Female sterile mutations and egg chamber development in
Drosophila melanogaster

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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
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 relocation 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
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, 1999; 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 gerarium 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

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, 1999; Rotoli et al., 1998; Gigliotti et al., 1998).

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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 hup2, 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/hup1 or hup2 females show an increased severity of the female sterility phenotype, in comparison with hup1/hup1 and hup2/hup2 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 hup1/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 hup1/hup1 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...
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 /Egrf. In Fig. 5 are shown some of the phenotypic defects observed in the egg chambers produced by hup1, topc2 double homozygous mutant females. Phenotypes not observed in hup or topc2 homozygous females ovaries appeared. Beside the presence of dicyclical 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 Egrf 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; Laemml 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.

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
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
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, underline 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.

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