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Review

Endocytosis, asymmetric cell division, stem cells and cancer: Unus pro omnibus, omnes pro uno

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ABSTRACT

Carcinogenesis can be initiated in adult stem cells, suggesting that tumours arise as a consequence of stem-cell dysfunction. In the fruitfly, cancer arises in stem cells that fail to undergo asymmetric cell division. In flies and mammals, a specific regulation of the endocytic trafficking machinery allows stem cells to self-renew and generate the differentiating cells required to form and maintain mature organs. We review recent findings suggesting that an understanding of the relationship between endocytosis, asymmetric cell division, stem cells and cancer will be crucial to unravel the cell biological basis of tumourigenesis.

1. Introduction

Cancer has long been seen as a disease that arises from mutations that impair the capacity of any cell within the organism to respond to the signals that regulate proliferation. More recently, this view has however been challenged by the so-called Cancer Stem Cell (CSC) hypothesis: According to this concept, cancer could arise only from very few cells that have the capacity to generate the numerous different cells types present in a tumour. This process would be similar to the formation of different cell types during normal embryogenesis (Clarke and Fuller, 2006). In this review, we would like to suggest that studying the mechanisms that regulate the formation of different cell types through Asymmetric Cell Division (ACD) will be crucial to understand stem cells and their role in tumourigenesis.

We start by reviewing some of the experiments that led the foundation for the CSC hypothesis, as well as recent data that suggest that cancer can arise from the dysfunction of stem cells that are naturally present in adult tissue (Section 2). The observation that cancer may be a stem-cell disease raises the question of how proliferation and function of these cells are controlled under wild-type conditions. In Section 3, we review recent evidence obtained largely in *Drosophila* that suggests that stem cells give rise to one new stem cell and one differentiating daughter through ACD. Failure to divide asymmetrically causes stem cells to overproliferate and become CSCs. We then describe findings that suggest that in the

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nervous system of the fly, ACD is largely controlled through a differential regulation of the endocytic trafficking of signalling molecules in the two daughter cells (Section 4). Finally, we discuss in Section 5 first evidence that asymmetric endocytosis may indeed be implicated in the control of adult stem cells in the mammalian haematopoietic system.

2. When two become one: cancer and stem cells

The development and adult life of multicellular organisms require a tight control of cell divisions to ensure a proper balance between different cell populations. Complex metazoan organisms have therefore evolved numerous mechanisms to prevent cancerous overproliferation. As a consequence, cells can only escape proliferation control after having acquired a series of deleterious mutations. Indeed, it has been known for a long time that the formation of malignant tumours represents the final stage of a multi-step process (Hanahan and Weinberg, 2000). One of the implications of this model is that in order to accumulate a critical number of harmful modifications, a cell would have to be long-lived. In many of the organs that give rise to the most frequent cancers, such as the haematopoietic system, the skin and the gut, the vast majority of the cells are however subject to very rapid turn-over, making it highly unlikely that they may acquire a full set of oncogenic modifications. In contrast, tumourigenic transformation could occur with much higher probability in ''immortal'' and repeatedly dividing stem-cell-like populations, a model that has become known as the ''Cancer Stem Cell'' hypothesis (Clarke and Fuller, 2006).

A number of studies in the last fifteen years have indeed proposed that only a very small percentage of the cells that compose a tumour can initiate cancer when transplanted into a healthy recipient host. Pioneering work in the haematopoietic system showed that only one out of 250 000 human Acute Myeloid Leukemia cells can initiate leukemia when transplanted into immune-deficient mice (Lapidot et al., 1994). Interestingly, further investigations revealed that the tumourigenic cells can be recognized by the presence of specific surface markers that are characteristic for a primitive precursor population, suggesting that the original leukemic transformation may have occurred in haematopoietic stem cells (Bonnet and Dick, 1997). Similar observations pointing at the existence of CSCs have been obtained more recently for solid tumours in breast (Al-Hajj et al., 2003), brain (Singh et al., 2004), skin (Schatton et al., 2008) and colon cancers (O'Brien et al., 2007; Ricci-Vitiani et al., 2007).

2.1. *Cancer stem cells: the chosen few or the vast majority?*

At this point it seems important to clarify the CSC concept, in order to avoid frequent confusions that arise from mixing the concepts of cancer, stem cells and Cancer Stem Cells. Currently, the consensus definition of a CSC is a cell within a tumour that can self-renew and form the different cell types present in the tumour (Clarke et al., 2006). As such, this definition makes no statement about either the abundance or the origin of CSCs.

One of the founding observations of the CSC concept was the finding that only a minute fraction of tumour cells can actually sustain cancer. Obviously, this has important consequences for the way we think about cancer: if only a small subset of cells is capable to initiate cancer formation, the crucial step on the way to successful therapy may actually be to understand the behaviour of these cells to ensure their elimination. Two recent studies suggest however that the potential to initiate cancer formation may be much more widespread among tumour cells than previously thought (Kelly et al., 2007; Quintana et al., 2008). The current gold standard for the identification of CSCs has been the serial implantation of human cancer cells into immune-deficient SKID/NOD mice in order to elicit tumour formation. Studies using this approach have suggested that 0.1–0.0001% of the cells of a tumour are actually ''tumourigenic''. In the new studies, cancer cells were transferred among more similar organisms by implanting murine tumour cells into mice (Kelly et al., 2007) or human tumour cells into even more severely immune-depleted mice (Quintana et al., 2008). Both of these approaches increase the cancer forming efficiency by several orders of magnitude, with up to 25% of the implanted cells initiating the formation of new tumours. It should however be noted that, per se, neither of these reports is incompatible with the CSC concept, although the two studies suggest that the abundance of these cells in malignant tumours may have been substantially under-estimated. In this context it is interesting to note that the specific introduction of oncogenic mutations in murine intestinal stem cells causes the formation of tumours that comprise about 7% CSCs (Zhu et al., 2009; Barker et al., 2009) (see below).

2.2. *Where do cancer stem cells come from?*

Besides their scarcity or abundance, a second important issue with respect to CSCs is their origin. The cells of most adult organs can be grouped in three classes: Stem cells, Transit Amplifying Cells (TACs) and differentiated cells. Stem cells are capable to form all the cell types that compose the mature organ. They divide throughout the life of the organism to replace dying cells and maintain tissue homeostasis. In many instances, the division of a stem cell gives rise to one new stem cell and one TAC. TACs undergo a limited number of cell divisions before giving rise to the differentiated cells that ensure organ function. The multi-step process of oncogenic transformation could therefore give rise to CSCs in two ways: According to a first scenario, TACs or terminally differentiated cells could acquire the infinite proliferative potential of stem cells, causing hence the excessive production of different cell types. Alternatively a second possibility could be that adult stem cells that are naturally present within a tissue accumulate mutations that allow them to escape the mechanisms that normally regulate their proliferation. While both of these scenarios are conceivable, recent work provides compelling evidence for the later hypothesis by demonstrating that tumour formation can arise from natural stem cells (Barker et al., 2007).

