

***Drosophila rhino* Encodes a Female-Specific Chromo-domain Protein That Affects Chromosome Structure and Egg Polarity**

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ABSTRACT

Here we describe our analyses of Rhino, a novel member of the Heterochromatin Protein 1 (HP1) subfamily of chromo box proteins. *rhino* (*rhi*) is expressed only in females and chiefly in the germline, thus providing a new tool to dissect the role of chromo-domain proteins in development. Mutations in *rhi* disrupt eggshell and embryonic patterning and arrest nurse cell nuclei during a stage-specific reorganization of their polyploid chromosomes, a mitotic-like state called the "five-blob" stage. These visible alterations in chromosome structure do *not* affect polarity by altering transcription of key patterning genes. Expression levels of *gurken* (*grk*), *oskar* (*osk*), *bicoid* (*bcd*), and *decapentaplegic* (*dpp*) transcripts are normal, with a slight delay in the appearance of *bcd* and *dpp* mRNAs. Mislocalization of *grk* and *osk* transcripts, however, suggests a defect in the microtubule reorganization that occurs during the middle stages of oogenesis and determines axial polarity. This defect likely results from aberrant Grk/Egfr signaling at earlier stages, since *rhi* mutations delay synthesis of Grk protein in germaria and early egg chambers. In addition, Grk protein accumulates in large, actin-caged vesicles near the endoplasmic reticulum of stages 6–10 egg chambers. We propose two hypotheses to explain these results. First, Rhi may play dual roles in oogenesis, independently regulating chromosome compaction in nurse cells at the end of the unique endoreplication cycle 5 and repressing transcription of genes that inhibit Grk synthesis. Thus, loss-of-function mutations arrest nurse cell chromosome reorganization at the five-blob stage and delay production or processing of Grk protein, leading to axial patterning defects. Second, Rhi may regulate chromosome compaction in both nurse cells and oocyte. Loss-of-function mutations block nurse cell nuclear transitions at the five-blob stage and activate checkpoint controls in the oocyte that arrest Grk synthesis and/or inhibit cytoskeletal functions. These functions may involve direct binding of Rhi to chromosomes or may involve indirect effects on pathways controlling these processes.

THE structure of chromatin powerfully influences gene expression and chromosome behavior in many systems (reviewed in SINGH and HUSKISSON 1998; PARO *et al.* 1998; STEGER *et al.* 1998). *Drosophila* oogenesis is an excellent model for examining the role of these processes in development. The egg chamber contains highly visible nurse cell chromosomes that undergo complex, dynamic changes throughout oogenesis. In addition, female-sterile mutations exist that alter nurse cell chromosome morphology, providing insight into the mechanisms that regulate these changes. Most such mutations cause egg chamber degeneration midway through oogenesis; a few, however, allow maturation of late-stage egg chambers that frequently exhibit specific eggshell

patterning defects (reviewed in SPRADLING 1993). Here we describe *rhino*, a new female-sterile gene required for normal nurse cell chromosome structural reorganization and for establishing egg polarity.

The *Drosophila* ovary consists of ovarioles where egg chambers develop in an assembly-line-like process. Each egg chamber contains 16 interconnected germline cells surrounded by a layer of somatically derived follicle cells. The first germline-derived cell becomes the oocyte while the other 15 become nurse cells. Early in oogenesis, nurse cell chromosomes undergo endoreplication, increasing in ploidy. During this time, homologous chromosomes remain paired. By stage four (S4), when the DNA content is $\sim 32C$, the polytene chromosomes show visible banding patterns. As endoreplication continues, homolog pairing weakens so that by S5, banding is lost but five distinct chromosome arms are still observable. This stage, called the five-blob stage, is transient. By S6, and for the rest of oogenesis, the chromosomes are diffuse and uniformly distributed throughout the nucleus. The nurse cell chromosomes continue endoreplicating until mid-oogenesis. By S10, the largest, most posterior nurse cells attain a DNA content of $\sim 1000C$ (KING

This article is dedicated to Joseph G. Gall, whose interest in chromosome behavior during development has inspired many scientists throughout their careers.

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1970; HAMMOND and LAIRD 1985; DEJ and SPRADLING 1999).

Some female-sterile mutations may provide clues to the mechanisms that regulate these dynamic changes in chromosome morphology and link these events to other aspects of egg chamber development, such as cell-cycle regulation, meiosis, eggshell synthesis, and the establishment of egg polarity. Certain alleles of the genes *ovarian tumor (otu)*, *suppressor of Hairy wing (su[Hw])*, *string of pearls (sop)*, *female sterile of Bridges (fs(2)B)*, *fs(2)cup (cup)*, and *morula (mr)* produce egg chambers in which nurse cell polytene chromosomes persist well past S4 (KING 1970; HEINO 1989; SPRADLING 1993; CRAMTON and LASKI 1994; HEINO *et al.* 1995; REED and ORR-WEAVER 1997). For example, mutations in *sop* arrest oogenesis at S5, when pairing has begun to break down but five distinct chromosome arms are still visible in the nurse cells. Prior to degeneration, several consecutive S5 egg chambers are present within each ovariole, leading to the name *string of pearls* (CRAMTON and LASKI 1994). *sop* encodes a ribosomal protein, but how this gene product affects chromosome structure is unclear. Similarly, elegant genetic studies reveal an interaction between *otu* and *cup* (KEYES and SPRADLING 1997), but molecular analyses do not provide a clear mechanism for their effect on chromosome morphology, since both genes encode novel cytoplasmic proteins (STEINHAEUER *et al.* 1989; KEYES and SPRADLING 1997). In contrast, studies on *morula* reveal a direct role in cell-cycle regulation and predict a distinct mitotic endo cell cycle at S5 (REED and ORR-WEAVER 1997), a prediction borne out by work of DEJ and SPRADLING (1999). Mutations in *morula* alter cell-cycle controls, allowing a mitotic-like state with condensed chromosomes and the formation of spindles (REED and ORR-WEAVER 1997). Unlike mutations in *otu* and *cup*, however, these defects do not alter egg polarity (T. L. ORR-WEAVER, personal communication).

Other mutations that affect nurse cell development or number eventually lead to egg chamber degeneration in which abnormal nuclear morphology, including highly condensed chromatin, may be observed (reviewed by SPRADLING 1993). Some such degenerating mutations are not fully penetrant and allow production of a few late-stage egg chambers that exhibit specific patterning defects. For example, abnormal chromosome morphology and eggshell defects are sometimes seen in *Bicaudal-C (Bic-C)*, *pipsqueak (psq)*, and *Dp* mutants (SIEGEL *et al.* 1993; SPRADLING 1993; MYSTER *et al.* 2000). SAFFMAN *et al.* (1998) propose a model in which Bic-C affects translation through its activity as an RNA binding protein. In contrast, *psq* encodes a protein with a BTB/POZ domain, a motif found in transcriptional activators (HOROWITZ and BERG 1996; LEHMANN *et al.* 1998), while *Dp* encodes a subunit of the E2F transcription factor (reviewed by LA THANGUE 1994).

How do these mutations affect patterning? Most such

mutations disrupt the synthesis or localization of Gurken (Grk), a TGF α -like molecule that plays a key role in establishing anterior-posterior and dorsoventral polarity in the egg and embryo (reviewed in NILSON and SCHÜPBACH 1999). Early in oogenesis *grk* mRNA is localized to the posterior of the oocyte. Signaling to posterior follicle cells through the epidermal growth factor receptor (Egfr) pathway determines posterior follicle cell fate. At \sim S7, a reciprocal signal from posterior follicle cells to the oocyte induces a microtubule rearrangement that leads to the correct localization of axis determinants: *oskar (osk)* at the posterior, *bicoid (bcd)* at the anterior, and *grk* in a dorsal anterior cap above the oocyte nucleus. Grk then participates in a second signaling pathway to the dorsal follicle cells, inducing a patterning process that establishes dorsal cell fates in the eggshell and eventually leads to correct ventral cell fates in the embryo. The eggshell patterning process involves an autocrine mechanism in which Argos mediates inhibition of Egfr activity in a midline domain between two populations of dorsal follicle cells (reviewed in STEVENS 1998). Since the dorsal follicle cells respond to the signaling cascade by migrating and secreting the chorion that forms the dorsal respiratory appendages of the eggshell, defects in dorsal-ventral determination can be readily observed by examining eggshell phenotypes.

Here we describe *rhino (rhi)*, a female-sterile mutant noted for its eggshell dorsal appendage defects. Our studies show that *rhi* mutants have an aberrant nurse cell chromosome structure and that *grk* and *osk* transcripts are mislocalized. Moreover, Grk protein first accumulates slowly but later is present in high amounts in the oocyte in large vesicles near the endoplasmic reticulum. We have cloned *rhi* and found that it encodes a protein with homology to members of the chromo-domain family of proteins. The best-characterized members of this family (*e.g.*, HPI, reviewed by EISSENBERG and ELGIN 2000) interact with other proteins in the formation of a heritable chromatin domain that represses gene transcription. Some members of this family are important for centromere function and also anchor chromatin to the inner nuclear membrane (reviewed in CAVALLI and PARO 1998). *rhi* is required for a specific developmental transition in chromosome reorganization and coordinates these nuclear morphological changes with egg chamber maturation and axis formation.

