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Review

Molecular genetics of oogenesis in *Drosophila melanogaster*

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Abstract

Gonadal development requires complex differentiation programs leading to the production of functional female and male gametes. Upon fertilization, while the male germ cell contributes to the newly formed zygote only its genetic material, the female germ cell also supplies its cytoplasmic components, including fundamental molecular cues on which early embryonic development will rely. Unravelling the mechanisms employed by animal species for building up their eggs is therefore a challenging task in developmental biology. As demonstrated by the impressive body of data produced in recent years, *Drosophila melanogaster* is a useful model system for attempting a step by step dissection of the whole oogenesis process. Remarkable opportunities for comparative analyses are in turn expected to be provided by these studies, since it is becoming evident that conserved themes underlie oogenesis in all animal species. In this review, we focus on few key differentiation events occurring during egg chamber development in *Drosophila*, outlining our interest in the mechanisms leading to egg polarity establishment, transfer of information between nuclear and cytoplasmic cell compartments and germ cell apoptosis.

Key words: oogenesis; *Drosophila;* egg polarity; nuclear pore; apoptosis.

Introduction

The production of functional gametes is essential for the propagation of all sexually reproducing metazoan species. The central biological importance of this process has for centuries stimulated a remarkable scientific interest towards the mechanisms underlying female germline establishment and development. An unanticipated aspect of these studies is the emerging evidence that evolutionarily distant animals make eggs using common strategies (for review, see Matova and Cooley, 2001). In higher insects, oogenesis starts with the formation of a group of interconnected cells known as germline cist, originated from a single germline progenitor cell that undergoes synchronous divisions followed by incomplete cytokinesis (Buning, 1994). Germ cell clusters have been more recently described also in primitive insect and vertebrate females, suggesting that early steps of germ line development involve a cyst stage also in these animals (for review, see Pepling ***et al***., 1999). A second conserved feature in oogenesis is programmed cell death. This process initiates in the germ cells when they are interconnected and requires the activation of a conserved intracellular program, according to a predictable temporal and spatial pattern. The assembly of ovarian follicles, where gonadal somatic cells envelope female germ cells to create a special compartment for their growth and differentiation, is a third characteristic shared by a number of species throughout the animal kingdom. Finally, in many invertebrates and vertebrates, the establishment of one or more body axes is already set up during oogenesis (Gerhart and Kirschner, 1997) and is based on common principles, such as cortical localization of key mRNAs and proteins (Palacios and St Johnston, 2001; Pellettieri and Seydoux, 2002; Vinot ***et al***., 2004). In this frame, the study of oogenesis in model organisms that have the entire genome sequenced and annotated can be viewed as a promising tool for identifying conserved players of basic developmental programs leading to germ cell differentiation and growth. A particularly useful

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paradigm for understanding these processes is provided by the *Drosophila* egg chamber. This structure is composed by one oocyte and 15 nurse cells surrounded by a monolayer of somatic follicle cells and represents the fundamental morphological and physiological unit where oogenesis takes place (King, 1970). Egg chambers are organized in linear arrays termed ovarioles. At the tip of each ovariole, in a specialized region called germarium, a germline stem cell divides asimmetrically to produce one cell that maintains the characteristics of stem cell and a cystoblast. This cell undergoes four rounds of synchronous mitotic divisions with incomplete cytokinesis, giving rise to a cluster of 16 cells interconnected by intercellular bridges called ring canals. Only one cell within a cyst adopts the oocyte fate, while the remaining 15 develop into nurse cells, which provide the synthetically quiescent oocyte with RNAs and proteins. After encapsulation of the germline cyst by follicle cell precursors originating from the asymmetric division of somatic stem cells, egg chamber assembly is complete (Margolis and Spradling, 1995; Spradling *et al.*, 1997). This new individualized structure buds off the germarium and undergoes maturation while moving toward the posterior of the ovariole.

In this review we will focus on few key aspects of *Drosophila* oogenesis, summarizing our studies on the differentiation events leading to: 1) definition of the egg chamber anterior-posterior polarity; 2) integration of nuclear and cytoplasmic developmental processes during egg chamber maturation; 3) activation of apoptosis in germline cells.

For a better understanding of each single topic, a brief outline including the most relevant and recent contributions given by the scientific community, is reported below together with our results.

Establishment of the egg chamber anterior-posterior polarity: the *hold hup* mutation

