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Female sterile mutations and egg chamber development in *Drosophila melanogaster*

SILVIA GIGLIOTTI, DEBORAH ROTOLI, ANDREA MANZI, FRANCO GRAZIANI and CARLA MALVA*

Istituto Internazionale di Genetica e Biofisica, CNR, Napoli, Italy.

ABSTRACT ***Drosophila*** **oogenesis provides an excellent opportunity to study fundamental aspects of developmental biology and to learn the importance of multiple signalling pathways in the regulation of cellular morphogenesis. Taking advantage of the genetic and molecular approaches extremely powerful in this organism, over the years an enormous collection of data has accumulated on the genes involved in important steps of egg chamber development, such as germline and somatic stem cell maintenance, division and differentiation; oocyte determination and positioning; establishment of follicle cell fate and axes formation. These different processes are mediated by a reciprocal cross-talk between germline and somatic follicle cells. Here, in a schematic and simplified form, we point out what we believe are the main recent results on the molecular and cellular mechanisms underlying ovarian development and outline our recent contribution to this field.**

KEY WORDS: *female fertility, oogenesis, follicle cell fate, axes formation, Drosophila melanogaster*

Introduction

The two ovaries of adult *Drosophila* females are each composed of 15 ovarioles. In a special region at the tip of each ovariole, the germarium (Fig. 1), stem cell division and cyst formation occur throughout the *Drosophila* adult life (Spradling, 1993; Spradling *et al.*, 1997). At the tip of each germarium (Fig. 1A,B), two to three large Germline Stem Cells (GSCs) are intimately associated with two subpopulations of somatic cells: the Basal Cells (BC) of Terminal Filament (TF) and the Cap Cells (CpC), that have been identified morphologically and by specific gene expression. Terminal filament and cap cell formation depends on the expression of various genes, including *hedgehog* (*hh*), which plays a role in regulating cell proliferation and specifying cell identity in diverse systems (Forbes *et al.*, 1996).

A great attention is dedicated to the molecular mechanisms involved in stem cell formation, maintenance, division and function (Lin and Spradling, 1997; Spradling *et al.,* 1997; Xie and Spradling, 1998; Cox *et al.,* 1998). Each germline stem cell divides asymmetrically so that one daughter cell continues as a stem cell and remains in contact with the basal and cap cells and the sister cell acquires a Cystoblast (CB) fate and expresses the *bag-of-marbles* gene, which is essential for the initiation of Cystocyte (CC) differentiation into functional egg chambers (Gonczy *et al.,* 1999). When the cystoblast moves posteriorly, it contacts the Inner Sheath Somatic Cells (IS) and undergoes four incomplete divisions forming a cluster of 16 cells interconnected by ring canals (Fig. 1C). The 16-cell cluster, passing throughout region 2 of germarium, contacts the two Somatic Stem Cells (SSC) and their progeny, the prefollicle cells (Fig. 1A).

In region 2b the germline cyst acquires a monolayer of follicle cells (FC) and buds from the germarium in region 3 as a new egg chamber. Before the egg chamber is formed, two of the 16 germline cells develop as pro-oocytes, and one of these two cells is selected to become the oocyte, the other 15 becoming nurse cells.

Oocyte specification and positioning

A great importance in cyst formation and oocyte specification is played by a special vesiculated cytoplasmic organelle, the fusome, which contains proteins related to most of the known membrane skeleton components. It traverses the ring canals linking individual cystocytes and helps establishing a system of directional transport between cystocytes that underlies oocyte determination (Lin and Spradling, 1995; Spradling *et al.,* 1997; de Cuevas and Spradling, 1998).

Genes of the *spindle* class, which encode double-strand break repair enzymes and RNA helicases, affect oocyte specification and polarity (Gonzalez-Reyes *et al.,* 1997; Ghabrial *et al.,* 1998). The fact that genes involved in meiotic DNA metabolism have specific effects on oocyte patterning suggests that the regulation of this key process is co-ordinated with progression of the meiotic cell cycle (Morris and Lehmann, 1999).

In the follicle, the oocyte always occupies a posterior position among the 16 germline cells. Recent work has begun to reveal how

***Address correspondence to:** Carla Malva. Istituto Internazionale di Genetica e Biofisica, CNR, Via Marconi 10, 80125 Naples, Italy. TEL: 081 7257275. FAX: 081 7257278.
e-mail: malva@iigbna.iigb.na.cnr.it

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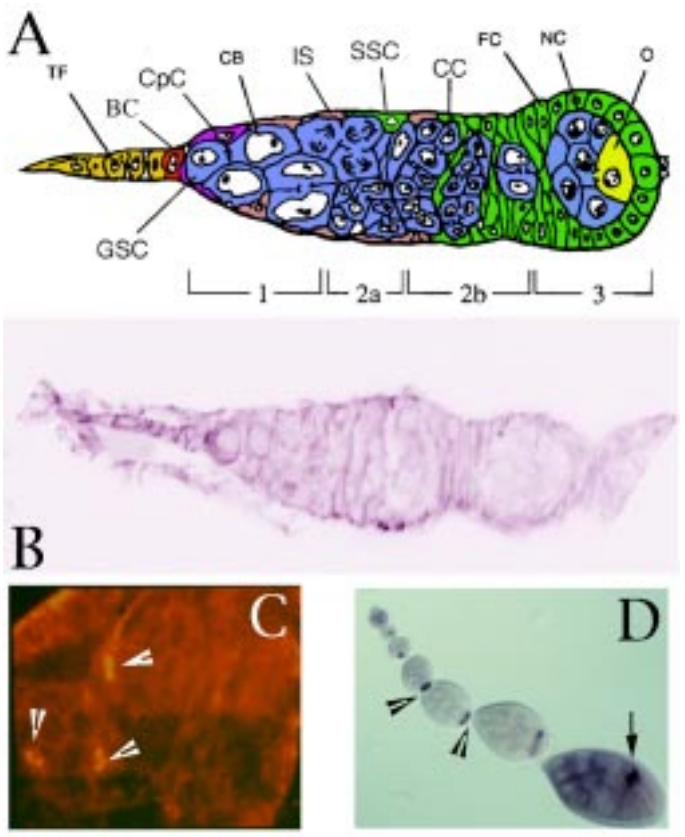


