Review Article

Neural Stem Cells in *Drosophila*: Molecular Genetic Mechanisms Underlying Normal Neural Proliferation and Abnormal Brain Tumor Formation

Nidhi Saini and Heinrich Reichert

Biozentrum, University of Basel, 4056 Basel, Switzerland

Correspondence should be addressed to Nidhi Saini, nidhi.saini@unibas.ch

Neural stem cells in *Drosophila* are currently one of the best model systems for understanding stem cell biology during normal development and during abnormal development of stem cell-derived brain tumors. In *Drosophila* brain development, the proliferative activity of neural stem cells called neuroblasts gives rise to both the optic lobe and the central brain ganglia, and asymmetric cell divisions are key features of this proliferation. The molecular mechanisms that underlie the asymmetric cell divisions by which these neuroblasts self-renew and generate lineages of differentiating progeny have been studied extensively and involve two major protein complexes, the apical complex which maintains polarity and controls spindle orientation and the basal complex which is comprised of cell fate determinants and their adaptors that are segregated into the differentiating daughter cells during mitosis. Recent molecular genetic work has established *Drosophila* neuroblasts as a model for neural stem cell-derived tumors in which perturbation of key molecular mechanisms that control neuroblast proliferation and the asymmetric segregation of cell fate determinants lead to brain tumor formation. Identification of novel candidate genes that control neuroblast self-renewal and differentiation as well as functional analysis of these genes in normal and tumorigenic conditions in a tissue-specific manner is now possible through genome-wide transgenic RNAi screens. These cellular and molecular findings in *Drosophila* are likely to provide valuable genetic links for analyzing mammalian neural stem cells and tumor biology.

1. Introduction

Stem cells play a central role in the process of growth and development in multicellular organisms in which they ensure the generation of a large and diverse set of cell types as well as provide for the maintenance of tissue homeostasis [1–3]. In recent years stem cells in the genetic model system *Drosophila* have become an excellent model for studying the cellular and molecular mechanisms that underlie stem cell function. Specifically, the neural stem cells in *Drosophila*, called neuroblasts for historical reasons, are currently one of the best and most extensively used model systems for understanding stem cell biology during normal development [4, 5]. Moreover, *Drosophila* neural stem cells have also become useful for understanding the cellular and molecular basis of stem cell-derived brain tumors that arise due to

loss of control of the stem cell divisions [6, 7]. In this review, we focus on the cellular mechanisms of neural stem cell proliferation in the central brain and optic lobes of *Drosophila* under normal conditions, present the current state of insight into the molecular elements that control the proliferative action of these neural stem cells during brain development, and discuss the alterations in the mechanisms of neural stem cell control that lead to overproliferation and brain tumor formation.

2. Neural Stem Cells in *Drosophila*: Neuroblasts of the Central Brain and Optic Lobe

The brain of *Drosophila* can be divided into the paired optic lobes and the central brain, and the neurons in both of these structures derive from neuroblasts. Of these two sets of

neuroblasts, the neuroblasts that give rise to the central brain have been studied in much greater detail (Figure 1). There are two kinds of central brain neuroblasts, type I and type II. The more abundant type I neuroblasts delaminate from the ventral cephalic neuroectoderm during embryogenesis and undergo up to 20 rounds of proliferative activity to generate the restricted number of neurons that make up the larval brain. Subsequently these neuroblasts enter quiescence by embryonic stage 16 and later during larval development in the second instar larval stage, and they re-enter the cell cycle to generate the vast majority neurons of the adult brain [8–15]. The proliferative activity of most central brain neuroblasts during embryonic and postembryonic stages is comparable and relies on asymmetric cell divisions by which the neuroblasts self-renew and also generate a smaller daughter called ganglion mother cell (GMC) which undergoes a single cell division to generate two postmitotic daughter cells that differentiate into neurons or glial cells [2, 16–18] (Figure 2(a)). Other specialized kinds of type I NB are found in the mushroom bodies and the optic lobes [19–21].

In addition to the majority of these so-called type I neuroblasts a smaller set of type II neuroblasts is located in the dorsoposterior and medioposterior region of each of the two central brain hemispheres (8 per hemisphere); these neuroblasts manifest a somewhat different proliferative activity that shows an interesting amplification of neural proliferation. Unlike the type I neuroblasts, in type II neuroblast proliferation the smaller daughter cell initiates expression of the proneural gene *asense* and becomes an intermediate neural precursor (INP), which undergoes a limited number of repeated self-renewing asymmetric divisions, with each division resulting in one INP and one GMC [22–26] (Figure 2(b)). Due to the amplification of proliferation through INPs, the type II neuroblasts can produce lineages of neurons which are markedly larger in size than those of type I neuroblasts.

The neuroblasts of the optic lobes also derive from neuroectodermal cells; however, the development of the optic lobe neuroectoderm and the manner in which the optic lobe neuroblasts differentiate from this neuroectoderm are different from the situation in the central brain. The optic lobes derive from an embryonic optic placode, which during larval stages form two proliferation centers adjacent to the central brain, the inner optic anlagen and the outer optic anlagen. In the inner optic anlagen, neuroepithelial cells initially divide symmetrically to expand the pool of potential precursor cells and later on transform into optic lobe neuroblasts in an ordered and highly localized manner in response to a wave of proneural gene expression that traverses the neuroepithelium [19, 35–37]. Subsequent to their formation, the optic lobe neuroblasts switch to a neurogenic mode and proliferate by undergoing a limited number of asymmetrical cell divisions which generate neuronal progeny in a manner that is similar, but not identical, to that of the asymmetrically dividing neuroblasts in the central brain [21, 27, 28, 38] (Figure 3).
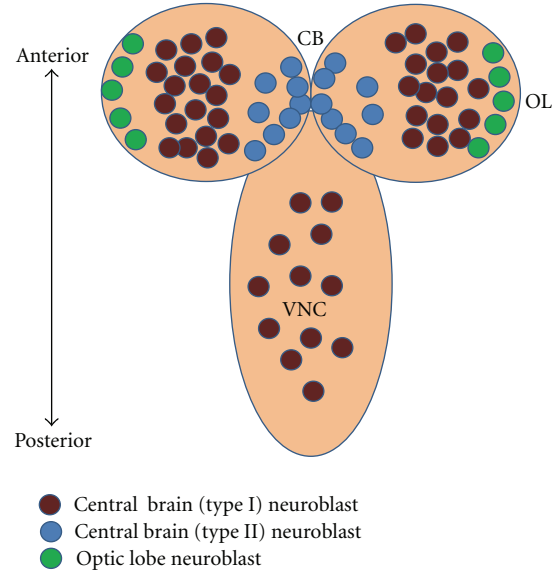

Figure 1: Schematic representation of development of the nervous system in the third instar *Drosophila* larval brain. During postembryonic neuroblast development, the brain of *Drosophila* can be divided into the paired optic lobes (OL) at the lateral surface of the two hemispheres, the central brain (CB), located medially to the OL, and the ventral nerve cord (VNC). The type I neuroblasts are the most abundant in the CB and VNC. The type II neuroblasts are located on the dorsomedial surface of the hemispheres.