The anterior-posterior polarity of the *Drosophila* oocyte is established by a multi-step process involving a complex sequence of interactions between somatic and germline cells. An essential early event taking place in the germarium is oocyte positioning at the posterior pole of the germline cyst, mediated by its association with posterior follicle cells (Gonzales Reyes and St Johnston, 1994). This association involves E-cadherin-based cell adhesion mechanisms and relies on a cascade of inductive signals that are transmitted from the older posterior egg chamber (Godt and Tepass, 1998; Gonzales-Reyes and St Johnston, 1998a; Torres *at al.*, 2003). These signals are believed to trigger only a temporary polarization of the follicle epithelium, required to induce E-cadherin upregulation in posteriorly located follicle cells. Once anchored at the posterior end of the germline cyst, the oocyte will become the source of a new inductive signal, driving the adjacent terminal follicle cells to adopt a posterior fate (Gonzales-Reyes *et al.*, 1995; Roth *et al.*, 1995). The two central players of this cell signaling event are the TGF-α like protein Gurken (Grk) produced by the oocyte and the EGF receptor (EGFr) homologue Torpedo (Top), that is expressed throughout the follicular epithelium, but is selectively activated only in the follicle cells that contact the oocyte and are therefore exposed to the Grk signal (Fig. 1A). At the time when this signal is produced, the follicle cells located at both termini of the egg chamber appear to be equivalent (Gonzales-Reyes and St Johnston, 1998b). Two pairs of so-called polar cells, one at each end of the egg chamber, have been induced to differentiate through Delta signaling from the germline cyst and have in turn acquired a distinctive organizer function. By generating a gradient of activation of the Janus kinase (JAK) pathway in adjacent follicle cells, polar cells are in fact able to specify their fate (Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001; Grammont and Irvine, 2002; Xi *et al.*, 2003). Therefore, as soon as it is triggered by Grk at the posterior pole of the egg chamber, EGFr signaling defines posterior terminal follicle cell identity overriding the default anterior fates specified by JAK activity alone (Xi *et al.*, 2003). Oocyte to follicle cell signaling is then followed by an unidentified back signal that induces an overall reorganization of the oocyte microtubule cytoskeleton. This eventually allows proper localization of the two key determinants of embryonic anterior-posterior axis formation: *bicoid* (*bcd*) and *oskar* (*osk*) mRNAs (Theurkauf *et al.* 1992; Pokrywka and Stephenson, 1995) (Fig. 1B, C). *grk* mRNA is in turn relocated, first, transiently, to the anterior margin of the oocyte and finally to the anterior corner where the nucleus has in the meantime migrated (Mac Dougall *et al.*, 2003) (Fig.1A). This process is tightly regulated by several factors and is coupled to translational control, ensuring that, when a second round of Gurken signaling is elicited, it will interest only the group of follicle cells that are next to the oocyte nucleus. These cells will acquire a dorsal identity, defining the egg chamber dorsal-ventral axis (Neuman-Silberberg and Schupbach, 1993).

Even if significant progresses have been made in the last years, a complete picture of the mechanisms defining the anterior-posterior polarity of the *Drosophila* egg is still missing. Since the whole process requires a tight integration between differentiation events taking place in somatic and germline cells, the identification of mutants displaying phenotypic alterations in both cell types might be useful in the search for gene functions involved in egg chamber polarization. On the basis of these considerations we have focused our studies on the *hold up* (*hup*) mutation (Sandler, 1977). A detailed phenotypic characterization carried out in our laboratory has in fact shown that this female sterile mutation causes several characteristic defects in oocyte and follicle cell behaviour and has a striking impact on egg chamber anterior-posterior axis formation (Rotoli *et al.*, 1998). The earliest morphologically visible alteration detectable in *hup* mutant egg chambers is oocyte mislocalization. In 15% of the germline cysts produced by females bearing the *hup* mutation in trans with a non-complementing chromosomal rearrangement, the oocyte was in fact randomly positioned. In Fig. 2A, where a *hup* mutant ovariole is shown, one of the egg chambers displays an anteriorly localized oocyte and is direcly joined to the following, older egg chamber. The absence of an intervening stalk suggests that the primary defect responsible for oocyte mispositioning in *hup* mutant

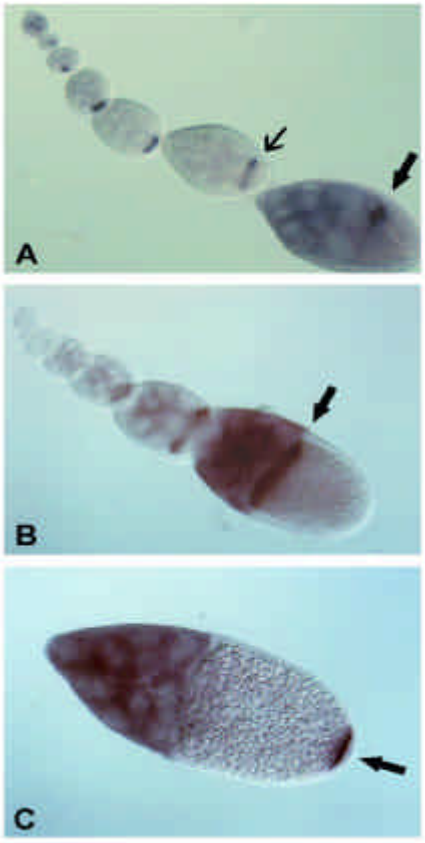

Fig. 1 Anterior-posterior and dorsal-ventral polarization of the *Drosophila* oocyte. The localization of key molecules involved in axes formation was visualized in wild type egg chambers by *in situ* hybridization. (A) Triphasic *grk* mRNA localization: at the posterior end of the oocyte in early egg chambers, at the anterior margin of the oocyte at stage 8 (thin arrow) and at the anterior-dorsal corner of the oocyte at stage 9. (B) *bcd* mRNA localization at the anterior margin of the oocyte (arrow). (C) *osk* mRNA localization at the posterior pole of the oocyte (arrow).