One of the main problems in studying the role of stem cells in tumourigenesis has been the lack of stem-cell markers. Recent work in the mouse has allowed major

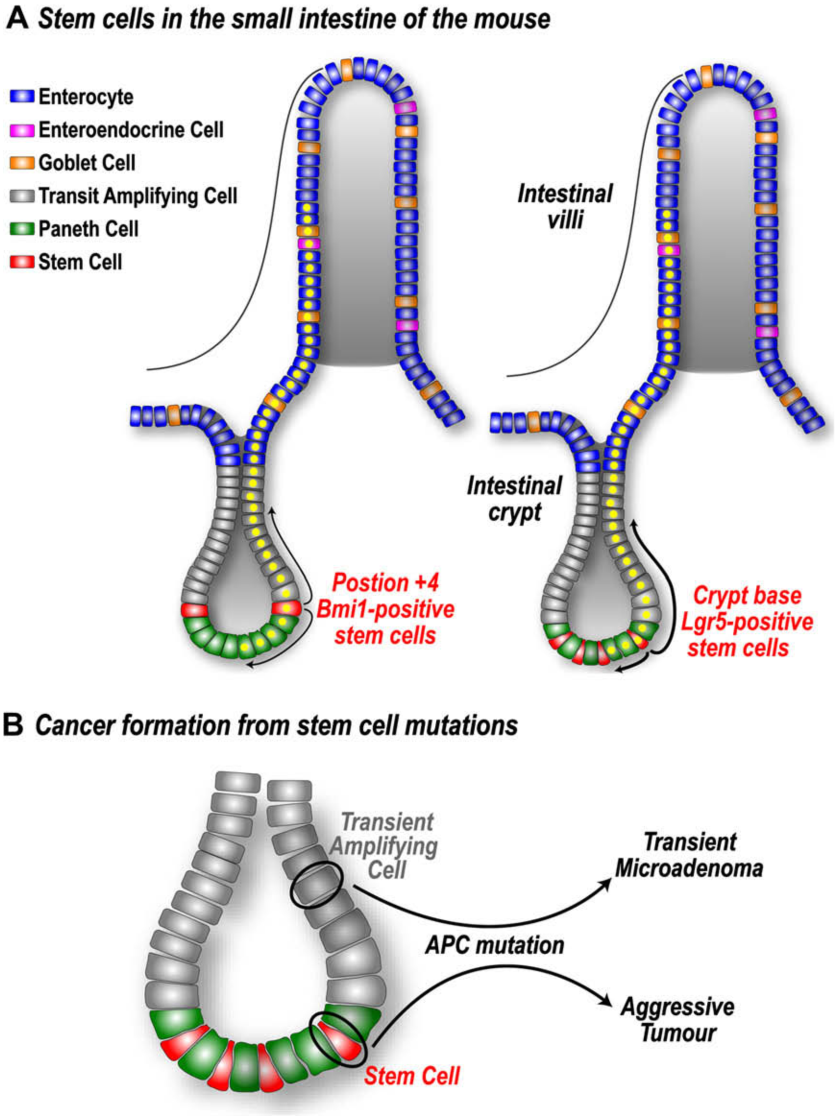

Figure 1 – Stem cells in the mammalian small intestine. A: The murine intestine harbours two stem-cell populations. Left: In 10% of the crypts of the anterior part of the small intestine, Bmi1-positive stem cells (red) are present at position +4 with respect to the crypt bottom. Right: Throughout the small intestine and colon, Lgr5 expression marks a population of wedge-shaped stem cells (red) that are found at the crypt bottom between Paneth cells (orange). If a stable lineage marker (yellow dots) is expressed in Bmi1- or Lgr5-positive cells, all cell types in the crypt and villus are labelled, demonstrating hence that the two stem-cell populations can indeed give rise to all the differentiated cells that compose the organ. B: Inactivation of APC causes cell autonomous activation of the Wnt signalling pathway. APC inactivation in transit amplifying cells (grey) causes only transient overproliferation. In contrast, a mutation in stem cells (red) causes the formation of aggressive adenomas.

progress along this line: The small intestine has long been known as an excellent model system for the study of stem-cell function. In this tissue the basal crypts act as proliferative compartments that harbour both stem and transit amplifying cells that give rise to the different types of differentiated cells that are present in the intestinal villi (Figure 1A). All cells of a villus are renewed every three to five days due to extensive crypt cell proliferation. Numerous studies throughout the last years have revealed that signalling by growth factors of the Wnt family plays a major role in the regulation of crypt cell proliferation (Radtke and Clevers, 2005). Indeed malignant mutations that lead to colorectal cancer target most frequently Adenomatous Polyposis Coli (APC), a negative regulator of Wnt signalling. A comprehensive characterisation of intestinal Wnt target genes has led to the identification of Lgr5, an orphan G-protein coupled receptor, as a specific marker of intestinal stem cells. According to their position and morphology, these cells have been termed Crypt Base Columnar cells (Barker et al., 2007). Genetic lineage tracing experiments reveal that Lgr5-positive cells are long-lived and can give rise to all the cell types of the small intestine, identifying them hence as bona fide intestinal stem cells (Figure 1A). Lgr5 is also expressed in stem cells of the murine hair follicle, suggesting that this gene may mark stem cells in numerous tissues (Jaks et al., 2008).

When the Lgr5 promoter was used to inactivate APC specifically in intestinal stem cells, it caused their rapid neoplastic transformation and the appearance of severe adenomas (Barker et al., 2009) (Figure 1B). A similar tumourigenic phenotype has been observed in an independent study that used the promoter of Prominin1/CD133, a gene co-expressed with Lgr5 in intestinal crypts, to promote the stem-cell specific activation of Wnt signalling (Zhu et al., 2009). Most importantly, tumour formation was not observed when APC was inactivated in transient amplifying cells instead of stem cells (Barker et al., 2009) (Figure 1B).

A second study has identified Bmi1, a member of the Polycomb group gene family involved in epigenetic gene silencing, as another stem-cell marker in the murine small intestine (Sangiorgi and Capecchi, 2008). Similar to what was observed for Lgr5-positive cells, Bmi1-expressing cells give rise to the entire spectrum of intestinal cells and activation of Wnt signalling in Bmi1-expressing stem cells causes cancer formation (Figure 1A, B). Surprisingly, Lgr5 and Bmi1 are not expressed in the same cells: while Lgr5 is detected throughout the small intestine in wedge-shaped cells at the crypt bottom, Bmi1 is transcribed only in the anteriormost small intestine in cells that occupy position +4 with respect to the crypt bottom (Figure 1A). Further studies will be required to investigate whether Lgr5- and Bmi1-positive cells are entirely distinct, or whether they might be related to each other.

The current observations show unambiguously that Wnt pathway activation can direct tumour formation in naturally occurring stem-cell populations. At the same time they highlight however an additional level of complexity arising from the unexpected presence of different stem-cell populations within the very same tissue. Moreover it should be noted that it can currently not be ruled out that mutations different from the ones used in the present studies (Zhu et al., 2009; Barker et al., 2009; Sangiorgi and Capecchi, 2008) might cause the tumourigenic transformation of non-stem-cell populations.

3. Asymmetric cell division in stem cells and cancer

The advent of the CSC hypothesis prompts two obvious questions: first, how is the proliferation and maintenance of stem cells controlled? Second, how can deviations from normal stem-cell behaviour lead to cancer? The main characteristic of stem cells is their mode of division which involves the formation of two different daughter cells: one that keeps a stem-cell identity, and a second one that is prompted to further differentiation. Depending on the tissue context, the formation of two different daughters is thought to be achieved by different mechanisms. According to a first scenario, the generation of different mitotic sister cells can be ensured through a cell-intrinsic process whereby cell fate determinants are unequally distributed during ACD. This type of mechanism is best known for its occurrence in the nervous system of flies and mammals (Knoblich, 2008), but its has also been described in other tissues such as the murine epidermis (Lechler and Fuchs, 2005). Alternatively, stem cells in organs like the *Drosophila* male or female gonads contact tissue-specific niche cells that emit signals to prevent differentiation and hence promote stem-cell identity (Fuller and Spradling, 2007). Following division, one of the two stem-cell daughters finds itself displaced from the signals emanating from the niche and initiates differentiation. It should however be noted that even in the presence of a morphologically identifiable niche, stem cells are polarized at the moment of division: In *Drosophila* male germ cells, APC is recruited to a specialized contact point between the stem cell and the niche in order to retain the mother centrosome in the stem cell and orient the mitotic spindle perpendicular to the niche (Yamashita et al., 2003, 2007).

