The Mystery of Rhomboid in Capicua mutant Fruit Flies

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Introduction

Drosophila melanogaster is a fruit fly that is one of the most commonly used animal model for genetic studies. Dorsal-ventral (dv) patterning, which is the body plan, of the Drosophila embryo is determined by the asymmetric positioning of different underlying cellular molecules. DV patterning of the Drosophila embryo is established well before fertilisation. The Drosophila egg develop within a cluster of cells consisting of an oocyte interconnected with fifteen sister nurse cells, which function as a source of RNAs and proteins for the developing oocyte and degenerate by the end of oogenesis. Surrounding this cluster is an epithelium of follicle cells, which provide yolk and secrete the eggshell (Fig1). Initial dv asymmetry is established at mid oogenesis and the information is then passed on to the surrounding follicle cells (3). The protein gurken (grk), in the oocyte, provides the initial polarizing signal to initiate the formation of the eventual polarity of the egg, and activates Drosophila epidermal growth factor receptor (EGFR) signaling in the dorsal follicle cells. Through positive and negative feedback mechanisms, this EGF signaling is refined and defines a precise pattern of cell fates within the follicular epithelium (3). This specific definition of fates will be used as a basis to understand the effects of the gene capicua (cic) on follicle cell fate patterning.

Key Terms

Follicle:
a small bodily cavity or sac, such as the oocyte.

Oogenesis :
the stages of development and maturation of the female reproductive gamete

Ectopic:
an abnormal location or position

Mapping Crosses:
Mating crosses designed to locate the chromosomal position of a given gene.

Mosaic Flies:
Flies that carry a mixture of mutant and wild-type cells

Cell Autonomous:
Expression in a given cell independent of other cells

RNA Probes:
Strands of RNA that are complementary to the RNA of study.

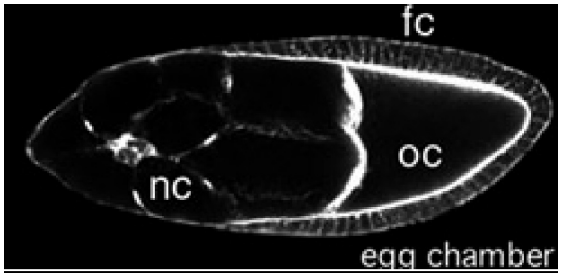

Figure 1. Drosophila egg chamber. The developing oocyte cell (oc) is interconnected with 15 nurse cells (nc). Surrounding these cells is an epithelium of follicle cells (fc)

Expression of rho in cic mutant ovaries

Follicle cells generate an eggshell embellished with external structures including the long respiratory appendages located dorsally at the anterior of the egg (Fig 2). Since external structures reflect the follicular patterning along the dv axis of the mature egg, any defect in the patterning will be reflected by anomalies in the resulting eggshell. Females lacking cic function lay eggs that display an expansion of the dorsal features to the ventral half of the egg. This phenotype, most prominently, includes broad dorsal appendages that are laterally shifted and a collar of ectopic dorsal appendage material near the ventral anterior circumference of the maturing oocyte (Fig 3, right panel). Typically this eggshell phenotype is linked to EGFR activation and of its subsequent target genes including mirror (mirr), kekkon 1 (kek1), and rhomboid (rho) (1). However in cic mutant ovaries analysis of mirr and kek 1 expression patterns reveals that mirr expression expands ectopically in the follicular epithelium leading to a dorsalized phenotype, but the expression of kek 1 remains unaffected (2) (Fig 2). This indicates that cic plays a role in regulating the expression of mirr but not that of kek 1, and that mirr is under the control of cic.

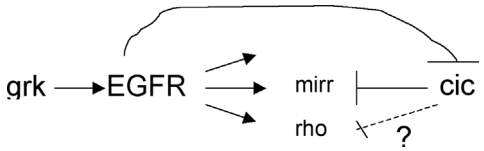

Figure 2. The above pathway is not absolute and only represents the important players for this study. grk protein in the oocyte activates the epidermal growth factor receptor (EGFR) which then goes on to activate kek 1, mirr, rho and inactivate suppress expression of cic in the dorsal half of the Drosophila egg. Manipulation of this expression allowing it to expand to the ventral half of the embryo will lead to a dorsalised embryo. Females mutant for the gene cic lay dorsalised embryos where the expression of mirr was expanded but not that of kek 1.

Interestingly, ectopic expression of rho in flies generates dorsalized eggs, with the characteristic broadened and laterally shifted dorsal appendages similar to those seen in cic mutant flies (1). One would then hypothesize that rho is also under the control of cic just like mirr.

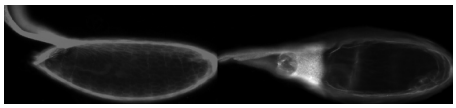

Figure 3. Wildtype (WT) egg (left) versus cic mutant egg (right). Notice the difference in shape of the egg and the dorsal appendages between WT and cic.

Materials and Methods

To test the above prediction, a Fluorescent In Situ Hybridisation (FISH) was performed to visualise rho mRNA expression in both cic mutant ovaries, and wildtype controls for comparison. FISH involves the use of RNA probes that are complementary to the mRNA in question. The RNA probes bind to the complementary mRNA in the tissue and with the use of fluorescent antibodies against the RNA probe one can visualise the location of the particular mRNA.

Moreover, the difference in appendage fate in cic mutant ovaries was investigated. Since cic mutant flies generate eggs that exhibit abnormal dorsal appendages, it can be concluded that cic is involved in defining the two populations of cells that make up the dorsal appendages. The transcription factor Broad-Complex (BR-C), expressed by the roof cell population, which go on to make the actual appendage, has been used as a visual marker to study the fate of these cells on a cic mutant background. Results have shown that BR-C expression expands to the ventral half of the follicular epithelium in ovaries lacking cic, forming a collar around the anterior-most circumference of the maturing oocyte (1). The roof cells make up only part of the dorsal appendages, and thus tell only part of the story. Thus, rhomboid-lacZ (rho-lacZ) was used to visualize changes in the floor cell fate patterning, which go on to make up the base of the appendages.

Results

The FISH technique worked against grk mRNA (positive control for technique) in wildtype ovaries (Fig 3). Unfortunately, similar results failed to be reproduced with the grk mRNA probes and the rho mRNA probes built for the purpose of this experiment. Troubleshooting the probes will be performed to determine the proper functioning of the technique.

Furthermore, the chromosomal position of the rho-lacZ transgene, which is an artificially inserted gene, in the Drosophila stock came into question. After resolving this issue through conducting mapping crosses, it will be possible to use the transgene as a reporter to visualize changes in floor cell fate patterning. Additionally, mosaic flies bearing clones of cic mutant follicle cells will be generated genetically to further characterize the expression of rho-lacZ. It will also be determined whether changes in the expression of rho-lacZ are cell autonomous. Lastly, the above experiments will be complemented by testing for the suppression of the cic mutant phenotype through reduction of the expression of rho and/or mirr, and in time by characterizing how cic regulates rho and/or mirr. This analysis of rho in a cic mutant background will further our understanding of the important pathway that regulates dv patterning of the Drosophila egg, and advance Drosophila developmental research a small step, with the final goal being application of this research to humans.

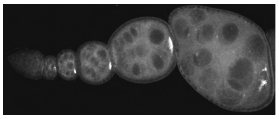

Figure 4. Grk mRNA FISH of single ovariole of a wildtype female ovary. The ovariole is like an assembly line with every subsequent egg chamber at a later stage of oogenesis than the egg chamber before it. The grk mRNA fluoresces red here and is seen localising near the maturing oocyte nucleus.

References

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