centrosome migrates away, thereby specifying the perpendicular orientation of the mitotic spindle even before the onset of mitosis [17]. In female GSCs, a germ line-specific organelle, called the spectrosome, appears to be essential for spindle orientation [18]. One pole of the mitotic spindle is anchored to the spectrosome, which is always located close to the cap cells. In both cases, E-cadherin-containing adherens junctions form between the niche component cells (hub cells or cap cells) and the GSCs. It is likely that these adherens junctions (and the cell cortex area that contains adherens junctions) provide polarity cues [19] in addition to mechanically supporting GSC–niche interactions.

Although neuroblasts do not rely on extrinsic fate determinants, the axis formation is somehow dependent on extrinsic information [20]. If neuroblasts are cultured, those that remain clustered with the epithelial cells due to the incomplete dispersal of cells maintain cell polarity, in accordance with their attachment to epithelial cells. Apical–basal polarity appears to be maintained throughout the cell cycle, and every time the neuroblast forms apical and basal polarity complexes, they form in a similar location to that in the previous cell cycle. In contrast, 'isolated' neuroblasts that do not have any contact with other cells do form polarity complexes, but they appear at random locations in every cell cycle. Yet, these isolated neuroblasts can coordinate the axis of apical and basal complexes and divide asymmetrically. This phenomenon suggests

a two-step polarization of the neuroblast. First, interactions between the neuroblast and epithelial cells instruct which way the neuroblast needs to polarize. Once established, the cell polarity dictates the orientation of the spindle through a mechanism independent of cell–cell contacts.

Asymmetric stem cell division and tumourigenesis

Although it has been long speculated, there is little direct evidence that a failure of asymmetric stem cell division leads to tumourigenesis. If stem cells rely on the niche for their stem cell identity, the limited availability of the stem cell niche can limit the expansion of the stem cell population, even if stem cell divides 'symmetrically'. *Drosophila* male GSCs from *cnn* mutants frequently divide symmetrically, since *cnn* mutants are defective in microtubule anchoring to centrosomes and cannot correctly orientate mitotic spindles toward the hub cells. Yet, the increase in stem cell number in *cnn* mutants is very mild (only ~20–30% increase), due to the fixed size of the hub [16]. In *cnn* mutant testes, GSCs become more 'crowded' around the hub, due to symmetrical divisions, but those crowded GSCs can attach to the hub with only a smaller cell cortex area and it appears that, if those cells try to divide symmetrically, some GSCs 'pop off' from the hub, presumably because they do not have enough cell surface to adhere to the hub cells. This suggests that a failure of asymmetric stem cell division (or uncontrolled symmetric stem cell division) is not enough to induce tumourigenesis. It is plausible that tumourigenesis requires additional key events, such as an expanded stem cell niche or mutations that free stem cells from niche dependence.

However, if stem cells do not rely on the stem cell niche, it might be possible that symmetric stem cell divisions lead to tumourigenesis. One interesting observation comes from the study of *Drosophila* neuroblasts. There is a subset of mutations that lead to loss of cell polarity and/or the asymmetric stem cell division of neuroblasts. In these mutants, including *lgl, dlg, numb* and *miranda*, neuroblasts often fail to divide asymmetrically and form malignant tumours (highly proliferative, invasive/metastatic and lethal to the host; reviewed in [21] and references therein). In the case of polarity mutants, such as *lgl* and *dlg*, neuroblasts divide symmetrically, in which both the apical complex and the basal complex are segregated equally into the two daughter cells. In the case of fate determinant mutants, such as *numb* and *miranda*, neuroblasts divide asymmetrically (in terms of the apical complex and spindle orientation) but the daughter cell that is supposed to differentiate fails to differentiate due to the lack of fate determinants. These studies suggest that a failure in fate determination can sometimes lead to tumourigenesis. It should be noted that neuroblasts do not appear to rely on the stem cell niche, and thus defective asymmetric stem cell division may be able to induce tumourigenesis on its own.

Recently it has been shown that multiple centrosomes can initiate tumourigenesis in *Drosophila* neuroblasts [22]. When multiple centrosomes (more than two) are induced by the over-expression of a kinase that promotes centrosome amplification (SAK–Plk4), the cells remain capable of forming bipolar spindles, with little evidence for genetic instability. However, neuroblasts with amplified centrosomes fail to orientate their mitotic spindles with respect to the apical–basal axis and divide symmetrically. Such neuroblasts can generate metastatic tumours upon transplantation. This is the first demonstration that centrosome amplification can initiate tumours, after numerous observations that tumour cells often contain more than two centrosomes. This raises an intriguing possibility that some tumours with amplified centrosomes became tumourigenic not due to genomic instability (as previously speculated), but due to a failure of asymmetric stem cell division. Strong support for this hypothesis was published by Castellanos *et al*, who demonstrated that neuroblast mutants that display genomic instability (such as ATM mutant defective in DNA damage checkpoint, and bubR1 mutant defective in spindle assembly checkpoint) do not generate metastatic tumours, in contrast to centrosome mutants [23].

Stem cells and ageing

Tissue degeneration is the opposite phenotype of tumourigenesis and once again, because of their fundamental role in providing new cells, stem cells have been suspected to be the culprits of many tissue-degenerative diseases and ageing. Thus far, there is no evidence to suggest that a failure of asymmetric stem cell division leads to tissue degeneration (in disease or ageing). Rather, the focus has been on stem cell function and environment. There is compelling evidence that multiple causes lead to lower production of new cells by stem cells, including decreases in stem cell number, stem cell function, function of the microenvironment and systemic environment (reviewed in [24]; see references therein).

It was recently demonstrated for *Drosophila* male and female GSCs that stem cell number decreases in aged flies after day 50. Such decreases in stem cell number appear to be due partly to the decreased function of niche-supporting cells [25,26] and partly to stem cell-intrinsic ageing, such as an accumulation of damages caused by reactive oxygen species [26]. However, the average life span of *Drosophila* is ~40 days and tissue degeneration is obvious at day 30, when the number of stem cells has not significantly decreased, suggesting that there are additional physiological causes for tissue degeneration that occur within the normal lifespan. Recently we have found that, with

age, *Drosophila* testes accumulate GSCs with misorientated centrosomes (in which neither of the two centrosomes is adjacent to the hub), and this 'misorientated' GSC population has low cell cycle activity, suggesting that GSC polarity is important for stem cell function and has an effect on tissue ageing [27].

Summary

The *Drosophila* model system has served as a powerful and genetically tractable system to study the regulation of stem cell behaviour. Many studies in mammalian systems have been triggered by the knowledge obtained from lower model organisms, such as *Drosophila* and *Caenorhabditis elegans*, and often reveal evolutionary conservation. Stem cell behaviour is tightly regulated via multiple layers of control, niche signalling and asymmetrical division. A better understanding of these features of stem cell biology will shed light on the mechanisms that, when misregulated, lead to diseases, such as tumourigenesis and tissue degeneration. Also, understanding how stem cells are controlled will allow us to better exploit their nature *ex vivo* for therapeutic usage.

Teaching Materials

Power Point slides of the figures from this Review may be found in the supporting information.

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