The Only Function of Grauzone Required for Drosophila Oocyte Meiosis Is Transcriptional Activation of the cortex Gene

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ABSTRACT

The grauzone and cortex genes are required for the completion of meiosis in Drosophila oocytes. The grauzone gene encodes a C2H2-type zinc-finger transcription factor that binds to the cortex promoter and is necessary for high-level activation of cortex transcription. Here we define the region of the cortex promoter to which Grauzone binds and show that the binding occurs through the C-terminal, zinc-finger-rich region of the protein. Mutations in two out of the five grauzone alleles result in single amino acid changes within different zinc-finger motifs. Both of these mutations result in the inability of Grauzone to bind DNA effectively. To determine the mechanism by which Grauzone regulates meiosis, transgenic flies were produced with an extra copy of the cortex gene in homozygous grauzone females. This transgene rescued the meiosis arrest of embryos from these mutants and allowed their complete development, indicating that activation of cortex transcription is the primary role of Grauzone during Drosophila oogenesis. These experiments further define a new transcriptional pathway that controls the meiotic cell cycle in Drosophila oocytes.

The female meiotic cell cycle is a complex process. Unlike meiosis in the male germline, which is continuous, meiosis in the female germline is discontinuous. Mechanisms must exist to arrest and restart the female meiotic cell cycle in a precisely timed manner to allow for the development of the oocyte and the nuclear fusion that occurs at fertilization. In the female Drosophila germline, the oocyte first arrests in prophase of meiosis I. The cycle then restarts after oocyte growth and arrests again at metaphase I. Once the egg becomes activated, the metaphase I arrest is released, and the meiotic divisions are completed (for review, see Page and Orr-Weaver 1997).

In Drosophila, the regulatory mechanisms necessary for the completion of meiosis after the metaphase I arrest are largely unknown. Two female sterile mutants, grauzone (grau) and cortex (cort), which arrest early in development (Schüpbach and Wieschaus 1989) and have multiple biological phenotypes, have been identified. Embryos from grau or cort homozygous females (subsequently referred to as grau or cort eggs/embryos) fail to translate bioid mRNA due to a defect in the cytoplasmic polyadenylation of this message (Salleés et al. 1994; Lieberfarb et al. 1996). These embryos also have abnormal cytoskeletal reorganization (Lieberfarb et al. 1996; Page and Orr-Weaver 1996) and show enhanced stability of certain maternal mRNAs (Bashirullah et al. 1999). Additionally, grau and cort eggs arrest inappropriately during the female meiotic cell cycle (Lieberfarb et al. 1996; Page and Orr-Weaver 1996).

Meiotic defects have not been observed in grau and cort eggs until after the metaphase I arrest. After this arrest is released, however, grau and cort eggs arrest aberrantly in meiosis II. Few genes that affect the second meiotic division are known. The analysis of these two genes, therefore, may provide useful information as to how this division is regulated.

The phenotypes of grau and cort mutant eggs are qualitatively indistinguishable, suggesting that these genes are involved in the same developmental pathway. Grauzone encodes a C2H2-type zinc-finger transcription factor that binds to the cort promoter and is necessary for high-level activation of cort transcription (Chen et al. 2000). The function of cort is not yet known, but it may act as a cell cycle regulator (T. Chu, unpublished observations).

The requirement for transcriptional regulation during meiosis has been demonstrated in yeast. In Saccharomyces cerevisiae, meiosis (sporulation) is characterized by the sequential transcription of meiosis-specific genes. These genes are placed into four classes: early, middle, mid-late, and late (Mitchell 1994). Many of the early genes are involved in meiotic prophase and are activated by a complex of two proteins, Ume6 and Ime1 (Rubin-Bejerano et al. 1996). A meiosis-specific transcription factor, Ndt80, is important for the transcription of middle genes at the prophase to metaphase transition (Xu et al. 1995; Chu and Herskowitz 1998; for review see Clancy 1998). Many genes that are involved in the exit...
from mitosis (metaphase to anaphase transition) are also induced midway through sporulation, suggesting that these genes may play a role in the exit from meiosis as well (Chu et al. 1998).