egg chambers might reside in the chain of events leading to somatic cell fate specification in the germarium. As mentioned above, oocyte positioning at the posterior pole of a newly formed egg chamber involves in fact a series of inductive signals that originate in the adjacent more mature follicle and eventually lead to the formation of a separating stalk.

hup gene function is required also for later somatic cell differentiation processes taking place during egg chamber development. Even when supporting correct oocyte positioning, nearly half of the mutant egg chambers were in fact unable to fully specify posterior follicle cell fates and displayed ectopic expression of anterior follicle cell markers in posteriorly located follicle cells. This striking defect had profound consequences on oocyte polarization and resulted in the abnormal distribution of several localized mRNAs, as shown in Fig. 2B, where *bicoid* (*bcd*) mRNA appears to be partially mislocalized to the posterior of the oocyte (Rotoli *et al.*, 1998). These results indicated that appropriate cross-talk mechanisms between oocyte and posterior follicle cells are not established in *hup* mutant egg chambers. In

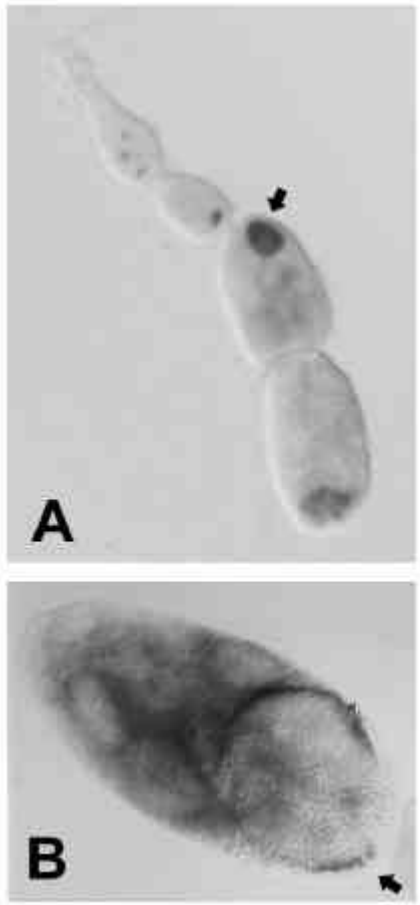

Fig. 2 Defective anterior-posterior axis formation in *hup* mutant egg chambers. *In situ* hybridization was used to visualize the localization of selected mRNA molecules in the oocyte. In (A) the position of the oocyte is marked by the accumulation of *osk* mRNA during previtellogenic stages of oogenesis. One of the egg chambers shows oocyte mislocalization to the anterior tip of the germline cyst (arrow). In (B) *bcd* mRNA is ectopically found also at the posterior pole of the oocyte in a stage 10 egg chamber (arrow).

agreement with this hypothesis, we found that *hup* genetically interacts with *top*. Interestingly, in 10% of double homozygous mutant egg chambers follicle cells formed multiple posterior layers and were in some cases engaged in a centripetal migration process, displaying a behaviour characteristic of anterior follicle cells. The finding that *hup* cooperates with *top* for the correct specification of posterior follicle cell identity suggested that it might be a crucial component of the molecular machinery that leads to the establishment of polarity in both the follicle cell layer and the oocyte. In this frame, two lines of evidence indicated that the primary defect due to the *hup* mutation does not reside in the oocyte. First of all, *grk* mRNA always accumulated at the posterior pole of the oocyte in *hup* mutant egg chambers where this cell was correctly localized. Second, mitotic recombination experiments demonstrated that *hup* mutant germline clones did not exhibit the *hup* mutant phenotype.

Up to now the molecular nature of the *hup* gene is not identified because only EMS-induced *hup* mutant

alleles are available and several screenings performed in our laboratory to isolate P element-induced *hup* mutations failed. To identify the *hup* gene we are focusing on the genes that have been annotated in the *Drosophila* genome sequence in the region where *hup* was genetically mapped. P element-induced transformation experiments with overlapping fragments containing these genes are currently in progress in the attempt to rescue the *hup* mutant phenotype.