3. Molecular Mechanisms for Neural Proliferation in Central Brain and Optic Neuroblasts

The molecular mechanisms that underlie the asymmetrical cell divisions by which neural stem cells self-renew and generate lineages of differentiating progeny have been studied extensively in the neuroblasts of the central brain [2, 39]. From a temporal point of view, each asymmetric cell division can be divided into three successive steps, namely, establishment of a polarity axis during interphase, followed by appropriate spindle orientation during the onset of mitosis and finally by asymmetric localization of cell fate determinants in the neuroblast and their inheritance by only one of the two daughter cells at the end of mitosis [40, 41]. From a molecular point of view these successive steps involve two major protein complexes: the apical complex and the basal complex.

Members of the apical complex include the PDZ domain-containing proteins PAR3 and PAR6 and the protein kinase atypical PKC (aPKC) [42–48] which accumulate at the apical cell cortex prior to mitosis and are also involved in the asymmetric partitioning of basal determinants [2, 49]. Other proteins constituting this complex are the adaptor protein, Inscuteable [50, 51] which links PAR3-PAR6-aPKC to a further protein complex containing the heterotrimeric G protein α_i-subunit, Gα_i [52–55], and the adaptor protein Partner of Inscuteable, PINS [56–58]. The PINS protein interacts with the microtubule-associated dynein-binding
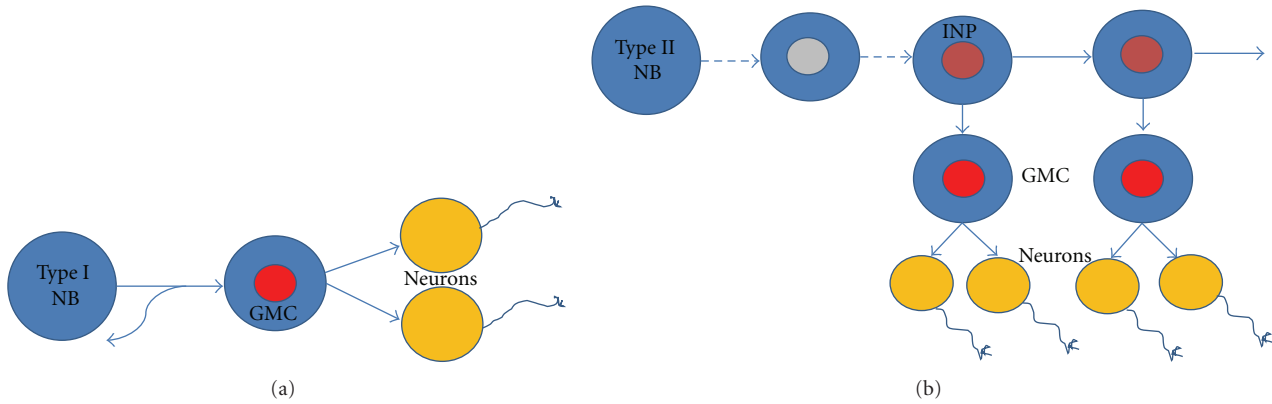
FIGURE 2: Neural stem cells/neuroblast (NB) undergo two types of self-renewing cell divisions: symmetric (proliferating) and/or asymmetric (differentiating). (a) Type I NB self-renew, and also generates a ganglion mother cell (GMC) which divides only once to generate two postmitotic daughter cells that differentiate into neurons or glial cells [2, 16–18]. (b) Type II NB initiates expression of the proneural gene *asense* and becomes an intermediate neural precursor (INP), which undergoes self-renewing asymmetric divisions, with each division resulting in one INP and one GMC [22–26]. Type II NB generates much larger lineages compared to type I NB.
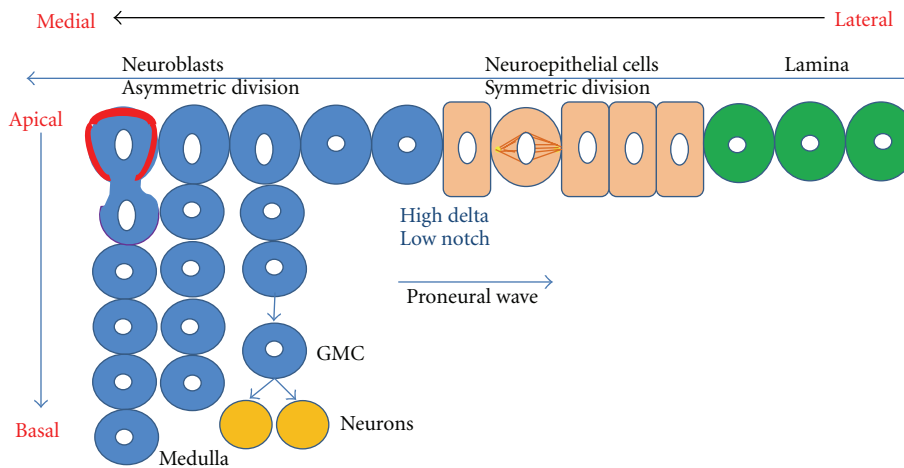

FIGURE 3: Schematic representation of neurogenesis in optic lobe development. During larval development transition from neuroepithelial (NE, orange) to neuroblast (NB, blue) takes place. NE cells undergo symmetric proliferation with a horizontal spindle orientation to expand the pool of precursor cells and give rise to asymmetrically dividing NB (green). This is in response to the proneural wave of *lethal of scute*. The median NB divides asymmetrically with a vertical spindle orientation, owing to the clear subcellular localization of the apical (polarity proteins, red boundary) and basal (cell-fate determinants and their adaptor proteins, purple boundary) complex, to give rise to the Ganglion mother cells (GMCs) and further, post mitotic neuronal daughter cells. Most central brain neuroblasts during embryonic and postembryonic stages undergo asymmetric cell divisions [21, 27, 28].

protein, MUD providing for a cortical attachment site for astral microtubules which maintains the apical-basal orientation of the mitotic spindle [59–61]. *Drosophila* neuroblasts have asymmetrically shaped mitotic spindles, where the apical microtubule asters are larger than their basal counterparts and this contributes to asymmetric cell division since it results in two different sized daughter cells [51, 62, 63]. Interestingly, the site of cytokinesis has recently been shown to be determined by another cortical pathway which is mediated by the apical PINS-Gα_i-MUD complex. Here the cleavage furrow proteins and the myosin segregates into the basal part of the cell even before the mitotic spindle assumes asymmetry. Moreover, in mutants with abnormal spindle orientation but normal cortical polarity, or in flies where

spindle formation is blocked, the cortical asymmetry and the resulting cleavage furrow still establishes itself normally [49, 64] (Figure 4).