The general requirement for transcriptional regulation during meiosis appears to be conserved in higher eukaryotes as well. In this article, we report that the primary role of Grau during Drosophila oogenesis is the regulation of cort transcription. Grau binds to a defined region of the cort promoter, and mutations that abolish grau function disrupt this protein-DNA interaction. It is possible that Grau activated other target genes important for meiosis. However, increasing the expression of cort rescues the meiosis arrest in eggs from grau homozygous females. This result suggests that the meiosis arrest in grau eggs is due to low levels of cort transcript and that the completion of meiosis does not require that Grau activate the transcription of other target genes.

**MATERIALS AND METHODS**

**Fly stocks:** The grau alleles, RM 61, QF31, QE70, QQ36, and RG1, and cort alleles, QWS5 and RH65, were generated by Schüpbach and Wieschaus (1989) in an EMS screen for female sterile loci on the second chromosome and were generously provided by T. Schüpbach. All other fly stocks were obtained from the Bloomington Stock Center.

**Construction and purification of glutathione S-transferase (GST)-tagged Grau fusion protein and derivatives:** The full-length GST-Grau fusion protein was constructed as described (Chen et al. 2000). The grau coding region used to construct GST-Grau fusion was amplified from a wild-type ovarian cDNA pool using the Expand Long Template PCR System (Boehringer Mannheim, Indianapolis). The amplification was performed using the 5′, 5′-CATGATAGTGGATATCT GGGCCCTCTTGG TCCAGACTTCTCCGCACTGTC-3′, and the 3′ primer, 5′-CATGATGGTCG GCCGCATTTGCTCTGC-3′, both of which contain EcoRI restriction sites at their ends. The PCR product was digested with EcoRI and inserted into EcoRI-digested pGEX-3X vector (Amersham Pharmacia Biotech, Piscataway, NJ), forming the GST-Grau fusion.

The wild-type GST-Grau coding region was also amplified by a wild-type ovarian cDNA pool as described above using the 5′, 5′-CATGATAGTGGATATCT GGGCCCTCTTGG TCCAGACTTCTCCGCACTGTC-3′, and the 3′ primer, 5′-CATGATGGTCG GCCGCATTTGCTCTGC-3′, both of which contain EcoRI restriction sites at their ends. The PCR product was digested with EcoRI and inserted into EcoRI-digested pGEX-3X vector (Amersham Pharmacia Biotech). All fusion plasmids were sequenced by automated sequencing (Applied Biosystems, Foster City, CA) to confirm that the fusion was in the correct reading frame and lacked PCR-induced mutations.

The fusion proteins were expressed in Escherichia coli BL21 cells according to the manufacturer’s instructions (Amersham Pharmacia Biotech) and purified as described (Chen et al. 2000).

**Gel-shift assays:** Oligonucleotides corresponding to regions of the cort promoter were annealed and radiolabeled with [γ-32P]-ATP using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). GST-Grau proteins are able to bind to a 32-bp oligo (5′-TATCGAGTGGTACTGTGTAACCTG TAAG-3′), but not to two smaller oligos (5′-CAGCTCT ATCGAGTGGTACTGTGTAACCTG AAG-3′). Nonspecific competitor was one of the previously mentioned regions of the cort promoter to which GST-Grau cannot bind. Antibodies used were anti-GST antibody (Z-5, Santa Cruz Biotech) and control rabbit IgG (Santa Cruz Biotech).

The gel-shift experiments were performed as described (Chen et al. 2000), with the following changes: 1 μg of GST or GST-Grau protein derivative was added to each gel-shift reaction. For the GST-Grau N E493K and GST-Grau N C298Y fusions, increasing amounts of protein were added (1 μg, 2 μg, and 4 μg per reaction). When used, cold competitor DNA was added at 20× the concentration of probe.

