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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 *bicoid* mRNA due to a defect in the cytoplasmic polyadenylation of this message (Sallés *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

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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, *RM61*, *QF31*, *QE70*, *QQ36*, and *RG1*, and *cort* alleles, *QW55* 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-GrauΔC 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′ primer, 5′-CATGAATTGAATGGATATCT GCCGCCTCTG-3′, and the 3′ primer, 5′-CATGAATTCGGC GAACACCTTTCCGCAGTC-3′, both of which contain *Eco*RI restriction sites at their ends. The PCR product was digested with *Eco*RI and inserted into *Eco*RI-digested *pGEX-3X* vector (Amersham Pharmacia Biotech, Piscataway, NJ), forming the *GST-GrauΔC* fusion.

The wild-type GST-GrauΔN coding region was also amplified from a wild-type ovarian cDNA pool as described above using the 5′ primer, 5′-CATGAATTCCCCGAGTACATC AAATGCAAG-3′, and the 3′ primer, 5′-CATGAATTCTTCC GGGCCTAACCCGAATAG-3′. GST-GrauΔN E493K and GST-GrauΔN C298Y coding regions were amplified with the same primers as the wild-type version of GrauΔN using *Pwo* polymerase (Boehringer Mannheim). The GST-GrauΔN E493K coding region was amplified from an ovarian cDNA pool prepared from females carrying the $grau^{QE70}$ allele *in trans* to *Df(2R)Pu-D17*, a deficiency that deletes the *grau* gene. The GST-GrauΔN C298Y coding region was amplified from an ovarian cDNA pool prepared from females hemizygous for the *QQ36* allele of *grau.* The PCR products were digested with *Eco*RI and inserted into *Eco*RI-digested *pGEX-1* vector (Amersham Pharmacia Biotech).

All fusion plasmids were sequenced by automated sequencing (Applied Biosystems, Foster City, CA) to confirm that the fusions were in the correct reading frame and lacked PCR-induced mutations.

The fusion proteins were expressed in *Escherichia coli* BL21 cells according to 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 [^{32}P]γATP using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). GST-Grau proteins are able to bind to a 32-bp oligo (5′-TATCGAGTGTTCACTGTACTTGTAACTG TAAG-3′), but not to two smaller oligos (5′-CACTCCT ATCGAGTGTTCACT-3′ and 5′-CACTGTACTTGTAACTGT 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-GrauΔN E493K and GST-GrauΔN 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 (*plChsπΔ2-3*; 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 Na_2HPO_4, 35 mm NaH_2PO_4) 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 with mitotically dividing nuclei were scored as developing embryos.

Analysis of *cort* expression levels by RT-PCR: Twenty females of each genotype (wild type, $grau^{QF31}/grau^{QF31}$, and $grau^{QF31}/grau^{QF31}$; P [w^+ *cort*]) were maintained on yeast paste for 2 days, at which time their ovaries were dissected and used as a source of RNA. Total ovarian RNA was isolated using Trizol Reagent (GIBCO BRL, Rockville, MD) according to manufacturer's instructions. The total RNA from each genotype was reverse transcribed using Superscript II enzyme (GIBCO BRL) according to manufacturer's instructions. cDNAs from each genotype were diluted to 1 ovary per microliter.

PCR was performed using *cort*-specific primers (T. Chu, unpublished results). For PCR, the cDNAs were further diluted 1:100, and increasing amounts (1, 2, 5, and 10 μl) were used as template. A 20-cycle PCR amplification was performed at 45 sec at 93°, 1 min at 60°, and 1 min at 72°, with a final extension of 7 min at 72°. The amplification products were run on a 1.5% agarose gel and visualized by staining with ethidium bromide.

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 (Heinemeyer *et al.* 1998; Akiyama, http://www.rwcp.or.jp/papia/). The cap signal lies within many initiator elements and contains the transcription initiation site (Larsen *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

