

# 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*.



Cayetano González

## Modelling cancer in *Drosophila*

We are starting to exploit *Drosophila* to study some basic principles of cell proliferation and malignant growth (Caussinus and González, 2005; Wodarz and González, 2006). This research focuses on the role of neural stem cells (larval neuroblasts) as the cell-of-origin of tumours.

Loss of cell polarity and cancer are tightly correlated; however, evidence of a causative relationship remains elusive. In stem cells, loss of polarity and impairment of asymmetric cell division could alter cell fates and thereby render daughter cells unable to respond to the mechanisms that control proliferation. To test this hypothesis, we generated larval neuroblasts from *Drosophila melanogaster* with mutations in various genes that control asymmetric cell division and then assayed the proliferative potential of these cells after transplantation into adult hosts. Larval brain tissue carrying neuroblasts with mutations in *raps* (also called *pins*), *mira*, *numb* or *pros* grew to more than 100 times its initial size, thereby invading other tissues and killing the host in two weeks. These tumours became immortal and could be retransplanted into new hosts for years. Six weeks after the first implantation, genome instability and centrosome alterations, two traits of malignant carcinomas, were detected in these tumours. Increasing evidence suggests that several tumours may be of stem cell origin. Our results show that loss-of-function of any of several genes that control the fate of stem cell daughters cause hyperproliferation, thus triggering a chain of events that subverts cell homeostasis in a general sense and leads to cancer.

Our recent findings include the observation that in stem cells, centrosomes are asymmetric in function and fate (Rebollo *et al*, 2007). Like the stem cells in other higher eukaryotes, *Drosophila* neural stem cells (NBs) undergo asymmetric division whereby one of the two daughters retains stem cell identity, while

the other enters a differentiation programme. The molecular mechanisms that drive asymmetric division in these cells have been the subject of intensive research effort in the last decade. Briefly, the localisation of the Par complex, which includes Bazooka (Baz), Par-6 and atypical protein kinase C (aPKC) at the apical cortex, drives the basal localisation of the adaptor proteins Miranda and Partner of Numb. These, in turn, mediate the accumulation of cell fate determinants such as Numb Prospero at the basal cortex of the NB. Consequently, upon cell division, the determinants end up mostly within the small ganglion mother cell that buds off the basal side of the NB, thereby resulting in the unequal developmental fate of the two daughters (González, 2007).

A key step in the asymmetric segregation of the determinants is spindle orientation, which must be in line with the polarity axis of the cell. This process is governed by Inscuteable, another protein of the apical complex that binds to Baz and aPKC. Inscuteable mediates the organisation of a complex that includes Pins Gal and Mushroom body defective (Mud), the protein thought to ultimately mediate spindle orientation through direct interaction with one of the two mitotic asters, which is thereby singled out as the apical aster. It is unclear, however, how the system discriminates between the two asters of the NB so that only one engages in this interaction. Unlike embryonic NBs where, after assembly, the spindle rotates 90 degrees to align along the polarity axis, larval NBs assemble the spindle already aligned and no rotation occurs. The contribution of aPKC to spindle orientation also differs between larval and embryonic NBs. Directed assembly without rotation has also been observed in grasshopper embryonic NBs and in *Drosophila* germline stem cells (González, 2007).

To study this key process, we have recorded *Drosophila* larval NBs engineered to express fluorescent reporters for microtubules, pericentriolar material



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(PCM), and centrioles. We have found that early in the cell cycle, the two centrosomes become unequal: one organises an aster that remains near the apical cortex for most of the cell cycle, while the other loses PCM and microtubule-organising activity, and moves extensively throughout the cell until shortly before mitosis when, located near the basal cortex, it recruits PCM and organises the second mitotic aster. Upon division, the apical centrosome remains in the stem cell, while the other goes into the differentiating daughter cell. Apical aster maintenance requires the function of Pins. These results reveal that spindle orientation in *Drosophila* larval NBs is determined very early in the cell cycle and is mediated by asymmetric centrosome function (Rebollo *et al*, 2007).

### Molecular analysis of centrosomes

We have recently cloned the gene that encodes a centriolar protein. We have mutant alleles, functional GFP fusions and antibodies that will be instrumental in the molecular dissection of this organelle (Lange *et al*, 2002; Tavasani and González, 2003). Centrosomes are the major organisers of the microtubule network in most animal cells, and comprise centrioles embedded in a web of PCM. Recruitment and stabilization of PCM on the centrosome is a centriole-dependent function. Compared to the considerable number of PCM proteins known, the molecular characterisation of centrioles is still very limited. Only a few centriolar proteins have been identified to date in *Drosophila*, most of these related to centriole duplication.

We have cloned asterless (*asl*) and found that it encodes a 120 kD highly coiled-coil protein that is a

constitutive pancentriolar and basal body component. Loss of *asl* function impedes the stabilization/maintenance of PCM at the centrosome. In embryos deficient for Asl, development is arrested immediately after fertilisation. Asl shares significant homology with Cep152, a protein described as a component of the human centrosome for which no functional data is yet available. In conclusion, the cloning of *asl* offers new insight into the molecular composition of *Drosophila* centrioles and a model for the role of its human homologue.