**Rescue of meiosis arrest by increased expression of cort:** A 2.7-kb genomic fragment containing the cort coding region was subcloned into a CaSpeR transformation vector and used to generate transgenic flies. Plasmid DNA at 0.4 mg/ml was coinjected with 0.1 mg/ml of helper plasmid (pichiRsΔ2-3; Spradling 1986) into early yw embryos. Transformed flies were crossed to both cort and grau mutant flies. Homozygous cort and grau females with and without the transgene were collected.

Females of the different genotypes were crossed to Canton-S male flies and maintained on apple juice plates. Embryos were collected over the course of 2 hr and aged for 1–2 hr at room temperature. The embryos were dechorionated in 50% Clorox bleach, devitellinized, fixed in methanol, and rehydrated by standard methods (Theurkauf 1994). The embryos were stained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI; Sigma, St. Louis) in phosphate-buffered saline (130 mm NaCl, 70 mm Na2HPO4, 35 mm NaH2PO4, containing 0.1% Triton X-100 (PBST) for 30 min, briefly washed in PBST, mounted in 70% glycerol, 30% PBST, and visualized immediately with a Nikon microscope with a DAPI filter. Embryos mitotically dividing nuclei were scored as developing embryos.

**Analysis of cort expression levels by RT-PCR:** Twenty females of each genotype (wild type, grauQF31/grauQF31, and grauHomozygous females. This result suggests that the meio-

**RESULTS**

Grau protein binds to a defined region of the cort promoter: In vitro gel mobility shift assays have shown...
that GST-tagged Grau protein binds to a 32-bp region of the cort promoter (Chen et al. 2000). This 32-bp sequence lies 56 bp upstream of the cort ATG. Using the TFSEARCH program, this region was found to include an eight-nucleotide motif that has an 85% match to the cap signal for transcription initiation (Hinemeyer et al. 1998; Akiyama, http://www.rwcp.or.jp/papia/). The cap signal lies within many initiator elements and contains the transcription initiation site (Larson et al. 1995).

To determine whether this eight-nucleotide motif was necessary for interaction with Grau, gel mobility shift assays were performed. Three double-stranded oligonucleotides were tested for interaction with GST-Grau, one that contained the entire eight-nucleotide cap motif and two that did not (Figure 1A). While GST-Grau was able to bind to the 32-bp region of the cort promoter that contained the entire cap motif (Figure 1B, lane 3), it was unable to bind to either of the two smaller oligos that did not contain the entire eight-nucleotide motif (Figure 1B, lanes 6 and 9). Thus, although the cap signal for transcription initiation is a loosely defined nucleic acid motif, gel-shift probes that bisected this eight-nucleotide motif were unable to be bound by GST-Grau protein in a gel-shift assay.

Grau protein binds DNA through its zinc-finger-rich C terminus: The Grau protein is a 570-amino-acid polypeptide that contains eight C2H2-type zinc-finger motifs scattered throughout its C terminus. The Grau protein also contains a patch of acidic residues within the N-terminal region of the protein (Asp146 to Asp172), which may function as its transcription activation region (Ptashne 1988; Chen et al. 2000). The DNA-binding domains and transcription activation domains of many transcription factors act as separable modules (Hope and Struhl 1986; Keegan et al. 1986). Given the modular nature of the Grau protein, we attempted to determine the region within the Grau protein that is required for DNA binding.

Three different forms of Grau (Figure 2A) were expressed in bacteria as GST-tagged fusion proteins, purified (Figure 2B), and used in gel mobility shift assays (Figure 2C). The GST-Grau protein has the 26-kD GST protein sequence fused at the N terminus to the full-length Grau protein (amino acids 1-570). The GST-GrauΔC protein contains amino acids 1-306 of the Grau protein, including the patch of acidic residues and one complete zinc-finger motif. The GST-GrauΔN protein contains amino acids 293-570 of the Grau protein, which contains seven of Grau’s eight C2H2-type zinc-finger motifs (Figure 2A).