Fig. 1. *Drosophila* germarium and steps of oogenesis. (A) *Schematic drawing of the Drosophila germarium, partially modified from Forbes et al. (1996). TF, terminal filament; CpC, cap cells; GSC, germline stem cells; SSC, somatic stem cells, BC, basal cells; CB, cystoblast; IS, inner sheath cells; CC, cystocyte; FC, follicle cells; NC, nurse cells; O, oocyte. Germarium regions 1, 2a, 2b and 3 are indicated below the drawing. A population of budding follicle cells separates the newly completed egg chamber from the germarium.* **(B)** *Confocal microscopy image of the germarium stained with rhodamine-conjugated phalloidin, which identifies the actin filament network in the different cell types.* **(C)** *Ring canals of the 16 cell cluster, as identified by rhodamine-conjugated phalloidin staining.* **(D)** *A single ovariole where the localisation of grk mRNA is shown in the growing egg chambers. The arrowheads indicate the posterior location of the oocyte in the egg chamber, allowing cross talk between oocyte and polar follicle cells. The arrow indicates the re-localisation of grk mRNA in the anterior dorsal corner of the growing oocyte.*

this first asymmetry in development arises and how this is linked to the later events that define the embryonic axes. It has been recently demonstrated that oocyte localisation is mediated by different cadherin molecules and that the highest concentration of DE-cadherin is found at the interface between oocyte and the posterior follicle cells to which the oocyte attaches itself selectively (Peifer *et al.,* 1993; Gonzalez-Reyes and St Johnston, 1998a). One of the mutations under study in our laboratory, *hold up* (*hup*) also affects oocyte positioning in the egg chamber (Rotoli *et al.,* 1998) and the consequences of this early defect on egg chamber development will be described below in the Results.

Early events in follicle cell fate and axes formation

Various genes act in processes that are common to oocyte determination, oocyte positioning and axes formation, all requiring signalling between germline and somatic cells. The two central components of this cross talk are the products of the genes *gurken* (*grk*) and *torpedo* (*top/Egfr*). *gurken* codes for a TGFα-like protein and its mRNA is localised within the developing oocyte (Fig. 1D). *torpedo/Egfr* codes for the *Drosophila* homologue of the EGF receptor, which is present throughout the follicular epithelium surrounding the oocyte. Grk-Egfr signalling is required early for specification of posterior follicle cell fate and later in oogenesis for dorsal follicle cell fate determination, thus establishing both axes of the egg and embryo (Gonzalez-Reyes and St Johnston, 1994; Ray and Schupbach, 1996; Gonzalez-Reyes and St Johnston, 1998b; van Eeden and St Johnston, 1999).

The final distribution of Gurken within the oocyte appears to be specified both by the localisation of the *gurken* RNA and by regulation of Gurken protein accumulation, possibly at the level of translation. A number of genes are involved in transcriptional, translational and post translational regulation and in localisation of *grk* mRNA and protein (Styhler *et al.,* 1998; Norvell *et al.,* 1999).

Posterior activation of the Egfr by Gurken within the follicular epithelium induces the subpopulations of follicle cells contacting the oocyte, the polar follicle cells, to acquire a posterior fate. Signalling from these follicle cells back to the oocyte in turn induces a polarization of the cortical cytoskeleton leading to the formation of a microtubule network with the plus end directed toward the posterior pole of the oocyte. This re-orientation of the microtubule network is involved in the intracellular transport and localisation of RNA and protein molecules (Theurkauf and Hazelrigg, 1998), as well as in the relocalisation of the oocyte nucleus and its associated *gurken* mRNA. As a consequence of *gurken* mRNA movement toward the anterior rim of the oocyte, the dorsal side of the egg and of the developing embryo becomes randomly determined (Roth *et al.,* 1999).

The observation that activation of Grk/Egfr pathway induces different cell fates in the overlying follicular epithelium and mediates both A/P and D/V patterning, raises the question of how two different responses are produced by the same molecules. It has been proposed that the strategies used to pattern posterior follicle cells are different from those used to pattern dorsal follicle cells (Nilson and Schupbach, 1999). In addition, Notch and other neurogenic genes (Ruohola-Baker *et al.,* 1994; Ray and Schupbach, 1996; Goode *et al.,* 1996) have been implicated in restricting the competence of follicle cells to respond to this signalling and in regulating epithelial development.

We are presently studying the role of the *hup* gene, which genetically interacts with *Egfr* (Rotoli *et al.,* 1998), in the specification of monolayer follicle epithelium integrity and in the establishment of both A/P and D/V axes.

Egg chamber development

The egg chambers leaving the germarium enter a growth phase and continue their development passing through 14 morphological stages (King, 1970; Mahowald and Hardy, 1985) as they move posteriorly. Adjacent egg chambers are separated by short chains of interfollicular stalk cells whose fates appear to be linked to that of polar cells.