Members of the basal complex include the cell-fate determinants Numb, Prospero, and Brat which are asymmetrically segregated into the GMC during neuroblast division [4, 5, 7, 65–67]. During mitosis, these cell-fate determinants are transiently concentrated in a basal cortical crescent in the neuroblast and are subsequently segregated asymmetrically into the GMCs. The endocytic protein Numb is a tissue-specific inhibitor of Notch-Delta signaling and was the first asymmetrically segregating cell fate determinant characterized in *Drosophila* [68–73]. The translational inhibitor Brat (brain tumor) as well as (in type I neuroblasts)
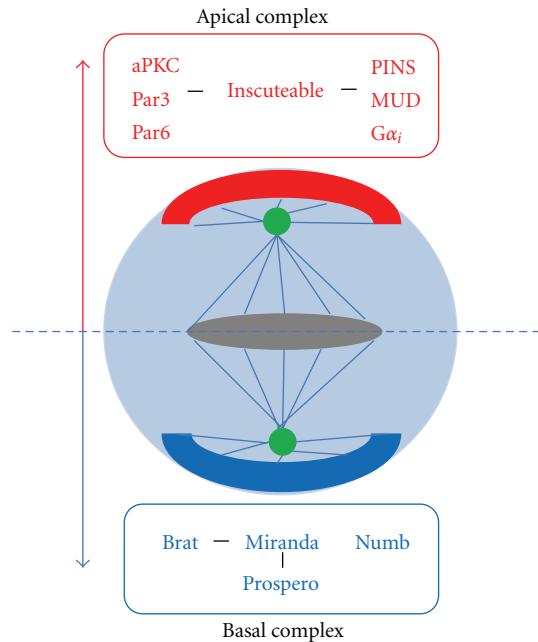
Apical complex

FIGURE 4: Asymmetric cell division in *Drosophila* neuroblasts. Apical (red) and basal (blue) proteins are asymmetrically segregated at cortical ends of the neuroblast at the time of mitosis. Members of the apical complex are involved in the asymmetric partitioning of basal determinants, in establishing cell polarity and in the correct orientation of the mitotic spindle. The apical complex consisting of aPKC, Par3, and Par6 is linked to the Gαi-PINS-MUD complex via Inscuteable. The basal complex consists of the cell-fate determinants, Miranda, Prospero, Brat, and Numb.

the homeodomain transcription factor Prospero is also asymmetrically segregated into the GMC aided by the adaptor protein Miranda [29–32, 74–80] (Figure 4). In the GMC, Prospero translocates to the nucleus where it represses the cell-cycle genes and induces neuronal differentiation genes. Brat is thought to act both as a translational repressor and an inhibitor of cell growth as well as a regulator of the transcription factor Myc and micro-RNAs; however, the precise mechanisms by which Brat regulates cell fate is not known [80–82]. In contrast to type I neuroblasts, the Asense-negative type II neuroblasts do not express Prospero, hence, Prospero is not segregated to the INP daughter cell during type II neuroblast division and this may contribute to the continued proliferative activity of INPs in these lineages. This restricted proliferative potential of INPs during limited rounds of asymmetric divisions is maintained by the transcription factor Earmuff [26, 75].

The molecular mechanisms that control the limited number of asymmetric proliferative divisions of the neuroblasts in the optic lobe are thought to be similar to those that operate in the INPs of type II neuroblast lineages in the central brain, however, this has not yet been studied in more detail. In contrast, a considerable amount of information is available on the molecular control of the neuroectoderm to neuroblast transformation occurring in the developing optic lobe. Initially and prior to neuroblast formation, the neuroectodermal cells are maintained in their expansive

symmetrical division mode by Notch signaling, which also prevents their transformation to neuroblasts [83, 84]. However, at the spatially dynamic transition zone between epithelial neuroectodermal cells and neuroblasts, Notch activity is reduced and high levels of Delta are observed [85–87]. The transition between neuroepithelial cells and neuroblasts takes place in response to a proneural wave of *lethal of scute* (*l'sc*) expression which sweeps across the neuroepithelium and leaves the asymmetrically dividing neuroblasts behind it [38, 88] (Figure 3). JAK/STAT and EGFR pathways are involved in the control of this wave's progression [85]. Moreover, the differentiation of neuroepithelial cells into neuroblasts at this zone has been shown to involve the Salvador-Warts-Hippo (SWH) signaling pathway [86]. It is noteworthy that this transition from symmetrically dividing neuroepithelial cells to asymmetrically dividing neuroblasts is similar to the transition from self-renewing to neurogenic neural stem cells in mammalian cortical development.

4. Abnormal Neuroblast Proliferation and Brain-Tumor Formation

Classical genetic screens have identified a number of genes such as *brat*, *l(2)gl*, *dlg*, *lethal (2) giant discs,* and *lethal (3) malignant brain tumor* as potent tumor suppressor genes. Flies mutated for any of these tumor suppressor genes develop a tumor-like overproliferation in tissues such as the brain or the imaginal discs [39, 89–93]. Building on these classical genetic studies, and based on the cellular and molecular analysis of the proliferation of neuroblasts under normal conditions, recent molecular genetic work has now established *Drosophila* neuroblasts as an excellent model system for understanding the mechanisms that underlie neural stem cell-derived tumors [4, 5]. Interestingly, these recent investigations have shown that both the molecular mechanisms that control asymmetric cell divisions of neuroblasts in the central brain and those that control the neuroectodermal expansion/transition in the optic lobes are prone to dysregulation which can lead to brain tumor formation.

