Light, flies and cell division

Workshop: Regulation of cell division in *Drosophila*

A meeting on the regulation of cell division in *Drosophila* took place in Cortona, Italy, June 10–15, 2000. This is the second of a series that started in 1995 in Loch Ranoch, Scotland, UK. This meeting was organized by Maurizio Gatti (Rome, Italy) as an EMBO workshop and had additional financial support from the University of Rome 'La Sapienza' and the 'Centro di Genetica Evoluzionistica del CNR'.

Introduction

During the past 10 years, *Drosophila* has revealed itself as a powerful system for studying the cell cycle, a field traditionally dominated by yeast and *Xenopus*. There are a number of reasons for this, including the fact that *Drosophila* is a well-established model system amenable to sophisticated genetic analysis and molecular biology techniques. In fact, it is actually the only genetically amenable, higher eukaryote that is being used extensively as a model system for cell-cycle research. Furthermore, as a higher eukaryote, *Drosophila* uses much of the same cell cycle machinery that is also used in vertebrates. The similarities go beyond ultrastructural detail, as shown by the recently published analysis of the *Drosophila* genome sequence, which revealed that half of the fly protein sequences identified by the *Drosophila* genome project show significant similarity to mammalian proteins and that the fly has orthologues of 61% of human disease genes and 68% of the cancer genes that were surveyed (Rubin *et al.*, 2000). The same applies to cell division; analysis of the *Drosophila* genome confirms previous suggestions of strong parallelism between flies and human cell cycle regulators. Last, but not least, *Drosophila* is much more amenable than other classical cell-cycle models to analysis with the new cell imaging techniques.

Live imaging

One of the most significant highlights of the meeting was the extensive use of live imaging, which is becoming commonplace

in the study of the cell cycle in *Drosophila*. Several presentations on cyclins, cell cycle checkpoints, cytokinesis and the behaviour of different centrosomal components substantiate this view. Live imaging is revealing many important features of all these processes that either had been missed or were simply unapproachable through observations carried out on fixed materials. Since space constraints limited us to describing only a fraction of the many exciting contributions presented at the meeting, we decided to follow this trend and concentrate mainly, although not exclusively, on some of the talks that were based on live imaging techniques. We apologize to our colleagues whose work we are not able to cover.

Although standard bright-field techniques like phase-contrast or Nomarski differential interference contrast (DIC) still provide the basis for many live microscopy applications, green fluorescent protein (GFP) technology is rapidly expanding. Indeed, GFP tagging is unique in that it affords the visualization of a particular protein, thus allowing very precise studies on its behaviour through time and space. The potential of this technique is expanding as more functional GFP fusion proteins become available. Of these, the GFP–Histone 2 (GFP–His2) produced by R. Saint (Clarkson and Saint, 1999) deserves special mention as it has become the label of choice for visualizing chromatin in a variety of tissues and developmental stages throughout the cell cycle.

Chromatin condensation, chromatid cohesion and telomeres

Proper chromosome condensation and the maintenance of sister chromatid cohesion are essential for faithful chromatid segregation during mitosis. A number of proteins and protein complexes required for these processes have been identified during the last few years. These include cohesins, structural maintenance of chromosomes (SMC) proteins, securins, separins and others. In yeast, cohesins remain bound to chromosomes until the metaphase-to-anaphase transition, when cleavage of the scc1/rad21 cohesin subunit promotes sister separation. Despite sequence similarities with their yeast homologues, the cohesins of *Xenopus* and mouse are only associated with chromosomes until prophase, raising the question of how cohesion is mediated between prophase and the onset of anaphase. W. Warren (Melbourne, Australia) has combined the RNA interference technique and video microscopy to perform a detailed study of the function of Drad21, the *Drosophila* homologue of the yeast mitotic cohesin scc1/rad21. Based on confocal time-lapse

observation of the dynamics of a GFP–Drad21 chimera, he has shown that while the bulk of Drad21 does indeed dissociate from the chromatin at prophase, a small pool remains bound to the centromeres until anaphase. This observation strongly suggests that the remaining Drad21 may be sufficient to maintain sister chromatid cohesion until anaphase, a mechanism that could be applicable to other higher eukaryotes. Warren and colleagues are now investigating whether proteolysis of this centromeric pool of Drad21 is able to drive sister chromatid separation at anaphase.