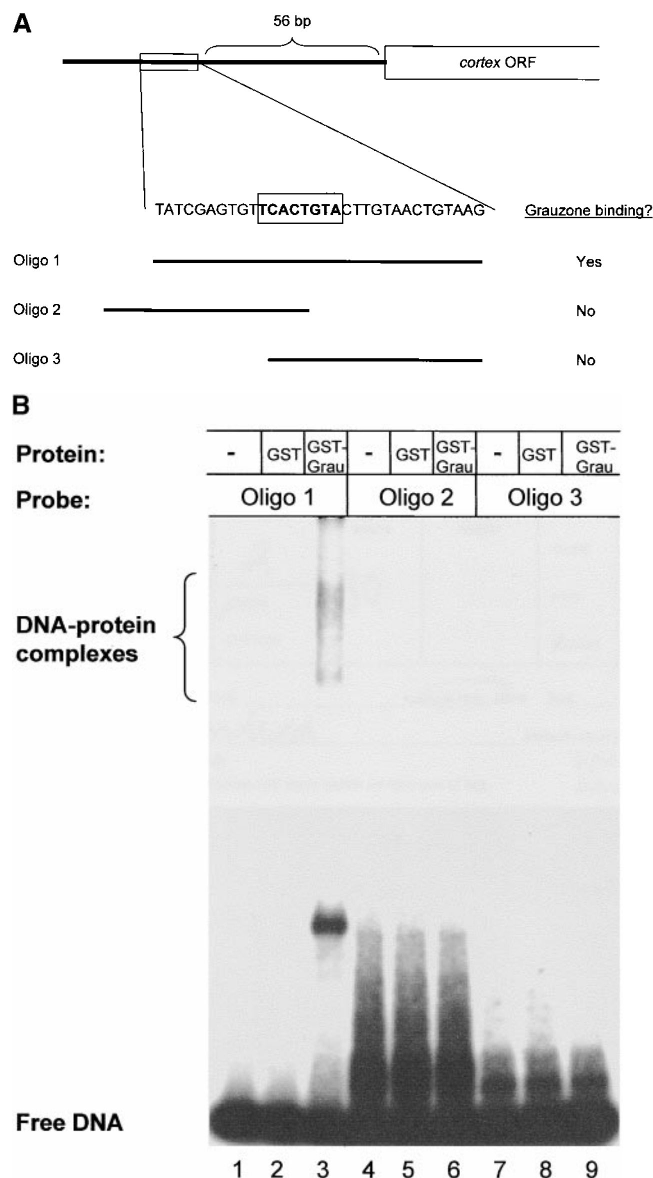

Figure 1.—Defining the Grau binding site. (A) Three different regions of the *cort* promoter were used as probes in gel mobility shift assays (oligos 1–3). Grau binds to a site 56 bp upstream of the *cort* open reading frame. By gel-shift assay, Grau binds to a 32-bp region of the *cort* promoter (oligo 1), which includes a putative cap signal for transcription initiation (boxed region). Gel-shift probes that bisect this motif (oligos 2 and 3) are unable to be bound by Grau in a gel-shift assay. A summary of the binding results is shown at the right. (B) GST-Grau was analyzed for its ability to bind to three different regions of the *cort* promoter by gel-shift assay. DNA probes used were oligo 1 (lanes 1–3), oligo 2 (lanes 4–6), and oligo 3 (lanes 7–9). DNA-binding reactions included either GST (lanes 2, 5, and 8) or GST-Grau protein (lanes 3, 6, and 9).