A firm link between dysregulated asymmetric cell division and brain tumor formation has been established for central brain neuroblasts (Figure 5(a)). Indeed, a number of regulators of asymmetric cell division act as tumor suppressors in *Drosophila* neuroblasts. Thus, mutations in any one of the key asymmetrically segregated cell-fate determinants Prospero, Numb, or Brat result in brain tumors, even if these mutations are restricted to individual neuroblast clones [24, 29–32]. In the absence of any of these cell-fate determinants, the sensitive balance between self-renewal and differentiation is thought to be perturbed in the neuroblasts, leading directly or indirectly to the generation of self-renewing "tumor neuroblasts." Uninterrupted divisions of these incorrectly specified "tumor neuroblasts" as well as failure to respond to signals that normally act in the termination of neuroblast proliferation at the end of the larval stage, result in indefinite proliferation [32–34]. Interestingly, the type II lineages which contain transit amplifying INPs appear to be especially vulnerable to tumor formation. Mutations in
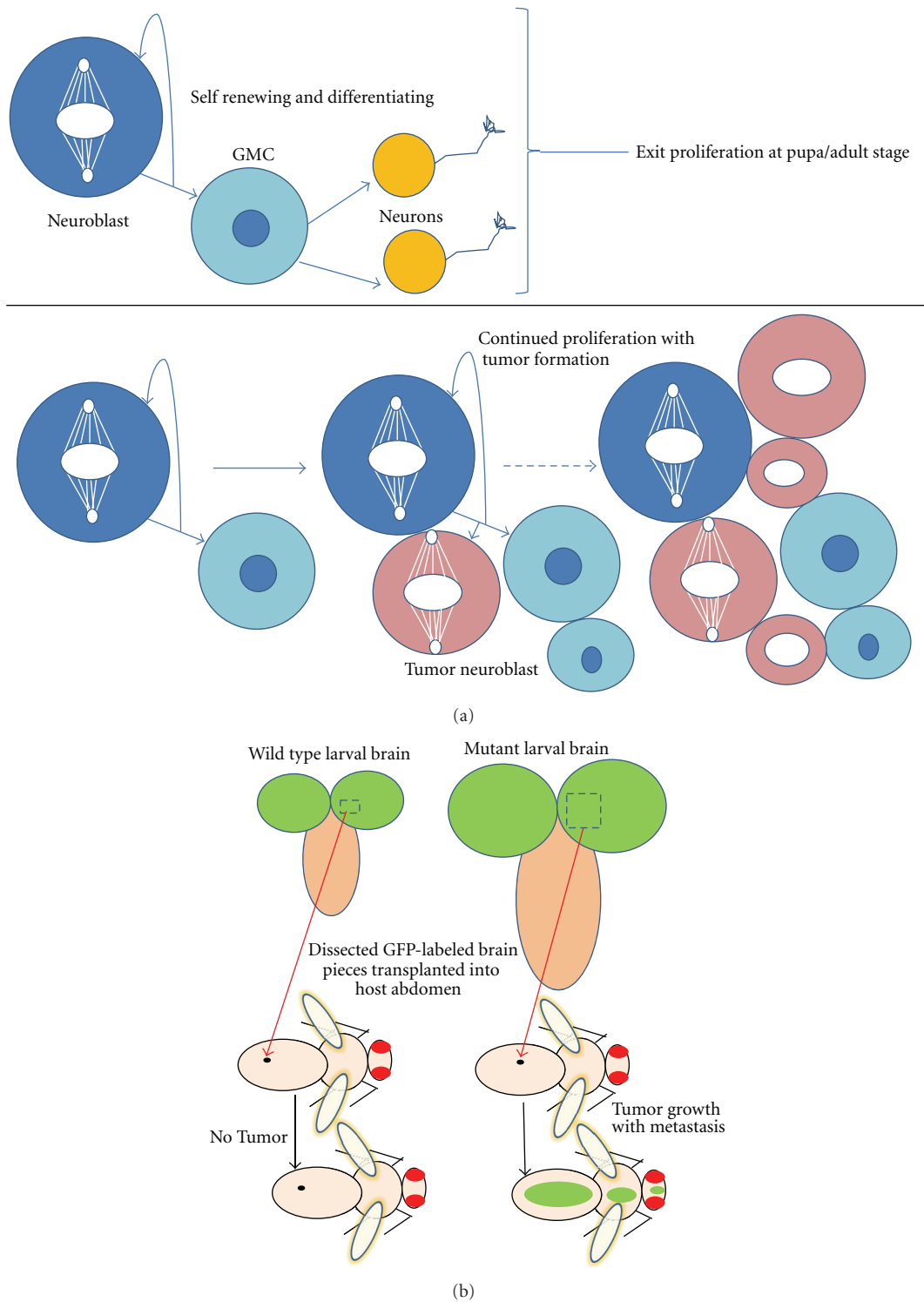
(a)

(b)

FIGURE 5: Abnormal neuroblast proliferation and brain tumor formation. (a) (Top), wild-type *Drosophilae* have "normal neuroblasts" which undergo a regulated self-renewal and differentiation process to generate neurons or glial cells. This proliferation exits at pupal stage. (Bottom), Dysregulated asymmetric cell division in central brain neuroblasts of larval brains with knockdown or knockout of cell-fate determinants results in brain tumor formation [24, 29–32]. The disturbed balance between self-renewal and differentiation results in the generation of self-renewing "tumor neuroblasts" and indefinite proliferation. (b) (Left) wild-type larval brain compared to (right) cell fate determinant (*brat/prospero/numb*) mutant, overproliferated brain. Transplantation of dissected GFP-labeled neuroblasts from the latter results in tumor formation in host flies and subsequent metastasis [32–34].

Brat, Numb and Earmuff in these lineages lead to a drastic and uncontrolled expansion in the number of proliferating "tumor neuroblasts." An important feature of the brain tumors induced by mutation of asymmetric cell division regulators in neuroblasts is that their uncontrolled overgrowth potential is maintained following transplantation of mutant brain tissue into normal hosts (Figure 5(b)). Indeed, upon transplantation into wild-type adult hosts, *prospero, numb,* and *brat* mutant brain tissue form malignant tumors and metastases, and these tumors can be maintained through subsequent re-transplantation into hosts [32, 33, 94]. In this respect it is interesting to point out that human homologs of Brat [95], Numb [96], and Prospero [97] have been shown to have connections to cancer formation, and thus results obtained with studies concerning *Drosophila* tumorigenesis can be relevant for understanding mammalian tumorigenesis as well.

Tumorigenic overgrowth due to mutation in tumor suppressor genes also takes place in the optic lobes. For example, mutation of the tumor suppressor *l(3)mbt* has recently been shown to result in optic lobe overgrowth [98–100]. However, in contrast to the situation in the central brain, the primary cause of this overgrowth is not due to dysregulated proliferation of the neuroblast, it is also not a result of the asymmetric segregation of cell-fate determinants in optic lobe neuroblasts of *l(3)mbt* mutants. Rather an overproliferation of the symmetrically dividing neuroepithelial cells during their expansion phase occurs in these mutants which in turn results in the generation of an uncontrolled number of optic lobe neuroblasts. At the molecular level, this unregulated overproliferation in the optic lobes of *l(3)mbt* mutants is caused, at least in part by derepression of the target genes of the SWH signaling pathway. Accordingly, experimental repression of SWH signaling or an increased expression of its downstream targets reproduces the massive proliferation of optic lobes similar to the *l(3)mbt* mutants [100]. While extensive studies point towards the importance of SWH pathway and its downstream targets in tumorigenesis of *l(3)mbt* mutants, the tumorigenic process is likely to involve the combined imbalance of several other signaling pathways like the Notch pathway [83, 85, 101–103], the JAK-STAT pathway [38], and other developmental control pathways, some of which operate in the germline [99]. Combined together with the studies of overproliferation in central brain neuroblasts, these studies clearly show that very different cellular and molecular events can lead to the formation of neural stem cell-derived brain tumors in *Drosophila*. Thus, a different cascade of initiating events in larval brain neuroblasts and optic lobe neuroblasts finally leads to a similar outcome of overproliferating cells resulting in brain tumor formation [104, 105].

5. Genome-Wide Screens for Neural Stem Cell Control Elements

Given the fundamental roles of the regulators of neural stem cell differentiation and maintenance that have been shown to operate in neuroblasts during normal brain development and during abnormal brain tumor formation, an in-depth analysis of their molecular mode of action and of their molecular interaction partners is of central importance. Several successful attempts have been made in the recent past, to identify novel candidate molecules involved in neural stem cell maintenance and differentiation at the genome-wide level using both microarray techniques and transcriptional target identification [31, 106, 107]. However, the functional relevance of most of these novel candidate molecules is still unknown. A useful approach to understanding the functional relevance of such identified candidate genes, is the targeted RNAi methods used to knock down the expression of their respective genes in neural stem cells, *in vivo*, where the immediate environment and the interactions with the surrounding niche are intact. This approach is eminently feasible in *Drosophila*, since genome-wide transgenic RNAi libraries are now available which allow for candidate gene functional analysis in a tissue-specific manner [108].