While GST protein alone showed no DNA-binding activity (Figure 2C, lane 2), GST-Grau and GST-GrauΔN bound to the cort promoter with high affinity (Figure 2C, lanes 3 and 5). The GST-Grau shift included a high molecular weight complex and a smaller complex. The smaller complex appears to be binding between the DNA and a degradation product of the fusion protein, since a portion of full-length GST-Grau becomes degraded during expression and purification (Figure 2B). The GST-GrauΔC protein exhibited only extremely weak binding. The minimal binding of the GST-GrauΔC protein to the cort promoter is probably due to the one
Figure 2.— Grau binds to DNA through its zinc-finger-rich C terminus. (A) Full-length and truncated versions of grau were cloned into bacterial expression vectors and used to express GST-tagged fusion proteins. Zinc-finger motifs are shown as shaded boxes, and amino acids are numbered. (B) A 10% SDS-PAGE gel stained with Coomassie blue shows the relative migration of the purified fusion proteins. Molecular mass is numbered at the left. (C) The bacterially expressed and purified GST-Grau proteins were analyzed for their ability to bind to a 32-bp region of the cort promoter by gel-shift assay. DNA-binding reactions included either GST (lane 2), GST-Grau (lane 3), GST-GrauΔC (lane 4), or GST-GrauΔN protein (lane 5). The DNA protein complexes were resolved by electrophoresis in a nondenaturing polyacrylamide gel and visualized by autoradiography.

The C terminus of Grau binds to the cort promoter sequence specifically: It was important to determine whether the high affinity binding of the C-terminal region of the Grau protein (GST-GrauΔN) to the cort promoter was specific. To test whether GST-GrauΔN binding to the cort promoter was sequence specific, competition experiments were performed. GST-GrauΔN bound to the cort promoter very efficiently (Figure 2, lane 5 and Figure 3, lane 3). The addition of excess cold cort probe to the gel-shift reaction competed away the mobility shift (Figure 3, lane 4). The addition of excess cold probe, corresponding to a region of the cort promoter to which Grau protein did not bind, failed to compete away the mobility shift (Figure 3, lane 5). GST-GrauΔN protein also failed to bind to several other unrelated DNA targets (data not shown). To test whether GST-GrauΔN binding to the cort promoter was specific for GST-GrauΔN protein, an antibody directed against the GST-tag was included in the gel-shift reaction. In the presence of this antibody, the mobility shift was supershifted (Figure 3, lane 6). The addition of a control antibody to the gel-shift reaction, however, was unable to supershift the DNA-protein complex (Figure 3, lane 7). Thus, GST-GrauΔN protein binds to the cort promoter in a sequence-specific manner.

Point mutations in the grauPE70 and grauQQ36 alleles interfere with DNA binding: All five of the grau alleles
Figure 3.—GST-GrauΔN binds to the cort promoter in a sequence-specific manner. The specificity of binding to the cort promoter by the GST-GrauΔN fusion protein was analyzed by gel-shift assay. All reactions used a 32-bp region of the cort promoter as probe. DNA-binding reactions included either GST (lane 2) or GST-GrauΔN protein (lanes 3–7). Competition reactions were incubated with 20× cold-specific competitor (+, lane 4) or 20× cold-nonspecific competitor (C, lane 5). Supershift reactions were incubated with a specific antibody directed against the GST tag (GST, lane 6) or a nonspecific control antibody (C, lane 7).

have been previously sequenced (Chen et al. 2000). These sequences provided information about which regions of the Grau protein are critical for its function. Two grau mutant alleles (grauE70 and grauG73) contain premature stop codons within their sequence. Both of these alleles would produce truncated proteins without a complete zinc-finger motif. Since the zinc-finger-rich C terminus of Grau is necessary to bind DNA efficiently, protein products from either of these two alleles would be unable to bind DNA.