3.1. *Cancer through defective asymmetric cell division*

Mutations that perturb cell polarity can cause neoplastic overproliferation (Bilder et al., 2000). The formation of these tumours has long been thought to arise from the perturbation of the mechanisms that control growth factor signalling in intact epithelia. Recent work performed in *Drosophila* brain stem cells suggests however an alternative explanation (Caussinus and Gonzalez, 2005). The larval neuroblasts of the fly have recently emerged as an excellent model system for the study of stem-cell biology. During larval development, neuroblasts act as stem cells to generate the different cells of the central nervous system. Through ACD neuroblasts self-renew and generate at the same time a ganglion mother cell that divides once again to form two terminally differentiated neurons (Wu et al., 2008) (Figure 2A). The observation that isolated larval neuroblasts can continue to generate their normal progeny shows clearly that in this system ACD can be achieved through cell-intrinsic mechanisms (Rebollo et al., 2007). Interestingly, the transplantation of neuroblasts that are mutant for known regulators of ACD causes the formation of tumours in wild-type hosts (Caussinus and Gonzalez, 2005), a situation perfectly analogous to the experiments that have led to the original identification of CSCs (Lapidot et al., 1994; Bonnet and Dick, 1997). Most importantly, tumours arise only following the implantation of mutant brain stem cells, but not upon the transplantation of equally mutant symmetrically dividing wing epithelial cells (Castellanos et al., 2008) (Figure 2C).

3.2. *The root of all evil: how can asymmetric cell division go wrong?*

The observations mentioned above suggest that a CSC is as stem cell that fails to undergo proper ACD. To understand the initial steps of tumourigenesis, we need therefore to understand the mechanisms regulating ACD. Three types of ACD defects can result in stem-cell derived cancers in *Drosophila*:

1. The function of ACD is to create two different daughter cells. One way to achieve this is the asymmetric segregation of so-called cell fate determinants. A cell fate determinant is a molecule that instructs the cell that inherits it to adopt a certain identity. In *Drosophila* Neuroblasts, several cell fate determinants are known to specify the formation of the differentiating daughter cell. In cell fate determinant mutants, no daughter cell is driven into differentiation and two stem cells are formed, a process that will eventually lead to a tumourous overproliferation of the stem-cell population (Figure 2B).
2. Successful asymmetric segregation of cell fate determinants depends on two processes: First, the determinant

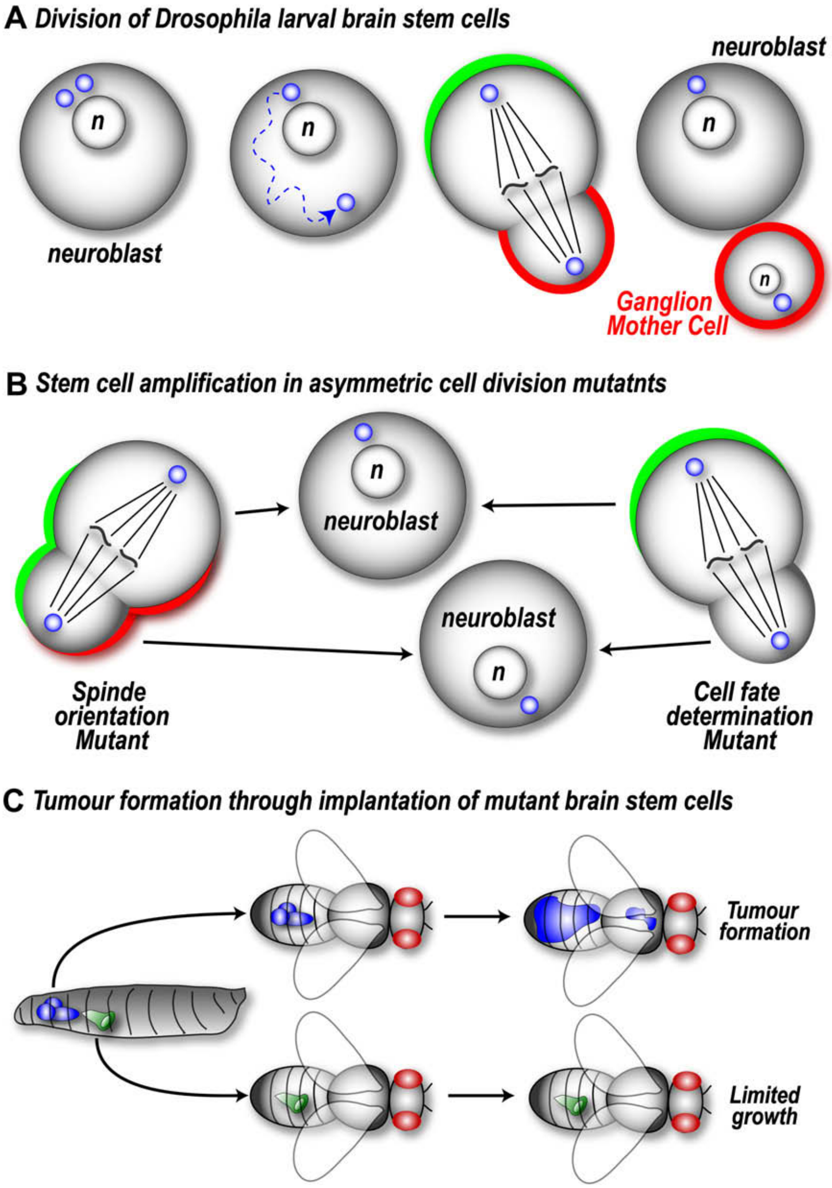

Figure 2 – Larval neuroblasts are *Drosophila* brain stem cells. A: Larval neuroblasts undergo asymmetric cell division to self-renew and generate one differentiating Ganglion Mother Cell. The axis of division is established through the unequal behaviour of the two centrosomes (blue): while one centrosome remains immobile, the second one migrates to the opposite pole of the cell to orient the axis of division. Asymmetric cell division involves the recruitment of aPKC (green crescent) to the apical cortex of the neuroblast while Numb, Prospero and Brat localise basally (red crescent) and segregate into the ganglion mother cell. n: nucleus. B: Left: Tumourigenic overproliferation of neuroblast stem cells can result from a failure to align the spindle so that cell fate determinants (red and green crescents) are now equally partitioned. As a consequence, both daughter cells adopt a neuroblast identity (middle), causing amplification of the stem-cell population. Right: Similar stem-cell overproliferation can arise from the lack of functional cell fate determinants. C: Upper row: Implantation of larval neuroblasts that fail to undergo asymmetric cell division (blue) elicits tumour formation in wild-type flies. Lower row: Tumour formation does not occur when symmetrically dividing wing imaginal disc cells that carry the same mutation (green) are implanted.

itself must be enriched in one part of the cell and depleted from the other (e.g. through recruitment to a certain domain at the cell cortex). Second, the mitotic spindle must be oriented in such a way, that one cell will inherit the polarized determinant, while its sister does not. If any of these two processes fails, determinants will partition equally among the two daughter cells, which will then adopt the same identity. If both daughters behave as stem cells, tumour formation will occur (Figure 2B).

3. The two daughters of an ACD can communicate with each other and the environment through the use of cell-cell signalling pathways. In some cases, the formation of different daughter cells relies on the differential activation of a signalling pathway in the two daughter cells. Consequently,

failure in the regulation of signalling activity can result in tumourous stem-cell self-amplification.

In the following, we discuss in some detail these three scenarios.

3.3. *Stem cells, cell fate determinants and cancer*

Studies in *Drosophila* larval brain stem cells (neuroblasts) have identified four cell fate determinants: Prospero, Brain tumour (Brat), Numb and atypical Protein Kinase C (aPKC). While inactivation of any of these genes will cause cancer, their functions are different. Prospero, Brat and Numb instruct the cell that inherits them to become a Ganglion Mother Cell (GMC), which divides once more to generate two neurons or a transient amplifying cell (termed secondary neuroblast (Choksi et al., 2006; Betschinger et al., 2006; Wang et al., 2006; Lee et al., 2006a; Bowman et al., 2008). In contrast, aPKC directs the other cell to retain its stem-cell identity (Lee et al., 2006b) by promoting the asymmetric segregation of Numb, an endocytic protein that negatively regulates the activity of the Notch signalling pathway (Wirtz-Peitz et al., 2008) (see Section 4).