Another group of proteins involved in regulating chromosome cohesion are SMC proteins. Collaborative work between the laboratories of C. Sunkel (Porto, Portugal) and M. Heck (Edinburgh, UK) has recently identified the *Drosophila* homologue of SMC4 as the product of the previously known gene *gluon* (*glu*). Antibodies raised against the Glu protein have been used to show that Glu is precisely localized along the inner chromosomal axis in cells arrested before the metaphase-to-anaphase transition. This coincides with the localization of Barren, another member of the condensing complex. Consistent with a role in the SMC complex, loss of Glu function results in abnormal chromosome condensation and segregation.

Although the *Drosophila* genome sequencing project has not identified any homologues of the securin proteins of yeast and vertebrates, C. Lehner (Bayreuth, Germany) discussed the possibility that the *Drosophila* protein Pimples (Pim) could be related to them at a functional level. Pim is required for sister chromatid separation and is rapidly degraded during mitosis through a cyclin B-like destruction box. High levels of non-degradable Pim, as well as overexpression of wild-type Pim, inhibit sister chromatid separation. Moreover, cells arrested in mitosis before sister chromatid separation fail to degrade Pim. Thus, although not related by sequence, the behaviour of Pim has intriguing similarities to the securin proteins of other species.

Taken together, the results presented above reveal a high degree of conservation among the molecules and mechanisms that bring about chromatin condensation, sister cohesion and sister chromatid release in higher eukaryotes. This is not the case for telomeres in spite of the fact that these *cis*-acting sequences are essential for chromosome stability and transmission. While telomeres in vertebrates are characterized by simple repeats maintained by telomerases, *Drosophila* telomeres lack this characteristic structure and are maintained by the frequent insertion of HeT and TART elements. The laboratory of M. Gatti (Rome, Italy) is carrying out a detailed analysis of the genes involved in the process of telomeric fusion. The end-to-end association of chromosomes through their telomeres has been observed in normal cells of certain organisms, as well as in senescent and tumour cells. It is very rare in wild-type *Drosophila*, but can be induced by mutation in a series of genes. The molecular mechanisms underlying this phenomenon are largely unknown. Two of the genes identified by Gatti and colleagues are the ubiquitin conjugating (E2) enzymes UbcD1 and pendolino (*peo*). This indicates that ubiquitin-mediated proteolysis is normally needed to ensure proper telomere behaviour and to avoid telomeric fusion during *Drosophila* cell division. More recently, in screening C. Zuker's (San Diego, CA) collection of EMS-induced lethals, M. Gatti and M. Goldberg (Ithaca, NY) have identified 10 additional loci that are involved in telomeric fusion. In some cases, including UbcD1 and *peo*, the ectopic association between telomeres is efficiently resolved during anaphase. Mutations in other genes, however, lead to

extensive chromosome bridges and fragments. These include *Su(var)²⁰⁵*, the gene that encodes Heterochromatic Protein 1, a protein that is highly enriched in regions of centromeric heterochromatin and telomeric DNA.

The spindle checkpoint

Two reports based on video microscopy of live spermatocytes were presented in the meeting. This type of study is still quite rare, the only one published so far being Church and Lin (1985). C. Rieder (Albany, NY) used this technique to study chromosome movement in Zeste-White 10 (ZW10) mutant spermatocytes. He showed that ZW10 is required for pole-ward chromosome movement during the anaphase of the first meiotic division.

ZW10 and the closely related protein Rod were a frequent focus of attention during the meeting. The behaviour of these two proteins, which associate with the kinetochore in a mutually dependent manner (R. Karess and M. Goldberg), is very dynamic. Using a GFP–Rod fusion, R. Basto and R. Karess (Gif-sur-Yvette, France) showed that Rod accumulates at the kinetochore after nuclear envelope breakdown (NEB). At prometaphase, the fusion protein appears to flow along kinetochore microtubules, and at the onset of anaphase the GFP–Rod signal quickly fades. Similar behaviour has been observed for the ZW10 protein in fixed cells. ZW10 and Rod are part of a large complex that is well-conserved in higher eukaryotes.

In addition to their roles in chromosome movement, both ZW10 and Rod appear to participate in the mitotic spindle checkpoint. This conclusion is based on the inability of colchicine to inhibit the onset of anaphase in cells mutant for either protein. Interestingly, the flowing of these two proteins along the microtubules appears to be sensitive to the tension exerted by the spindle microtubules on the kinetochore. One of the recently identified functions of this complex is to target dynein to the kinetochore. The extension of Rod along the spindle microtubules is dependent on dynein, suggesting an involvement of this motor molecule in the checkpoint mechanism (T. Hays, Minneapolis, MN).