Two additional grau alleles, grauE70 and grauG036, contain single base-pair mutations within individual zinc-finger motifs (Chen et al. 2000). The grauE70 allele contains a missense mutation leading to the conversion of Glu493 to Lys within the seventh of the eight zinc-finger motifs. The grauG036 allele also contains a missense mutation, this one leading to the conversion of Cys298 to Tyr.

Eggs from mothers homozygous for any of the five grau alleles, or hemizygous for any allele over a deficiency that deletes the grau gene, have very similar phenotypes (Page and Orr-Weaver 1996). This result suggests that all of the grau alleles are null and that an amino acid change within a single zinc-finger motif has the same functional consequence as lacking zinc fingers altogether.

To determine whether the DNA-binding activity of the protein products from the grauE70 and grauG036 alleles was compromised, GST-tagged fusion proteins were produced that contained the single amino acid changes corresponding to these two alleles. These proteins were then used in a gel mobility shift assay. GST-GrauΔN/E493K contains the C terminus of Grau with the single amino acid change found in the grauE70 allele, while GST-GrauΔN/C298Y contains the single amino acid change found in the grauG036 allele (Figure 4A). The two mutant proteins were expressed in bacteria and purified side by side with the wild-type protein. All three fusion proteins (GST-GrauΔN, GST-GrauΔN/E493K, and GST-GrauΔN/C298Y) were expressed at similar levels and ran at the same relative mobility on an SDS-PAGE gel (Figure 4B).

The wild-type and mutant GST-GrauΔN proteins were tested for their ability to bind to the cort promoter in a gel-shift assay at increasing concentrations. The wild-type version of GST-GrauΔN bound DNA efficiently, even at the lowest concentration tested (Figure 4C, lane 3). In addition, this binding appears to be near saturation, as an increasing amount of protein does not appear to increase proportionately the amount of DNA shifted (Figure 4C, lanes 3–5). GST-GrauΔN/E493K did not bind to the cort promoter, even at the highest protein concentration tested (Figure 4C, lanes 6–8). Amino acid E493, although not conserved within the zinc-finger structure, lies adjacent to the first histidine of the finger and is one of the amino acids involved in sequence-specific DNA contacts (Klevit 1991; Bernstein et al. 1994).

GST-GrauΔN/C298Y bound to the cort promoter only weakly, and the amount of DNA shifted by the highest concentration of GST-GrauΔN/C298Y protein was still significantly less than the amount of DNA shifted by the lowest concentration of wild-type GST-GrauΔN (Figure 4C, lanes 9–11, darker exposure shown at the bottom). Cys298 is within the second zinc-finger motif and is one of the conserved cysteines within the C2H2-type zinc-finger structure. Changes in the conserved amino acids that coordinate the zinc atom within the finger may destabilize the structure. The interaction of this protein with the cort promoter may be so weak that it is not able to sufficiently activate cort transcription to allow the progression through meiosis. Thus, both the grauE70 and grauG036 alleles produce protein products that are defective in their ability to bind to the cort promoter.