Prospero and Brat both bind to the adapter molecule Miranda so that all three proteins form a basal crescent during ACD and are subsequently inherited by the differentiating daughter cell, the GMC (Betschinger et al., 2006; Bello et al., 2006; Lee et al., 2006c; Shen et al., 1997). Prospero acts directly on cell-cycle genes and appears to be important to limit the cell-cycle progression of GMCs and promote the neuronal differentiation of their progeny (Choksi et al., 2006; Li and Vaessin, 2000)

Brat, a member of the TRIM-NHL protein family, acts as a posttranscriptional repressor of Myc and thereby prevents GMC growth. In Brat mutants, GMCs reinitiate growth after division and behave like neuroblast stem cells (Betschinger et al., 2006; Bello et al., 2006; Lee et al., 2006c). Interestingly, TRIM-NHL proteins appear to play a similar role in different stem cell populations. As second family member, Mei-P26 is required for germ cell differentiation following ovarian stem-cell division (Neumuller et al., 2008). The murine Brat orthologue TRIM32 has moreover recently been shown to encode a factor that is asymmetrically inherited during neural progenitor division (Schwamborn et al., 2009). Once again, one function of TRIM32 is to prevent growth of the differentiating daughter cell by promoting the ubiquitination and degradation of Myc.

3.4. *Orienting the mitotic spindle: from centrosomes to cancer*

As mentioned already above, ACD requires that the mitotic spindle is positioned in such a way that polarized cell fate determinants are only inherited by one of the two daughter cells. The positioning of the mitotic spindle relies on the activity of the centrosomes that act as Microtubule Organizing Centers (MTOC) (Doxsey et al., 2005). Interestingly centrosome abnormalities have long been known as one of the hallmarks of cancer. Already a century ago, this led Theodor Boveri to propose that centrosome dysfunction may play a causative role in tumour formation. Indeed centrosomal aberrations can affect chromosome segregation by the mitotic spindle and hence cause aneuploidy (Ganem et al., 2009). Studies in flies suggest however that this genomic instability is not the primary cause of tumour formation (Castellanos et al., 2008).

In contrast, centrosome dysfunction perturbs the asymmetric segregation of cell fate determinants during the division of neuroblast stem cells (Castellanos et al., 2008; Basto et al., 2008). Consequently, "centrosome-deficient" neuroblasts elicit tumourous overgrowth when transplanted into a wild-type host, meeting hence the golden CSC standard. As no defects in symmetric cell division are observed in these animals, this suggests that centrosomes play a specific role in ACD. In agreement with this, centrosomes themselves display an asymmetric behaviour during the cell cycle of the neuroblast stem cell (Figure 2A): while one centrosome remains mostly immobile until the next division, the second one moves directionally to the opposite pole of the cell to set up the proper orientation of the mitotic spindle (Rebollo et al., 2007) (Figure 2A). A similar difference in the behaviour of the two centrosomes has been observed during the cell cycle of fly male germline stem cells: The centrosome that is inherited from the parental cell during mitosis (the "mother centrosome") remains close the point of contact between the stem cell and its niche. In contrast, the newly synthesized centrosome (the "daughter centrosome") migrates to the opposite pole of the cell (Yamashita et al., 2007).

These observations have led to a re-interpretation of Boveri's hypothesis (Gonzalez, 2007): rather than interfering with chromosome segregation, centrosomal malfunction could lead to a misorientation of the mitotic spindle during asymmetric stem-cell division, preventing hence the proper segregation of cell fate determinants. Genomic instability would then arise only after tumour initiation, and appear more as a consequence than as cause of cancer. It should however be noted that these observations do not rule out an important role for genomic alterations in the progression towards later stages of malignancy.

4. Signalling in asymmetric cell division: the role of endocytosis

Once cell fate determinants have been localized, the spindle oriented and division completed, the two stem-cell daughters can communicate with each other and their environment through cell-cell signalling pathways. Delta/Notch signalling among mitotic sister cells is crucial for the specification of different ACD daughter cells in numerous biological contexts, including the regulation of intestinal stem cells in flies as well as in mice (Ohlstein and Spradling, 2006; Micchelli and Perrimon, 2006; Crosnier et al., 2006). Notch is a transmembrane receptor that elicits signalling upon binding to membrane-bound ligands of the Delta/Serrate/Lag2 (DSL) family ligand (for review see Bray, 2006). This interaction triggers two sequential proteolytic cleavages of the receptor, causing ultimately the release of the Notch Intra-Cellular Domain (NICD) into the cytoplasm. From there, NICD translocates to the nucleus where it associates with cofactors of the CBF1-Su(H)-Lag1 (CSL) family to activate the transcription of target

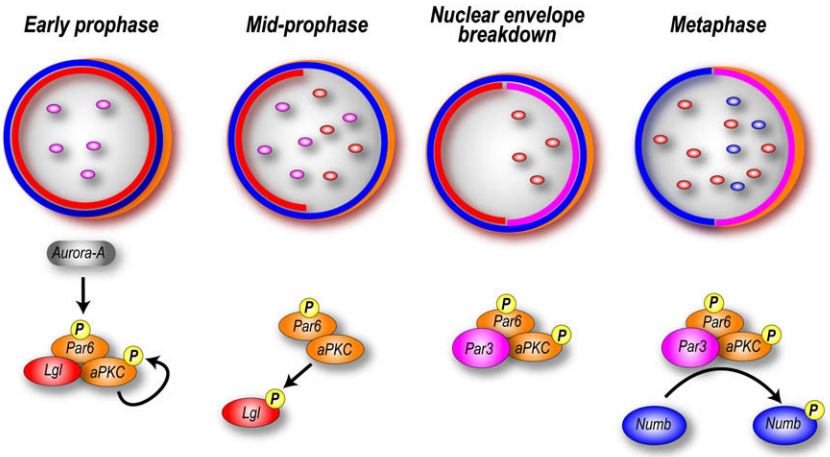

Figure 3 – Molecular basis of the asymmetric segregation of Numb. Upper row: localisation of cell fate determinants in a sensory bristle precursor cell at different stages of mitosis (anterior to the left). Lower row: Stage-dependent composition and activity of the Par-complex. At the beginning of mitosis, Numb (blue) and Lgl (red) decorate the entire cell cortex. The kinase Aurora-A (grey) is activated at the beginning of prophase and phosphorylates Par6 which localizes together with aPKC to the posterior cell cortex (orange). Phosphorylated Par6 can no more inhibit aPKC which activates itself by autophosphorylation. Activated aPKC then phosphorylates Lgl, causing its release into the cytoplasm. Once Lgl is released, Par3 (purple) forms a novel complex with Par6 and aPKC. This changes the substrate specificity of aPKC, allowing it to phosphorylate Numb (blue). As Par3/Par6/aPKC are restricted to the posterior cell cortex, Numb now forms a crescent at the anterior cell cortex.

genes. The differential activation of Notch in one of the two daughters derived from an ACD is achieved essentially, if not exclusively through an asymmetric regulation of the endocytic trafficking of both ligand and receptor molecules (Le Borgne et al., 2005a; Fürthauer and Gonzalez-Gaitan, 2009).

Accordingly Numb, one of the cell fate determinants that segregates asymmetrically during the division of *Drosophila* neuroblast stem cells, is an endocytic protein that regulates the trafficking of Notch and other cell surface receptors (Santolini et al., 2000; Berdnik et al., 2002; Nishimura and Kaibuchi, 2007). Similar to Prospero and Brat, Numb protein forms a basal crescent during neuroblast ACD and segregates into the differentiating GMC daughter (Wang et al., 2006; Lee et al., 2006a; Bowman et al., 2008). The analysis of *numb* mutant animals suggests that Numb inhibits neuroblast self-renewal and promotes GMC differentiation. Conversely, Notch receptor activation promotes stem self-renewal and prevents differentiation, suggesting that Numb affects neuroblast stem-cell behaviour by antagonizing Notch. Similar to the situation in the fly, Numb is unequally partitioned during the asymmetric divisions of neural progenitors in the mouse (Shen et al., 2002). In contrast to their *Drosophila* homologues, the role of murine Numb and Numb-like is however to promote stem-cell renewal and inhibit neuronal differentiation (Petersen et al., 2002).

4.1. *Coordinating cell division and asymmetric localisation of cell fate determinants*

Numb is localized all around the cell cortex in interphase cells. As cells enter mitosis and the nuclear envelope is disassembled, Numb localisation changes rapidly so that the protein forms a basal crescent outlining the cortex of the future GMC. The localisation of Numb must therefore involve spatial as well as temporal (i.e. cell-cycle-dependent) cues.