During the growth phase, both nurse cells and oocyte undergo changes in their nuclear organization (Spradling, 1993; Keyes and Spradling, 1997). By stage 3 of egg chamber development, the oocyte chromosomes condense into a karyosome and the nucleus remains in meiotic prophase until the end of oogenesis. On the other

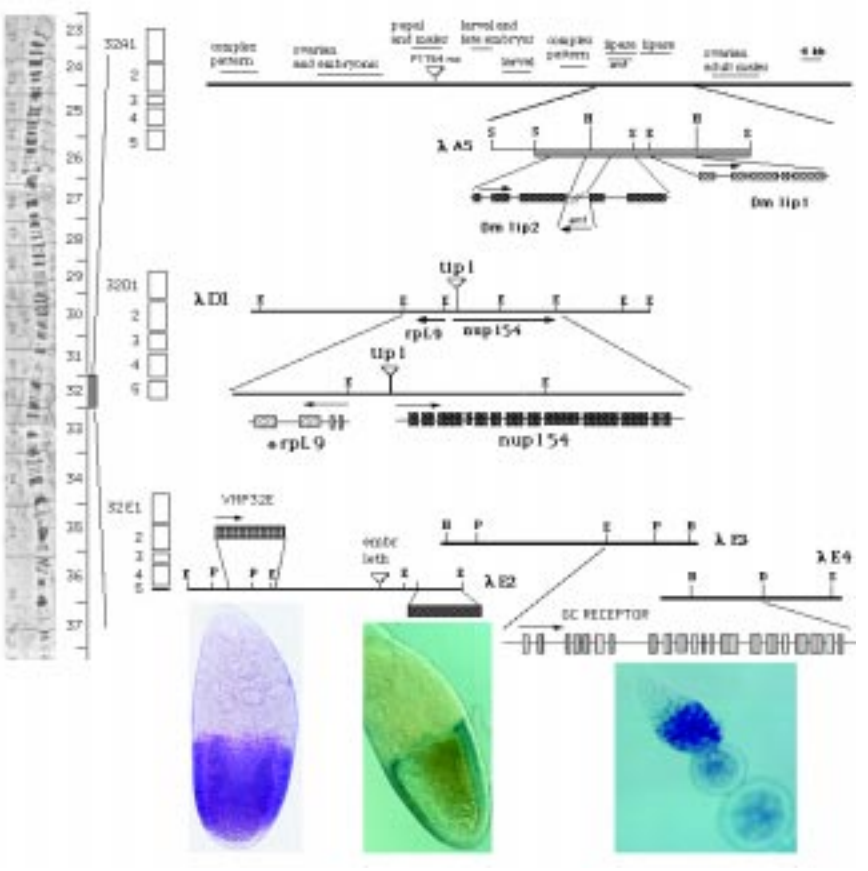


Fig. 2. The left arm of the *Drosophila* second polytene chromosome with a schematic representation of those bands in region 32 where the genes under study in our laboratory have been mapped. *Upper part*: *In region 32A, a schematic drawing of the chromosome walking and of the genes and stage specific transcripts so far identified is shown. P1764ms is the P-element-induced male sterile mutation, isolated by the group of Steve Wasserman (Castrillon et al., 1993). The lambda A5 phage contains the two lipase genes that we have recently identified (Pistillo et al., 1998).* ***Central part*:** *In region 32D the P element insertion that induced the tulipano mutation is indicated in the lambda phage D1. This P-insertion allowed the identification of the first Drosophila nucleoporin mutant (Gigliotti et al., 1998) and was located downstream the rpL9 gene (indicated by the asterisk) isolated by the group of U. Schafer (Schmidt et al., 1996).* ***Lower part*:** *The genes that we have identified in region 32E, with their expression pattern in the ovary. Three overlapping phages are indicated, lambda E2, E3 and E4 (Lavorgna et al., 1989). In phage lambda E2, the Vitelline Membrane Protein gene (VM321) was identified (Gargiulo et al., 1991; Gigliotti et al., 1989). In the overlapping phages lambda A2 and A3, the gene coding for the receptor form of guanylate cyclase (GC) has been described (Gigliotti et al., 1993). The new P-element insertion inducing embryonic lethality is indicated between the VM321 and the GC genes. Using the region overlapping the P-element insertion, we have isolated cDNAs and rescued the lethality induced by mutations in this new gene. Under the cDNA, the β-galactosidase activity in an egg chamber isolated from a heterozygous mother is shown. For further information see text.*

hand, 10-12 rounds of endoreplication take place in nurse cells, along with a massive chromatin reorganisation process. During the first four endocycles, nurse cell chromosomes are paired and undergo progressive condensation. This leads to the appearance of "blob-like" chromosomes, characterized by highly compacted chromatin organization. The association between homologues is then lost and each polytene chromosome breaks down into 32 chromatid pairs. This event is coupled with the sudden transition to a highly decondensed chromatin structure. During the subsequent endocycles new polytene chromosomes are produced, starting from each chromatid pair, but the dispersed chromatin organization is maintained. Several genes are required to attain normal nurse cell chromosome organization and to sustain normal oocyte growth, including *fs(2)B* (King *et al.,* 1957), *ovarian tumor* (*otu*) (King and Storto, 1988), *D-elg* (Schulz *et al.,* 1993), and *cup* (Keyes and Spradling, 1997). These genes encode either for cytoplasmic proteins or transcriptional regulators. *fs(2)B*, *otu* and *cup* genetically interact with each other and it has been proposed that they take part in a common pathway required for egg chamber development (Keyes and Spradling, 1997). The nucleoporin gene *Nup154*, that we have recently isolated (Gigliotti *et al.,* 1998) also seems to be involved, among other functions, in chromosome organization and interacts with some of these genes (unpublished).