Integration of nuclear and cytoplasmic differentiation events during egg chamber maturation: the *tulipano* mutation and the Nup154 nucleoporin gene

In the *Drosophila* ovary, germline derived nurse cells differentiate while undergoing 10-12 cycles of endoreplication. This process is characterized by morphologically visible changes in both chromosome architecture and chromatin configuration (Dej and Spradling, 1999). During the first four endocycles, homologous chromosomes are paired and progressively condense acquiring the banding pattern characteristic of polytene chromosomes. During the fifth endocycle homolog pairing loosens but chromatin compaction increases. Five chromatin masses corresponding to individual chromosome arms transiently appear as a sort of "blobs" inside nurse cell nuclei. At the end of this endocycle, each polytene chromosome dissociates into 32 chromatid pairs. Their chromatin becomes uniformly distributed throughout the nucleus, acquiring a decondensed structure that is maintained also during the following endocycles, when each chromatide pair is used as template to generate new polytene chromosomes (Fig. 3A). The mechanisms responsible for the transition from polytene to dispersed chromosomes in the nurse cells are poorly understood. It has been proposed that this process takes place during a mitosis-like phase and is therefore carried out through the control of crucial cell cycle regulators (Dej and Spradling, 1999).

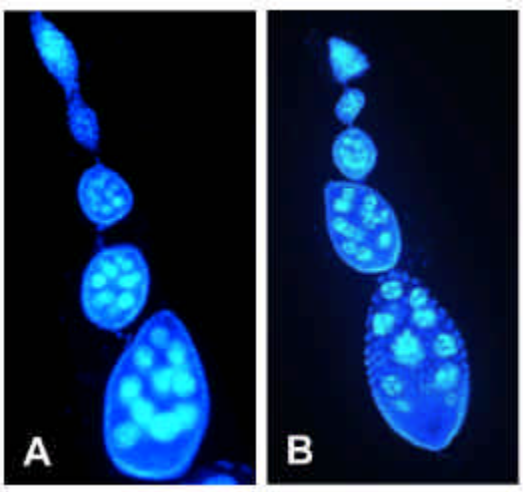

Fig. 3 Abnormal nurse cell chromosome dispersal in *tlp* mutant egg chambers. The egg chambers were stained with DAPI to visualize the DNA. (A) Wild type ovariole, showing homogeneous DNA distribution in the nurse cells of post-stage 5 egg chambers. (B) *tlp* mutant ovariole displaying persistent "blob like" nurse cell chromosome conformation throughout oogenesis.

This hypothesis has been corroborated by the finding that the activity of the anaphase-promoting complex/cyclosome is required for nurse cell polytene chromosome breakdown (Kashewski *et al.*, 2002). It is however not known how cell cycle regulation can be affected by developmental cues. Appropriate genetic programs must be in fact responsible for triggering nurse cell chromosome dispersal in a spatially and temporally regulated manner. Several putative members of these genetic programs have been identified by phenotypic characterization of female sterile mutations altering nurse cell chromosome morphology. These mutations, called *rhino* (*rhi*), *half pint* (*hfp*), *hrb27C*, *squid* (*sqd*), *ovarian tumor* (*otu*) and *cup* cause the persistence of "blob like" chromosomes in late stage egg chambers (King, 1970; King and Storto, 1988; Keyes and Spradling, 1997; Volpe *et al.*, 2001; Van Buskirk and Schüpbach, 2002; Goodrich *et al.*, 2004).

A similar phenotype is shown by the *tulipano* (*tlp*) mutation, isolated in our laboratory (Gigliotti *et al.*, 1998). *tlp* female sterile alleles displayed striking defects in both nurse cell chromatin organization and egg morphology and have been grouped into two general classes. Stronger alleles caused egg chamber developmental arrest during mid-oogenesis, while weaker alleles sustained egg chamber maturation till complete egg assembly (Gigliotti *et al.*, 1998; Kiger *et al.*, 1999). Morphological features characteristic of early developmental stages persisted in nurse cell nuclei of both types of mutant alleles, even if with different degrees of penetrance (Fig. 3B).

We have cloned the gene affected by the *tlp* mutation and identified its protein product as the *Drosophila* homolog of yeast nucleoporin Nup170 and Nup157 and mammalian Nup155 (Radu *et al.*, 1993; Aitchison *et al.*, 1995; Zang *et al.*, 1999). These proteins are structural components of the nuclear pore complexes, the gated channels that perforate the nuclear envelope and mediate nucleo-cytoplasmic transport in all eukaryotes. Accordingly, the *Drosophila* protein, named Nup154 with reference to its deduced molecular weight, was found to be localized at the nuclear periphery in both somatic and germline cells of the ovary (Fig. 4). The Nup154 gene was also shown to be expressed in other tissues and at all developmental stages (Gigliotti *et al.*, 1998; Kiger et al., 1999). This finding, together with the identification of strong loss of function lethal alleles suggested that Nup154 is required in all cell types, confirming that nuclear pore complex components play essential roles in different aspects of cell physiology (Fahrenkrog *et al.*, 2004).