MATERIALS AND METHODS

Fly stocks: Canton-S and *cn; ry⁵⁰⁶* were used as wild-type controls. *rhi¹* [originally called *fs(2)ryI*], was generated in a *P[ry⁺]* screen described in BERG and SPRADLING (1991) and is carried as a *cn rhi¹/CyO, cn²; ry⁵⁰⁶* stock. *rhi²*, formerly *rhi²⁰⁸⁶*, was generated in a *P[lacZ, ry⁺]* mutagenesis screen described in KARPEN and SPRADLING (1992) and is carried as a *cn rhi²/CyO, cn²; ry⁵⁰⁶* stock. Fly stocks were maintained at 24° on standard cornmeal/yeast/molasses medium.

Excision screens: Excision alleles were generated as de-

scribed previously (HOROWITZ and BERG 1995) except that background mutations were eliminated by employing isogenized lines generated after outcrossing to *cn*; *ry*⁵⁰⁶ flies, the starting strain for both original mutagenesis screens. Several screens yielded 169 independent *ry*⁻ lines consisting of 7 fertile lines, 114 sterile lines, and 48 lethal or semilethal lines. This frequency of fertile revertants is unusually low and may be due to the insertion of both *P* elements in coding regions (see RESULTS). Presumably, perfect double-strand break repair must occur with the wild-type homolog to generate precise excisions and restore a valid open reading frame (ENGELS *et al.* 1990).

The extent of the deletions in nine female-sterile lines (four derived from *rhi*¹ and five derived from *rhi*²) was mapped at the molecular level. Six contained internal *P*-element deletions, including one line, *rhi*^{2S17}, in which virtually all of the *P*-element had been deleted, leaving only ~50 bp of 5' *P*-element end. In three lines, the ends of the *P* element and the flanking DNA remained intact (data not shown); in these cases, a small internal deletion was inferred by the *ry* phenotype. Forty-eight lines were either lethal or semilethal and nine were chosen for complementation analysis. Four excision alleles of the *PZ* element (*rhi*^{2L12}, *rhi*^{2L13}, *rhi*^{2dL14}, and *rhi*^{2dL15}) belonged to the same complementation group. Southern blot analyses of DNA from *rhi*^{2L13} and *rhi*^{2dL15} revealed that these lines retain intact *P*-element ends, with no apparent loss of flanking DNA detected ~12 kb 5' and 8.5 kb 3' to the insertion site. These results, along with transcript mapping and expression data (see below), suggest that the lethality is not due to disruption of the *rhi* gene. We speculate that a nearby essential gene contains a hotspot for *P*-element insertion and local hopping into this gene is responsible for the lethality.

4',6-Diamidino-2-phenylindole and Sytox green staining of ovaries: Ovaries were dissected in phosphate-buffered saline (PBS, 130 mM NaCl, 7 mM Na₂HPO₄, 3 mM NaH₂PO₄, pH 7.0) and fixed for 20 min at room temperature in 4% paraformaldehyde in PBTE (PBS plus 0.2% Tween-20, 1 mM EDTA). Fixed ovaries were rinsed once with PBTE, permeabilized in PBTE plus 1% TRITON X-100 for 1 hr at room temperature and rinsed again. They were then stained with 0.2 μg/ml 4',6-diamidino-2-phenylindole (DAPI) in PBTE for at least 30 min at room temperature, rinsed several times, and mounted in PBTE plus 50% glycerol. Microscopy was carried out with a Nikon Microphot FXA microscope; photographs were digitally scanned and then manipulated in Adobe Photoshop.

For confocal microscopy, egg chambers were dissected in PBS and fixed as above in PBTE/4% paraformaldehyde. Fixed egg chambers were then washed three times for 5 min in TBST (25 mM Tris, 140 mM NaCl, 2.6 mM KCl, 0.2% Tween-20, pH 7.4). Sytox green (Molecular Probes, Eugene, OR) was added to a final concentration of 20 μM in TBST, incubated at room temperature for 1 hr, and washed five times 5 min with TBST. Egg chambers were mounted in 50% glycerol/TBST plus Vectashield antifade reagent (Vector, Burlingame, CA) and examined on a Bio-Rad (Hercules, CA) MRC600 laser scanning confocal microscope. Images were transferred to Adobe Photoshop and adjusted for optimal brightness and contrast.

Whole mount *in situ* hybridization: cDNA *in situ* hybridizations were carried out as described previously (GILLESPIE and BERG 1995). Digoxigenin probes were prepared using a Boehringer Mannheim (Indianapolis) DNA labeling and detection kit. To prepare probes, we used a *rhi* cDNA (this article), a *grk* cDNA (provided by Trudi Schüpbach), a *dpp* cDNA (provided by Rick Fehon), an *osk* cDNA (provided by Ruth Lehmann), and a *bcd* cDNA (provided by Markus Noll). To allow direct comparison of expression levels in wild type and mutant, we set up equivalent conditions within these samples by opti-

mizing several aspects of the protocol. Newly eclosed females were aged 2 days on wet yeast to ensure a distribution of stages similar to wild type. An equal volume of tissue was fixed and all samples were treated identically throughout the procedure. Equimolar amounts of probes were used, resulting in 5-min staining reactions for *grk* and *osk*, 15 min for *bcd*, and 60 min for *dpp*.

Immunohistochemistry: Analysis of Grk protein levels and distribution was carried out as described by QUEENAN *et al.* (1999). Monoclonal anti-Grk antibody 1D12 was provided by Trudi Schüpbach and used at a dilution of 1:10. Alexa-488 conjugated anti-mouse secondary antibody (Molecular Probes) was used at a dilution of 1:500. Ovaries were triple stained with rhodamine-phalloidin (2 units/ml) and DAPI (0.2 μg/ml) and examined using a Nikon Microphot FXA. Confocal images were collected using a Bio-Rad MRC600 microscope.

Molecular characterization of *rhino*: Cloning DNA flanking the *P* elements: Genomic DNA flanking the *ry11* *P* element in *rhi*¹ was cloned as follows: Total genomic DNA from *rhi*¹ adults was cut to completion with *Bam*HI, ligated into λ-arms (Stratagene, La Jolla, CA), and packaged using the Gigapack Gold commercial extract from Stratagene. The resulting library was screened with a probe to the 5' *P*-element end and phage containing an insert composed of *P*-element sequences plus 1.8 kb of genomic DNA was isolated. The entire 5.6-kb insert was subcloned into pBluescript KS(+) (Stratagene) to create pCG5'-5.

Genomic DNA flanking the *PZ* element in *rhi*² was cloned by plasmid rescue (ASHBURNER 1989) using the restriction enzymes *Xba*I and *Nhe*I. The resulting plasmid, pR1, contains 2.3 kb of genomic DNA flanking the 5' end of the *PZ* insertion.

The sites of *P*-element insertion for *rhi*¹ and *rhi*² were determined by sequencing pCG5'-5 and pR1 using the 5' *P*-element primer IRXb: 5'-GCTCTAGACGGGACCACCTTATGT-3' and comparing the resulting sequence to *rhi* cDNA and genomic sequence (see below).

Isolation and characterization of *rhino* cDNA and genomic clones: Seven *rhi* cDNAs of 1.6 kb length were isolated from an ovarian λgt22 library (gift of Peter Tolias, Public Health Research Institute, New York; see STROUMBAKIS *et al.* 1994) by screening with the 1.8-kb *Hind*III genomic fragment from clone pCG5'-5. All had similar restriction maps; one was subcloned into pBluescript KS(+) to create pBSB2.

pBSB2 was sequenced by the dideoxy chain termination method using reagents supplied by United States Biochemical (Cleveland). Sequence data were compiled and organized using the IntelliGenetics program. BLASTp from National Institutes of Health (ALTSCHUL *et al.* 1990) and Genetics Computer Group (University of Wisconsin, Madison, WI) were used for database searches and alignments of homologous protein sequences. The *rhi* cDNA matches the Berkeley *Drosophila* Genome Project gene CG10683.

The cosmid 13H6, which maps to 54C, was obtained from the *Drosophila* Genome Mapping Project, courtesy of Dr. Inga Siden Kiamos (Foundation for Research and Technology-Hellas, Crete, Greece; see SIDEN-KIAMOS *et al.* 1990 and KAFATOS *et al.* 1991). Southern hybridization demonstrated that sequences homologous to the *rhi* cDNA were present within this genomic clone. A 6.5-kb *Eco*RI fragment that hybridized to the *rhi* cDNA was subcloned from cosmid 13H6 into pCaSpeR4 to create pHH76. Sequence analysis of this subclone permitted identification of the two introns within the *rhi* gene. This clone and cosmid 13H6 do not contain the final (third) exon of *rhi*. The absence of other introns within this final exon was established by its identification as one contiguous stretch of sequence within the bacterial artificial chromosome (BAC) clone, BACR12023 (accession no. 007697) of the Berkeley *Drosophila* Genome Project (<http://flybase.bio.indiana.edu>).