Results and Discussion

Our interest in *Drosophila* oogenesis started with the isolation and characterisation of the first gene coding for a vitelline membrane protein (Gigliotti *et al.,* 1989; Gargiulo *et al.,* 1991) in region 32E. To obtain greater insight into the functions present in region 32, several years ago we performed chromosome walking in 32D-32E-F (Lavorgna *et al.,* 1989) and more recently in 32A-B (unpublished). We molecularly identified various genes with maternal expression patterns, and some are illustrated in Fig. 2. We have also performed genetic screens in region 32 and have isolated various female-sterile mutations and their corresponding genes (Malva *et al.,* 1991; Gigliotti *et al.,* 1993; Malva *et al.,* 1994; Pistillo *et al.,* 1998; Rotoli *et al.,* 1998; Gigliotti *et al.,* 1998).

In the lower part of Fig. 2 some of the genes identified in region 32E, together with their expression pattern in the ovary, are illustrated schematically. The VM32E gene (left) shows a peculiar pattern of expression in the follicular epithelium, where it is highly transcribed in the cells surrounding the oocyte, with the exception of the most anterior and posterior ones. Its fine regulation has been defined by Cavaliere *et al.,* (1997).

The gene coding for the receptor form of Guanylate Cyclase, GC, (Fig. 2, lower part, right), was the first gene belonging to this interesting family of receptors isolated in *Drosophila* (Gigliotti *et al.,* 1993). It is expressed early in germarium and later in stage 10 egg chambers. The VM32E and the GC genes are separated by a genomic region of 10 kb, and for neither of the two genes mutations are yet available. We have performed various attempts, by PZ-element mobilisation and local transposition, to isolate mutations in these genes. Unfortunately, up to now, we have been unable to recover mutations in the VM32E or GC genes. Instead, a new P element insertion has been recovered in

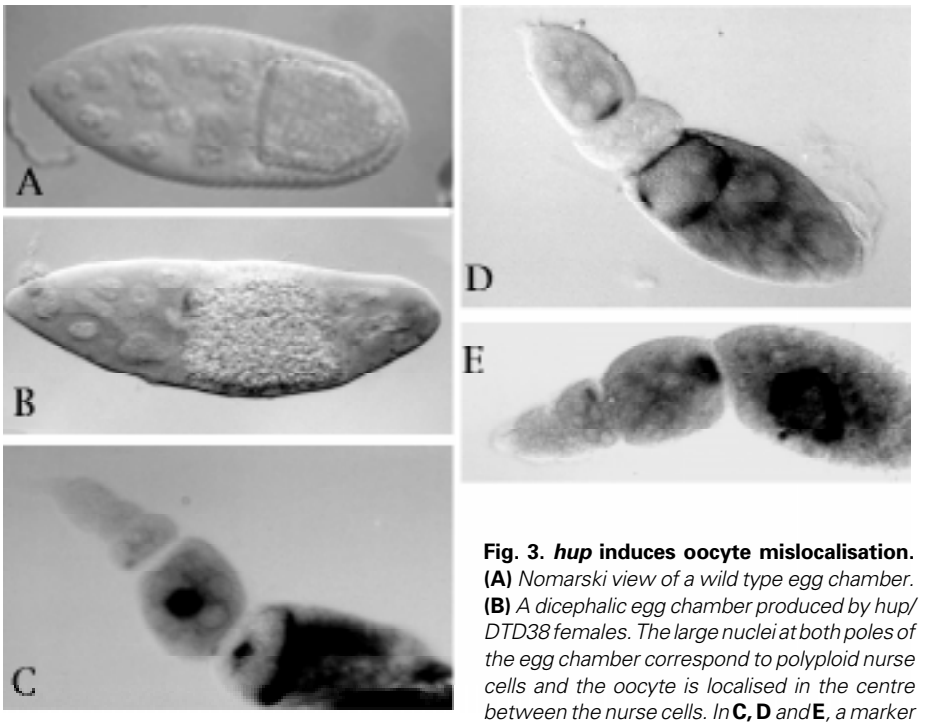


Fig. 3. *hup* induces oocyte mislocalisation. **(A)** *Nomarski view of a wild type egg chamber.* **(B)** *A dicephalic egg chamber produced by hup/DTD38 females. The large nuclei at both poles of the egg chamber correspond to polyploid nurse cells and the oocyte is localised in the centre between the nurse cells. In* **C, D** *and* **E**, *a marker that localises to the oocyte is used to show the oocyte mislocalisation. In C and D, two egg chambers are shown where the oocyte acquires an anterior, instead of a posterior position.*

the region between the two genes, inducing an embryonic lethal phenotype and identifying another gene (middle). Using the region overlapping the P-element insertion we have identified the genomic segment able to rescue the lethality induced by this new mutation and isolated the corresponding cDNA, downstream the P-element insertion (unpublished). An egg chamber isolated from females heterozygous for this lethal mutation is shown in the figure, under the corresponding cDNA. The highest β-galactosidase activity of the PZ element inserted in the gene is observed in the dorsal anterior follicle cells.

A more detailed analysis on the genes isolated in 32D and 32A is reported below, with a special emphasis on the *hup* mutation and our attempts to identify the corresponding gene and on the *tulipano* mutation and the nucleoporin *Nup154* gene.

The hold up *mutation*

The first *hold up* (*hup*) mutation was isolated by Sandler many years ago; it induces female and male semi-sterility and affects wing morphology (Sandler, 1977). We isolated another EMS induced *hup* allele, called hup^2, while the attempts to isolate P-induced mutations have been, so far, without success. We have genetically mapped the *hup* mutation between two chromosomal rearrangements: *T(2;4)DTD38* and *Df(2L)DerJ2*. Both rearrangements have a breakpoint mapped in 32A and trans-heterozygous flies *T(2;4)DTD38/Df(2L)DerJ2* are viable but female-sterile. *T(2;4)DTD38* does not complement any phenotypic aspects of the *hup* mutation, while *Df(2L)DerJ2* complements the wing adult phenotype and the male sterility but not the *hup* induced female sterility. Finally *Def(2L)DerJ2/*hup^1 or hup^2 females show an increased severity of the female sterility phenotype, in comparison with hup^1/hup^1 and hup^2/hup^2 females.