However, the peculiar ovarian phenotype of *tlp* hypomorphic alleles indicated that Nup154, probably in association with other ovarian proteins, might also play cell-type specific functions. One possibility is that Nup154 is directly implicated in the nurse cell chromosome dispersal process. It has in fact been proposed that, serving as anchorage sites for chromatin, several nucleoporins might be involved in chromatin organization (Ishii *et al.*, 2002; Feuerbach *et al.*, 2002). It is also conceivable that cytoplasmic and/or nuclear components of the molecular pathway

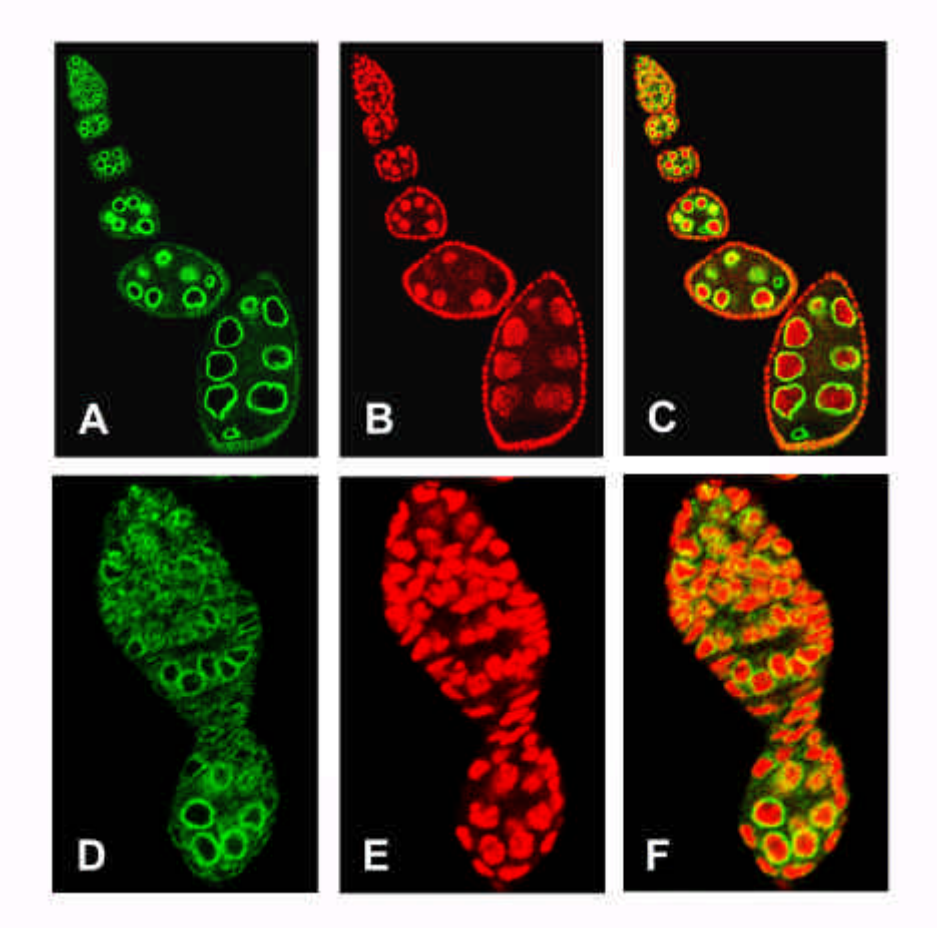

Fig. 4 Immunolocalization of the Nup154 protein during oogenesis. Confocal microscope images of wild type ovaries stained with anti-Nup154 antibodies (A,D) and the TOTO-3 nucleic acid dye (B,E). Nup154 is present throughout egg chamber development (A) and is localized in both germline and follicle cells all along the perimeter of the nuclei (B), as best seen in the merged image (C). In (D,E merged in F) high magnification of a germarium shows that Nup154 is already detectable during germline cyst formation.

regulating nurse cell chromosome dynamics during oogenesis require Nup154 function for moving in and/or out of the nuclei. A possible list of candidates includes the proteins coded by the genes listed above, whose mutant alleles closely resemble the *tlp* mutant phenotype. Interestingly, Cup, that is a cytoplasmic protein, is transiently localized at the nuclear envelope of the nurse cells as long as their chromosomes remain condensed (Keyes and Spradling, 1997) and is able to shuttle between the nucleus and the cytoplasm, at least in transfected Schneider cells (Zappavigna *et al.*, 2004). Hrb27C and Sqd belong to the heterogeneous nuclear ribonucleoprotein (hnRNP) A/B family of RNA-binding proteins, involved in various aspects of RNA metabolism, including nuclear transport (for review, see Dreyfuss *et al.*, 2002).

The phenotype induced by *tlp* mutations not only affected nurse cell chromosome dispersal, but also the dorsal-ventral patterning of the eggs, which, when produced by weak alleles, displayed fused or missing dorsal appendages (Fig. 5). Remarkably, dorsal-ventral defects are also shown by mutations in *hrb27C, sqd* and *otu* (Goodrich *et al.*, 2004). Genetic and biochemical data have suggested that the protein products of these genes can form a complex regulating the localization and/or the translation of specific mRNA targets. One of these targets is *grk* mRNA: it has been proposed that the association of Hrb27C and Sqd with this transcript already occurs in the oocyte nucleus and persists during its translocation to the cytoplasm. Here, the two proteins are involved together with Otu in *grk* mRNA localization and translation (Goodrich *et al.*, 2004). We are currently testing the hypothesis that Nup154 can participate to this process and preliminary results indicated that both *grk* mRNA localization and translation are affected also in our mutant (unpublished results).