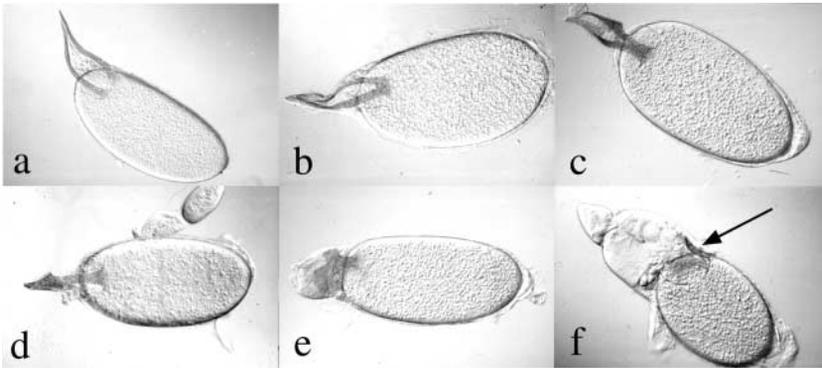


FIGURE 1.—Eggshells from *rhino* females display a range of dorsal-ventral defects. All alleles produce each phenotype although frequencies differ depending on allele strength. Anterior is to the left and dorsal is facing out of the page. (a) A wild-type S14 egg chamber has two dorsal appendages equidistant from the dorsal midline. (b) A weak *rhino* phenotype in which the appendages are fused at their bases only. (c) A forked dorsal appendage is typical of a ventralized eggshell. (d) Stronger allelic combinations produce partially dorsalized, short eggs; the appendages are broadly fused on the midline and carry extra spade-like material at the tip. (e) A large paddle of chorionic appendage material is observed here atop nurse cell remnants. (f) A strong dumpless phenotype in which shortened dorsal appendages (arrow) extend out over nurse cells, which failed to transfer their cytoplasmic contents into the oocyte and then undergo apoptosis.

Analysis of deletions in *rhino* excision alleles: A genomic restriction map for the *rhi* locus was deduced from restriction digest analysis of the cosmid 13H6 and Southern analysis of total fly DNA isolated from wild-type and *rhi* mutant flies. DNA deletions present within the *rhi* excision alleles were mapped by Southern analysis of total fly DNA using *rhi* and *P*-element probes.

Northern blot analysis: RNA was prepared from adult flies, ovaries, and all developmental stages by the hot phenol method (JOWETT 1986). Hybridization was performed as described in GILLESPIE and BERG (1995). All probes were labeled by the random hexamer-primed method (FEINBERG and VOGELSTEIN 1983).

The GenBank accession number for the *Drosophila melanogaster rhino* cDNA is AF411862.

RESULTS

Isolation of *rhino* mutations: *rhi*¹ and *rhi*² are female-sterile mutations that were isolated in two independent, large-scale *P*-element mutagenesis screens. *rhi*¹ contains an insertion of a γ 11 element and *rhi*² contains an insertion of a *P*[*lacZ*, γ ⁺] (*PZ*) element (BERG and SPRADLING 1991; KARPEN and SPRADLING 1992). Both *P* elements map to the 54C/D border by *in situ* hybridization (data not shown; FlyBase places this locus at 54D5). Females homozygous for *rhi*¹ laid fewer eggs than wild-type flies do. Almost all laid eggs exhibited dorsal appendage defects (Figure 1; Table 1), and many had the single dorsal appendage for which *rhino* is named. *rhi*² homozygotes laid more eggs than did *rhi*¹ flies and almost half looked wild type. *rhi*² mutants also exhibited a mild dumpless phenotype; in some egg chambers, the nurse cells failed to transfer their contents into the oocyte at S11 (for review see COOLEY and THEURKAUF 1994). Finally, DAPI staining and other lines of evidence suggested that eggs laid by *rhi* mutant females either are not fertilized or arrest early in embryonic development (data not shown).

We generated new *rhi* alleles through transposase-induced excision of each *P* element, using Δ 2-3 transposase (ROBERTSON *et al.* 1988) and scoring for loss of the γ ⁺ eye-color marker. This approach also allowed isolation of deficiencies for the region, since no dele-

tions had been described. Multiple excision screens resulted in 169 independent γ ⁻ lines. Precise excision of the *P* element produced 7 fertile revertant lines, indicating that the female sterility of the starting lines was indeed caused by the insertions. Most γ ⁻ excision lines (114) were female sterile, however, and exhibited eggshell phenotypes similar to those of their respective starting lines. Molecular analyses of a subset of these lines revealed that imprecise excision had left a portion of the *P* element within the *rhi* gene. Two of these female-sterile excision lines, *rhi*^{1-S6} and *rhi*^{2-S18}, are described in more detail here (Table 1). The remaining 48 lines were either lethal or semilethal. Complementation testing and Southern blot analyses of a subset of these lines, together with transcript mapping and expression data (see below), suggested that the lethality was not due to disruption of the *rhi* gene. One possibility is that a nearby essential gene contains a hotspot for *P*-element insertion and local hopping into this gene is responsible for the lethality. Finally, one lethal excision line, *rhi*^{2-L12}, contains a deletion of genomic DNA extending at least 11 kb from the insertion point, removing the presumptive start site for *rhi* transcription (see below) as well as a putative neighboring gene of unknown function, CG18186. Thus, *rhi*^{2-L12} is deficient for the region and can be considered null for *rhi* function. We rename this deletion *Df*(2R)*rhi*^{2-L12}.

Mutations in *rhino* disrupt dorsal appendage structures of the eggshell: Eggs laid by *rhi* mutants displayed a range of late-stage eggshell phenotypes (Figure 1; Table 1). A wild-type S14 egg has two dorsal anterior respiratory appendages that are equidistant from the dorsal midline (Figure 1a). *rhi*¹ females laid a few wild-type eggs (5%) but most eggs had a single dorsal appendage fused on the dorsal midline. Some eggs carried two appendages that emanated from one base; such phenotypes are produced by weak ventralizing mutations. Other eggs had a single dorsal appendage with extra appendage material; the eggs themselves were shorter than wild-type eggs. These characteristics are produced by dorsalizing mutations. This variability in patterning

TABLE 1
Molecular lesions and eggshell phenotypes of *rhino* mutants

Genotype	Molecular lesion	Eggshell phenotype ^a				<i>n</i> ^b
		% wild type	% weak	% strong	% dumpleless	
<i>cn; ry</i>	None	99	1	0	0	568
<i>rhi</i> ¹	<i>P[ry11]</i> insertion at nucleotide 55	5	26	66	3	100
<i>rhi</i> ¹⁻⁵⁶	Internal deletion of <i>rhi</i> ¹ <i>P</i> -element insertion	9	40	27	24	125
<i>rhi</i> ²	<i>P[ry</i> ⁺ ; <i>lacZ</i>] insertion at nucleotide 267	50	14	25	11	296
<i>rhi</i> ^{2-SL15}	Internal deletion of <i>rhi</i> ² <i>P</i> -element insertion	16	21	38	25	113
<i>rhi</i> ^{2-S18} / <i>rhi</i> ²	Internal deletion of <i>rhi</i> ² <i>P</i> -element insertion	15	5	77	3	70
<i>Df(2R)rhi</i> ^{2-L12}	Deletion of 5' start site	NA	NA	NA	NA	NA

NA, not available.

^a "Weak" refers to an eggshell in which fusion of the dorsal appendages occurs solely at the bases; all other aberrant phenotypes are considered "strong."

^b Total number of eggshells scored.

defects typifies mutations that disrupt the synthesis or distribution of Grk; small changes in the concentration of morphogen lead to dramatic differences in eggshell structures (ROTH and SCHÜPBACH 1994; GHABRIAL *et al.* 1998). Eggs laid by *rhi*² mothers also displayed diverse eggshell phenotypes but the range of phenotypes clearly differed from that caused by the *rhi*¹ mutation. Fifty percent of eggs laid by *rhi*² mothers were indistinguishable from wild-type eggs and 11% exhibited some degree of incomplete cytoplasmic transfer from the nurse cells. Dorsal appendage defects observed in *rhi*² eggs again varied greatly, resembling those of *rhi*¹ eggs. Excision alleles and heteroallelic combinations displayed highly variable eggshell phenotypes similar to those seen in the starting lines; examples are shown in Figure 1. Most alleles also produced a variable number of dumpleless egg chambers (Table 1). Figure 1b shows a weakly ventralized eggshell with two appendages fused at their bases; forked dorsal appendages were also common (Figure 1c). Appendages that were fused along their entire length usually displayed a spade-like tip (Figure 1d), unusual for most ventralizing mutants but typical for *rhi* and similar to defects produced by gain-of-function alleles of *psq* (HOROWITZ and BERG 1996). Excess chorion material on a single broad dorsal appendage coupled with a truncated egg shape was also observed and represents a weakly dorsalizing phenotype (Figure 1e). Some eggs failed to transfer their nurse cell contents into the oocyte, due either to a direct dumping defect or to premature migration of centripetal follicle cells. These eggs exhibited remnants of degenerating nurse cells (Figure 1, e and f). Rarely, we observed egg chambers with two micropyles (data not shown). Finally, females carrying any allele *in trans* to *Df(2R)rhi*^{2-L12} laid few or no eggs. Dissected ovaries revealed a higher incidence of degenerating egg chambers and a higher frequency of strong eggshell phenotypes compared with phenotypes produced by homozygotes or mutants carrying various heteroallelic combinations (data not shown).

The increased severity of phenotypes in hemizygous animals demonstrates that these *P*-element mutations are not null.