Increasing the expression of cort rescues the meiosis arrest of grau mutants: Ovaries from females hemizygous...
Figure 4.—Point mutations in the grau<sup>DN</sup> and grau<sup>CN</sup> alleles interfere with DNA binding. (A) Point mutations corresponding to those found in two of the mutant grau alleles were introduced into the GST-Grau<sup>DN</sup> construct and used to express GST-tagged fusion proteins. Single amino acid changes are labeled at the position where they occur. Zinc-finger motifs are shown as shaded boxes, and amino acids are numbered. (B) A 10% SDS-PAGE gel stained with Coomassie blue shows the relative migration of the purified fusion proteins. Molecular mass is numbered at the left. (C) The DNA-binding activity of the GST-Grau<sup>DN E493K</sup> and GST-Grau<sup>DN C298Y</sup> fusions was analyzed by gel-shift assay. All reactions used a 32-bp region of the cort promoter as probe. DNA-binding reactions included either GST (lane 2), wild-type GST-Grau<sup>DN</sup> (lanes 3-5), GST-Grau<sup>DN E493K</sup> (lanes 6-8), or GST-Grau<sup>DN C298Y</sup> (lanes 9-11). Each version of the GST-Grau<sup>DN</sup> fusion protein was used in three different reactions. Equal amounts of protein (1×) were used in lanes 3, 6, and 9, with 2× this amount of protein being added in lanes 4, 7, and 10, and 4× this amount of protein in lanes 5, 8, and 11. A darker exposure of the shifted region (8× the original exposure) is shown at the bottom.

gous for any of the five grau alleles (each grau mutant allele in trans to Df(2R)Pu-D17, a deficiency that deletes the grau gene) have severely reduced levels of cort transcript abundance (Chen et al. 2000). To determine whether the meiotic arrest in grau eggs was due to the reduction of cort transcript, we increased the copy number of cort in a grau mutant background and determined whether this increase could rescue the mutant phenotype.

Flies that carried a transgene on the third chromosome containing the cort genomic region were crossed into a grau mutant background. These flies contained three copies of the cort gene (two endogenous copies plus one copy supplied by the transgene). Embryos from homozygous grau females that carried the transgene were collected, stained with DAPI, and the percentage of embryos that had progressed through meiosis was determined. Embryos from sibling grau homozygous females that lacked the transgene were analyzed as a control. In addition, the cort genomic transgene was crossed into a cort mutant background and subjected to the same analysis to ensure that the cort transgene was functional.

For each of two transgenic lines analyzed, the presence of the cort transgene in a grau mutant background increased the percentage of developing embryos from ~3 to ~88-95% (Table 1). This was similar to the per-
Overexpression of cort rescues the meiosis arrest of grau mutant embryos

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<th>Total no. of embryos</th>
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\(^a\) The transgene contains the cort genomic region in a P-element vector.

centage of embryos that developed when the cort transgene was present in a cort mutant background (\(\sim 93-95\%), Table 1). The embryos laid by grau homozygous females that carry the cort transgene not only completed meiosis, but developed into adult flies.

This rescue could have resulted from exceedingly high levels of cort transcript being produced by the transgene. Therefore, cort mRNA abundance in the rescued flies was analyzed by RT-PCR (Figure 5). Total ovarian RNA was isolated from an equal number of wild-type, grau, and grau + transgene females, and cDNA was prepared. PCR with cort-specific primers was then performed on varying amounts of these cDNAs. This analysis showed that the amount of PCR products was dose dependent. As previously demonstrated (Chen et al. 2000), the level of cort transcript in homozygous grau females was dramatically reduced from the wild-type level. The cort transgene in homozygous grau females increased this level, but the amount was less than that found in wild-type flies (Figure 5). This result was confirmed by Northern analysis (data not shown), but the low abundance of cort transcript in a grau mutant background made detection difficult. These results show that a modest increase in cort mRNA can rescue grau mutant embryos.

**DISCUSSION**

grau mutants produce protein products that are unable to bind DNA: All of the characterized grau mutations inhibit the ability of the Grau protein to interact with the promoter region of its target gene, cort. Single amino acid changes within two of Grau's eight zinc-finger motifs interfere with DNA binding. It would be interesting to investigate whether a mutation within each of Grau's eight zinc fingers would perturb DNA binding or whether any of the zinc fingers may serve other functions, such as mediating protein-protein interactions. The identification of which zinc fingers are required for DNA binding could be accomplished by mutagenesis in vitro. In addition, it would be useful to identify regions of the Grau protein that are critical to its function, but which do not interfere with DNA binding. The generation of additional alleles of grau (especially those that retain the ability to bind DNA efficiently) may provide insight into the mechanism by which Grau protein regulates meiosis.