We investigated the ovarian phenotype induced by the *hup* mutation when placed in trans with the *T(2;4)DTD38* translocation, which is the genetic combination showing the strongest effect on female fertility. In dissected hup^1*/DTD38* mutant ovaries, the oocyte is displaced from its posterior localisation in 15.5% of the egg chambers. In Fig. 3 oocyte positioning in mutant egg chambers (B-E), with respect to the normal egg chamber (A), is shown by the use of molecular markers that localise to the oocyte. The oocyte can acquire a central (Fig. 3 B,C), lateral (Fig. 3E), or even anterior (Fig. 3C,D) localisation. Dicephalic egg chambers were never observed in hup^1/hup^1 ovaries, while oocytes with an anterior localisation were occasionally detected. As discussed in the introduction, oocyte positioning in the egg chamber is the crucial step allowing cross talk between oocyte and polar follicle cells. Therefore this early defect observed in *hup* egg chambers induces a series of consequences on egg chamber development. By the use of appropriate markers we have demonstrated that in the *hup* background a subset of the polar follicle cells at the posterior end of the egg chamber seems to adopt an anterior fate. We have observed that this occurs in egg chambers with an aberrant oocyte position, but also in egg chambers where the oocyte is normally positioned at the posterior end. In addition, the distribution of *oskar*, *bicoid* and *gurken* mRNAs and of kinesin-β-galactosidase activity (Clark *et al.,* 1994) are abnormal in mutant egg chambers (Rotoli *et al.,* 1998).

Another effect of the *hup* mutation on follicle cells is shown in Fig. 4, where the same ovariole from mutant females is shown after staining with rhodamine-conjugated phalloidin (A) and with DAPI (B). In this ovariole, the stalk cells, separating adjacent egg chambers, are missing. As discussed in the introduction, the most anterior and most posterior follicle cells, called polar cells, are considered as different from the other follicle cells surrounding the oocyte, called main body follicle cells. Recently, by generating dominantly marked mitotic clones, it has been proposed that both

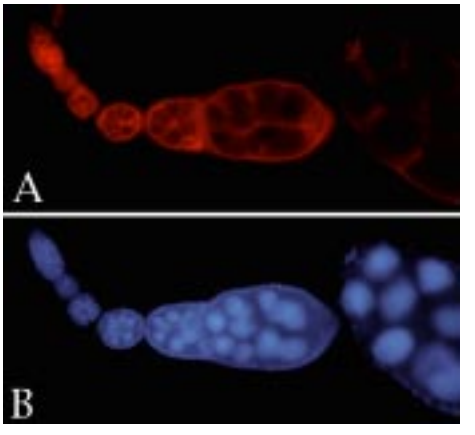


Fig. 4. The induction of stalk cell fate requires *hup*. *In* **(A)** *an ovariole from a* hup^2/hup^2 *mutant female is stained with rhodamine-conjugated phalloidin and in* **(B)** *the DAPI staining of the same egg chamber is shown. The stalk cells, which separate egg chambers in the wild type, are missing.*

stalk cells and polar cells are derived from a precursor population that is distinct from the precursors of other follicle cells (Tworoger *et al.,* 1999). The phenotypic defects that we have shown in *hup* follicles, affecting both posterior and stalk follicle cell fate, are in agreement with these data.

We are investigating the possible interaction of *hup* with a number of other mutants including *top*/*Egfr*. In Fig. 5 are shown some of the phenotypic defects observed in the egg chambers produced by hup^{1}, top^{CJ} double homozygous mutant females. Phenotypes not observed in *hup* or top^{CJ} homozygous females ovaries appeared. Beside the presence of dicephalic egg chambers and egg chambers showing degenerated nurse cells (not shown), the most striking phenotype was the presence, in 10% of the egg chambers, from stage 9 to stage 12, of multiple posterior layers of follicle cells (Fig. 5C). In some cases, these follicle cells in the posterior region of the egg chamber try to migrate centripetally, splitting the posterior cytoplasm of the oocyte (Fig. 5A,B). These results indicate that *hup* cooperates with *Egfr* in the specification of monolayer follicle epithelium integrity. The molecular nature of this interaction, as well as the possible involvement of Grk or other known molecules, cannot be elucidated until the *hup* gene will be molecularly identified. With this aim, we have performed a chromosome walk in region 32A (Fig. 2, upper part) and have identified the transcripts present in the region with respect to the position of some P element insertions and the two rearrangement breakpoints. We have isolated candidate genomic regions that will be used in transformation experiments to rescue the *hup* mutation. During this analysis we have identified two lipase genes, *lip1* and *lip2*, whose schematic structure is also reported in the figure (Pistillo *et al.,* 1998).

The tulipano *mutation and* Nup154 *gene*

The *tulipano* (*tlp*) mutation was isolated in a genetic screen for P-element induced female sterile mutations in region 32D (Fig. 2, central part). The affected gene turned out to be the first *Drosophila* homologue of known nucleoporin genes (Gigliotti *et al.,* 1998) and has been isolated also by Margaret Fuller's group , in Stanford (Kiger *et al.,* 1999). Nucleoporins are protein components of the nuclear pore complex, which is a highly conserved structure in eucaryotes, playing a fundamental role in regulating the bi-directional trafficking of RNA and proteins through the nuclear envelope (Gorlich and Mattaj, 1996; Laemmli and Tjian, 1996).