These data suggest an allelic series in which *rhi*² and sterile *rhi*² excision alleles are weaker than *rhi*¹ and its sterile derivatives. Further, all *P* alleles provide more function than the deficiency chromosome. Interestingly, all *P* alleles also produce a range of eggshell phenotypes. This variability could be due to the molecular mechanism that allows some function from these *P* alleles or to a partial redundancy in the process in which Rhi functions.

Molecular structure and expression of the *rhino* gene:

To characterize the *rhi* gene (Figure 2A), we cloned DNA sequences flanking the 5' ends of the two *P*-element insertions. We used the 1.8 kb of DNA adjacent to *ry11* in *rhi*¹ to probe a Northern blot of RNA from wild-type females, wild-type males, and *rhi*¹ females. In wild-type females, a highly abundant 1.6-kb transcript was present and enriched in ovaries. This same transcript was not detected in wild-type males nor in *rhi*¹ females (data not shown). We refer to this 1.6-kb transcript as the *rhi* transcript. We observed a less abundant >9-kb transcript in RNA from all three sources. Using this same genomic probe, we isolated a 1.6-kb cDNA from an ovarian cDNA library (STROUMBAKIS *et al.* 1994). When this cDNA was used to probe Northern blots (Figure 2B), it hybridized to the same 1.6-kb ovary-enriched transcript and to a higher molecular weight transcript of >9 kb present in wild-type female and male RNA. We detected this large message in all mutants tested; the *P* elements do not appear to disrupt this transcript. Although the 1.6-kb transcript was highly enriched in ovaries, some *rhi* transcript was also observed in RNA prepared from *osk*³⁰¹ flies, which lack a germline (EPHRUSSI *et al.* 1991). By this criterion, *rhi* is expressed in both germline and somatic cells.

The *rhi* transcript was not detected in RNA prepared from *rhi*¹ and *rhi*² homozygous females (Figure 2B) nor

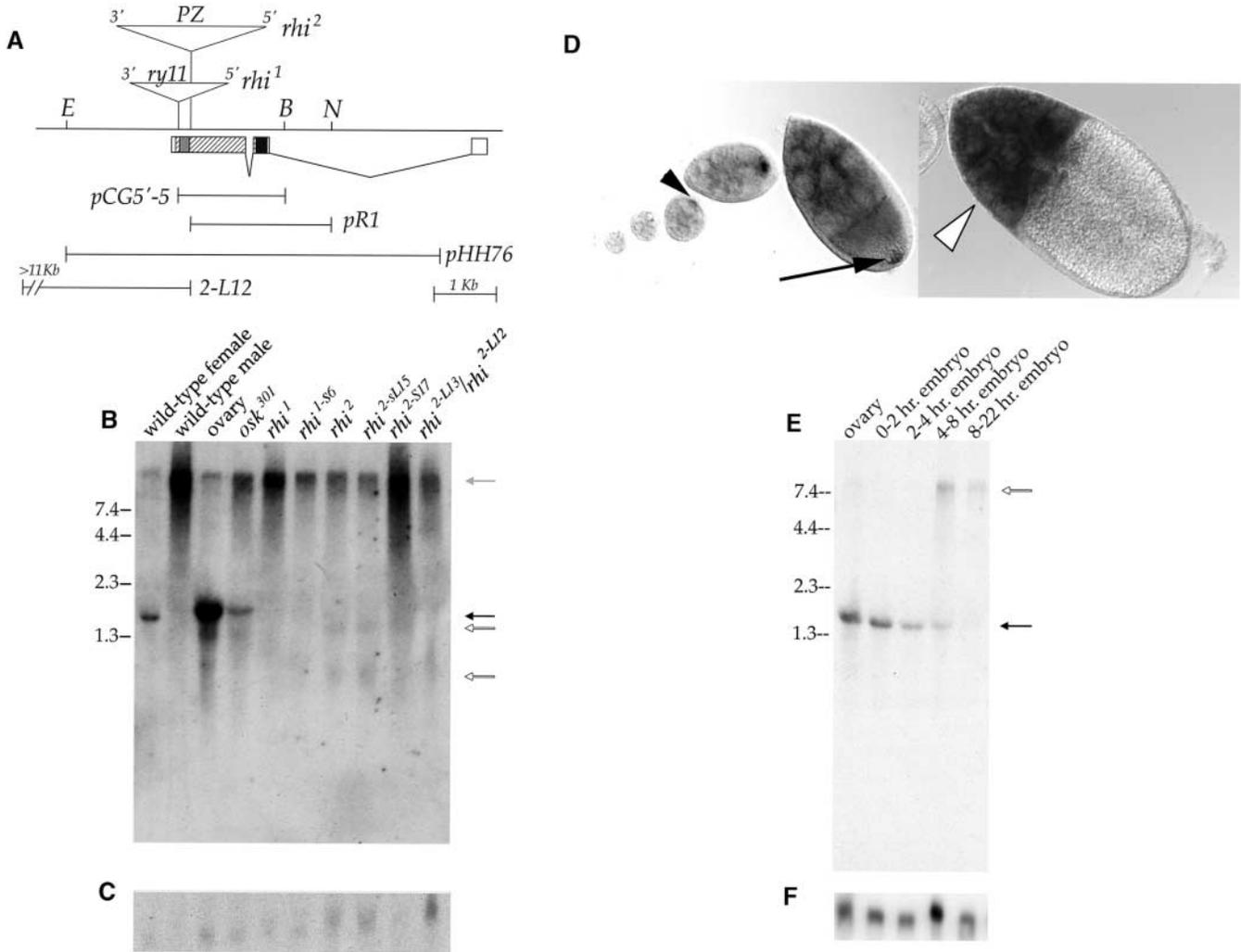


FIGURE 2.—Molecular and expression analyses of *rhino*. (A) Molecular map of *rhi* locus based on genomic blots of *rhi*¹ and *rhi*² flies and DNA from cosmid 13H6 (EDGP). The *rhi* transcript is indicated by boxes connected with thin lines; the hatched regions encode protein, the gray box represents the chromo domain, the black box represents the chromo-shadow domain. The *P* elements inserted in *rhi*¹ and *rhi*², shown above the line that indicates genomic DNA, are not diagrammed to scale. pCG5'-5 denotes a plasmid containing DNA flanking *rhi*¹; pR1 contains DNA flanking *rhi*². pHH76 is a subclone made from cosmid 13H6 (not shown). The deletion in *Df(2R)rhi*^{2-L12} is indicated by thin lines; a notch marks the 3' deletion endpoint; the 5' endpoint is unknown but resides at least 11 kb 5' to the region shown here. Restriction sites correspond only to the genomic map: E, *Eco*RI; B, *Bam*HI; N, *Nhe*I. (B) Northern blot probed with the 1.6-kb *rhi* cDNA. Lanes contain 35 μ g of total RNA. The 1.6-kb *rhi* transcript is female specific and is highly enriched in the ovary (solid arrow). *osk*³⁰¹ females have no germline and produce very low levels of the 1.6-kb transcript. This transcript is apparently absent in RNA from *rhi* homozygous females. Low levels of truncated transcripts are visible in *rhi*² and *rhi*^{2-S15} females (open arrows). These truncated transcripts also hybridize to sequences present in the *l(3)S12* gene (data not shown). A >9-kb transcript is not affected in any *rhi* mutants (gray arrow). (C) The same blot reprobed with the ribosomal gene *rpS5* as a loading control. (D) *rhi* cDNA used as a probe to wild-type ovaries. The *rhi* transcript is present at low levels in the germarium (not shown) and begins to accumulate in the oocyte at S5 (arrowhead). *rhi* mRNA is localized to the posterior of the oocyte at S9 (arrow) but is no longer localized by S10. At S10B, *rhi* transcription is strongly induced in the nurse cells (open arrowhead). (E) Developmental Northern blot probed with the *rhi* cDNA. The 1.6-kb transcript is indicated with a solid arrow. It is abundant in ovaries and its level gradually tapers off throughout embryogenesis. The >9-kb transcript is predominant in late embryogenesis (open arrow). (F) The same blot reprobed with the ribosomal gene *rp49* as a loading control.

in RNA from flies carrying any of the sterile excision alleles. *rhi*² and *rhi*^{2-S15} females produced two lower molecular weight transcripts of \sim 1.3 and 0.5 kb; these messages were observed with long exposures and appeared somewhat heterogeneous in length. These messages also hybridized to a probe for the putative *l(3)S12* gene

(data not shown), the 3' end of which is contained in the *PZ* element (HOROWITZ and BERG 1995). These RNAs most likely are truncated *rhi* transcripts that terminate in and/or splice into the *l(3)S12* gene present in the *PZ* element. Consistent with this hypothesis, the *rhi*^{2-S17} mutation deletes the *l(3)S12* sequences and RNA

from flies carrying this allele does not exhibit the 1.3- and 0.5-kb transcripts.

To determine when and where *rhi* is expressed during oogenesis, we performed *in situ* hybridizations to ovaries using a digoxigenin-labeled *rhi* cDNA probe (Figure 2D). *rhi* transcript is first detectable at low levels in region I of the germarium, where it is localized perinuclearly in the germ cells (data not shown). By S5, *rhi* transcript accumulates in the oocyte and is later found at the posterior of S8–10A egg chambers (Figure 2D). By S10B, this posterior localization disappears. Finally, transcript levels increase dramatically in the nurse cells at S10 (Figure 2D) and *rhi* mRNA is loaded into the oocyte as maternal message (Figure 2E, 0–2 hr lane). We also performed Northern analysis on RNA isolated from embryonic stages (Figure 2E). The 1.6-kb *rhi* transcript was present in high levels in ovaries and in 0- to 2-hr embryos and then tapered off to low but detectable levels later in embryogenesis. This expression profile is typical for maternal transcripts that are deposited into the embryo.

