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Cell division

Our goal is to elucidate the mechanisms of cell division. We apply a multidisciplinary approach that combines genetics, molecular biology and advanced in vivo *microscopy. We use* Drosophila *as well as cultured cells derived from vertebrates as model systems. Current on-going projects include the study of the mechanisms of spindle assembly, the characterisation of new centrosomal proteins and the modelling of cancer in* Drosophila *to determine the functional connections between stem cell polarity and tumour growth.*

## Cancer stem cells and asymmetric division in Drosophila

During the last few years, we have been exploiting *Drosophila* to study some of the basic principles of cell proliferation and malignant growth (Causinus and González, 2005; Wodarz and González, 2006). This research focuses on the role of larval neural stem cells (Neuroblasts: NBs) as the cell of origin of tumours.

Drosophila *as a model for cancer research*

The first observations of deadly tumours in *Drosophila* were made almost one hundred years ago, but experiments in this field started in earnest four decades ago. The result of this effort has been the identification of dozens of genes whose function is required to prevent tissue overgrowth and which are collectively referred to as *Drosophila* tumour suppressors (TSs). To date, all TSs known in this fly model identify functions that are essential for cell differentiation and development. Many of

them have homologues in vertebrates, thus opening up the possibility of using *Drosophila* to further characterise the pathways in which they operate. Moreover, some of these homologues have been reported to be mutated in human cancers, thus strengthening the possible relevance of the fly model in cancer research.

The first *Drosophila* TSs were identified *in situ* by the growth of massive neoplasms in mutant third instar larvae (Gateff, 1978). The best established assay to discern between benign and malignant growth in *Drosophila* is to implant the affected tissue in a healthy host. This kind of allograft, or 'dauer' culture, is now a standard technique in our laboratory (González, 2007). Upon implantation, wild-type tissue never overgrows, and benign hyperplasias grow slowly, do not invade other tissues, and retain their capacity to differentiate. Malignant neoplasms, in contrast, display autonomous growth, the capacity to migrate
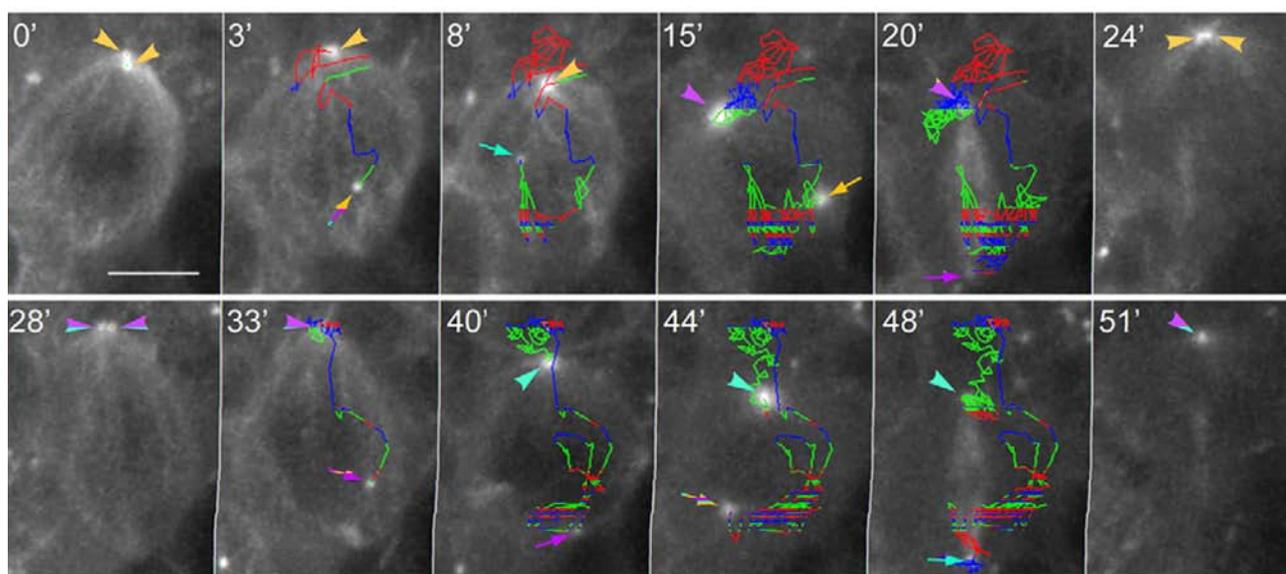
Figure 1. *Asymmetry of centriole motility in an embryonic neuroblast (NB). Centrosome movement was recorded during two consecutive cell cycles in a delaminated embryonic NB expressing YFP-Asl and GFP-α-TUB84B. Movements of the active apical centrosome (arrowhead) and the motile centrosome (arrow) are traced in red and green, respectively. Scale bar: 5 μm. Taken from: Rebollo E* et al, *2009.*

to and colonise distant organs, and lethality to the host. Moreover, malignant neo-plasms frequently become immortal and can be expanded limitlessly through succes-sive rounds of implantation into healthy hosts.