The *Drosophila* Nup154 protein displays similarity with Nup170 and Nup157, two central scaffold proteins of the yeast nuclear pore complex and with rat Nup155 (Radu *et al.,* 1993; Aitchison *et al.,* 1995). Very recently, the human homologue has also been cloned (Zang *et al.,* 1999). In pairwise alignments, the *Drosophila* protein shows a more extensive homology with the mammalian than with the yeast counterparts. 33.3% identity along its entire length is obtained with the human homologue (Fig. 6), indicating that a significant degree of conservation has been maintained during evolution. The C-terminal region contains a GVRLFF motif (bold in the figure) present in the yeast, rat and human proteins, although the *Drosophila* protein contains an F rather than a Y in the fifth position of the motif. Like all the other homologues, Nup154 belongs to the nucleoporin family that does not contain FG repeats.

Consistent with the assumption that nucleoporins play a pivotal function in cell physiology, the *Nup154* gene is expressed at all developmental stages and strong loss of function alleles are lethal.

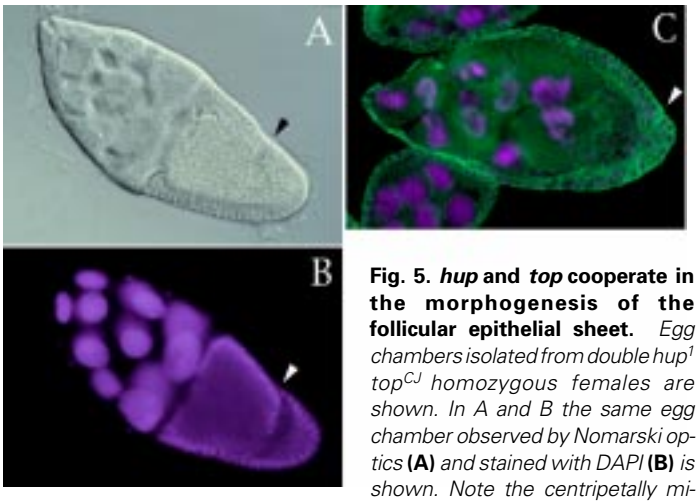
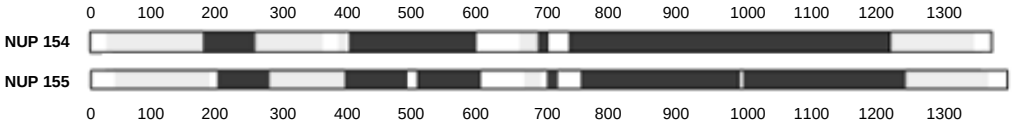


Fig. 5. *hup* and *top* cooperate in the morphogenesis of the follicular epithelial sheet. *Egg chambers isolated from double* hup^{1} top^{CJ} *homozygous females are shown. In A and B the same egg chamber observed by Nomarski optics* **(A)** *and stained with DAPI* **(B)** *is shown. Note the centripetally migrating posterior follicle cells trapping a small portion of the oocyte cytoplasm at the posterior end.* **(C)** *Confocal image of an egg chamber, stained with DAPI (nuclei in blue), and an anti-α-tubulin antibody (yellow-green), showing the multiple layers of follicle cells at the posterior end (arrowhead).*

On the other hand, the analyses of hypomorphic alleles revealed a requirement for *Nup154* gene function at specific steps of female and male gametogenesis. Accordingly, *Nup154* is transcribed in both ovaries and testes. During oogenesis, expression starts in the region 1 of the germarium and persists in all egg chamber developmental stages, in both nurse and follicle cells (Fig. 7B), according with the β-galactosidase expression pattern of the PZ element, driven in the *tlp* alleles by gene specific enhancer elements (Fig. 7A). In spermatogenesis, *Nup154* is transcribed in all germline cells while it seems to be inactive in somatic cells, since *Nup154* mRNA was not detected in agametic testes from adult sons of *osk* mothers (Kiger *et al.,* 1999). The fact that *Nup154* expression is germline dependent in testes but is ubiquitous in ovaries, suggests that the gene function may be differentially required and/or regulated in specific cell-types and this might in turn explain why *tlp* mutations have distinct phenotypic consequences on male and female gametogenesis. For example, while the somatic cells of the ovary display altered migration patterns, the somatic component of the testes does not show any evident abnormal behaviour. Moreover, even if both sperm and oocyte development are affected, meiosis normally occurs in female mutant germ cells, while it is completely prevented in male mutant spermatocytes (Gigliotti *et al.,* 1998).

Egg chamber development in *tlp* mutant ovaries is blocked in early vitellogenic stages, which display clear defects in nurse cell chromatin organization (Fig. 8). These defects can be interpreted as the result of the failure to decondense nurse cell chromosomes. Of the three genes demonstrated to be required in the modulation of nurse cell chromatin structure, *cup*, *otu* and *fs(2)B*, the first has been shown to encode for a cytoplasmic protein that is transiently localised at the periphery of nurse cell nuclei. It has been proposed that it may indirectly influence chromatin organization by acting in a pathway that affects the nuclear envelope (Keyes and Spradling, 1997). It is interesting in this context that mutations in the *Nup154* gene show an ovarian phenotype closely resembling the *cup* mutant phenotype. In addition, Nup154 protein is not only localised, as expected, at the periphery of nurse cell nuclei, but is also

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Nup154         25 ERNKPGLLELTGVSQHGRATMSGLNDYDYQSLSFLKSDRTHNLQQMRTVTKSAIPNEILE
Nup155         37 DRMYPDLSELLMVSAPNNPTVSGMSDMDYP-LQGPGLLSVPNLPEISSIRRVPLPPELVE
                   *  * * **  **     * **  * **  *          **           * *  *

