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.

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 line of research focuses on the role of larval neural stem cells (Neuroblasts: NBs) as the origin of tumours.

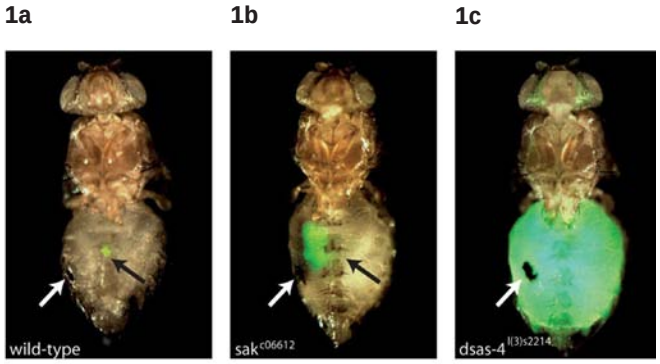

Figure 1. Benign and malignant growth following allograft culture. *(a) A piece of GFP-labelled wild-type larval brain (black arrow) implanted into the abdomen of an adult host (white arrows point to the scar produced by the needle at the point of injection) does not show any significant growth in two weeks. (b) In the same period, a mutant implant of the same size that grows significantly. This type of growth is graded as benign as it does not notably compromise the survival of the host. (c) An implant from a distinct mutant tissue that grows and spreads throughout the entire abdominal cavity (green), severely compromising the viability of the host. Extracted from Castellanos et al, 2008.*

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. This research effort has led to 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). All the TSs identified in *Drosophila* to date are essential for cell differentiation and development. Many of them have homologues in vertebrates, thus opening up the possibility of using this model system 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 relevance of the fly model in cancer research.

The first *Drosophila* TSs were identified *in situ* by observation of 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. Such an 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 to and colonise distant organs, and lethality to the host. Moreover, malignant neoplasms frequently become immortal and can expand limitlessly through successive rounds of implantation into healthy hosts.

Drosophila neural stem cells

Neurons and glia in the developing central nervous system of *Drosophila* are generated by the self-renewing asymmetric division of neural stem cells, called neuroblasts (NBs). Acquisition of

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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 intermediate progenitors -to use the terminology that is common in vertebrates- that divide, normally just once, to generate cells that eventually differentiate into neurons or glia. Therefore, some of the key processes that characterise stem cells occur in *Drosophila* NBs. Thus this model system is probably the best model to study asymmetric division in animal stem cells.

Self-renewing asymmetric division of *Drosophila* NBs relies on the tight coordination 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 segregation of cortical protein complexes between the daughter cells (reviewed in González, 2008). Clearly, both processes are necessary for self-renewing asymmetric division of these cells, but neither is sufficient.

How polarity is re-established in each cell cycle is still unclear. Polarised cortical markers are not detected after mitosis, and the first sign of them returning to the cortex occurs very late in the cell cycle, when mitosis starts. A tantalizing hypothesis, derived from results obtained recently in our lab, is that polarity information is kept by the asymmetric structure of the NB cytoskeleton (Rebollo *et al*, 2007). Data from live imaging of microtubules and centrosomes in these cells shows that soon after cytokinesis both centrosomes migrate to the nearest cortex, which roughly coincides with the region where apical markers were last localised. A few minutes later, the two centrosomes start to display markedly asymmetric behaviour. One stays fixed at the apical cortex, organising an aster that will be the main

microtubule network during most of the NB interphase. The orientation of the future mitotic spindle and the future localisation of the apical complexes can be accurately predicted from the position of this apical aster, long before any of the known polarised markers can be seen at the apical cortex of the cell (González, 2007). The second centrosome, which has little, if any, PCM and does not display any significant MTOC activity, moves extensively throughout the cytoplasm, mainly in the apical side first, more basally later, until shortly before mitosis when it slows down near the basal cortex, recruits PCM and organises the second mitotic aster. Thus, the structure and function of the two centrosomes of a NB differ greatly. They are also unequal in fate since the apical centrosome remains in the stem cell, while the other centrosome goes into the differentiating daughter cell.

Therefore, while we cannot discard that unknown cues might guide centrosomes to the apical cortex, it is also possible that it is the positioning of the centrosome itself at the apical side of the NB that contributes to triggering the sorting of the apical markers (Januschke and González, 2008). Interestingly, cultured individual NBs show the same asymmetric centrosome behaviour as NBs observed "in toto". This observation therefore strongly suggests that regulation of such stereotyped behaviour does not depend on the crosstalk between NBs and their neighbouring cells (Januschke and González, 2008).

Self-renewing asymmetric division in NBs and tumour suppression

Loss of cell polarity and malignant transformation are tightly correlated in human carcinomas. There are several hypotheses to explain how 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 stem cells, impair the fate of the daughter cells, rendering them unable to respond to the mechanisms that control proliferation in the wild-type lineage and initiating tumour growth. The possible functional link between failed NB asymmetry and tumour growth was first proposed after 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, which kill the implanted hosts in about two weeks. Moreover, these tumours 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 be involved in NB polarity and tumour suppression in these cells, including cell fate determinants, some elements of the apical cortex complexes, and kinases that regulate stem cell 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 become malignant cells by disrupting their delicately balanced process of self-renewing asymmetric division. This finding provides additional support to the general hypothesis proposing that malfunction of the asymmetric cell division machinery of stem cells contributes to their transformation (For 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 the genetic material—aneuploidies, polyploidies, deficiencies, translocations, and inversions. This correlation suggests that GI might not merely be a consequence of transformation, but a contributing factor to it. However, causality has not been unequivocally established between GI and tumour progression.

Interestingly, GI is observed in all types of *Drosophila* tumours that arise as a result of the deregulation of the mechanisms that drive asymmetric stem cell division. When grown in allograft culture, all these tumours display significant levels of chromosomal alterations that affect both chromosome integrity and number, regardless of whether the mutation that initiated the tumour causes a certain level of GI or none at all (Castellanos *et al*, 2008; Caussinus and González, 2005). Moreover, we have recently shown that GI is not an efficient tumorigenic condition in *Drosophila* NBs (Castellanos *et al*, 2008), thereby suggesting that GI is 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.

SCIENTIFIC OUTPUT

Publications

Castellanos E, Dominguez P and González C. Centrosome dysfunction in *Drosophila* neural stem cells causes tumors that are not due to genome instability. *Curr Biol*, **18**(16), 1209-14 (2008)

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Research networks and grants

Ajut per a grups de recerca consolidats
Agency for Administration of University and Research Grants (AGAUR), 2005-SGR-00821 (2006-2008)
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
Spanish Ministry of Science and Innovation, CSD2006-23 (2006-2011)
Principal investigator: Cayetano González

Identificación mediante análisis genético y farmacológico de proteínas esenciales para prevenir la transformación maligna de células madre en Drosophila
Oncostem Pharma SL, Cibasa (2006-2008)
Principal investigator: Cayetano González

Identification of pathways that are relevant for the malignant transformation of stem cells in Drosophila
Spanish Ministry of Science and Innovation, BFU2006-05813-BMC (2007-2009)
Principal investigator: Cayetano González

Collaborations

Co-evolution of the chaperonin CCT and tubulins from antarctic fishes; United States Antarctic Programme expedition
William Dietrich, Northeastern University (Massachusetts, USA)

Control of asymmetric division in cancer stem cell
Marcos González, University of Geneva (Geneva, Switzerland)