The spatial cues for the asymmetric division of neuroblast stem cells derive from their embryonic origin. Neuroblasts arise through a process of delamination from the embryonic neuroepithelium. Through an unknown mechanism, neuroblasts are able to remember the apico-basal polarity cues of the emryonic epithelium and reuse them subsequently to orient the axis of the asymmetric stem-cell divisions. The Par complex proteins Par3, Par6 and aPKC are located apically in epithelial cells and mark the “stem cell side” of the division axis. Conversely Numb, which localizes baso-laterally in epithelial cells (Smith et al., 2007), is confined to the pole at which the GMC will form (for review see Betschinger and Knoblich, 2004).

The cell-cycle dependent regulation of Numb localisation is achieved through the interaction of Par complex proteins with the kinases Aurora-A and Polo, two important mitotic regulators that have been implicated in mammalian cancer formation (Wirtz-Peitz et al., 2008; Wang et al., 2007). Prior to the onset of mitosis the kinase activity of aPKC is suppressed by its association with Par6 and a second protein, the tumour suppressor Lethal giant larvae (Lgl) (Figure 3). As a neuroblast enters mitosis, Aurora-A is activated and phosphorylates Par6. In a way that is not yet perfectly understood, this causes the activation of aPKC which then phosphorylates Lgl. As a consequence, phosphorylated Lgl is displaced from the complex and released from the cell cortex into the cytoplasm. The displacement of Lgl then allows Par3 to enter a new complex with aPKC and Par6. The association with Par3 appears to change the substrate specificity of aPKC, so that it can now phosphorylate membrane-associated Numb, a modification that is known to trigger its release into the cytoplasm (Wirtz-Peitz et al., 2008; Nishimura and Kaibuchi, 2007; Smith et al., 2004).

While this model provides an attractive explanation for the coordination between cell-cycle progression and the asymmetric recruitment of cell fate determinants, we are still far from a comprehensive understanding of this intriguing process: aPKC is localized to the posterior/basal cortex, but Lgl is released from the entire cortex as the cell enters mitosis. A potential explanation for this might be that Lgl diffuses laterally in the membrane so that localized aPKC activity may cause the progressive release of Lgl from the entire cell cortex. But how can this be reconciled with the fact that Numb is not released from the entire cell cortex, but maintained as a crescent opposite to the zone of aPKC activity? One possible explanation for this could be that the lateral mobility of Numb is substantially lower than the one of Lgl. While some observations suggest that this might indeed be the case (Wirtz-Peitz et al., 2008), the molecular basis underlying such a different behaviour remains to be established.

In this context, it may be worthwhile to reconsider the role of Partner-of-Numb (Pon), a protein whose localisation during ACD closely matches the one of Numb (Lu et al., 1998). Pon binds directly to Numb and contributes to its asymmetric localisation during the ACDs of neural as well as muscle precursor cells. Interestingly, Pon is itself phosphorylated by the cell-cycle dependent kinase Polo, and this phosphorylation is essential for the timely asymmetric recruitment of Pon at the onset of mitosis (Wang et al., 2007). In *polo* mutants, asymmetric Numb localisation is defective and supernumary neuroblasts form at the expense of differentiated neurons. While it is tempting to speculate that the aberrant localisation of Numb is a direct result of the lack of Pon phosphorylation, loss of Polo function also affects the localisation of aPKC and the orientation of the mitotic spindle so that it is difficult to estimate the specific contribution of each of these different defects to the final phenotype. Clearly further work will be required to determine how the activity of Aurora-A and Polo coordinates the timely asymmetric recruitment of cell fate determinants.

4.2. *Regulation of receptor activity through asymmetric endocytosis*

How does Numb modulate Notch activity? While Numb has been shown to regulate the intracellular trafficking of cell surface receptors in several systems (Santolini et al., 2000; Nishimura and Kaibuchi, 2007), the importance of regulated endocytic trafficking for the ACD of *bona fide* stem cells remains to be established. In contrast, numerous studies have established that asymmetric endocytic trafficking is crucial to regulate the activity of the Delta/Notch signalling pathway during the asymmetric division of *Drosophila* Sensory Organ Precursor (SOP) cells (Fürthauer and Gonzalez-Gaitan, 2009). SOP cells undergo a series of ACDs to give rise to the four different cells that compose the mechanosensory bristles of the fly (Figure 4A). After each ACD, differential activation of the Delta/Notch signalling pathway assigns different identities to the two mitotic sister cells. While SOP cells are not self-renewing stem cells, the mechanisms that govern the asymmetric divisions are very similar in larval neuroblasts and SOP cells (Knoblich, 2008). It is therefore likely that many of the mechanisms that ensure an endocytic regulation of Delta/Notch signalling in the SOP will also be conserved in neuroblast stem cells.

Numb directly binds the Notch receptor (Guo et al., 1996). In addition, Numb binds α-Adaptin, an endocytic adapter protein that plays an important role in the Clathrin-mediated internalization of cell surface molecules (Santolini et al., 2000). Accordingly, Numb and α-Adaptin co-segregate during the asymmetric SOP division to become inherited by the anterior pIIb cell where they act to suppress Notch signalling (Berdnik et al., 2002) (Figure 4C). Numb has therefore been proposed to bridge Notch and α-Adaptin. The latter protein would then promote preferential receptor internalization in one of the two daughter cells. The selective removal of Notch from the surface of the anterior cell would render it refractory to receptor activation. Inactivation of Numb or α-Adaptin results in the ectopic activation of Notch in the anterior daughter cell, and prompts it to adopt the same Notch-induced cell fate as its posterior pIIa sibling. While this model provides a very attractive explanation for the molecular function of Numb, it has to face the caveat that there is presently no evidence that the levels of Notch receptor present on the surface of the two daughter cells are actually different.

In addition, Numb interacts directly with Sanpodo (Spdo), a four-pass transmembrane protein that binds to Notch and promotes its signalling activity (O'Connor-Giles and Skeath, 2003). In contrast to α-Adaptin, the Spdo protein displays a uniform cytoplasmic localisation during cell division. Following the completion of cell division, Spdo localisation becomes however very different in the two daughter cells: In pIIa, the daughter cell in which Notch signalling is active, Spdo is detected at the plasma membrane. In contrast, the protein displays a mostly endosomal localisation in pIIb, where Notch activation is suppressed (Figure 4C). These observations have led to the proposal that Numb could inhibit Notch activation in pIIb by removing actually not the receptor itself but its essential cofactor Sanpodo from the cell surface (Hutterer and Knoblich, 2005). Interestingly, both Numb and Sanpodo regulate Notch signalling only in asymmetrically dividing cells, but not in other biological contexts. The reason for this specific requirement is a fascinating but totally open question.

4.3. *Localizing functional ligand molecules through asymmetric endocytosis*

The observation that asymmetric internalization contributes to the regulation of Notch activation by removing the receptor and/or its cofactor Spdo from the cell surface conforms to the common conception that endocytic trafficking is important to down-regulate biological signalling pathways. Perhaps more surprisingly, studies in the fly have also led to suggest an essential positive role of the endocytic pathway in the Delta/Notch signal transduction. Already more than 10 years ago, Dynamin-dependent endocytosis had been suggested to be crucial for both ligand and receptor activity (Seugnet et al., 1997). More recently it was shown that Neuralized, a specific E3-Ubiquitin-ligase promoting the internalization of Delta is required to allow successful activation of the Notch receptor by its ligand (Pavlopoulos et al., 2001; Lai and Rubin, 2001; Le Borgne and Schweisguth, 2003).