In conclusion, Nup154 is required, probably in association with some of the gene products listed above, for both nuclear and cytoplasmic differentiation events taking place during oogenesis. This dual role could be explained by postulating that Nup154 is able to mediate the nucleocytoplasmic transport of specific regulatory factors independently involved in nurse cell chromosome dispersal and dorsal-ventral polarity establishment. To test this hypothesis we are currently performing genetic interaction tests of *tlp* with *cup*, *otu*, *hrb27C* and *sqd* and employing overexpression approaches aimed at investigating the effects induced by Nup 154 overproduction and/or mislocalization.

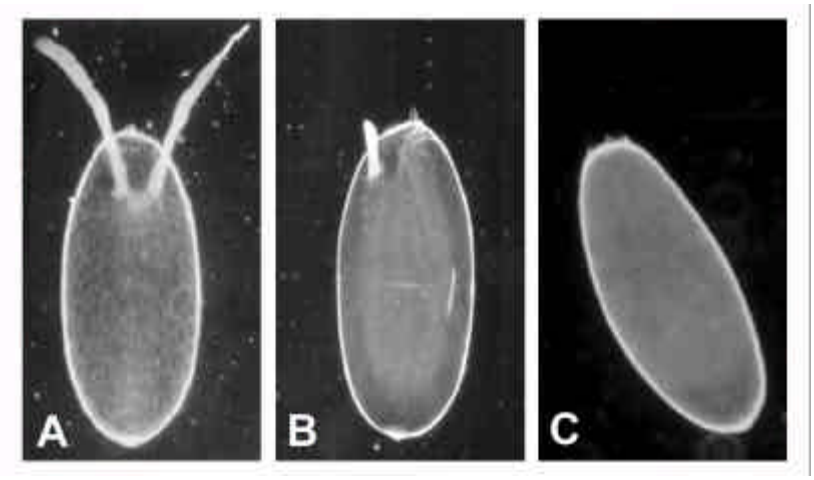

Fig. 5 Altered egg morphology in *tlp* mutant females. Wild type egg with two chorionic appendages that mark the dorsal surface. In eggs laid by *tlp* mutant females, these structures are reduced, fused (B) or completely missing (C).

Apoptosis in the germ cells: analysis of dumpless mutants

Programmed cell death during *Drosophila* oogenesis occurs at distinct stages and is triggered by both developmental and environmental stimuli.

At late stages of oogenesis, developmentally regulated cell death is responsible for the removal of the nurse cells after their function of supplying nutrients to the oocyte has been fulfilled, and is necessary for the proper development of every oocyte. During the first ten stages of oogenesis, a flux of cytoplasm gradually streams from the nurse cells into the oocyte through the ring canals. Starting from stage 10B, a fast phase of cytoplasmic transfer occurs and outcomes of this transport into the oocyte are the regression of the nurse cell cluster and the doubling of the oocyte volume. The cytoplasmic dumping is preceded by dramatic rearrangements of the actin cytoskeleton. During stage 10B, filamentous actin forms bundles extending from the plasma membrane toward the envelope of nurse cell nuclei (Fig. 6A-C), preventing the movement of these nuclei during the dumping of the cytoplasm. Coincident with actin bundle formation, the nurse cell nuclei begin to break down, lose their lamin staining, show gaps in their envelope and become permeable (Smith and Fisher, 1989; Cooley *et al*., 1992; McCall and Steller, 1998; Matova *et al*., 1999). Following the massive cytoplasm dumping, that has been attributed to the myosin-based contraction of subcortical actin (Wheatley *et al*., 1995), the nurse cells dye by apoptosis (Cavaliere *et al*., 1998; Foley and Cooley, 1998; McCall and Steller, 1998). The nurse cell remnants are phagocitized by the follicle cells that in turn, at the completion of oogenesis, show sign of apoptosis and are reported to be engulfed by epithelial cells of the oviducts (Nezis *et al*., 2000).

The programmed cell death of nurse cells shows distinct differences compared to cell death in other *Drosophila* tissues. The formation of actin bundles, the cytoplasmic transfer to the oocyte and the protection of the connected oocyte from the death process of nurse cells are cellular events not seen in apoptotic death occurring in other *Drosophila* cells. In addition, germline cell death at late-oogenesis does not require the three pro-apoptotic proteins Reaper, Hid and Grim that promote caspase activation in the vast majority of cell deaths occurring during *Drosophila* development (Foley and Cooley, 1998).

The upstream signals that induce developmentally regulated cell death are still largely unknown (for review, see McCall, 2004). Some evidences point to the involvement of the ecdysone signaling pathway in controlling nurse cell death at late-oogenesis. Injection of 20-hydroxyecdysone, the biologically active ecdysteroid, in virgin females leads to premature nurse cell death suggesting that ecdysone may normally act to promote nurse cell death (Soller *et al*., 1999).