***rhino* (CG10683) encodes a novel member of the chromo-domain family:** Sequence analysis of the 1.6-kb cDNA revealed a single open reading frame that on conceptual translation encodes a protein of 418 amino acids with a predicted molecular weight of ~46 kD (Figure 3). The cDNA possesses a short 5' untranslated region (UTR) containing an in-frame stop codon just upstream of the predicted translational start site. A nuclear localization signal is present in the C-terminal portion of the protein. The *rhi* cDNA matches the Berkeley *Drosophila* Genome Project gene CG10683.

Database searches performed with the BLASTp and GCG programs revealed that Rhi contains a chromo domain (*chromatin organization modifier*; PARO and HOGNESS 1991), a motif found in proteins involved in transcriptional repression via gross chromatin reorganization (reviewed in CAVALLI and PARO 1998). At least 70 members of the chromo-domain family have been discovered to date in many organisms, including yeast and humans. Two general types of chromo-domain motif subdivide this family into two classes, HP1-like and Pc-like, named after two *Drosophila* family members Heterochromatin Protein 1 (HP1) and Polycomb (Pc). HP1 binds to heterochromatin and is involved in position-effect variegation while Pc heritably silences transcription of the homeotic genes (LEWIS 1978; JAMES and ELGIN 1986; PARO and HOGNESS 1991). Rhi is a member of the HP1-like class (Figure 4). Across the 40-amino-acid chromo domain, Rhi is 48% identical and 73% similar at the amino acid level to its closest homologs, the murine and human HP1 α proteins (also called CBX5; SAUNDERS *et al.* 1993; LE DOUARIN *et al.* 1996). Slightly less similarity in this region (68%) is found when Rhi is aligned with a human protein, p25 β (also called CBX1; SINGH *et al.* 1991). Rhino's chromo do-

main shares less homology with analogous regions in Polycomb and Polycomb-homologous proteins.

Rhino also shares a region of homology at its carboxyl terminus with a subset of the HP1-like chromo-domain proteins. This C-terminal motif has been named the "chromo shadow domain," since it has a low level of homology to the chromo domain (AASLAND and STEWART 1995; KOONIN *et al.* 1995). The shadow domain is found at the C-terminal ends of *Drosophila melanogaster* and *D. virilis* HP1 and in two human, two murine, one *Xenopus*, and one chicken HP1 homologs (SINGH *et al.* 1991; SAUNDERS *et al.* 1993). Although significant homology between Rhi and other subfamily members exists in the chromo-shadow domain, Rhi sequence diverges to a greater extent (Figure 4B). Finally, the central region of Rhi contains weak homology to mouse and human HP1 α chromo-domain proteins (CBX5), suggesting a more recent evolutionary relationship with these proteins.

We determined the positions of the two *P* elements relative to the *rhi* coding region by sequencing the flanking DNA cloned from the *rhi*¹ and *rhi*² flies (Figures 2A and 3). The *P* element in *rhi*¹ is inserted within the coding region at nucleotide 55 of the cDNA, 10 amino acids downstream from the conceptual start site of the protein. Since *rhi*¹ hemizygotes produce a phenotype more severe than that of *rhi*¹ homozygotes, we speculate that rare splicing events produce low quantities of a functional Rhi protein. Thus, *rhi*¹ is a strong hypomorphic mutation of the gene. In contrast, the *PZ* element in *rhi*² is inserted 81 amino acids from the protein start site, potentially permitting translation of the entire chromo domain and thereby producing a partially functional protein. Chromo domains are involved in protein:protein interactions and are included in large multiprotein complexes (SINGH *et al.* 1991; COWELL and AUSTIN 1997; HUANG *et al.* 1998; YAMADA *et al.* 1999); thus, a truncated Rhino protein potentially present in *rhi*² flies could induce a mutant phenotype by interfering with proper complex formation. Since *rhi*² alleles overall produce phenotypes weaker than those of *rhi*¹ alleles, however, we favor an alternative hypothesis, that the Rhi chromo domain alone provides some aspects of normal Rhi function.

Mutations in *rhino* affect chromosome structure or organization in the nurse cell nucleus: *rhi* encodes a putative chromatin-binding protein. We therefore stained ovaries with the DNA dyes DAPI or Sytox green to ask if mutations in *rhi* produce visible effects on chromosome structure. We used females hemizygous for each *rhi* allele to decrease the gene dose and to eliminate the contributions of potential background mutations. Although some ovariole degeneration was seen in these heteroallelic combinations, including loss of germline stem cells and their derivatives, the most striking feature of the egg chambers was their aberrant nurse cell chromosome configuration (Table 2; Figure 5). Normally,

▼P_{ryl1}

1 CGGCGTCTCTGAGCGGATAAGTTATGTCTCGCAACCATCAGCGACCAAACTCTGGTCTGGTTCGATGCACCGCCTAATGATCATGTCGAA
M S R N H Q R P N L G L V D A P P N D H V E

91 GAATATGTAGTGGAGAAAATCTTAGGCAAACGGTTTGTTAATGGGCGTCCCCAGGTTCTGGTGAAGTGGAGCGGTTTTCCGAACGAGAAC
E Y V V E K I L G K R F V N G R P Q V L V K W S G F P N E N

▼PZ

181 AACACCTGGGAACCATTTGGAAAACGTAGGCAACTGCATGAAGCTGGTCTCCGACTTCGAGTCGGAGGTCTTCAGGCTGCATCGGAAGGCC
N T W E P L E N V G N C M K L V S D F E S E V F R L H R K A

271 GCGGCTAAATCTGTCTGGGAAATCGAAGTCAAGTCTTAGCAGCAGCGGTCCATTGATCACCGAGAATGGCCCCAGCAGCTCTAAGAAAACA
A A K S V G K S K S S P S S S G P L I T E N G P S S S K K T

361 CAACAACATTTCCAAGTCCGGTGCAGGCAAAAAACACAGCTGGAATGTCGAAGATGAATCAGAAAAAGGAAAGAATATTAAGAAAACGGCG
Q Q H S K S V Q A K N T A G M S K M N Q K K G K N I K K T A

451 GGAAAAATCAAGATATTGAAAATATCCAAAACACTCAGATGCCTAGTACTAGCCAAGTCTCCACAGATCAACTGAAGTTTTTCGACGGC
G K I K D I E N Y P K T Q M P S T S Q V S T D S T E V F D G

541 AATCCATCAGCCACAACGACGAACATGATTAAGAGTCCCAGGATTCAAAGCCTTTTCAGTGATCTGAACTTGATCGAACCCACTAAAGAC
N P S A T T T N M I K S P R I Q S L F S D L N L I E P T K D

631 AAAGATGTGGGGACACATCTTTAAAAACGCCCTCCCAAATCGCGCCGGCTTATTGAATTTCCCTCAACGGGAAGACGCACCATTGTCTTCG
K D V G D T S L K T P P K S R R L I E F P Q R E D A P L S S

721 AAACATGTGTCTCCTATGCTGATTTCGTAAGAATCCCAACCATTCAGAGCTCATGTACTGATGATTCCGGATTTGGGTGAATCCTCTTCC
K H V S P M L I R K E S Q P L Q S S C T D D S D L G E S S S

811 AGTATGTGCTTCCTACAGTATCATCGACCAGCTCTGAGAAATCGATCAAGGTCACCAAATCGGAACCTAAAACGCTCGGCCAGATAAAG
S M S L P T V S S T S S E K S I K V T K S E P K T L G Q I K

901 TTTTCGTCTAGGTCATCCGATGGTGGTTCATGCGCTGGGCGCACAAAAGAAGGGGACATTGGCTTGGATTTATCTGGCAGT
F S S R S S D G G H A A S S L G A P K E G D I G L D L S G S

Intron 1

991 GATTCCATGGATTCCGAAGTGGAGAGCTATGCGCCGCTGTCCGCGCCGCAAGCGTAAAAAACTTACCCGGATTGGAAATTCCCGGAGATG
D S M D S E V E S M R R C P **R R K R K K** T Y P D W K F P E M

1081 ACGAAAACCTTCGGTGTGAATCGCGGACTGGATCTGGATAAAAATCTCCACTGCTACCAGATGAATGACGATCTATTCATGTTTGTACAG
T K P F G V N R G L D L D K I L H C Y Q M N D D L F M F V T

1171 TGGAAGGGCTGCTCGTCCATCGATGCAGTGCATATTAATGACATTAAGGAAGCGTATCCATTGCAGATAATTAAGTACTTCGAGAGCTTG
W K G C S S I D A V H I N D I K E A Y P L Q I I K Y F E S L

Intron 2

1261 AGGATCATTGTGCCCAAGTAAAGATGCCTAGTACCGGTTTGTGACAACGTTTTGGCCAAAAGAAAATTCACATCACACAACATAATCG
R I I V P K

1351 ACTCCAAGTTCAAGGTCATCGCATCTAAGCTGGGTAACCTCCAGCTGACCCATCAAATTGATCTATTCAAAGGAAAAACACCGAGTTGTG
1441 TTTATACTATGTTTATGTTTTCTCCGTACATGTTGAAAAATGTACATAATCAATGGTTTCAAGTAACCTGATATACTAACCTTTTTCCCC
1531 **ATAAA**TTTTGTTTTGGCCTGACAAAAA

FIGURE 3.—Sequence of *rhino* cDNA and predicted sequence of Rhino protein. Numbers correspond to nucleotide sequence. Inverted triangles indicate the insertion points of the two *P* elements. The chromo domain is boxed and the chromo-shadow domain is underlined. The intron positions are indicated by T-bars. A putative nuclear localization signal is shown in boldface. The polyadenylation signal is both underlined and in boldface type.

a specific progression of changes in higher-order chromosome structure occurs during oogenesis (Figure 5a; KING 1970; HAMMOND and LAIRD 1985; DEJ and SPRADLING 1999). Early endoreplicating nurse cell chromosomes are paired and as the DNA content increases, a polytene banding pattern becomes visible. At S5, pairing breaks down and the chromosomes assume a stereotypic five-lobed or wagon-wheel morphology (Figure 5d). This morphology is transient, however, and after S5 the chromosomes assume a fairly uniform distribution throughout the nucleus (Figure 5, g and j). The nurse

cell chromosomes then retain this morphology for the rest of oogenesis.