Drosophila *neural stem cells and controlled spindle orientation*

Neurons and glia in the developing central nervous system of *Drosophila* are generated by the self-renewing asymmetric division of neural stem cells (SCs), called neuroblasts (NBs). Acquisition of NB identity imposes a self-renewing asymmetric division mode whereby each of the two daughter cells acquires one of two possible developmental fates: NB or ganglion mother cell (GMC). GMCs can be considered as intermediate pro-genitors -to use the terminology that is common in vertebrates- that divide, normally just once, to generate cells that eventually differentiate into neurons or glia. There-fore, some of the key processes that characterise SCs occur in *Drosophila* NBs, which are probably the best understood models for animal SC asymmetric division.

The self-renewing asymmetric division of NBs in this model relies on the tight coor-dination of two processes: (i) the differential sorting of the Pins and Par complexes to the apical cortex and the Mira and Pon complexes to the basal cortex, and (ii) the controlled positioning of the plane of cytokinesis, which leads to the unequal seg-regation of these cortical protein complexes between daughter cells (reviewed in González, 2008). Clearly, both of these processes are necessary, but neither of them is sufficient.

Pioneering live microscopy studies carried out on embryos demonstrated the first re-ported mechanism of spindle alignment in *Drosophila* NBs: spindles assemble at an angle that is almost perpendicular to the apicobasal axis of the cell and later rotate to align with it (Kaltschmidt *et al*, 2000). More recent studies carried out on larval NBs revealed a different mechanism, by which spindles assemble already closely aligned along the cortical polarity axis of the NB and only minor rotations refine their align-ment before division occurs (Rebollo *et al*, 2007; Rusan and Peifer, 2007). This sec-ond mechanism relies on the differential spatiotemporal control of the activity of the microtubule-organising center (MTOC) activity of the NB centrosomes (Rebollo *et al*, 2007; Rusan and Peifer, 2007). Because larval NBs originate from quiescent embryonic NBs, these observations raise the question of when the switch from the rotational to the predetermined spindle alignment mode occurs during development. We have

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recorded embryonic NBs that express centrosome and microtubule reporters, from delamination up to the fourth cell cycle, by two-photon confocal microscopy, and have found that the switch between these two modes occurs in the second cell cycle of the NB, the first one taking place after delamination. Therefore, predetermined spindle orientation is not restricted to larval NBs. On the contrary, this phenomenon occurs in all but the first cell cycle of embryonic NBs (Rebollo *et al*, 2009).

As we have discussed in a recent article published in collaboration with S. Tajbakhsh (Stem Cells and Development Department of Developmental Biology, Institut Pasteur, CNRS, Paris, France), given that old and newly synthesized centrosomes differ in their microtubule nucleating capacity, the asymmetric localisation of epigenetic marks and kinetochore proteins could lead to the differential recognition of sister chromatids and the biased segregation of DNA strands to daughter cells during cell division (Tajbakhsh and González, 2009). Such asymmetric localisation could be linked to biased chromatid segregation, which might also be associated with the acquisition of distinct cell fates after mitosis (Tajbakhsh and González, 2009).

Self-renewing asymmetric division in neural SCs and tumour suppression

Loss of cell polarity and malignant transformation are tightly correlated in human carcinomas. There are several hypotheses to explain how the loss of polarity contributes to neoplastic transformation. Most of these call on models in which changes in cellular architecture impinge directly on the cell cycle either by inhibiting cell proliferation restraints or by enhancing mitogenic pathways. Alternatively, loss of polarity might, if affecting asymmetrically dividing SCs, impair the fate of daughter cells, rendering them unable to respond to the mechanisms that control proliferation in the wild-type lineage and thus initiating tumour growth. The possible functional link between failed NB asymmetry and tumour growth was first suggested by the identification of known TS genes as key regulators of NB asymmetry. However, direct demonstration of this link came from results published by our laboratory showing that pieces of larval brain tissue mutant for any of several elements that regulate NB asymmetry develop as tumours when transplanted to the abdomen of adult hosts (Caussinus and González, 2005; Clevers, 2005). We found that these tumours grow unrestrained and often give rise to the development of tumour colonies dispersed around the body, killing the implanted hosts in about two weeks. Moreover, they can be re-transplanted into healthy hosts and survive for years, thereby showing that the transformed cells become immortal (Caussinus and González, 2005; Castellanos *et al*, 2008). Therefore, these tumours fulfill the criteria for neoplastic growth: invasiveness and metastasis, lethality to the host and autonomous, limitless growth.