Further uses of live imaging in exploring the spindle checkpoint mechanism were illustrated by C. Rieder (Albany, NY) and C. Gonzalez (Heidelberg, Germany), who tracked the activity of the meiotic spindle checkpoint during male meiosis-I. The possible existence of a spindle checkpoint in *Drosophila* meocytes has remained unclear until now, with different pieces of evidence seeming to contradict one another. Direct observation of meiotic spermatocytes has now revealed that meiotic progression is transiently, but significantly, delayed under conditions that are likely to trigger the checkpoint. Such conditions include treatments with drugs that alter microtubule dynamics or the presence of multiple misaligned chromosomes introduced by genetic means. Microtubule depolymerization or overstabilization has much more severe effects during any of the four mitotic divisions that precede meiosis in *Drosophila* than during the meiotic divisions themselves. These observations are consistent with the activity of a spindle checkpoint in *Drosophila* meocytes, albeit a far less efficient one than that of their precursor cells. C. Rieder reported that microtubule overstabilization of ZW10 mutant meocytes does not delay meiosis to any significant extent, showing that ZW10 is required for the function of this spindle checkpoint.

Centrosomes

The centrosome session included numerous presentations based on new live imaging approaches that are quickly changing the way we see this fascinating organelle. G. Sluder (Worcester, MA) showed data obtained from time-lapse video recording of vertebrate BSC-1 cells whose centrosomes had previously been ablated by micromanipulation. These centrosome-less cells enter mitosis at about the same time as control cells, suggesting the absence of a centrosome duplication or centrosome integrity checkpoint. Subsequently, in the following cell cycle most of the acentrosomal cells arrest before DNA replication and fail to enter a second mitotic division. This observation suggests that a functional centrosome is required for somatic cells to enter S phase.

Making use of a GFP-centrin fusion, which concentrates in the lumen of both centrioles, M. Bornens (Paris, France) reported on time-lapse observations of centrioles in vertebrate cells. The dramatic differences in the behaviour of mother and daughter centrioles that were observed had escaped countless previous studies carried out on fixed material. The centriole pair inherited after mitosis splits during or just after telophase. The mother centriole remains near the cell centre while the daughter migrates extensively throughout the cytoplasm. The motion of the daughter centriole persists, although modified, in the absence of either microtubules or actin, but is arrested when both filaments are disrupted. As the centrioles replicate at the G₁/S transition, the movements exhibited by the original daughter become progressively attenuated. While both centrioles nucleate similar numbers of microtubules, only the mother centriole is located at the focus of the microtubule array. Based on differences in microtubule anchoring and release by the mother and daughter centrioles, a model was proposed to explain how the centrosome activity and that of the plasma membrane are coupled during cell division as well as during cell locomotion. A role for the centrosome in controlling the ultimate step of cell division is suggested by extensive centriole movements towards the midpiece before the end of cytokinesis.

Centriole duplication and particularly the roles of known cell cycle regulators in controlling this process during embryonic development were discussed by P. O'Farrell (San Francisco, CA). Cdc25 function appears to be essential for maturation of daughter centrioles, and APC activation seems to be required for splitting of the two centrioles into a pair of singlets. Moreover, initiation of daughter centriole assembly requires the degradation of mitotic cyclins. D. Parry and P. O'Farrell also reported that the behaviour of individual sister chromatids following mitotic arrest produced in cells by the introduction of stable cyclin B is much more dynamic than was originally thought. Live imaging has revealed that, instead of 'getting stuck' somewhere along their pole-ward migration, as suggested by the studies using fixed preparations, the sister chromatids continuously move back and forth between the metaphase plate and the spindle poles.

Additional data on centrosome replication were presented by I. Belec and J. Szabad (Szeged, Hungary), who showed that LaborcD, one of the dominant female sterile mutations that they identified some time ago, is a dominant-negative mutation in the *Drosophila* cytoplasmic dynein heavy chain (Dhc64C). As in many other species, *Drosophila* wild-type oocytes inactivate their centrosome midway through oogenesis and remain acentri-

olar until fertilization. Consequently, the first centrosome of the zygote is organized around the paternally derived centriole from the basal body of the sperm, a mechanism thought to play an essential role in ensuring syngamy by preventing parthenogenesis. Unlike unfertilized wild-type eggs, those derived from LaborcD heterozygous mothers contain numerous centrosomes. Although it is not clear whether these are formed *de novo* or are derived from the oocyte's centrioles, this observation suggests a role for dynein in the mechanism that preserves the acentriolar nature of wild-type unfertilized eggs.