Several models have been put forward to explain how the internalization of Delta could promote Notch activation

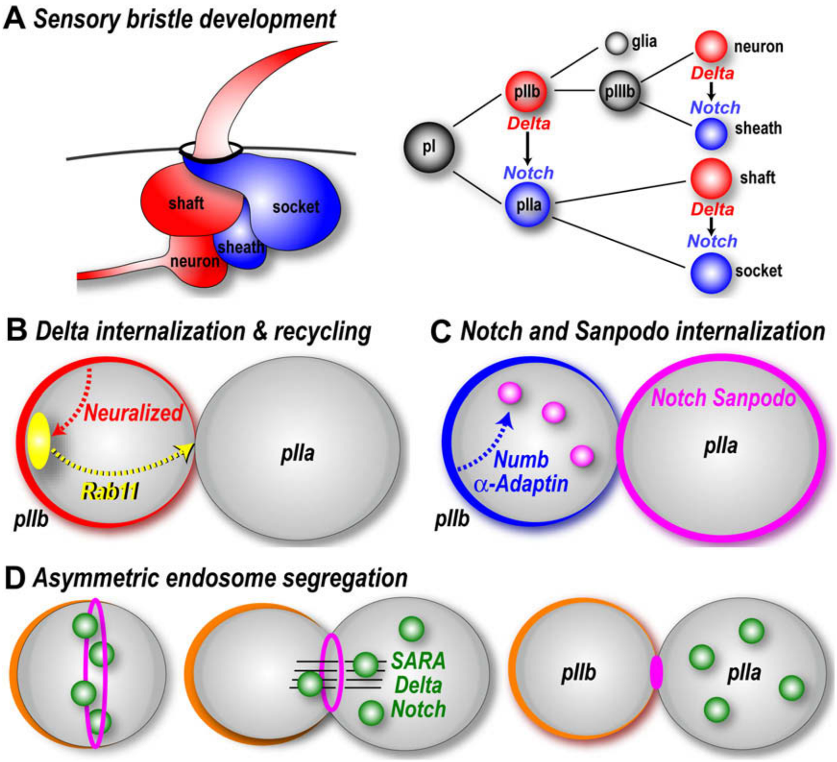

Figure 4 – Endocytosis in asymmetrically dividing sensory organ precursor cells. A: Left: *Drosophila* mechanosensory bristles are composed of two external (shaft and socket) and two internal (neuron and sheath) cells. Right: The four cells arise through a series of asymmetric cell divisions from a sensory organ precursor cell (also called pI). After the divisions of pI, pIIa and pIIIb, directional Delta/Notch signalling causes the two daughter cells to adopt different fates. The division of the pIIb generates a small glial cell that undergoes apoptosis soon afterwards. B: During asymmetric cell division Neuralized (red) is segregated into the anterior pIIb cell, resulting in the preferential internalization of Delta in this daughter cell. Shortly after division, a Rab11-positive recycling compartment (yellow) is established in pIIb, promoting the recycling of ligand molecules to the pIIb cell surface. C: Sensory precursor division results in asymmetric inheritance of Numb and α-Adaptin (blue) by the pIIb daughter cell. Numb and α-Adaptin act together to promote the internalization of Notch receptor and its cofactor Sanpodo (purple). The posterior pIIa cell lacks Numb and α-Adaptin so that Notch/Spdo remain at the cell surface. D: Delta and Notch molecules internalized before mitosis traffic to endosomes marked by the TGFβ signalling adpater SARA (green). Left: In metaphase, SARA endosomes line up at the edge of the crescent formed by Partner-of-Numb (orange), at the contractile actin ring (purple). Middle: During cytokinesis SARA endosomes are associated with the central spindle (black) and move into the posterior daughter cell so that immediately after division most SARA endosomes are found in pIIa.

(Le Borgne et al., 2005a; Fürthauer and Gonzalez-Gaitan, 2009 ; Chitnis, 2006). According to a first scenario; the internalization of Delta-ligands bound to Notch would change the receptor conformation in the extra-cellular juxta-membrane domain. This would expose the Notch S2 cleavage site, which would then be recognized by Metallo-proteases of the ADAM (for A Disintegrin And Metalloprotease) family that initiate Notch activation by mediating the cleavage and release of the Notch extra-cellular domain. Alternatively, it has been proposed that Delta internalization may allow its activating modification within the endocytic pathway or its preferential recycling to a sub-cellular micro-environment that is prone to productive ligand–receptor interactions. A recent study suggests that these different mechanisms may well not be mutually exclusive (Heuss et al., 2008): In mammalian cell culture, the ligand Delta-like 1 undergoes a first internalization to acquire affinity for its receptor and be recycled to a specific, lipid-ordered, membrane environment. A second round of endocytosis is then however necessary to activate the receptor, a process that is accompanied by the trans-endocytosis of the Notch extra-cellular domain, in accordance with a model where Delta pulls on Notch to activate it.

Studies in *Drosophila* (Le Borgne et al., 2005b), zebrafish (Itoh and Chitnis, 2001; Itoh et al., 2003) and mouse (Koo et al., 2007) suggest that the internalization of Delta/Serrate/Lag2 family ligands is essential for their productive interaction with Notch receptors in all biological contexts. While Delta endocytosis appears therefore to play a conserved role in Notch signalling, the analysis of *Drosophila* sensory bristle formation has revealed how a specific control of the endocytic machinery contributes to the formation of two different daughter cells following ACD. As the sensory precursor enters mitosis, the polarity cues than emanate from the Par-complex direct the asymmetric localisation of Neuralized at the anterior cell cortex (Langevin et al., 2005). As a consequence, Neuralized protein is inherited essentially by the anterior pIIb, promoting the preferential internalization of Delta molecules in this cell (Le Borgne and Schweisguth, 2003) (Figure 4B). Genetic analysis reveals that Neuralized is required in pIIb to activate Notch signalling in its pIIa sister cell.

Not only the rate of internalization but also the extent of recycling of Delta ligands differs among the two daughter cells: A prominent but transient Rab11-positive recycling compartment is established in the centrosomal region of the pIIb daughter cell a few minutes after the completion of cytokinesis (Figure 4B, Emery et al., 2005). In contrast to Neuralized and Numb, the asymmetric clustering of Rab11 endosomes does not depend on the Par-complex but on independent, currently unknown polarity cues. The presence of the asymmetric Rab11 compartment favours the recycling of Delta towards the plasma membrane of pIIb. Conversely, the absence of a similar compartment in pIIa causes internalized Delta molecules to be routed for degradation. Taken together, Numb-dependent removal of Notch and/or Spdo, Neuralized-dependent Delta internalization and Rab11-dependent Delta recycling predispose pIIb for ligand presentation and pIIa for signal reception.

4.4. *Asymmetric endosomes in asymmetric cell division*

The asymmetric distribution of Numb, Neuralized and Rab11 imparts a different trafficking behaviour on Delta and Notch in the two SOP daughter cells *after* division. But what is the behaviour of ligand and receptor molecules themselves *during* ACD A recent study addressed this issue through live imaging of Delta and Notch during asymmetric SOP division. The two signalling molecules were labelled with fluorophore-coupled antibodies, so that endocytic vesicles carrying Delta/Notch could be followed through mitosis (Coumailleau et al., 2009). Both the ligand Delta and its receptor Notch were found to traffic to a subpopulation of Rab5-positive early endosomes that can be specifically identified by the presence of the TGFβ signalling adapter SARA (for Smad Anchor for Receptor Activation). In the course of mitosis, SARA endosomes and Delta/Notch therein are first targeted to the contractile actin ring and then transferred to the mitotic central spindle which may serve as a template for their directional transport into pIIa, the posterior SOP daughter cell in which Notch signalling will be activated (Figure 4D). Asymmetric segregation of Notch has also been noted during cell division in the developing mammalian brain cortex, suggesting that this mechanism may be evolutionarily conserved (Chenn and McConnell, 1995). Moreover asymmetric partitioning of endosomal subpopulations has also been observed during the first cleavage of the C. *elegans* embryo, although the biological significance of this process remains to be established (Andrews and Ahringer, 2007).