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

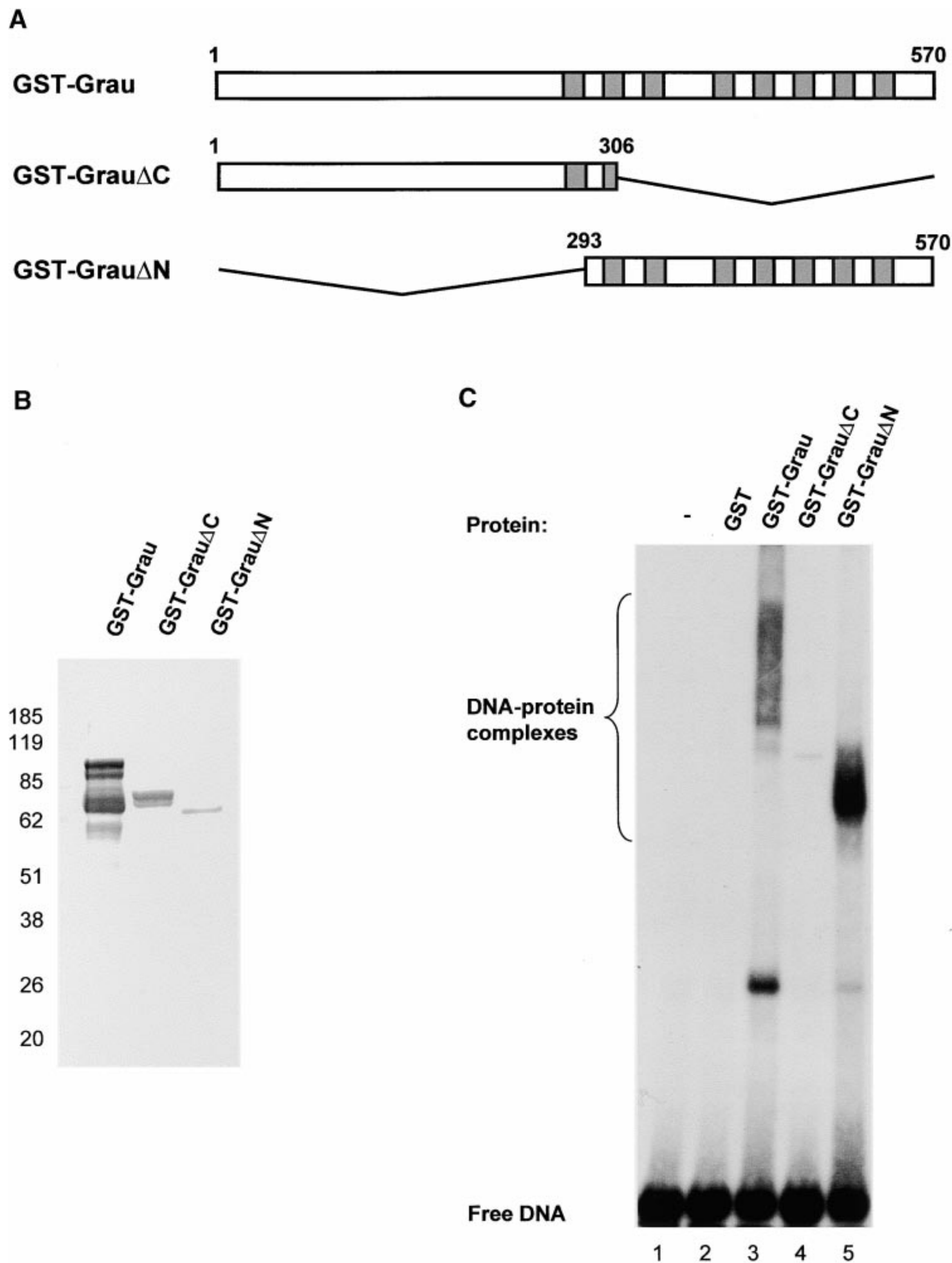

Figure 2.—Grau binds to DNA through its zinc-finger-rich C terminus. (A) Full-length and truncated versions of *grau* were cloned into bacterial expression vectors and used to express GST-tagged fusion proteins. Zinc-finger motifs are shown as shaded boxes, and amino acids are numbered. (B) A 10% SDS-PAGE 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.

zinc-finger motif contained in this fusion protein. These results suggest that Grauzone binds to its target DNA via the zinc-finger motifs.

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 *grau^{QE70}* and *grau^{QQ36}* alleles interfere with DNA binding: All five of the *grau* alleles