More than one signaling pathway may contribute to the regulation of nurse cell death. Genetic analyses of the Decapentaplegic (DPP) signaling pathway suggest that this pathway is involved in nurse cell death. Conditional mutations in the *dpp* gene, which encodes a TGF-β family member, give rise to small eggs at non permissive temperature and mutations in *saxophone*, a gene encoding a TGF-β receptor also cause defects in nurse cell dumping and nurse cell death (Twombly *et al*., 1996; Myster *et al*., 2000; Royzman *et al*., 2002). Recently, it has been reported that lipid composition may influence the timing of nurse cell death (Buszczak *et al*., 2002). Mutations in the *midway* gene, which encodes a diacylglycerol acyltransferase, result in premature nurse cell death and degeneration.

We have studied the nature of the death process of the nurse cells after their function has been fulfilled. Our studies started with the analysis of nuclear DNA integrity, since DNA fragmentation is a diagnostic hallmark of apoptotic death. We have analyzed DNA from egg chambers isolated at relevant steps of oogenesis and from whole ovaries of wild-type females and found that DNA extracted from late-stage egg chambers is fragmented into nucleosomal-size

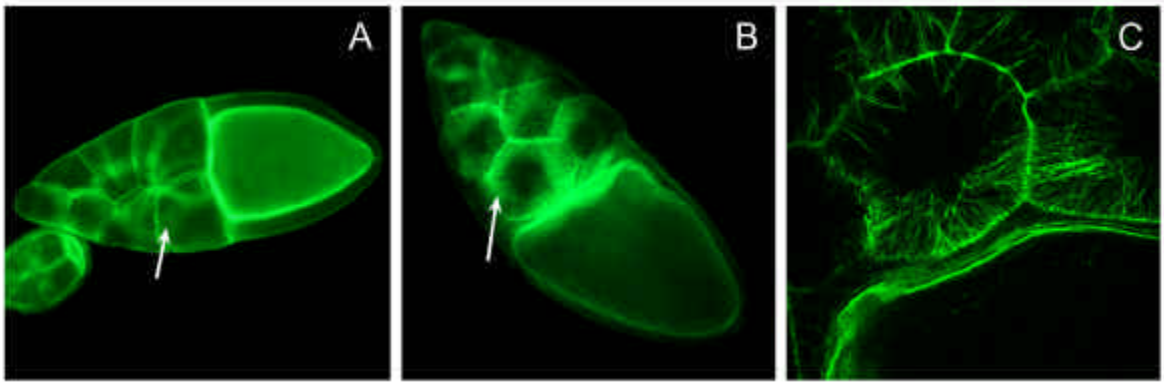

Fig. 6 Morphological changes associated with rapid cytoplasm transport from nurse cells to oocyte. The egg chambers were stained with FITC-conjugated phalloidin to visualize the actin cytoskeleton. In stage 10A egg chambers (A) filamentous actin localizes to the cortex of nurse cells (see arrow). At stage 10B a network of actin filaments polymerizes around the nurse cell nuclei (B, see arrow) and actin bundles extend from the plasma membrane to the internal region of nurse cells (C) containing the nuclei.