SARA marks a subpopulation of MultiVesicular Endosomes (MVE) (Bokel et al., 2006). MVEs comprise notably the so-called Multi-Vesicular Bodies (MVB), that are ‘‘involved in the transport from early to late endosomes’’ (Gruenberg and Stenmark, 2004) in order to ensure the lysosomal degradation of ubiquitinated cargo molecules (Williams and Urbe, 2007). Interestingly, mutations in the *Drosophila* ESCRT (Endosomal Sorting Complex Required for Transport) complex perturb MVE biogenesis and cause the Notch-dependent tumourigenic overgrowth of (symmetrically dividing) epithelial cells in the developing wing and eye (Moberg et al., 2005; Vaccari and Bilder, 2005; Thompson et al., 2005) (For review, see the paper by Vaccari and Bilder 2005). In the case of SARA-positive MVEs, several studies have however indicated a role of these endosomes in signalling rather than degradation: Mammalian SARA is required for Smad2/3-mediated TGFβ signalling (Tsukazaki et al., 1998). During *Drosophila* wing development, SARA endosomes ensure the equal partitioning of activated TGFβ pathway signalling components among mitotic sister cells (Bokel et al., 2006).

The analysis of SARA endosomes in the context of *Drosophila* sensory bristle development also supports a positive role of these organelles in Delta/Notch signalling. If SARA endosomes are mistargeted to the wrong (anterior) SOP daughter, Notch is ectopically activated in this cell, causing it to adopt the same fate as its posterior pIIa sister (Coumailleau et al., 2009). This suggests that SARA endosomes or the Delta/Notch molecules therein regulate Notch activation in the cell that inherits them. The former of these (not mutually exclusive) possibilities would be consistent with studies that have suggested that γ-Secretase, the enzyme that performs the final activating proteolytic cleavage in the process of Notch activation, is preferentially active in the acidic environment of the endosomal system (Pasternak et al., 2003). Asymmetric segregation of Sara endosomes would enrich the organelle carrying functional γ-Secretase in pIIa, favouring hence Notch activation in this cell.

Alternatively, it could be envisaged that Delta and Notch contained in SARA endosomes interact within this organelle to elicit signalling (Fürthauer and Gonzalez-Gaitan, 2009). While this scenario is certainly counter-intuitive, it should be noted that a recent biochemical study has suggested that a productive interaction between Delta and Notch is not only possible, but actually favoured under acidic pH conditions such as those encountered within the endosomal lumen (Pei and Baker, 2008). Irrespective of the underlying molecular mechanism the present data suggests that the directional transport of Delta/Notch in SARA endosomes *during* ACD promotes biased Notch activation *after* division.

4.5. *Direct control of tumour suppressor activity by Numb*

Cancer research has traditionally focused on lesions that perturb the function of the core cell-cycle machinery. In contrast, the CSC concept puts the spotlight on the factors that regulate asymmetric stem-cell divisions. A recent study has identified a direct molecular interaction between Numb and the tumour suppressor p53, suggesting that these two types of defects could possibly be linked (Colaluca et al., 2008).

p53, which is sometimes termed the guardian of the genome, exerts a crucial role in the coordination of the cellular DNA damage response through its capacity to induce cell-cycle arrest, DNA repair or apoptosis in response to genotoxic stress (Vousden and Lu, 2002). Accordingly, the loss of p53 is a major step on a tumour cell's progression towards malignancy and p53 is mutated in 50% of all human cancers. MDM2 negatively regulates both the transcriptional activity and the turn-over of this major tumour suppressor. The latter is achieved through MDM2's E3-Ubiquitin-ligase activity which induces p53degradation (Haupt et al., 1997; Kubbutat et al., 1997). A new study now reveals that Numb can form a trimeric complex with MDM2 and p53 and prevent the ubiquitination and degradation of the tumour suppressor (Colaluca et al., 2008). While Numb exerts its protective activity on p53 independently of Notch, Numb mutant tumours also show an increased Notch activity. Loss of Numb impairs therefore the function of the tumour suppressor p53 while promoting at the same time the oncogenic activation of Notch. Accordingly, loss of Numb expression correlates with a poor clinical prognosis.

These results highlight a novel and unexpected implication of Numb in the regulation of the DNA damage response by p53. An intriguing question for the future will be to investigate whether conversely p53 may play a role in Numb-dependent ACDs. If this was the case, it would prompt the question whether the oncogenic inactivation of p53 might transform normal stem cells into CSCs.

5. Asymmetric endocytosis in adult stem cells

In the preceding sections we have attempted to review the current knowledge of the mechanisms that govern ACD during the development of the nervous system of the fruitfly. Human cancer arises however most often during adult life. It will therefore be important to study the importance of endocytic trafficking for the ACD of *bona fide* adult stem cells. Once more, novel insights are likely to arise from the use of genetically tractable model organisms such as *Drosophila*. An important step towards this end was the recent identification of adult stem cells in the midgut (Ohlstein and Spradling, 2006; Micchelli and Perrimon, 2006), hindgut (Takashima et al., 2008) and the malpighian tubules (Singh et al., 2007) (the equivalent of the kidney) of the fly.

The proliferation and differentiation of midgut stem cells is regulated by Delta/Notch signalling (Ohlstein and Spradling, 2006; Micchelli and Perrimon, 2006). Interestingly, stem cells can be identified by a high abundance of endocytic vesicles carrying internalized Delta. In contrast to the situation encountered in sensory bristle precursor cells (Coumailleau et al., 2009), the accumulation of Delta-containing endosomes does not result from their asymmetric segregation during mitosis, but rather from specific degradation in one of the two daughter cells following mitosis (Ohlstein and Spradling, 2007). While the mode of division of these stem cells remains to be established, these observations provide a first hint that endocytosis may indeed be relevant for the control of stem-cell behaviour in the adult fly gut.

5.1. *Asymmetric division of haematopoietic stem cells*

In mammalian systems, the actual importance of endocytic trafficking for the ACD of normal and cancer stem cells remains to be established. Studies performed in Haematopoietic Stem Cells (HSCs) suggest however that this issue is likely to be highly relevant: While the existence of asymmetric HSC divisions has long been inferred from clonal analysis (Giebel, 2008), recent work has now provided direct evidence for this process (Beckmann et al., 2007). In contrast to other stem-cell populations, HSCs have been shown to migrate in and out of their niche in the bone marrow. Even when the stem cells circulate outside of the niche, they are highly polarized and display an amoeboid behaviour. At this stage, the chemokine receptor CXCR4 is specifically enriched at the leading edge of the migratory cell, while the adhesion molecules CD43, CD44, ICAM-1 and ICAM-3 and the stem-cell marker Prominin-1/CD133 are enriched in the uropod, a leucocyte-specific cellular process at the trailing end (Figure 5). In addition the Tetraspanins CD53 and CD63 as well as the Transferrin Receptor CD71 are found in vesicular structures at the base of the uropod. Tetraspanins are known to play a role in the organization of the endo-lysosomal system (Berditchevski and Odintsova, 2007) and the Transferrin receptor is a classical marker of recycling compartments, suggesting that these vesicles are of endocytic nature. In confirmation of this hypothesis, they can be labelled through the internalization of antibodies directed against CD63 or CD71.

As cells progress through mitosis, the localisation of the markers that were previously detected at the trailing edge changes (Figure 5). Prominin-1, CD43, CD44, ICAM-1 and ICAM-3 are enriched at the mitotic midbody. In contrast, CD53-, CD63- and Transferrin receptor-positive vesicles are asymmetrically segregated into one of the two daughter cells in 20% of the HSC divisions. While the functional relevance of this process remains to be established, these observations provide first direct evidence for the occurrence of asymmetric endosome segregation during HSC division (Beckmann et al., 2007).

5.2. *SARA endosomes and the haematopoietic stem cell niche*

Interestingly, the endosomal compartment marked by the TGFβ signalling adapter SARA that ensures the directional transport of Delta/Notch during sensory bristle formation (Coumailleau et al., 2009) also plays a role in HSCs. HSC proliferation and survival depend on their interaction with osteoblasts within the bone marrow niche (Nagasawa, 2006). A recent study suggests that a special trans-endocytosis event may regulate signalling at the HSC/osteoblast interface (Gillette et al., 2009; Figure 5A). As an HSC migrates into the niche, it establishes contacts with the osteoblast through a specialized membrane domain enriched for the adhesion molecule VLA-4, the tetraspanins CD63 and CD81 as well as Prominin1 (Gillette et al., 2009; Giebel et al., 2004). The contact zone between the two cells is further characterized by the presence of extensive microvilli that extend from the HSC towards the osteoblast. Following the establishment of the contact zone; the specialized membrane domain of the HSC is trans-endocytosed into the osteoblast in a Dynamin-dependent process (Figure 5A). The trans-endocytosed material traffics into SARA endosomes where it remains concentrated, rather than being degraded or diluted in the endomembrane system of the osteoblast. Following the internalization of HSC material into SARA endosomes, Smad2/3-signalling (which is usually thought to depend on SARA, Tsukazaki et al., 1998) is down-regulated while the expression of SDF-1/CXCL12, a chemokine promoting HSC homing, is increased.