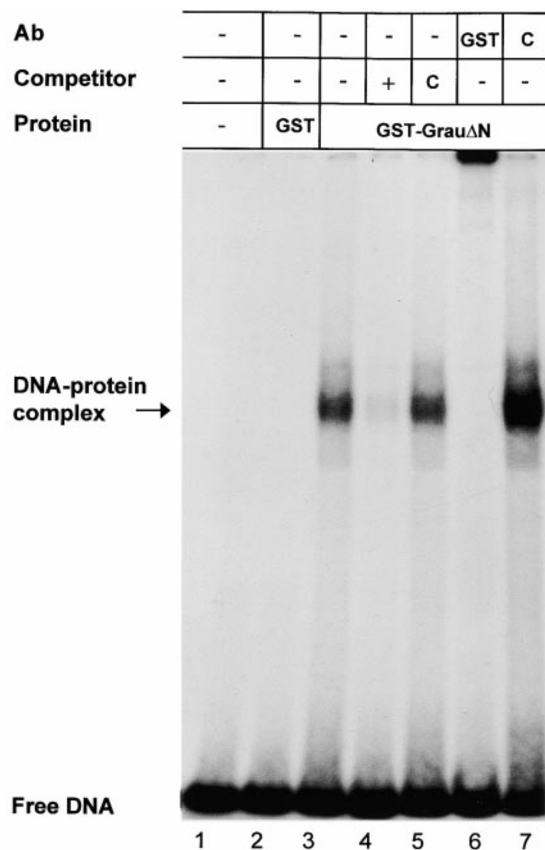

Figure 3.—GST-GrauΔN binds to the *cort* promoter in a sequence-specific manner. The specificity of binding to the *cort* promoter by the GST-GrauΔN fusion protein was analyzed by gel-shift assay. All reactions used a 32-bp region of the *cort* promoter as probe. DNA-binding reactions included either 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 (*grau*^{RM61} and *grau*^{QF31}) contain premature stop codons within their sequence. Both of these alleles would produce truncated proteins without a single 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, *grau*^{QE70} and *grau*^{QQ36}, contain single base-pair mutations within individual zinc-finger motifs (Chen *et al.* 2000). The *grau*^{QE70} allele contains a missense mutation leading to the conversion of Glu493 to Lys within the seventh of the eight zinc-finger motifs. The *grau*^{QQ36} 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 *grau*^{QE70} and *grau*^{QQ36} 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 *grau*^{QE70} allele, while GST-GrauΔN/C298Y contains the single amino acid change found in the *grau*^{QQ36} 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 *grau*^{QE70} and *grau*^{QQ36} 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 hemizy-