In a recent genome-wide study of self-renewal in *Drosophila* neuroblasts, transgenic RNAi targeted by the binary Gal4-UAS system was used to investigate the role of all known *Drosophila* genes in neuroblasts [30, 109]. In *Drosophila,* the GAL4-UAS system [110, 111] is routinely used to analyze the function of newly found developmental genes. The technique is based on the interaction of two different kinds of transgenic strains, activator and effector lines. In an activator line the gene for the yeast transcriptional activator GAL4 is placed under the control of a specific promoter, while in the effector line the gene of interest is fused to the DNA-binding motif of GAL4 (Upstream Activating Sequences, UAS). The effector gene becomes transcriptionally active only when the flies carrying it are crossed to those of an activator line, and thereby the effector gene is directed by the pattern of expression of GAL4 in the activator. This, of course, permits the controlled ectopic expression of the effector gene. In the study by Neumüller et al., out of a total of over 12,000 analyzed genes, around 600 candidate genes, showed RNAi-dependent defects in neuroblast self-renewal or in differentiation of their neural progeny. Based on precise quantification of the resulting loss-of-function phenotype and the hierarchical clustering as well as molecular interaction data, a set of functional networks representing the molecular elements involved in the control of neuroblast self-renewal and differentiation was established. Analysis of these networks reveals key roles of interacting sets of transcriptional regulators and chromatin remodeling complexes for the control of asymmetric cell division, cytokinesis, cell growth, and differentiation in the *Drosophila* brain. It is noteworthy that the dataset obtained from this RNAi screen is highly enriched for genes expressed in mammalian stem cells and thus is likely to provide valuable genetic links for analyzing mammalian stem cells and tumor biology [109].

6. Conclusions

A great deal of progress has been made in understanding the cellular and molecular mechanisms that underlie proliferation and cell-fate decision in the *Drosophila* brain neuroblast

model. A surprising aspect of this progress is the recent demonstration that key molecular control elements involved in asymmetric cell-fate determination in normal neuroblast lineages are also central elements in neuroblast-derived brain tumor formation. Further research in *Drosophila* and in other model systems is required to determine how the process of self-renewal and differentiation operates in normal neural stem cells and neural stem cell-derived cancer. Fortunately, the remarkable conservation of major transcriptional control and signaling pathways between flies and humans makes these studies of neural stem cells in *Drosophila* highly valuable for human stem cell biology. Thus, investigations in mammalian systems focused on the roles of those key factors for neural stem cell proliferation that have been identified in *Drosophila* are likely to be a gateway for a better understanding of many human cancers and further for developing therapeutic designs. Moreover, a sound understanding of the mechanisms underlying tumorigenic perturbations of neural stem cells is clearly a prerequisite for any potential development of neural stem cell-based therapy in humans.

Acknowledgments

References

[1] Y. M. Yamashita, D. L. Jones, and M. T. Fuller, "Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome," *Science*, vol. 301, no. 5639, pp. 1547–1550, 2003.

[2] J. Betschinger and J. A. Knoblich, "Dare to be different: asymmetric cell division in *Drosophila*, C. elegans and vertebrates," *Current Biology*, vol. 14, no. 16, pp. R674–R685, 2004.

[3] T. Lechler and E. Fuchs, "Asymmetric cell divisions promote stratification and differentiation of mammalian skin," *Nature*, vol. 437, no. 7056, pp. 275–280, 2005.

[4] C. Q. Doe, "Neural stem cells: balancing self-renewal with differentiation," *Development*, vol. 135, no. 9, pp. 1575–1587, 2008.

[5] R. A. Neumüller and J. A. Knoblich, "Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer," *Genes and Development*, vol. 23, no. 23, pp. 2675–2699, 2009.

[6] T. D. Southall, B. Egger, K. S. Gold, and A. H. Brand, "Regulation of self-renewal and differentiation in the *Drosophila* nervous system," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 73, pp. 523–528, 2008.

[7] P. S. Wu, B. Egger, and A. H. Brand, "Asymmetric stem cell division: lessons from *Drosophila*," *Seminars in Cell and Developmental Biology*, vol. 19, no. 3, pp. 283–293, 2008.

[8] V. Hartenstein, E. Rudloff, and J. A. Campos -Ortega, "The pattern of proliferation of the neuroblasts in the wild-type embryo of *Drosophila* melanogaster," *Roux's Archives of Developmental Biology*, vol. 196, no. 8, pp. 473–485, 1987.

[9] J. W. Truman and M. Bate, "Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila* melanogaster," *Developmental Biology*, vol. 125, no. 1, pp. 145–157, 1988.

[10] A. Prokop and G. M. Technau, "The origin of postembryonic neuroblasts in the ventral nerve cord of *Drosophila* melanogaster," *Development*, vol. 111, no. 1, pp. 79–88, 1991.

[11] K. Ito and Y. Hotta, "Proliferation pattern of postembryonic neuroblasts in the brain of *Drosophila* melanogaster," *Developmental Biology*, vol. 149, no. 1, pp. 134–148, 1992.

[12] J. W. Truman, B. J. Taylor, and T. A. Awad, "Formation of the adult nervous system," in *The Development of Drosophila Melanogaster*, M. Bate and A. Martinez-Arias, Eds., pp. 1245–1275, Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY, USA, 1993.

[13] R. Urbach and G. M. Technau, "Molecular markers for identified neuroblasts in the developing brain of *Drosophila*," *Development*, vol. 130, no. 16, pp. 3621–3637, 2003.

[14] C. Maurange and A. P. Gould, "Brainy but not too brainy: starting and stopping neuroblast divisions in *Drosophila*," *Trends in Neurosciences*, vol. 28, no. 1, pp. 30–36, 2005.

[15] J. Morante, T. Erclik, and C. Desplan, "Cell migration in *Drosophila* optic lobe neurons is controlled by eyeless/Pax6," *Development*, vol. 138, no. 4, pp. 687–693, 2011.

[16] T. Lee and L. Luo, "Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development," *Trends in Neurosciences*, vol. 24, no. 5, pp. 251–254, 2001.

[17] J. B. Skeath and S. Thor, "Genetic control of *Drosophila* nerve cord development," *Current Opinion in Neurobiology*, vol. 13, no. 1, pp. 8–15, 2003.

[18] A. Wodarz and W. B. Huttner, "Asymmetric cell division during neurogenesis in *Drosophila* and vertebrates," *Mechanisms of Development*, vol. 120, no. 11, pp. 1297–1309, 2003.

[19] A. Hofbauer and J. A. Campos-Ortega, "Proliferation pattern and early differentiation of the optic lobes in *Drosophila* melanogaster," *Roux's Archives of Developmental Biology*, vol. 198, no. 5, pp. 264–274, 1990.

[20] K. Ito, W. Awano, K. Suzuki, Y. Hiromi, and D. Yamamoto, "The *Drosophila* mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells," *Development*, vol. 124, no. 4, pp. 761–771, 1997.

[21] B. Egger, J. Q. Boone, N. R. Stevens, A. H. Brand, and C. Q. Doe, "Regulation of spindle orientation and neural stem cell fate in the *Drosophila* optic lobe," *Neural Development*, vol. 2, no. 1, article 1, 2007.

[22] B. C. Bello, N. Izergina, E. Caussinus, and H. Reichert, "Amplification of neural stem cell proliferation by intermediate progenitor cells in *Drosophila* brain development," *Neural Development*, vol. 3, no. 1, article 5, 2008.