Live imaging is also revealing the dynamic nature of the pericentriolar material (PCM), the electron-dense material that surrounds the centrioles and mediates microtubule polymerization. Time-lapse microscopy of GFP-centrosomin chimeras (GFP-Cnn; T. Kaufman, Bloomington, IN) and GFP-D-TACC, the *Drosophila* member of the mammalian transforming, acidic, coiled-coil-containing family of proteins (J. Raff, Cambridge, UK), shows that these two centrosomal proteins form long flare-like projections that emanate from the centrosome during mitosis. Thread-like structures containing Cnn can be observed connecting the centrosomes as they move to the poles of the interphase nucleus. Cnn is a centrosomal component that appears to be essential for the assembly of this organelle. D-TACC, on the other hand, is thought to be involved in mediating microtubule stability; dosage studies have shown that increasing the amount of D-TACC in the centrosome leads to an increase in the number of microtubules that interact with that centrosome. The C-terminal region of D-TACC has a microtubule stabilizing effect, even when it is outside the centrosome.

The loss of either D-TACC or Cnn results in anastral spindles during early cleavage divisions. Paradoxically, homozygous Cnn null flies develop seemingly normally, despite the absence of the centrosomal proteins CP190, CP60 and Cbs at the poles of the spindles in these mutant flies. These observations suggest that Cnn and a normal PCM are dispensable for mitosis after cleavage. This conclusion is consistent with previous reports suggesting that *Drosophila* larval neuroblasts and ganglion mother cells can form functional anastral spindles (Bonaccorsi *et al.*, 2000).

As mentioned before, oocytes in *Drosophila* are acentriolar and organize anastral meiotic spindles. Although it is clear that these spindles are organized in a centrosome-independent manner, we still do not know whether the organization of such spindles requires the contribution of any of the components of the pericentriolar material. One such component may be minispindles (msps). H. Okhura (Edinburgh, UK) showed that Msps, the *Drosophila* homologue of XMAP215, is present in both the mitotic centrosomes and the poles of the female meiotic spindle. Moreover, a newly generated mutant allele results in meiotic tripolar spindles that are similar to those observed in mitotic cells homozygous for the other msps mutant alleles.

Cytokinesis

Following mitosis 13 in the *Drosophila* embryo, the syncytial nuclei are compartmentalized into individual cells in a process known as cellularization. Cytokinesis and cellularization are similar processes, and many common proteins are required for both. One such protein is spaghetti squash (sqh), the regulatory light chain subunit of myosin II. Using a GFP-Sqh chimera,

A. Rayou and R. Karess (Gif-sur-Yvette, France), in collaboration with B. Sullivan (Santa Cruz, CA), have studied the behaviour of this protein during the slow phase of cellularization. They reported that the accumulation of myosin at the leading edge of the invagination furrow appears due, at least in part, to numerous myosin-containing particles that are pulled towards and fuse with the advancing furrow in a microtubule-dependent manner. This accumulation is dependent upon the function of the anillin protein (C. Field, Boston, MA), which itself accumulates at the base of the cellularization front. Anillin function is also required to accumulate actin, the septin Peanut and the Formin-Homology (FH) protein Diaphanous. Interestingly, Diaphanous is also required for the localization of anillin (S. Wasserman, San Diego, CA).

Results presented by R.M. Farkas (Stanford, CA), M. Fuller (Stanford, CA) and M. Gatti (Rome, Italy) suggest that cytokinesis and polarized cell growth involve similar mechanisms. This conclusion is based on the analysis of a collection of mutants in which both processes are defective. The cloning of one of these (*four way stop*, *fws*) has revealed that it is homologous to GTC-90, a protein implicated in vesicle trafficking, and suggests that it facilitates membrane addition at both the cytokinesis furrow of spermatocytes, and the leading edge of elongated spermatids. B. Sullivan (Santa Cruz, CA) reported the identification of MMAP370, a new microtubule/microfilament-associated protein that is required for both cytokinesis and cellularization. Unlike other proteins implicated in cytokinesis, MMAP370 is Golgi associated, suggesting that it may play a role in the Golgi-mediated membrane export essential for invagination of the cellularization furrow.