Nup154         85 HFKHIKCHCTMGLFPEIGRAWLTIDSEIYIWTFNQTRDVAYYDGLSHLIVSVGLVKPKPG
Nup155         96 QFGHMQCNCMMGVFPPISRAWLTIDSDIFMWNYEDGGDLAYFDGLSETILAVGLVKPKAG
                   * *  * * ** ** * ******** *  *      * ** ****  *  ******* *

Nup154        145 VFVQDVKYLLVLTTPIEVIVLGVTFG-----ESSYNEMQLMNRPVFVIGTDNVSI
Nup155        156 IFQPHVRHLLVLATPVDIVILGLSYANLQTGSGVLNDSLSGGMQLLPDPLYSLPTDNTYL
                   *   *  **** **     **               *    ***   *     ***

Nup154        195 SVIKGTDDGRIFLGGRDGCLYEIYYQAESSWFGKRCKKINLSQGLVSYMVPSFLK-VFSE
Nup155        216 LTITSTDNGRIFLAGKDGCLYEVAYQAEAGWFSQRCRKINHSKSSLSFLVPSLLQFTFSE
                    *  ** ***** * ******  ****  **  ** *** *    *  *** *   ***

Nup154        254 VDPIEHIEIDNSRKLLYVLTEKGVIEAWDISTSYTTARRLGRITQNDITNQAVSLITTVD
Nup155        276 DDPILQIAIDNSRNILYTRSEKGVIQVYDLGQDGQGMSRVASVSQNAIVSAAGNIARTID
                   ***  * *****  **   *****   *         *     ** *   *     * *

Nup154        314 PSIFKSVKAICPLSADDADKLHLVAVTQC GVRLFF STT //
Nup155        336 RSVFKPIVQIAVIENSESLDCQLLAVTHA GVRLYF STC //
                   * **    *            * ***  **** ***
Nup154       1003 EAHTLKVKDPLIHITLYEWLLAHDMLKELLDVVEPSLGEFLRRSVSQNVDNVVLIDLLWK
Nup155       1024 LKLSQRSKDELFSIALYNWLIQVDLADKLLQVASPFLEPHLVRMAKVDQNRVRYMDLLWR
                        ** *  * ** **   *    ** *  * *   * *        *   ****

Nup154       1063 YYEKNSHHSQAAHILDNLAMTRSENINLEQRIEYLVRAVMCMRNGNVGSSLS-NGIFLKE
Nup155       1084 YYEKNRSFSNAARVLSRLADMHSTEISLQQRLEYIARAILSAKSSTAISSIAADGEFLHE
                  *****   * **  *  **   *  * * ** **  **          **    * ** *

Nup154       1122 LEDKLDIARVQKSVLAAMTELASDKLEAATAVKELNYALYDITQLYQHFAEPFDLWECQL
Nup155       1144 LEEKMEVARIQLQIQETLQRQYSHHSSVQDAVSQLDSELMDITKLYGEFADPFKLAECKL
                  ** *   ** *          *       **  *   * *** **  ** ** * ** *

Nup154       1182 SILNCSHHNDPLLIESVWGQIINSVVDKPGT-TSERCNRLFTKIEILVREYGESGVCFP
Nup155       1204 AIIHCAGYSDPILVQTLWQDIIEKELSDSVTLSSSDRMHALSLKIVLLGKIYAGTPRFFP
                   *  *    ** *    *  **        *   * *   *  **  *   *      **

Nup154       1240 FAFLIRELEVKACQLRFPEGIVPEKLVSMNLDIELLLEYYSRMISMNERVWANEGNEWHL
Nup155       1264 LDFIVQFLEQQVCTLNWDVGFVIQTMNEIGVPLPRLLEVYDQLFKSRDPFWNRMKKPLHL
                    *    **   * *    * *            *** *          *       **

Nup154       1300 IQSVIRVVSLLADNAQSIWYRSKRRIVGKAQDIVAGCL
Nup155       1324 LDCIHVLLIRYVENPSQVLNCERRRFTNLCLDAVCGYL
                          *         **      * * * *
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Fig. 6. Alignment of *Drosophila* Nup154 with the related human nucleoporin protein Nup155. *The bar diagrams of complete predicted proteins show conservation between the two proteins, with black boxes corresponding to the most conserved regions. The alignment shown below the diagrams is for the C-terminal and N-terminal regions only. The sequence of the Drosophila gene is from Gigliotti et al., (1998) and that of the human homologue is from Zang et al., (1999). In pairwise alignments, the Drosophila protein shows 33.3% identity along its entire length with the human homologue, indicating that a significant degree of conservation has been maintained during evolution. The GVRLFF motif present in the yeast, rat and human proteins, is in bold.*

present in the nucleoplasm, in a pattern coinciding with DNA. Both localisations are very reduced in *tulipano* mutant egg chambers showing morphological alterations (Gigliotti *et al.,* 1998). We have proposed that the protein coded by the *Nup154* gene could act as a functional link between cytoplasmic factors, such as Cup, and nuclear factors, in the regulation of nurse cell chromosome dynamics during oogenesis. In this regard, it is worth mentioning that the role of nucleoporins in organising nuclear functions is largely unknown, but one hypothesis just predicts that the components of the nuclear pore complex, together with the peripheral nuclear lamina, could be important players in the maintenance and the alteration of the three-dimensional chromosome structure during cell life (Blobel, 1985).

Conclusions

In this paper we have limited our analysis to the involvement in egg chamber development of some of the genes most recently identified by our group , mainly *hup* and *Nup154*, as data obtained on other mutants and genes have been published long ago or are too preliminary to be reported here.