[23] J. Q. Boone and C. Q. Doe, "Identification of *Drosophila* type II neuroblast lineages containing transit amplifying ganglion mother cells," *Developmental Neurobiology*, vol. 68, no. 9, pp. 1185–1195, 2008.

[24] S. K. Bowman, V. Rolland, J. Betschinger, K. A. Kinsey, G. Emery, and J. A. Knoblich, "The tumor suppressors brat and numb regulate transit-amplifying neuroblast lineages in *Drosophila*," *Developmental Cell*, vol. 14, no. 4, pp. 535–546, 2008.

[25] O. A. Bayraktar, J. Q. Boone, M. L. Drummond, and C. Q. Doe, "*Drosophila* type II neuroblast lineages keep Prospero levels low to generate large clones that contribute to the adult

brain central complex," *Neural Development*, vol. 5, no. 1, article 26, 2010.

[26] M. Weng, K. L. Golden, and C. Y. Lee, "dFezf/Earmuff maintains the restricted developmental potential of intermediate neural progenitors in *Drosophila*," *Developmental Cell*, vol. 18, no. 1, pp. 126–135, 2010.

[27] A. H. Brand and F. J. Livesey, "Neural stem cell viology in vertebrates and invertebrates: more alike than different?" *Neuron*, vol. 70, no. 4, pp. 719–729, 2011.

[28] B. Egger, K. S. Gold, and A. H. Brand, "Regulating the balance between symmetric and asymmetric stem cell division in the developing brain," *Fly*, vol. 5, no. 3, pp. 237–241, 2011.

[29] B. Bello, H. Reichert, and F. Hirth, "The brain tumor gene negatively regulates neural progenitor cell proliferation in the larval central brain of *Drosophila*," *Development*, vol. 133, no. 14, pp. 2639–2648, 2006.

[30] J. Betschinger, K. Mechtler, and J. A. Knoblich, "Asymmetric segregation of the tumor suppressor brat regulates self-renewal in *Drosophila* neural stem cells," *Cell*, vol. 124, no. 6, pp. 1241–1253, 2006.

[31] S. P. Choksi, T. D. Southall, T. Bossing et al., "Prospero acts as a binary switch between self-renewal and differentiation in *Drosophila* neural stem cells," *Developmental Cell*, vol. 11, no. 6, pp. 775–789, 2006.

[32] C. Y. Lee, B. D. Wilkinson, S. E. Siegrist, R. P. Wharton, and C. Q. Doe, "Brat is a Miranda cargo protein that promotes neuronal differentiation and inhibits neuroblast self-renewal," *Developmental Cell*, vol. 10, no. 4, pp. 441–449, 2006.

[33] E. Caussinus and C. Gonzalez, "Induction of tumor growth by altered stem-cell asymmetric division in *Drosophila* melanogaster," *Nature Genetics*, vol. 37, no. 10, pp. 1125–1129, 2005.

[34] C. Gonzalez, "Spindle orientation, asymmetric division and tumour suppression in *Drosophila* stem cells," *Nature Reviews Genetics*, vol. 8, no. 6, pp. 462–472, 2007.

[35] K. White and D. R. Kankel, "Patterns of cell division and cell movement in the formation of the imaginal nervous system in *Drosophila* melanogaster," *Developmental Biology*, vol. 65, no. 2, pp. 296–321, 1978.

[36] P. Green, A. Y. Hartenstein, and V. Hartenstein, "The embryonic development of the *Drosophila* visual system," *Cell and Tissue Research*, vol. 273, no. 3, pp. 583–598, 1993.

[37] J. Ceron, C. González, and F. J. Tejedor, "Patterns of cell division and expression of asymmetric cell fate determinants in postembryonic neuroblast lineages of *Drosophila*," *Developmental Biology*, vol. 230, no. 2, pp. 125–138, 2001.

[38] T. Yasugi, D. Umetsu, S. Murakami, M. Sato, and T. Tabata, "*Drosophila* optic lobe neuroblasts triggered by a wave of proneural gene expression that is negatively regulated by JAK/STAT," *Development*, vol. 135, no. 8, pp. 1471–1480, 2008.

[39] C. Y. Lee, K. J. Robinson, and C. Q. Doe, "Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation," *Nature*, vol. 439, no. 7076, pp. 594–598, 2006.

[40] J. A. Knoblich, "Asymmetric cell division during animal development," *Nature Reviews Molecular Cell Biology*, vol. 2, no. 1, pp. 11–20, 2001.

[41] M. Glotzer, "Cleavage furrow positioning," *Journal of Cell Biology*, vol. 164, no. 3, pp. 347–351, 2004.

[42] M. Schober, M. Schaefer, and J. A. Knoblich, "Bazooka recruits inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts," *Nature*, vol. 402, no. 6761, pp. 548–551, 1999.

[43] A. Wodarz, A. Ramrath, U. Kuchinke, and E. Knust, "Bazooka provides an apical cue for inscuteable localization in *Drosophila* neuroblasts," *Nature*, vol. 402, no. 6761, pp. 544–547, 1999.

[44] A. Wodarz, A. Ramrath, A. Grimm, and E. Knust, "*Drosophila* atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts," *The Journal of Cell Biology*, vol. 150, no. 6, pp. 1361–1374, 2000.

[45] M. Petronczki and J. A. Knoblich, "DmPAR-6 directs epithelial polarity and asymetric cell division of neuroblasts in *Drosophila*," *Nature Cell Biology*, vol. 3, no. 1, pp. 43–49, 2001.

[46] M. M. Rolls, R. Albertson, H. P. Shih, C. Y. Lee, and C. Q. Doe, "*Drosophila* aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia," *Journal of Cell Biology*, vol. 163, no. 5, pp. 1089–1098, 2003.

[47] T. Yamanaka, Y. Horikoshi, N. Izumi, A. Suzuki, K. Mizuno, and S. Ohno, "Lgl mediates apical domain disassembly by suppressing the PAR-3-aPKC-PAR-6 complex to orient apical membrane polarity," *Journal of Cell Science*, vol. 119, no. 10, pp. 2107–2118, 2006.

[48] S. X. Atwood and K. E. Prehoda, "aPKC phosphorylates miranda to polarize fate determinants during neuroblast asymmetric cell division," *Current Biology*, vol. 19, no. 9, pp. 723–729, 2009.

[49] C. Cabernard and C. Q. Doe, "Apical/basal spindle orientation is required for neuroblast homeostasis and neuronal differentiation in *Drosophila*," *Developmental Cell*, vol. 17, no. 1, pp. 134–141, 2009.

[50] R. Kraut and J. A. Campos-Ortega, "inscuteable, a neural precursor gene of *Drosophila*, encodes a candidate for a cytoskeleton adaptor protein," *Developmental Biology*, vol. 174, no. 1, pp. 65–81, 1996.

[51] R. Kraut, W. Chia, L. Y. Jan, Y. N. Jan, and J. A. Knoblich, "Role of inscuteable in orienting asymmetric cell divisions in *Drosophila*," *Nature*, vol. 383, no. 6595, pp. 50–55, 1996.