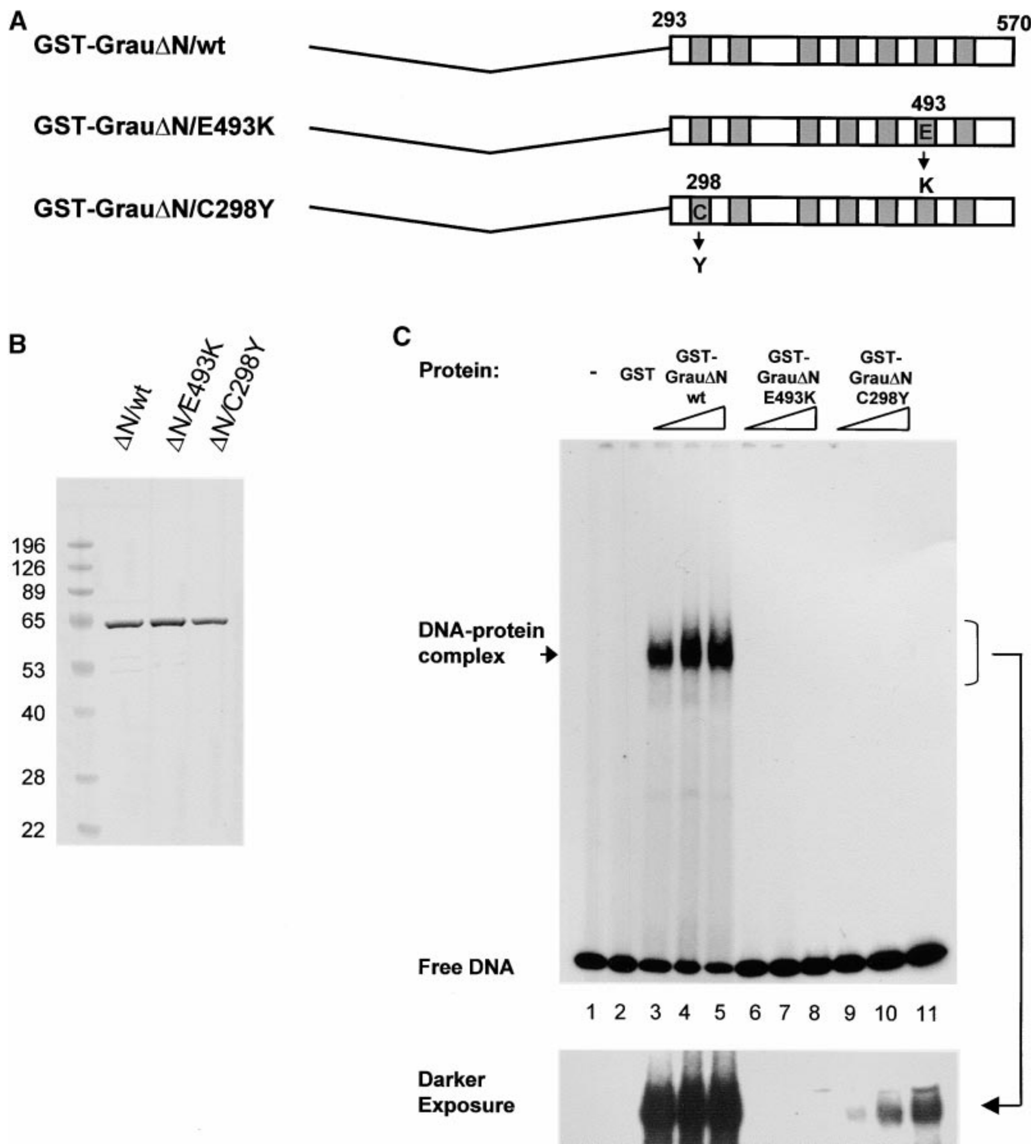

Figure 4.—Point mutations in the $grau^{QE70}$ and $grau^{QQ36}$ alleles interfere with DNA binding. (A) Point mutations corresponding to those found in two of the mutant *grau* alleles were introduced into the GST-GrauΔN construct and used to express GST-tagged fusion proteins. Single amino acid changes 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-GrauΔN E493K and C298Y 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-GrauΔN (lanes 3–5), GST-GrauΔN E493K (lanes 6–8), or GST-GrauΔN C298Y (lanes 9–11). Each version of the GST-GrauΔN 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-

TABLE 1

Overexpression of *cort* rescues the meiosis arrest of *grau* mutant embryos

Transgenic line	Maternal genotype	No. of embryos developing	Total no. of embryos	% of embryos developing
1	$grau^{QF31}/grau^{QF31}$	24	904	2.6
	$grau^{QF31}/grau^{QF31}$; *cort* transgene[a]	789	832	94.8
	$cort^{RH65}/cort^{RH65}$	0	872	0
	$cort^{RH65}/cort^{RH65}$; *cort* transgene	454	479	94.7
2	$grau^{QF31}/grau^{QF31}$	24	792	3.0
	$grau^{QF31}/grau^{QF31}$; *cort* transgene	555	628	88.4
	$cort^{RH65}/cort^{RH65}$	0	549	0
	$cort^{RH65}/cort^{RH65}$; *cort* transgene	437	472	92.6

[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 (~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.

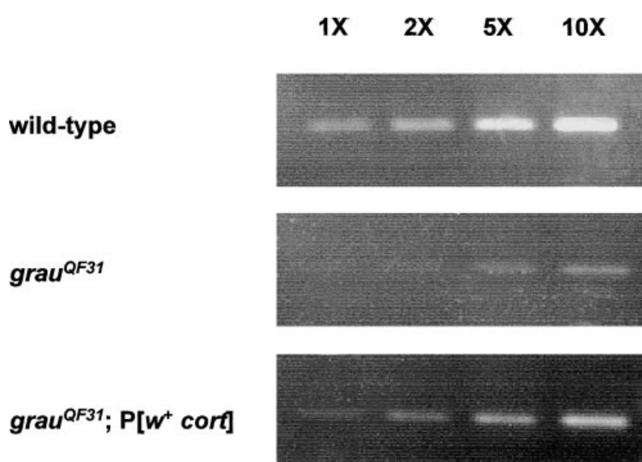

Figure 5.—*Grau* mutant ovaries containing a *cort* transgene produce only slightly more *cort* mRNA than *grau* mutant ovaries without the transgene. The amount of *cort* transcript present in *grau* mutant flies with and without the *cort* transgene was analyzed by RT-PCR. RNA was isolated from an equal number of flies for each genotype (wild type, $grau^{QF31}/grau^{QF31}$, and $grau^{QF31}/grau^{QF31}$; P [w^+ *cort*]), reverse transcribed, and amplified by PCR using *cort*-specific primers. Amplification was shown to be dose dependent by using increasing amounts of template DNA (1, 2, 5, and 10 μl).

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 ovaries 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 *grau* and *cort* should provide valuable information about this pathway that specifically regulates the female meiotic cell cycle.