In *rhi* mutants, chromosome structure was normal up to S5. In egg chambers from each of the *rhi* alleles, however, the wagon-wheel morphology persisted well after this stage; the chromosomes did not assume a uniform distribution. Nevertheless, DNA replication apparently continued in these nuclei since the intensity of DAPI staining increased in older egg chambers (Figure 5, b and c).

Females carrying alleles derived from the *rhi*¹ *P* ele-

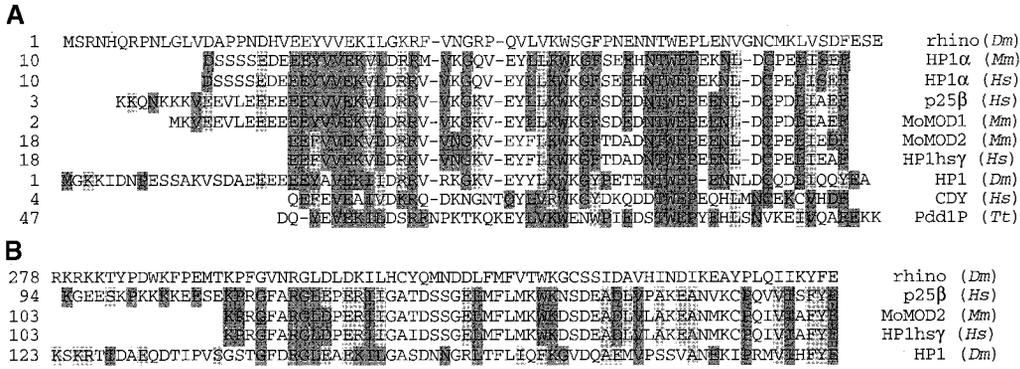


FIGURE 4.—Alignments of the Rhino chromo domain and chromo-shadow domain with the closest chromo-domain-family homologs. (A) Chromo domain. (B) Chromo-shadow domain. Numbers correspond to amino acid sequence. Identical amino acids are shaded in dark gray; similarities are indicated in light gray. Homologies depicted are: murine

HP1α (CBX5), human HP1α (CBX5), human p25β (CBX1), murine MoMOD1 and MoMOD2 (CBX3), human HP1hsy, Drosophila HP1, human Y chromosome CDY, and Tetrahymena Pddp1. *Dm*, *D. melanogaster*; *Mm*, *Mus musculus*; *Hs*, *Homo sapiens*; *Tt*, *Tetrahymena thermophila*.

ment (*rhi¹* and *rhi¹⁻⁵⁶*) produced egg chambers with a distinctive five-lobed phenotype (Figure 5, b and e). In S10 egg chambers from *rhi¹/Df(2R)rhi^{2L12}* females, the lobes of chromosomes were at the nuclear periphery, leaving a central “hole” that lacked DAPI staining (Figure 5, h and k). Alleles generated from the *rhi²* P-element insertion (*rhi²* and *rhi^{2-SL15}*) also resulted in the persistent wagon-wheel phenotype (Figure 5, c, f, and i). The chromosome lobes were larger and more broadly distributed throughout the nucleus, however, compared to chromosome defects produced by the *rhi¹*-based alleles (Figure 5, b and c).

Females hemizygous for each of the *rhi* alleles occasionally produced egg chambers with improper numbers of nurse cells (Table 2 and Figure 5i). These egg chambers usually contained 32 cells, indicative of either an extra round of mitosis or a follicle-cell encapsulation event involving two germline-derived cysts. Less frequently, egg chambers were produced that had too few nurse cells. In older females (~10 days), germaria usually contained fewer numbers of germline cysts. Ultimately, most egg chambers hemizygous for the various *rhi* alleles degenerated before completing oogenesis. Notably, chromosomes in *rhi¹⁻⁵⁶/Df(2R)rhi^{2L12}* nuclei broke up into unusually small and numerous fragments before degenerating completely (Figure 5l). The morphology

of the chromosomes in the oocyte nucleus and follicle-cell nuclei was not visibly altered in any of the *rhi* mutants (data not shown). These data reveal the need for Rhi in restructuring or reorganizing nurse cell chromosomes during the unique S5 endoreplication cycle. It is possible that *rhi* mutations affect chromosome structure in other ovarian cell types but defects are not detectable in the mutants due to the lower ploidy of these cells.

***rhino* mutations affect transcripts that are important for early axis establishment:** The similarity of Rhi to chromo-domain proteins and the aberrant nurse cell chromosome morphology and eggshell phenotypes produced by *rhi* alleles suggested three hypotheses for the role of *rhi* during oogenesis.

First, Rhino could bind specific sites on the chromosome as part of a multiprotein chromatin-binding complex, regulating expression of a variety of genes including key genes required for patterning. In this scenario, loss-of-function mutations would disrupt chromatin conformation and alter expression of the target genes. If Rhi binds a large number of sites on these highly polyploid chromosomes, visible changes in chromosome structure might be observed in *rhi* mutants. Alternatively, Rhi might be required at the end of endocycle 5 to repress expression of specific genes whose products inhibit chromosome dispersal; loss of Rhi would there-

TABLE 2
Chromatin phenotype of *rhino* mutant egg chambers

Genotype	Chromatin phenotype					n ^a
	% normal stage 4 chromatin	% Normal other stage	% persistent five-lobed chromatin	% degenerating egg chamber	% abnormal nurse cell number	
+ / <i>Df(2R)rhi^{2L12}</i>	20	74	0.1	6	0.5	833
<i>rhi¹ / Df(2R)rhi^{2L12}</i>	18	2	67	10	3	377
<i>rhi¹⁻⁵⁶ / Df(2R)rhi^{2L12}</i>	17	5	52	15	10	279
<i>rhi² / Df(2R)rhi^{2L12}</i>	19	11	58	7	4	422
<i>rhi^{2-SL15} / Df(2R)rhi^{2L12}</i>	9	20	58	6	7	392

^a Total number of egg chambers between stage 4 and stage 11 scored.

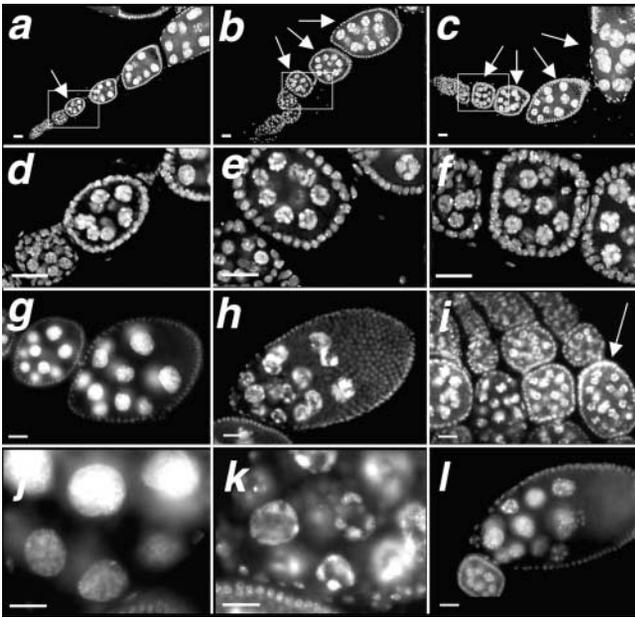


FIGURE 5.—*rhino* mutants exhibit altered chromosomal morphology. (a, d, g, and j) Wild-type ovarioles containing a normal five-lobed S5 egg chamber (arrow, a, enlarged in d), and later-staged egg chambers with uniformly distributed chromatin (a and g). A magnified view of early S10 nurse cell nuclei is shown in j. (b, e, h, and k) Similarly staged egg chambers from *rhi¹/Df(2R)rhi^{2L12}* females, exhibiting a five-lobed chromosome morphology in S5 and persisting into later stages (arrows, b, enlarged in e). Note the close association of the chromatin with the nuclear periphery in S10 nurse cells (k), leaving a hole in the center of the nucleus. (c and f) The five-lobed phenotype also persists in *rhi²/Df(2R)rhi^{2L21}* egg chambers (arrows), but the chromatin lobes are slightly larger and more evenly distributed in the later stages compared to *rhi¹/Df(2R)rhi^{2L12}* egg chambers. (i) *rhi^{2SL15}/Df(2R)rhi^{2L12}* ovarioles contain egg chambers with abnormal numbers of germline-derived cells (arrow); similar egg chambers were observed in other *rhi* allelic combinations. (l) Nurse cell chromosomes in *rhi^{1S6}/Df(2R)rhi^{2L1}* females break into fragments before degeneration. Regions enclosed by boxes in a–c are expanded in d–f. Bars, 25 μ m.

fore prevent the normal chromosome structural transitions. At the same time, eggshell defects might result if, for example, Rhino normally represses transcription of *gurken* in the nurse cells; lack of Rhino would allow overexpression and generate dorsalized egg chambers.