In terms of the roles that centrioles play during development, our results show that the first zygotic division never occurs in a cytoplasm deficient for Asl. This observation strongly suggests that functional centrosomes are required for embryogenesis in *Drosophila*. The finding that the first mitotic division does not take place in eggs derived from females lacking PCM components, like gTUB37C or D-TACC, leads to the same conclusion. However, the possibility remained that this early developmental arrest in gTUB37C or D-TACC-deficient embryos could be a downstream consequence of the meiotic defects caused by mutations in these genes. This caveat is now largely circumvented by the phenotype of embryos derived from *asl* mutant females in which both meiotic divisions proceed normally. Thus, although we cannot rule out a possible non-centrosomal function of Asl, the pheno-

type of embryos derived from *asl* mutant females is consistent with the hypothesis that centrosomes are required for *Drosophila* embryo development. Previous reports have shown that zygotic loss of key centrosomal proteins such as D-plp, Sas4, Sak/Plk4, or Cnn does not block progression of development into adult flies. However, the centrosome-less females that hatch are sterile, which strongly suggests that eggs defective for these centrosomal components do not support embryogenesis. How development can proceed in zygotic loss-of-function conditions for these genes is not clear. However, the initial stages of development of individuals homozygous for mutations in these centrosomal proteins are likely to be sustained by the wild-type RNA/protein contributed to the egg by the heterozygous females from which they derive. Thus, until a certain stage, which is hard to specify, development in these mutant individuals occurs when cells still have centrosomes.

The hatching of adults that have undergone the last stages of development without centrosomes proves a certain level of centrosome dispensability in *Drosophila* development, even though these adults are uncoordinated and sterile and die only hours after eclosion. Loss of centrosome function has been reported to impair a number of developmental stages in vertebrates. In humans, for instance, abnormal centrosomes have been linked to impaired neuronal migration, hereditary spastic paraplegia, Bardet-Biedl syndrome, the development of cystic kidneys, perturbed left-right asymmetry, microcephaly, and cancer. In mice, loss-of-function for Sak/Plk4 is a lethal condition, and haploinsufficiency for this gene results in a high incidence of tumours. The molecular dissection of centrioles in *Drosophila* may help to model the cellular basis of some of these processes.

## Publications

Dyer N, Rebollo E, Domínguez P, Elkhathib N, Chavrier P, Daviet L, González C and González M. Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. *Development*, 134(24), 4437-47 (2007)

González C. Spindle orientation, asymmetric division and tumour suppression in *Drosophila* stem cells. *Nat Rev Genet*, 8, 462-472 (2007)

Rebollo E, Sampaio P, Janushke J, Llamazares S, Varmark H and González C. Functionally unequal centrosomes drive spindle orientation in asymmetrically dividing *Drosophila* neural stem cells. *Dev Cell*, 12, 467-474 (2007)

Varmark H, Llamazares S, Rebollo E, Lange B, Reina J, Schwarz H and González C. Asterless is a constitutive centriolar protein required to organise functional centrosomes and essential to trigger zygotic development in *Drosophila*. *Curr Biol*, 17(20), 1735-45 (2007)

## Spindle assembly and cell division *in vivo*

Direct visualisation is becoming mandatory to unravel the complex processes that occur within the living cell. Using protocols developed in our laboratory, we are obtaining new information on the behaviour of specific proteins labelled with fluorescent tags (Rebollo and González, 2000; Lange *et al*, 2002; Sampaio *et al*, 2001; Rebollo *et al*, 2004).

Through this technology, we have unveiled a new role for *arf6* during cytokinesis. The dramatic morphological changes during cell cytokinesis requires the interplay between microtubules and the actomyosin contractile ring, and addition of membrane to the plasma membrane. Numerous membrane-trafficking components localise to the central spindle during cytokinesis, but it is still unclear how this machinery is targeted there and how membrane trafficking is coordinated with cleavage furrow ingression. Here we use an *arf6* null mutant to show that the endosomal GTPase ARF6 is required for cytokinesis in *Drosophila* spermatocytes. ARF6 is enriched on recycling endosomes at the central spindle, but it is required neither for central spindle nor actomyosin contractile ring assembly, nor for targeting of recycling endosomes to the central spindle. However, in *arf6* mutants the cleavage furrow regresses because of a failure in rapid membrane addition to the plasma membrane. We propose that ARF6 promotes rapid recycling of endosomal membrane stores during cytokinesis, which is critical for rapid cleavage furrow ingression (Dyer *et al*, 2007).

## Research Networks and Grants

*Alteraciones en la localización subcelular y transformación cancerosa: Determinación de su utilidad diagnóstica*  
Fundación Mutua Madrileña Automovilística: 2004-2007  
**Research Director:** Cayetano González

*CENTROSOMA 3D; Hacia la comprensión estructural y funcional del centrosoma*  
Ministerio de Educación y Ciencia, Consolider Ingenio 2010, CSD2006-23: 2006-2011  
**Research Director:** Cayetano González

*Desarrollo de modelos tumorales en Drosophila melanogaster*  
Generalitat de Catalunya, 2005SGR-00821: 2006-2010  
**Research Director:** Cayetano González

*Identification of pathways that are relevant for the malignant transformation of stem cells in Drosophila*  
Ministerio de Educación y Ciencia, BFU2006-05813: 2007-2009  
**Research Director:** Cayetano González

*Integrative approach to cellular signalling and control processes: bringing computational biology to the bench, COMBIO.*

European Commission, VI Framework Programme, STREP LSH-2004-503568: 2004-2007

**Research Director:** Cayetano González

*ONCASYM – Cancer stem cells and asymmetric division*

European Commission, VI Framework Programme, STREP LSH-2005-2205: 2006-2009

**Research Director:** Cayetano González

#### **Other Funding Sources**

Oncostem. Collaboration agreement, University of Geneva

#### **Collaborations**

*The role of arf6 in spermatocyte cytokinesis*

Marcos González-Gaitán, Département de Biochimie, Sciences II (Geneva, Switzerland)