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LITERATURE CITED

Bashirullah, A., S. R. Halsell, R. L. Cooperstock, M. Kloc, A. Karaiskakis *et al.*, 1999 Joint action of two RNA degradation pathways controls the timing of maternal transcript elimination at the midblastula transition in Drosophila melanogaster. EMBO J. **18:** 2610–2620.

Bernstein, B. E., R. C. Hoffman and R. E. Klevit, 1994 Sequence-specific DNA recognition by Cys2, His2 zinc fingers. Ann. NY Acad. Sci. **726:** 92–102.

Chen, B., E. Harms, T. Chu, G. Henrion and S. Strickland, 2000 Completion of meiosis in Drosophila oocytes requires transcriptional control by Grauzone, a new zinc finger protein. Development **127:** 1243–1251.

Chu, S., and I. Herskowitz, 1998 Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. Mol. Cell **1:** 685–696.

Chu, S., J. Derisi, M. Eisen, J. Mulholland, D. Botstein *et al.*, 1998 The transcriptional program of sporulation in budding yeast. Science **282:** 699–705.

Clancy, M. J., 1998 Meiosis: step-by-step through sporulation. Curr. Biol. **8:** R461–R463.

Heinemeyer, T., E. Wingender, I. Reuter, H. Hermjakob, A. E. Kel *et al.*, 1998 Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. Nucleic Acids Res. **26:** 362–367.

Hope, I. A., and K. Struhl, 1986 Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell **46:** 885–894.

Keegan, L., G. Gill and M. Ptashne, 1986 Separation of DNA

binding from the transcription-activating function of a eukaryotic regulatory protein. Science **231:** 699–704.
Klevit, R. E., 1991 Recognition of DNA by Cys2,His2 zinc fingers. Science **253:** 1367, 1393.
Larsen, N., J. Engelbrecht and S. Brunak, 1995 Analysis of eukaryotic promoter sequences reveals a systematically occurring CT-signal. Nucleic Acids Res. **23:** 1223–1230.
Lieberfarb, M. E., T. Chu, C. Wreden, W. Theurkauf, J. P. Gergen *et al.*, 1996 Mutations that perturb poly(A)-dependent maternal mRNA activation block the initiation of development. Development **122:** 579–588.
Mitchell, A. P., 1994 Control of meiotic gene expression in Saccharomyces cerevisiae. Microbiol. Rev. **58:** 56–70.
Morgan, D. O., 1999 Regulation of the APC and the exit from mitosis. Nat. Cell Biol. **1:** E47–E53.
Page, A. W., and T. L. Orr-Weaver, 1996 The *Drosophila* gene *grauzone* and *cortex* are necessary for proper female meiosis. J. Cell Sci. **109:** 1707–1715.
Page, A. W., and T. L. Orr-Weaver, 1997 Stopping and starting the meiotic cell cycle. Curr. Opin. Genet. Dev. **7:** 23–31.
Ptashne, M., 1988 How eukaryotic transcriptional activators work. Nature **335:** 683–689.
Rubin-Bejerano, I., S. Mandel, K. Robzyk and Y. Kassir, 1996 Induction of meiosis in Saccharomyces cerevisiae depends on conversion of the transcriptional repressor Ume6 to a positive regulator by its regulated association with the transcriptional activator Ime1. Mol. Cell. Biol. **16:** 2518–2526.
Sallés, F. J., M. E. Lieberfarb, C. Wreden, J. P. Gergen and S. Strickland, 1994 Coordinate initiation of Drosophila development by regulated polyadenylation of maternal messenger RNAs. Science **266:** 1996–1999.
Schüpbach, T., and E. Wieschaus, 1989 Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. Genetics **121:** 101–117.
Spradling, A. C., 1986 P element-mediated transformation, pp. 175–197 in *Drosophila: A Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.
Theurkauf, W. E., 1994 Immunoflourescence analysis of the cytoskeleton during oogenesis and early embryogenesis, pp. 489–505 in *Methods in Cell Biology*, edited by L. S. B. Goldstein and E. A. Fyrberg. Academic Press, New York.
Xu, L., M. Ajimura, R. Padmore, C. Klein and N. Kleckner, 1995 NDT80, a meiosis-specific gene required for exit from pachytene in Saccharomyces cerevisiae. Mol. Cell. Biol. **15:** 6572–6581.
Zachariae, W., and K. Nasmyth, 1999 Whose end is destruction: cell division and the anaphase-promoting complex. Genes Dev. **13:** 2039–2058.

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