Regarding the mechanism that signals the position of the cleavage furrow, S. Bonaccorsi and M. Gatti (Rome, Italy) reported that, in the acentrosomal spindles organized in *asterless* (*asl*) neuroblasts, functional cytokinetic structures are formed around the central spindle midzone. Based on this observation, it was concluded that the location of the cytokinesis furrow is determined by the central spindle midzone and does not require asters. This conclusion is also supported by the analysis of new male sterile mutants affecting cytokinesis during male meiosis. Unequal cytokinesis in *asl* cells is proposed to be mediated by interactions between the central spindle, the nascent ganglion mother cell (GMC) nucleus and the polar cortex, the combination of which shifts the position of the central spindle towards the side on the GMC.

Many components of the cytokinesis machinery are tightly localized to different organelles before cytokinesis. Proteins that are nuclear during interphase, like *pebble*, which could be required in the upstream signaling pathway of the initiation of the assembly of the contractile ring, could be simply sequestered into the nucleus as a means to remove this potential signaling activity from the cytoplasm (R. Saint, Adelaide, Australia). Others, like the inner centromere protein, INCENP, could coordinate the chromosomal and cytoskeletal events from metaphase to cytokinesis (M. Carmena and B. Earnshaw, Edinburgh, UK). The kinesin-like protein, Pavarotti (Pav-KLP), is centrosomal early in mitosis, and moves to the midbody region of the spindle in late anaphase and telophase. Pav-KLP is related to the mammalian MKLP-1 and is thought to be required for the assembly of the contractile ring. The subcellular localization of Pav-KLP is controlled by the Polo kinase, with which it shows an

overlapping pattern of subcellular localization during the mitotic cycle (D. Glover, Cambridge, UK).

Conclusions and perspectives

Drosophila has turned out to be a remarkably powerful model system for the study of cell division. Genetic analysis in the fly has been successful in identifying numerous critical players in the game. Many of these have subsequently been found to be conserved in other eukaryotes, and the combined findings can now be subjected to a refined analysis of mutant phenotypes by the use of live imaging and tagged proteins. Moreover, as expected from a holometabolous organism that undergoes extensive metamorphosis, some features of *Drosophila* development show rather unique cellular remodeling. Indeed, some division mutant phenotypes have no equivalents in vertebrates so far. These are also fairly valuable tools as they will help to reveal how flies use generic mechanisms for cell division in a specific way and, hopefully, will help in developing an understanding of the diverging evolution of cellular processes in insects and vertebrates.

Beyond mitosis, during which chromosome condensation and segregation take place, cell division is a tremendously complex process, which requires the participation of all cellular compartments, as well as the coordination of cytoplasmic and nuclear division. Like good cuisine, the preparation is very long but the consumption is rather rapid. And thus, understanding the basic principles controlling such a rapid and dramatic reshaping of cell organization in time and space is a difficult task. From what we learned at the Cortona meeting, it looks as if *Drosophila* can help us to fly through it.

References

- Bonaccorsi, S., Giansanti, M.G. and Gatti, M. (2000) Spindle assembly in *Drosophila* neuroblasts and ganglion mother cells. *Nature Cell Biol.*, **2**, 54–56.
- Church, K. and Lin, H.-P.P. (1985) Kinetochore microtubules and chromosome movement during prometaphase in *Drosophila melanogaster* spermatocytes studied in life and with the electron microscope. *Chromosoma*, **92**, 273–282.
- Clarkson, M. and Saint, R.A. (1999) *His2AvDGFP* fusion gene complements a lethal *His2AvD* mutant allele and provides an *in vivo* marker for *Drosophila* chromosome behavior. *DNA Cell Biol.*, **18**, 457–462.
- Gatti, M. and Baker, B.S. (1989) Genes controlling essential cell-cycle functions in *Drosophila melanogaster*. *Genes Dev.*, **3**, 438–453.
- Rubin, G.M. *et al.* (2000) Comparative genomics of the eukaryotes. *Science*, **287**, 2204.

Cayetano Gonzalez⁺ and Michel Bornens¹

Cell Biology and Biophysics Programme, EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany and ¹Institut Curie, UMR 144-CNRS, 26 Rue d'Ulm, 75248 Paris, Cedex 05, France

Received September 1, 2000; revised September 29, 2000; accepted October 2, 2000

⁺Corresponding author. Tel: +49 6221 387292; Fax: +49 6221 387306; E-mail: gonzalez@embl-heidelberg.de

DOI: 10.1093/embo-reports/kvd099