In the second hypothesis, Rhino does not act as a transcriptional regulator but rather participates in chromosome structural changes during specific transitions of the cell cycle, such as the chromosome reorganizational events that take place at S5 of oogenesis. The ordered progression of chromosome modifications that occur during the cell cycle would be monitored and this information then integrated with patterning processes to ensure coordinated egg chamber maturation. Schüpbach and colleagues have described a link between cell-cycle checkpoint functions that occur in the oocyte and pathways that regulate *grk* mRNA translation (GHABRIAL *et al.* 1998; GHABRIAL and SCHÜPBACH 1999).

This hypothesis would extend that connection by suggesting that cell cycle events in the nurse cells are also integrated with patterning mechanisms. Thus, in this scenario, *rhi* mutations would lead to defects in S5 chromosome structural transitions and these defects would then affect Grk protein synthesis through putative checkpoint controls. Transcription of most genes, however, would be normal.

Finally, the third hypothesis states that Rhino regulates the synthesis or activity of cytoskeletal or transport proteins that control the distribution of chromatin modifying proteins, nuclear import/export functions and/or microtubule organizing center components. Loss-of-function mutations in *rhi* would then indirectly lead to defects in nurse cell chromosome conformation and egg chamber polarity. A similar scenario is proposed for *cup* and *otu*, which encode cytoplasmic proteins and interact genetically to regulate nurse cell chromosome conformation and overall egg chamber maturation (KEYES and SPRADLING 1997). Since *rhi* mutants exhibit only a subset of the phenotypes produced by mutations in *otu* or *cup*, *rhi* may affect only a specific branch of the overall regulatory process. Nevertheless, this third hypothesis predicts that *rhi* mutants would exhibit defects in the subcellular distribution of proteins that regulate chromosome structure and mRNAs that determine axial patterning.

To distinguish between these hypotheses, we tested if *rhi* mutations resulted in gross changes in gene expression for four genes whose expression levels and mRNA localization patterns are required for and/or reveal the establishment of egg polarity.

We first examined *grk* mRNA because of its primary role in dorsal-ventral patterning. In wild-type ovaries, *grk* message is localized to the posterior of the oocyte early in oogenesis and then becomes concentrated in a dorsal anterior cap over the oocyte nucleus at S9 and S10 (Figure 6a and NEUMAN-SILBERBERG and SCHÜPBACH 1993). We observed this pattern of expression in 93.6% of Canton-S egg chambers ($n = 218$). In contrast, only about one-third of *rhi¹/Df(2R)rhi^{2L12}* and half of *rhi²/Df(2R)rhi^{2L12}* egg chambers exhibited this pattern (32.5%, $n = 77$ and 50.7%, $n = 67$, respectively). The remaining egg chambers mislocalized the mRNA laterally in early stages (Figure 6b) or in a ring at late stages (Figure 6c) or lacked detectable *grk* transcripts (not shown). We did not see excess *grk* mRNA, as expected if Rhi acted to repress *grk* transcription. In some S13 egg chambers, we did see residual *grk* mRNA in nurse cell remnants; this late expression could contribute to the variety of unusual dorsal appendage shapes of *rhi* mutants but is not consistent with a general role in *grk* transcriptional repression. Finally, we observed defects in *grk* mRNA localization before S6, the time at which we detected aberrant nurse cell chromosome morphology. Normally, *grk* mRNA is localized at the posterior of the oocyte in early stages; we observed *grk* mRNA in lateral

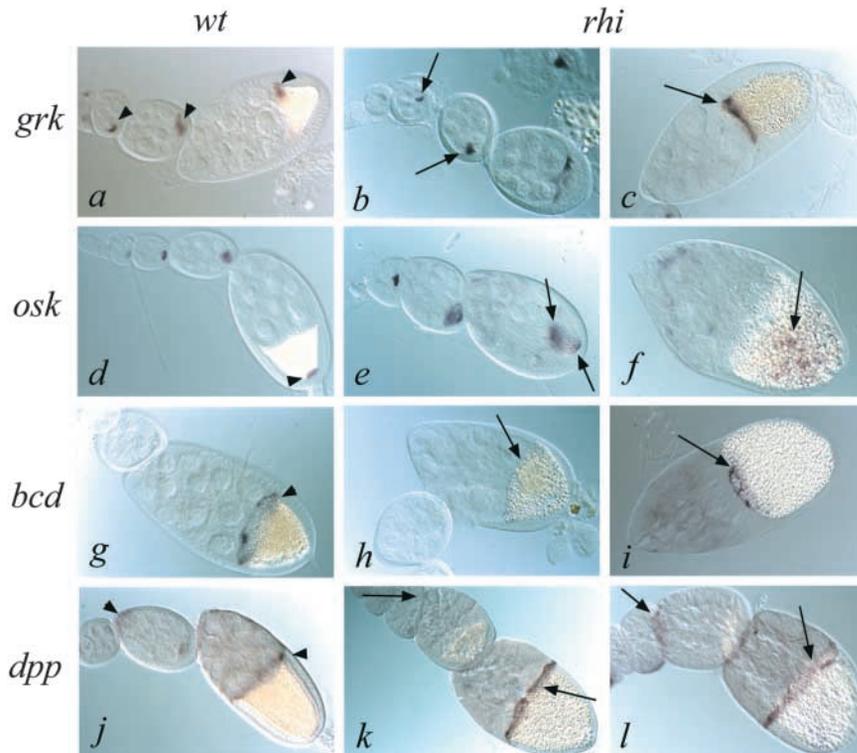


FIGURE 6.—Expression patterns in *rhino* mutants reveal normal transcript levels but some mislocalized mRNAs. Wild-type (a, d, g, and j) and *rho/Df(2R)rho^{2.112}* (b, d, e, f, h, i, k, and l) egg chambers probed with *grk* (a–c), *osk* (d–f), *bcd* (g–i), and *dpp* (j–l) cDNAs. (a) In the wild type, *grk* mRNA (arrowheads) is localized to the posterior of the oocyte in early egg chambers (first in line), transiently in a ring at S7 or S8 (middle egg chamber), and then in a dorsal anterior cap above the oocyte nucleus by S9 (last in line). (b) In *rho* mutants, *grk* mRNA is frequently mislocalized to the side of the egg chamber in early stages (arrows; compare first egg chamber in a to indicated egg chambers in b). (c) In S10 *rho* egg chambers, *grk* remains localized in a ring at the anterior of the oocyte (arrow). (d) In wild type, *osk* mRNA is localized to the posterior of the oocyte in early egg chambers, diffusely throughout the oocyte at S7, and then again at the posterior by S9 (arrowhead). (e and f) *osk* mRNA (arrows) may be found at anterior and posterior (e) or diffuse throughout the oocyte (f) in *rho* mutant egg chambers. (g) *bcd* message (arrowhead) is localized to the anterior cortex by S9 in wild-type egg chambers. (h) In some

S9 *rho* egg chambers, *bcd* expression is absent (arrow). (i) By S10, however, *rho* egg chambers express *bcd* normally (arrow). (j) In wild type, *dpp* (arrowheads) is expressed in anterior follicle cells at S8 and then more dramatically in a ring of centripetally migrating cells at S10. (k and l) In some *rho* egg chambers, *dpp* expression is delayed (arrows, k, indicate lack of expression in early egg chamber yet normal expression in late egg chamber). In most mutant egg chambers, however, *dpp* expression is normal (arrows, l, highlight expression in both egg chambers).

or anterior regions of the oocyte (arrows, Figure 6b). Taken together, these results suggest that Rhi does not act as a direct regulator of *grk* gene expression but rather affects eggshell patterning at least in part by disrupting the processes that localize *grk* transcripts.

To test the generality of this result, we examined the expression of three other genes, *oskar* (*osk*), *bicoid* (*bcd*), and *decapentaplegic* (*dpp*). Like *grk*, *osk* mRNA is localized to the posterior of the oocyte in early egg chambers. During microtubule reorganization at S7, *osk* transcripts are uniformly distributed within the oocyte or are transiently localized in an anterior ring. By late S8, however, *osk* mRNA once again is localized to the posterior (Figure 6d and EPHRUSSI *et al.* 1991; Canton-S 94.5%, $n = 71$). In ovaries from either *rho¹* or *rho²* hemizygous females, only half the egg chambers exhibited this pattern (*rho¹* 49.2%, $n = 130$; *rho²* 52.6%, $n = 213$). Mislocalization in early stages and diffuse localization into late stages were common phenotypes (Figure 6, e and f). Virtually all S8–11 egg chambers exhibited mislocalized *osk* mRNA. No defects in level of expression were detected. These results reveal a defect in the establishment of posterior polarity within the oocyte and support the hypothesis that *rho* mutations disrupt a mRNA localization process common to *grk* and *osk* transcripts.