The role of grau during oogenesis and beyond: Although the mutant phenotypes for which grau is recognized only occur during oogenesis and early embryogenesis, the grau transcript is expressed throughout development (Chen et al. 2000). The cort transcript, on the other hand, is only expressed maternally (T. Chu, unpublished result). These observations raise two questions: (1) How is Grau protein activity regulated so that...
it activates cort transcription only in the oocyte and early embryo? and (2) What role does Grau play during later developmental stages?

The first question has many possible answers. It is possible that Grau must be post-translationally modified to be active or that Grau requires a cofactor that is only present maternally. Conversely, an inhibitor of Grau may be present at later developmental stages, but is absent early in development. The discovery of the mechanism by which Grau is regulated would enhance our understanding of the role grau and cort play in the female meiotic cell cycle.

What role grau may play at later developmental stages remains unclear. Homozygous grau flies are viable, with no apparent defect. Only the eggs and embryos derived from homozygous females display a mutant phenotype. It is possible that Grau regulates the transcription of target genes later in development, but there are other genes with overlapping function. In the absence of transcriptional activation by Grau, these other gene(s) may be able to compensate.

While the role of grau during later developmental stages remains obscure, the role of grau during oogenesis is clearer. Increasing the expression of cort in grau homozygous females rescues the meiosis arrest seen in grau eggs and even allows these eggs to develop into adult flies.

It was unclear whether a cort genomic transgene (which is expressed under the normal cort regulatory sequences) would be able to rescue the grau mutant phenotype. The cort transcript is barely expressed in oocytes from grau mutant females (Chen et al. 2000), and the genomic transgene would also be expected to produce little transcript in the absence of grau. However, there is evidence that the amount of cort transcript expressed in grau homozygous females is poised on the threshold of what is required to drive the completion of meiosis. A small amount of cort transcript is expressed in grau mutant females, and ~3% of the embryos laid by these females are able to develop (Chen et al. 2000). Decreasing the dosage of wild-type cort in grau mutant females by half (homozygous grau, heterozygous cort) abolishes any development (Chen et al. 2000). These experiments suggest that the small amount of cort transcript produced in a grau mutant background (and therefore transcribed in a Grau-independent manner) accounts for the 3% development. Additionally, if a slight reduction in the amount of wild-type cort expressed in grau mutant females reduces the viability of their progeny to 0%, a slight increase in cort expression in grau mutant females may increase the viability of their progeny significantly.

Ovaries from flies homozygous for grau and carrying one copy of either of the two cort transgenic lines tested have more cort transcript than homozygous grau mutants that lack the transgene (Figure 5), but less than wild-type flies. This small increase in cort allows a much larger percentage of them to progress through meiosis. If grau were required for the expression of other target genes during this time, the increase in expression of cort would only be expected to (at most) partially rescue the defects seen in grau eggs. This does not exclude that grau plays a role in the transcriptional activation of other target genes during oogenesis, but indicates that the completion of meiosis does not require these gene products.

Regulation of the female meiotic cell cycle: Although the cloning of grau has revealed the requirement for transcriptional regulation during female meiosis in Drosophila, there is still much we do not know about the mechanisms that drive the second meiotic division. What is the biological function of cort, and how does it trigger progression through the metaphase-anaphase transition? In yeast, exit from mitosis requires proteolysis mediated by the anaphase promoting complex (APC) and its activator cdc20 (for reviews, see Morgan 1999; Zachariae and Nasmyth 1999). Many APC components are also upregulated during meiosis, suggesting that APC-mediated proteolysis may be required for exit from meiosis as well (Chu et al. 1998). The recent cloning of cort has revealed that the cort gene product may act as a cell cycle regulator (T. Chu, unpublished result).

Further characterization of cort and grau should provide valuable information about this pathway that specifically regulates the female meiotic cell cycle.

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