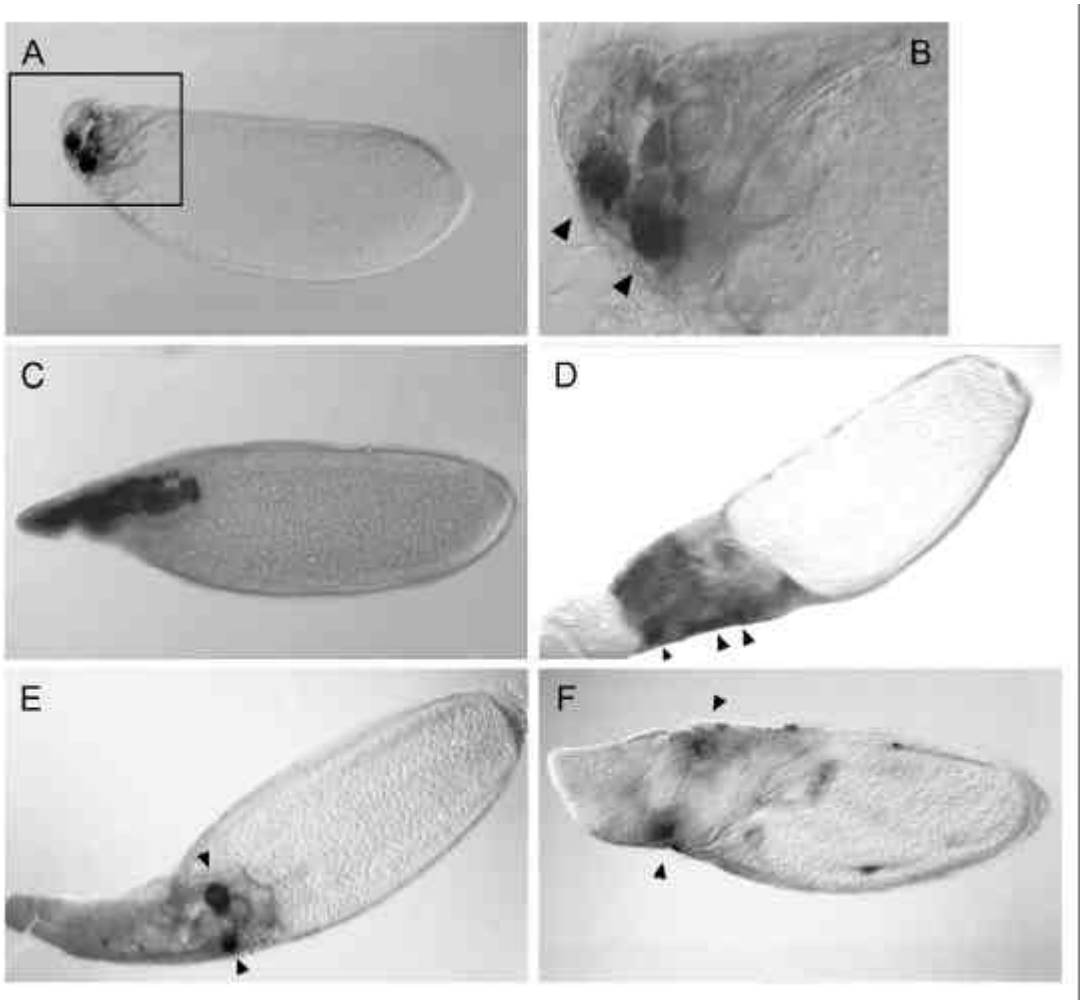

Fig. 7 *In situ* detection of DNA fragmentation by TUNEL labeling. The TUNEL technique labels the 3'-end of DNA witnessing its fragmentation. In wild type ovaries TUNEL staining starts to appear at stage 12 (A) and two darkly stained nuclei are visible at the ventral side of the egg chamber (see arrowheads in higher magnification B). At stage 13 extensive TUNEL labeling is observed in nurse cells (C). Differing from the wild type egg chambers at a comparable degree of maturation, egg chambers from *chickadee* (D), *quail* (E) and *singed* (F) mutants show few apoptotic nuclei, visible as darker brown spots (see arrowheads) in the nurse cell compartment.

fragments (Cavaliere *et al*., 1998). The TUNEL technique labels the 3'-end of DNA, witnessing its fragmentation (Gavrieli *et al*., 1992). Thus we have analyzed wild-type egg chambers by this assay and we found that starting from stage 12, the nurse cell nuclei become TUNEL positive (Fig. 7A-C) indicating that cell death by apoptosis is responsible for the removal of the nurse cells at the end of oogenesis.

In order to gain insights into the signals that induce nurse cell death, we investigated if the dumping process *per se* could trigger the apoptotic program and we extended our analyses to dumping defective mutants. Mutations in *chickadee*, *quail* and *singed* genes cause a defect of the dumping process due to the absence of actin filament bundles (Cooley *et al*., 1992; Cant *et al*., 1994; Mahajan-Miklos and Cooley, 1994). As a consequence, the nurse cell nuclei lodge into the ring canals, block nurse cell cytoplasm transport and the resulting dumpless egg chambers contain oocytes smaller than normal.

Total DNA extracted from *quail* and *singed* mutant ovaries showed the typical oligonucleosomal DNA ladder (Cavaliere *et al*., 1998). TUNEL analysis of egg chambers from *chickadee*, *quail* and *singed* mutants showed that the apoptotic process is activated and proceeds even if not all nuclei appeared to be in an apoptotic state (Fig. 7D-F), differently from the wild-type egg chambers at a comparable degree of maturation. The TUNEL positive nuclei were found to be well surrounded by a relevant amount of cytoplasm (Fig. 7D, F). This indicates that the apoptotic process program in the nurse cells can be activated and proceeds even if their cytoplasm is retained.

In conclusion our results showed that, during the normal development of the egg chamber, the exhausted nurse cells dye by apoptosis at the late stage of oogenesis. In wild type egg chambers the timing of nurse cell death follows the massive cytoplasm transport into the oocyte, but the finding of apoptotic nuclei in *chickadee*, *quail* and *singed* egg chambers indicated that apoptosis is not initiated by cytoplasm dumping.

Conclusions and perspectives

Advances in *Drosophila* research have placed this organism in a unique position to contribute a detailed understanding of the cellular and molecular mechanisms underlying oogenesis. Many important insights have been provided by the analysis of female sterile mutants, used as invaluable tools for the identification of essential gene functions involved in egg chamber development. More recently this forward genetic approach has been joined by other experimental strategies such as cell specific gene inactivation and RNA interference. Our knowledge of the overall complexity of the developmental pathways involved in oogenesis is therefore expected to greatly increase in the next future. This will in turn provide remarkable opportunities for studying comparative aspects of oogenesis. In this frame, the availability of the complete sequence of the *Drosophila* genome will contribute an essential basis for the identification of homologous proteins in different species. Investigating the existence of functionally conserved roles of these molecules is therefore the next, intriguing perspective.

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