[52] M. Schaefer, M. Petronczki, D. Dorner, M. Forte, and J. A. Knoblich, "Heterotrimeric G proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system," *Cell*, vol. 107, no. 2, pp. 183–194, 2001.

[53] N. Fuse, K. Hisata, A. L. Katzen, and F. Matsuzaki, "Heterotrimeric G proteins regulate daughter cell size asymmetry in *Drosophila* neuroblast divisions," *Current Biology*, vol. 13, no. 11, pp. 947–954, 2003.

[54] F. Yu, F. Cai, R. Kaushik, X. Yang, and W. Chia, "Distinct roles of Gαi and Gβ13F subunits of the heterotrimeric G protein complex in the mediation of *Drosophila* neuroblast asymmetric divisions," *Journal of Cell Biology*, vol. 162, no. 4, pp. 623–633, 2003.

[55] Y. Izumi, N. Ohta, A. Itoh-Furuya, N. Fuse, and F. Matsuzaki, "Differential functions of G protein and Baz-aPKC signaling pathways in *Drosophila* neuroblast asymmetric division," *Journal of Cell Biology*, vol. 164, no. 5, pp. 729–738, 2004.

[56] M. Schaefer, A. Shevchenko, A. Shevchenko, and J. A. Knoblich, "A protein complex containing inscuteable and the Gα-binding protein pins orients asymmetric cell divisions in *Drosophila*," *Current Biology*, vol. 10, no. 7, pp. 353–362, 2000.

[57] F. Yu, X. Morin, Y. Cai, X. Yang, and W. Chia, "Analysis of partner of inscuteable, a novel player of *Drosophila*

asymmetric divisions, reveals two distinct steps in inscuteable apical localization," *Cell*, vol. 100, no. 4, pp. 399–409, 2000.

[58] F. Yu, H. Wang, H. Qian et al., "Locomotion defects, together with Pins, regulates heterotrimeric G-protein signaling during *Drosophila* neuroblast asymmetric divisions," *Genes and Development*, vol. 19, no. 11, pp. 1341–1353, 2005.

[59] S. K. Bowman, R. A. Neumüller, M. Novatchkova, Q. Du, and J. A. Knoblich, "The *Drosophila* NuMA homolog mud regulates spindle orientation in asymmetric cell division," *Developmental Cell*, vol. 10, no. 6, pp. 731–742, 2006.

[60] Y. Izumi, N. Ohta, K. Hisata, T. Raabe, and F. Matsuzaki, "*Drosophila* Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization," *Nature Cell Biology*, vol. 8, no. 6, pp. 586–593, 2006.

[61] K. H. Siller, C. Cabernard, and C. Q. Doe, "The NuMA-related Mud protein binds Pins and regulates spindle orientation in *Drosophila* neuroblasts," *Nature Cell Biology*, vol. 8, no. 6, pp. 594–600, 2006.

[62] J. A. Kaltschmidt, C. M. Davidson, N. H. Brown, and A. H. Brand, "Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system," *Nature Cell Biology*, vol. 2, no. 1, pp. 7–12, 2000.

[63] E. Rebollo, P. Sampaio, J. Januschke, S. Llamazares, H. Varmark, and C. González, "Functionally Unequal Centrosomes Drive Spindle Orientation in Asymmetrically Dividing *Drosophila* Neural Stem Cells," *Developmental Cell*, vol. 12, no. 3, pp. 467–474, 2007.

[64] C. Cabernard, K. E. Prehoda, and C. Q. Doe, "A spindle-independent cleavage furrow positioning pathway," *Nature*, vol. 467, no. 7311, pp. 91–94, 2010.

[65] W. Chia, W. G. Somers, and H. Wang, "*Drosophila* neuroblast asymmetric divisions: cell cycle regulators, asymmetric protein localization, and tumorigenesis," *Journal of Cell Biology*, vol. 180, no. 2, pp. 267–272, 2008.

[66] P. Gönczy, "Mechanisms of asymmetric cell division: flies and worms pave the way," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 5, pp. 355–366, 2008.

[67] J. A. Knoblich, "Mechanisms of asymmetric stem cell division," *Cell*, vol. 132, no. 4, pp. 583–597, 2008.

[68] M. S. Rhyu, L. Y. Jan, and Y. N. Jan, "Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells," *Cell*, vol. 76, no. 3, pp. 477–491, 1994.

[69] E. P. Spana, C. Kopczynski, C. S. Goodman, and C. Q. Doe, "Asymmetric localization of numb autonomously determines sibling neuron identity in the *Drosophila* CNS," *Development*, vol. 121, no. 11, pp. 3489–3494, 1995.

[70] M. Guo, L. Y. Jan, and Y. N. Jan, "Control of daughter cell fates during asymmetric division: interaction of Numb and Notch," *Neuron*, vol. 17, no. 1, pp. 27–41, 1996.

[71] E. Santolini, C. Puri, A. E. Salcini et al., "Numb is an endocytic protein," *Journal of Cell Biology*, vol. 151, no. 6, pp. 1345–1352, 2000.

[72] D. Berdnik, T. Török, M. González-Gaitán, and J. A. Knoblich, "The endocytic protein α-adaptin is required for numb-mediated asymmetric cell division in *Drosophila*," *Developmental Cell*, vol. 3, no. 2, pp. 221–231, 2002.

[73] K. M. O'Connor-Giles and J. B. Skeath, "Numb inhibits membrane localization of sanpodo, a four-pass transmembrane protein, to promote asymmetric divisions in *Drosophila*," *Developmental Cell*, vol. 5, no. 2, pp. 231–243, 2003.

[74] J. Hirata, H. Nakagoshi, Y. Nabeshima, and F. Matsuzaki, "Asymmetric segregation of the homeodomain protein Prospero during *Drosophila* development," *Nature*, vol. 377, no. 6550, pp. 627–630, 1995.

[75] J. A. Knoblich, L. Y. Jan, and Y. N. Jan, "Asymmetric segregation of Numb and Prospero during cell division," *Nature*, vol. 377, no. 6550, pp. 624–627, 1995.

[76] E. P. Spana and C. Q. Doe, "The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*," *Development*, vol. 121, no. 10, pp. 3187–3195, 1995.

[77] H. Ikeshima-Kataoka, J. B. Skeath, Y. I. Nabeshima, C. Q. Doe, and F. Matsuzaki, "Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions," *Nature*, vol. 390, no. 6660, pp. 625–629, 1997.

[78] F. Matsuzaki, T. Ohshiro, H. Ikeshima-Kataoka, and H. Izumi, "miranda localizes staufen and prospero asymmetrically in mitotic neuroblasts and epithelial cells in early *Drosophila* embryogenesis," *Development*, vol. 125, no. 20, pp. 4089–4098, 1998.

[79] A. J. Schuldt, J. H. J. Adams, C. M. Davidson et al., "Miranda mediates asymmetric protein and RNA localization in the developing nervous system," *Genes and Development*, vol. 12, no. 12, pp. 1847–1857, 1998.

[80] J. Sonoda and R. P. Wharton, "*Drosophila* brain tumor is a translational repressor," *Genes and Development*, vol. 15, no. 6, pp. 762–773, 2001.