Delta/Notch signalling has been shown to be important for the communication between HSCs and the bone marrow niche osteoblasts (Hoflinger et al., 2004). As SARA endosomes have been shown to regulate Delta/Notch signalling during the ACD of *Drosophila* bristle precursor cells, it would be

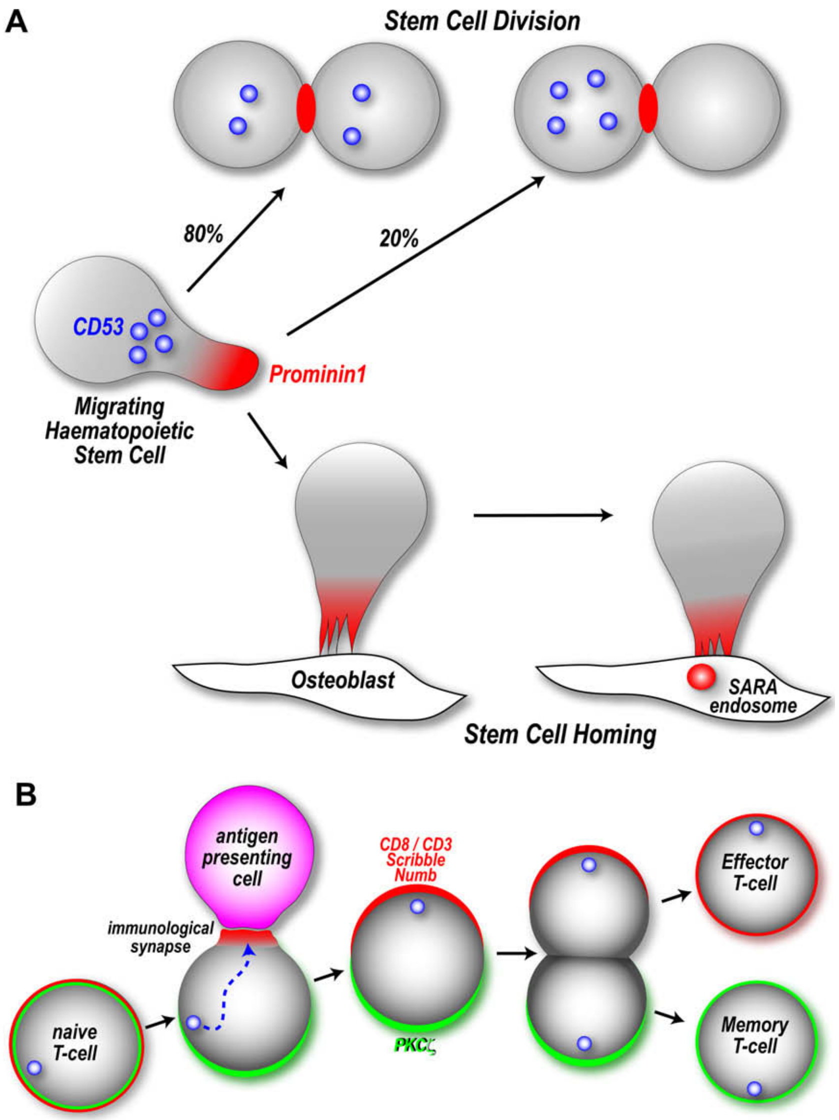

Figure 5 – Asymmetric cell division and endocytosis in haematopoietic stem cells. A: Migrating haematopoietic stem cells are highly polarized. Specific membrane proteins accumulate at the uropod, an extension at the trailing end of the cell (e.g. Prominin1, red). A subset of endocytic proteins accumulates in vesicles at the base of the uropod (e.g. CD53, blue). As the stem cell divides, proteins that were located at the uropod decorate the cytokinetic midbody (red). In 20% of the divisions, CD53-positive vesicles are asymmetrically segregated into one of the two daughter cells. When haematopoietic stem cells home into their niche, they contact osteoblasts through microvilli that originate from the uropod. After stem-cell/osteoblast contact is established, material from a specialized membrane domain positive for the stem-cell marker Prominin1 is trans-endocytosed into the osteoblast and transferred into SARA endosomes (red). B: Asymmetric cell division following T-cell activation. Naive T-cells display no overt sign of polarity. The encounter with an antigen presenting cell causes the cell surface molecules CD8/CD3 as well as Numb and the junctional protein Scribble (all indicated by red colour) to accumulate at the synapse. Moreover the centrosome migrates towards the synapse, so that the next division will occur perpendicular to the synaptic cleft. At the same time PKCζ accumulates at the pole opposite the synapse. After loosing contact with the antigen presenting cell, the activated T-cell divides asymmetrically to give rise to one effector and one memory T-cell.

interesting to investigate whether the cellular cytophagocytosis event at the HSC/osteoblast interface affects the activity of this signalling pathway. Conversely it will be instructive to determine whether a similar phenomenon might occur following the ACD of fly sensory precursors, when Delta/Notch signalling is likely to occur at the contact site between daughter cells. The observation that SARA endosomes are asymmetrically segregated during the division of *Drosophila* larval neuroblasts (Coumailleau et al., 2009) further supports a potential role of these vesicular carriers in the regulation of stem-cell behaviour.

5.3. *Asymmetric cell division during the immune response*

ACD is not only involved in the regulation of haematopoietic progenitor cells, but plays also an important role during their actual immune response. Following the encounter with a pathogenic agent, an antigen presenting cell establishes a prolonged contact with a naive T-cell, leading to the formation of a structure known as the immunological synapse. Following their activation, T-cells divide to give rise to both cytotoxic effector T-cells that mediate the acute immune response as well as memory T-cells that serve to improve immune function during a subsequent re-infection with the same pathogen. A very elegant study shows that the formation of the immunological synapse polarizes the localisation of the centrosome and of several immune response factors in T-cells undergoing activation (Chang et al., 2007) (Figure 5B). Similar to the situation in *Drosophila* neuroblast stem cells, T-cell polarization involves the localized recruitment of the Par-complex component PKCζ (the vertebrate counterpart of aPKC). As a result of this polarization, activated T-cells divide asymmetrically, resulting in the unequal partitioning of markers for effector- or memory-cell fate into the two daughter cells. Endocytosis and ACD appear therefore to be important for the formation as well as the function of immune cells.

6. Conclusion and outlook

In this review we have discussed how findings from different research fields ranging from oncology to developmental biology via cell biology have considerably reshaped our current understanding of cancer. The observation that cancer can arise from normal adult tissue stem cells suggests that we may have to consider cancer as a stem-cell disease. While it is not yet possible to visualize the divisions of mammalian cancer stem cells, studies in flies suggest that tumourous overproliferation of stem cells results from their failure to undergo asymmetric cell division. One of the major features of asymmetric cell divisions appears to be a differential regulation of endocytosis that biases the activity of cell signalling pathways among the two daughter cells. Evidence obtained in the mammalian immune system suggests that specific modulations of the intracellular trafficking machinery are likely to be of general importance for asymmetric stem-cell divisions.

In the light of these results, we believe that the study of endocytosis in the context of the asymmetric cell division of adult stem cells is going to yield important insights into the cell biological basis of cancer formation. Current work has highlighted unexpected links between endocytosis, asymmetric cell division, stem cells and cancer. The next step will be to establish model systems that allow merging these research areas, in order to be able to visualize *in vivo* endocytic trafficking events during the asymmetric division of normal and cancer stem cells.

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