Requirement of the *hup* product seems essential in different steps of oogenesis. *hup* acts in germarium for the correct positioning of the oocyte at the posterior end of the cyst and for the specification of posterior follicle cells. Due to the fact that the *hup* alleles are hypomorph, some mutant cysts are able to overcome the first defect, but other processes appear to be later impaired and

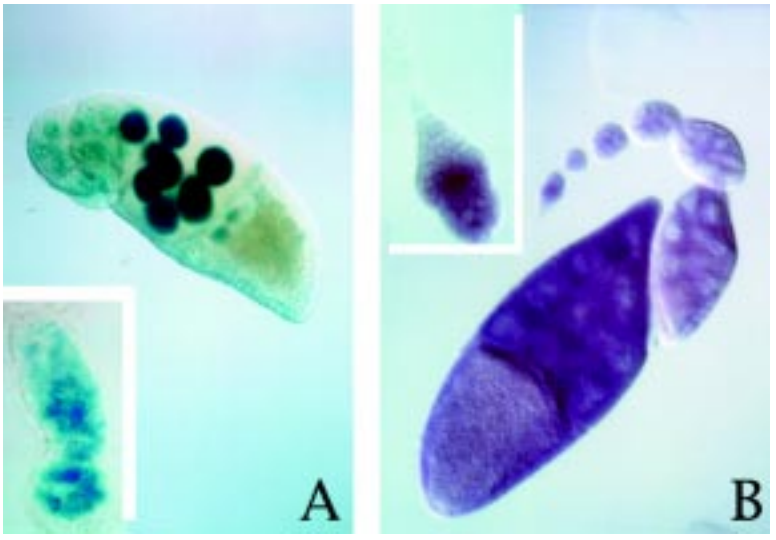

Fig. 7. Expression pattern of the *Nup154* gene in the ovary. (A) *β-galactosidase activity generated by the PZ element inserted in the Nup154 gene, in egg chambers of tlp heterozygous females. The activity is observed in germarium (small inset), and later, in stage 10 egg chambers. The nuclear localisation of β-galactosidase activity is due to the type of construct used.* **(B)** *Whole-mount in situ hybridisation showing the pattern of expression of Nup154 in a wild type ovariole. The hybridisation signal first appears in the germarial region 1 (enlarged in the small inset) but becomes prominent in region 2. After a marked decrease in RNA accumulation in the first stages of egg chamber development, a new peak is reached in stage 10, where the gene is clearly expressed in both nurse and follicle cells.*

localisation of selected mRNAs is affected. Thus, as described for various other genes, also the *hup* phenotypes confirm the link between oocyte determination, oocyte positioning and axis formation. The observation of stronger phenotypes in the double *hup top* mutant seems to indicate that *hup* and *top* genetically interact for A/P and D/V patterning. They can be involved in the same Gurken/Egfr pathway or in parallel and/or overlapping pathways. Until the *hup* gene will be isolated, it is impossible to unravel the hierarchical relationships between the genes showing genetic interactions. We have candidates for the *hup* gene and hope to characterise them in the next future.

Regarding the nucleoporin *Nup154* gene, we believe that the availability of the first *Drosophila* mutants in a gene showing homology with known nucleoporins provides a useful genetic tool to investigate the physiological and developmental role of nucleoporins in metazoa. Its future study will be important for progress in understanding how nuclear pore components regulate nuclear and cellular physiology, also in relation to specialised developmental processes such as oogenesis and spermatogenesis. The results so far obtained indicate that Nup154 is an essential protein involved in nuclear envelope structure. In addition, its dual localisation, both at the nuclear envelope and in the nuclear interior, suggests multiple roles in nuclear functions. The multiplicity of phenotypes observed in *tlp* mutants could be attributed to pleiotropic effects, as well as to the hypomorphic nature of our alleles. In any case, *Nup154* is required not only for female and male germline growth and development but also in other tissues and developmental stages, as demonstrated by the existence of lethal alleles and the observation of complex embryonic phenotypes. The most interesting aspect, in our opinion, is the different effects that impairment of this gene function has on female versus male fertility and on germline versus soma. This finding argues that distinct regulatory mechanisms, differentially involving *Nup154* function, underlie these differences. Nup154 could interact with sex and tissue specific proteins and this interaction could establish sex and tissue specific functions, or could be involved in the nuclear cytoplasmic traffic of sex specific molecules. We are presently investigating if differences exist between sexes in transcription, splicing or translation products. Even minor differences could have relevant consequences on protein function and protein-protein interactions.

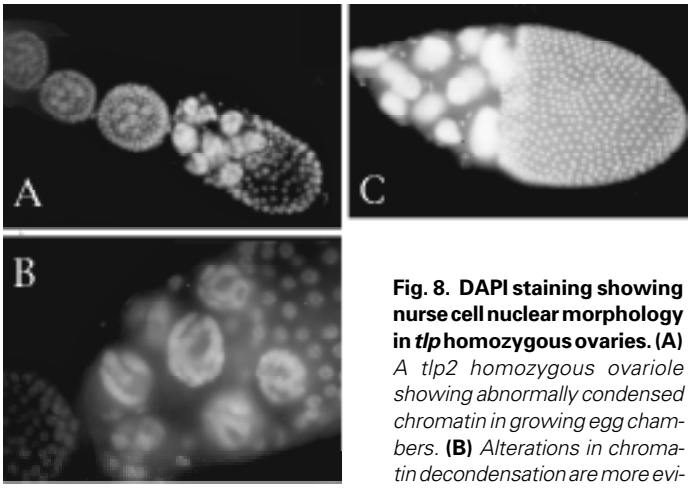

Fig. 8. DAPI staining showing nurse cell nuclear morphology in *tlp* homozygous ovaries. (A) *A tlp2 homozygous ovariole showing abnormally condensed chromatin in growing egg chambers.* **(B)** *Alterations in chromatin decondensation are more evident in the few egg chambers which reach later developmental stages.* **(C)** *Nuclear morphology of a control normal egg chamber*

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