[81] R. A. Neumüller, J. Betschinger, A. Fischer et al., "Mei-P26 regulates microRNAs and cell growth in the *Drosophila* ovarian stem cell lineage," *Nature*, vol. 454, no. 7201, pp. 241–245, 2008.

[82] J. C. Schwamborn, E. Berezikov, and J. A. Knoblich, "The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors," *Cell*, vol. 136, no. 5, pp. 913–925, 2009.

[83] B. Egger, K. S. Gold, and A. H. Brand, "Notch regulates the switch from symmetric to asymmetric neural stem cell division in the *Drosophila* optic lobe," *Development*, vol. 137, no. 18, pp. 2981–2987, 2010.

[84] W. Wang, W. Liu, Y. Wang, L. Zhou, X. Tang, and H. Luo, "Notch signaling regulates neuroepithelial stem cell maintenance and neuroblast formation in *Drosophila* optic lobe development," *Developmental Biology*, vol. 350, no. 2, pp. 414–428, 2011.

[85] K. T. Ngo, J. Wang, M. Junker et al., "Concomitant requirement for Notch and Jak/Stat signaling during neuroepithelial differentiation in the *Drosophila* optic lobe," *Developmental Biology*, vol. 346, no. 2, pp. 284–295, 2010.

[86] B. V. V. G. Reddy, C. Rauskolb, and K. D. Irvine, "Influence of Fat-Hippo and Notch signaling on the proliferation and differentiation of *Drosophila* optic neuroepithelia," *Development*, vol. 137, no. 14, pp. 2397–2408, 2010.

[87] D. Sprinzak, A. Lakhanpal, L. LeBon et al., "Cis-interactions between Notch and Delta generate mutually exclusive signalling states," *Nature*, vol. 465, no. 7294, pp. 86–90, 2010.

[88] T. Yasugi, A. Sugie, D. Umetsu, and T. Tabata, "Coordinated sequential action of EGFR and Notch signaling pathways regulates proneural wave progression in the *Drosophila* optic lobe," *Development*, vol. 137, no. 19, pp. 3193–3203, 2010.

[89] E. Gateff, "Malignant neoplasms of genetic origin in *Drosophila* melanogaster," *Science*, vol. 200, no. 4349, pp. 1448–1459, 1978.

[90] E. Gateff, "Tumor suppressor and overgrowth suppressor genes of *Drosophila* melanogaster: developmental aspects," *International Journal of Developmental Biology*, vol. 38, no. 4, pp. 565–590, 1994.

[91] T. Ohshiro, T. Yagami, C. Zhang, and F. Matsuzaki, "Role of cortical tumour-suppressor proteins in asymmetric division of *Drosophila* neuroblast," *Nature*, vol. 408, no. 6812, pp. 593–596, 2000.

[92] C. Y. Peng, L. Manning, R. Albertson, and C. Q. Doe, "The tumour-suppresor genes lgl and dlg regulate basal protein targeting in *Drosophila* neuroblasts," *Nature*, vol. 408, no. 6812, pp. 596–600, 2000.

[93] A. Wodarz, "Molecular control of cell polarity and asymmetric cell division in *Drosophila* neuroblasts," *Current Opinion in Cell Biology*, vol. 17, no. 5, pp. 475–481, 2005.

[94] E. Castellanos, P. Dominguez, and C. Gonzalez, "Centrosome dysfunction in *Drosophila* neural stem cells causes tumors that are not due to genome instability," *Current Biology*, vol. 18, no. 16, pp. 1209–1214, 2008.

[95] J. L. Boulay, U. Stiefel, E. Taylor, B. Dolder, A. Merlo, and F. Hirth, "Loss of heterozygosity of TRIM3 in malignant gliomas," *BMC Cancer*, vol. 9, article 71, 2009.

[96] S. Pece, M. Serresi, E. Santolini et al., "Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis," *Journal of Cell Biology*, vol. 167, no. 2, pp. 215–221, 2004.

[97] T. V. Petrova, A. Nykänen, C. Norrmén et al., "Transcription factor PROX1 induces colon cancer progression by promoting the transition from benign to highly dysplastic phenotype," *Cancer Cell*, vol. 13, no. 5, pp. 407–419, 2008.

[98] E. Gateff, T. Loffler, and J. Wismar, "A temperature-sensitive brain tumor suppressor mutation of *Drosophila* melanogaster: developmental studies and molecular localization of the gene," *Mechanisms of Development*, vol. 41, no. 1, pp. 15–31, 1993.

[99] A. Janic, L. Mendizabal, S. Llamazares, D. Rossell, and C. Gonzalez, "Ectopic expression of germline genes drives malignant brain tumor growth in *Drosophila*," *Science*, vol. 330, no. 6012, pp. 1824–1827, 2010.

[100] C. Richter, K. Oktaba, J. Steinmann et al., "The tumour suppressor L(3)mbt inhibits neuroepithelial proliferation and acts on insulator elements," *Nature Cell Biology*, vol. 13, no. 9, pp. 1029–1039, 2011.

[101] K. Harvey and N. Tapon, "The Salvador-Warts-Hippo pathway—an emerging tumour-suppressor network," *Nature Reviews Cancer*, vol. 7, no. 3, pp. 182–191, 2007.

[102] L. J. Saucedo and B. A. Edgar, "Filling out the Hippo pathway," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 8, pp. 613–621, 2007.

[103] B. V. V. G. Reddy and K. D. Irvine, "The fat and warts signaling pathways: new insights into their regulation, mechanism and conservation," *Development*, vol. 135, no. 17, pp. 2827–2838, 2008.

[104] C. J. Potter, G. S. Turenchalk, and T. Xu, "*Drosophila* in cancer researchan expanding role," *Trends in Genetics*, vol. 16, no. 1, pp. 33–39, 2000.

[105] H. Harris, "A long view of fashions in cancer research," *BioEssays*, vol. 27, no. 8, pp. 833–838, 2005.

[106] T. Loop, R. Leemans, U. Stiefel et al., "Transcriptional signature of an adult brain tumor in *Drosophila*," *BMC Genomics*, vol. 5, no. 1, article 24, 2004.

[107] T. D. Southall and A. H. Brand, "Neural stem cell transcriptional networks highlight genes essential for nervous system development," *The EMBO Journal*, vol. 28, no. 24, pp. 3799–3807, 2009.

[108] G. Dietzl, D. Chen, F. Schnorrer et al., "A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*," *Nature*, vol. 448, no. 7150, pp. 151–156, 2007.

[109] R. A. Neumüller, C. Richter, A. Fischer, M. Novatchkova, K. G. Neumüller, and J. A. Knoblich, "Genome-wide analysis of self-renewal in *Drosophila* neural stem cells by transgenic RNAi," *Cell Stem Cell*, vol. 8, no. 5, pp. 580–593, 2011.

[110] J. A. Fischer, E. Giniger, T. Maniatis, and M. Ptashne, "GAL4 activates transcription in *Drosophila*," *Nature*, vol. 332, no. 6167, pp. 853–856, 1988.

[111] A. H. Brand and N. Perrimon, "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes," *Development*, vol. 118, no. 2, pp. 401–415, 1993.