Subsequent reports from several laboratories have confirmed our results and expanded the number of what is now a long list of genes known to play a role in neural SC polarity and tumour suppression in these cells, including cell fate determinants, some elements of the apical cortex complexes, and kinases that regulate SC polarity like AurA and Polo (Betschinger *et al*, 2006; Lee *et al*, 2006; Wang *et al*, 2006; Wang *et al*, 2007; Bowman *et al*, 2008; Knoblich, 2008; Castellanos *et al*, 2008). The main conclusion derived from these observations is that NBs can be transformed into malignant cells by disrupting their delicately balanced process of self-renewing asymmetric division. This provides additional support to the general hypothesis that malfunction of the asymmetric cell division machinery of SCs contribute tá their transformation. (For a review, see: Januschke and González, 2008.)

Origin and functional relevance of genome instability in Drosophila *tumour models*

In most solid tumours in humans, malignancy is often correlated with genome instability (GI), defined as quantitative and/or qualitative changes in genetic material -aneuploidies, poliploidies, deficiencies, translocations, and inversions. This correlation suggests that GI might not merely be a consequence of transformation, but a factor that contributes to it. However, causality has not been unequivocally established between GI and tumour progression.

Interestingly, GI is observed in all types of *Drosophila* tumours originated from the deregulation of the mechanisms that drive asymmetric SC division, regardless of whether the mutation that initiated the tumour causes a certain level of GI, or none at all. When grown in allograft culture, all these tumours display significant levels of chromosomal alterations that affect both chromosome integrity and number (Castellanos *et al*, 2008; Caussinus and González, 2005). Moreover, recently our laboratory has shown that GI is not an efficient tumorigenic condition in *Drosophila* neural SCs (Castellanos *et al*, 2008), thereby suggesting that GI is in fact a downstream effect of transformation and leaving open the question of whether or not it plays an active role in the progression of these tumours towards malignancy.

Other TSs in Drosophila

In collaboration with AM Martinez and G Cavalli (Institut de Génétique Humaine, France), we have studied the tumorigenic potential of loss-of-function conditions in the PcG gene polyhomeotic (ph). Polycomb Group (PcG) proteins silence critical developmental genes and modulate cell proliferation. Using the *Drosophila* eye as a model system, we show that cells mutant at the PcG polyhomeotic (ph) locus overproliferate, and lose the capacity to differentiate and also their normal polarity. They invade neighbouring tissues and when combined with an activated form of the Ras proto-oncogene they trigger metastasis formation. PcG proteins bind to multiple genes in the Notch pathway and control their transcription as well as Notch signalling. The massive cell-autonomous overproliferation of ph mutant cell clones can be rescued by ectopic expression of a dominant negative form of Notch or by RNAi-mediated Notch repression. Conversely, overexpression of ph induces a small eye phenotype that is rescued by activation of Notch signalling. These data show that ph is a TS locus that controls cellular proliferation by silencing multiple Notch signaling components (Martinez *et al*, 2009).

Scientific output

Publications

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Research networks and grants

Acción complementaria
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Principal investigator: Cayetano González

Ayuda complementaria al proyecto europeo 'An integrative approach to cellular signalling and control processes: bringing computational biology to the bench'
Spanish Ministry of Science and Innovation, BFU2005-24117 (2006-2009)
Principal investigator: Cayetano González

Cancer stem cells and asymmetric division (ONCASYM)
European Commission, STREP LSHC-CT-2006-037398 (2006-2009)
Principal investigator: Cayetano González

Centrosoma 3D: Hacia la comprensión estructural y funcional del centrosoma. Consolider Ingenio 2010
Spanish Ministry of Science and Innovation, CENTROSOME 3D, CSD2006-23 (2006-2011)
Principal investigator: Cayetano González

Grup reconegut de la Generalitat
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Principal investigator: Cayetano González

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Spanish Ministry of Science and Innovation, *Oncológica* (2009-2012)
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Collaborations

Cancer stem cell and asymmetric cell division (ONCASYM)
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