To test if genes normally expressed only after S5 are

regulated correctly and to ask if the mislocalization defects were a general phenomenon, we examined expression of *bcd*, whose mRNA is localized to the anterior of the oocyte beginning at S8 (Figure 6g and ST. JOHNSTON *et al.* 1989; Canton-S 98.2%, $n = 218$). We observed no defects in early *bcd* expression in *rho¹* and *rho²* hemizygotes but did find some S8 and S9 egg chambers with little or no *bcd* message, suggesting a delay in the onset of *bcd* transcription or transport. Later-stage egg chambers either exhibited normal *bcd* transcript levels and distribution or were degenerating. Overall 69.7% of *rho¹* hemizygous ($n = 98$) and 82.2% of *rho²* hemizygous ($n = 135$) egg chambers exhibited mid-oogenesis defects. The normal distribution of *bcd* RNA in late-stage egg chambers demonstrates that some aspects of RNA localization function properly in *rho* mutant ovaries. The delay in *bcd* mRNA expression and localization, however, suggests a defect in the coordination between patterning processes and other aspects of egg chamber differentiation, such as yolk uptake and follicle-cell movements that define particular stages of egg chamber development. Finally, we tested if *rho* mutants misregulate expression of *dpp*. *dpp* is normally expressed only in the follicle cells and affects eggshell structures by determining anterior follicle cell fate (TWOMBLY *et al.* 1996; DENG and BOWNES 1997; XIE and SPRADLING 1998; PERI and ROTH 2000).

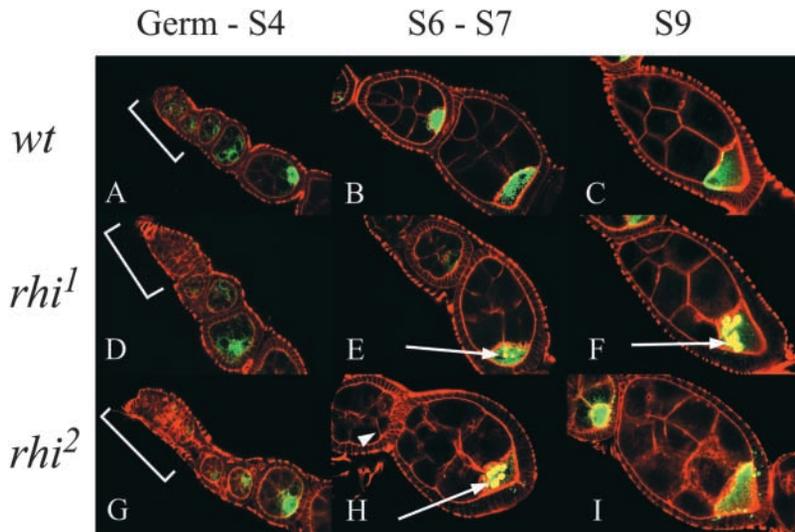


FIGURE 7.—Gurken protein accumulation is aberrant in *rhino* mutants. $+/Df(2R)rhi^{2L12}$ (A, B, and C), $rhi^1/Df(2R)rhi^{2L12}$ (D, E, and F), and $rhi^2/Df(2R)rhi^{2L12}$ (G, H, and I) egg chambers probed with monoclonal antibody 1D12 against Gurken protein (green) and counter-stained with rhodamine-phalloidin to highlight the actin cytoskeleton (red). In all panels, anterior is to the upper left. (A) In the germarium (bracket), Grk protein is detectable in region II, accumulating in the oocyte and two neighboring nurse cells by S2. Young egg chambers contain higher quantities of Grk protein, especially in a cortical strip at the posterior of the oocyte. (B) Upon a signal from posterior follicle cells at S6, microtubule reorganization leads to redistribution of Grk protein; a transient uniform localization throughout the oocyte is followed by accumulation at the nurse cell/oocyte boundary. (C) In early S9 egg chambers, Grk is present in a dorsal anterior cap above

the oocyte nucleus. (D) rhi^1 hemizygous egg chambers lack detectable Grk expression in the germarium (bracket) but contain approximately normal levels of protein by S4. (E) As early as S6, Grk protein accumulates in large vesicles caged by actin (arrow). (F) In this S9 egg chamber, most Grk protein resides in vesicles (arrow). (G) In rhi^2 hemizygous egg chambers, a low level of Grk protein is present in the germarium (bracket). (H) The S5 egg chamber lacks Grk protein (arrowhead) while the S7 egg chamber contains large, Grk-filled vesicles (arrow). (I) A late S8/early S9 rhi^2 hemizygous egg chamber in which Grk protein is present in a diffuse band at the anterior of the oocyte. In all panels, punctate dots over the follicular epithelium indicate secreted Grk protein.

Ectopic expression of *dpp* in the germline or misexpression in the follicle cells could cause altered eggshell morphology in *rhi* mutants. In wild-type egg chambers, *dpp* is expressed at very low levels in the germarium and then at more readily detectable levels in anterior follicle cells beginning at S8. This expression resolves into a ring of staining in centripetally migrating cells at S9 and S10 (Figure 6j and TWOMBLY *et al.* 1996; Canton-S 98.4%, $n = 126$). Almost all rhi^1 and rhi^2 hemizygous egg chambers exhibited normal *dpp* expression (Figure 6i; rhi^1 91.5%, $n = 130$; rhi^2 95.9%, $n = 271$). A few S8 and S9 egg chambers produced no detectable *dpp* transcript (S9 egg chamber in Figure 6k). Thus, the eggshell phenotypes we observe are likely due to defects in *grk* mRNA localization and not to defects in *dpp* expression.

In summary, these results suggest that Rhi does not affect eggshell patterning by regulating transcription of *grk* or *dpp*, key genes required for determining the fate of follicle cells that synthesize eggshell structures. Moreover, it is likely that Rhi does not act as a general transcriptional repressor in oogenesis. Severe hypomorphic alleles still allow development of most egg chambers to S10 and some egg chambers to S14. Although our survey of genes was limited, none of the genes we assayed exhibited altered levels of gene expression. Rather, mutations in *rhi* disrupt *grk* and *osk* mRNA localization in a large fraction of S8–10 egg chambers. Thus, Rhi likely acts upstream in a pathway controlling the microtubule reorganizations prerequisite for establishing axial polarity. In addition, our *rhi* results contrast with those of Heino and colleagues, who found that mutations in *otu*

disrupt chromosome conformation and lead to aberrant accumulation of *bcd* and other mRNAs in nurse cell nuclei. They found no defect in *osk* mRNA localization or in the localization of other transcripts involved in egg and embryonic patterning (HEINO *et al.* 1995). Thus, *rhi* and *otu* share some common phenotypes but differ in their mechanism of action.

Gurken protein accumulates slowly and associates with vesicles in *rhino* mutants: The *grk* and *osk* mRNA localization defects observed in *rhi* mutants suggested that the S6–S8 oocyte microtubule rearrangement that ensures proper mRNA distribution was defective. This cytoskeletal reorganization is directed by a signal from posterior follicle cells, which rely on earlier Grk signaling to determine their posterior fate (reviewed by NILSON and SCHÜPBACH 1999). *rhi* mutations could disrupt this earlier Grk patterning process, leading to microtubule defects as a downstream consequence, or they could affect a S5/S6 regulatory process that integrates nurse cell chromosome transitions with microtubule functions. If acting early in oogenesis, Rhi might be required for proper expression of any gene involved in Grk signaling (*e.g.*, *homeless*, *vasa*, *cornichon*, etc., but not *grk*) or it might regulate meiotic chromosome structural changes that occur in the oocyte and are then linked to Grk translation (GHABRIAL *et al.* 1998; GHABRIAL and SCHÜPBACH 1999). To begin to address these possibilities, we asked if Grk protein is expressed correctly in *rhi* mutant egg chambers.

In wild-type egg chambers, Grk protein is present in a punctate pattern in region II of the germarium and is localized to the oocyte in subsequent stages (Figure

7A; ROTH *et al.* 1995; NEUMAN-SILBERBERG and SCHÜPBACH 1996). S1 egg chambers frequently exhibit α -Grk staining mainly in the oocyte with cortical staining in neighboring nurse cells (Figure 7A). During S2–S5, Grk protein is tightly localized to the posterior of the oocyte (Figure 7A) but becomes diffuse throughout the oocyte during microtubule reorganization at S6 and S7 (Figure 7B). Transient localization of Grk protein in a ring at the anterior of the oocyte may occur in early S8 egg chambers but by S9, all Grk protein is found in a dorsal anterior cap outlining the oocyte nucleus (Figure 7C). Secretion of Grk protein may be monitored by visualization of punctate dots in the overlying follicular epithelium (*e.g.*, Figure 7B; QUEENAN *et al.* 1999). We observed this precise pattern of staining in 89.2% ($n = 158$) of $+/Df(2R)rhi^{2-L12}$ egg chambers (Figure 7, A–C). The remaining egg chambers exhibited subtle defects in the timing at which Grk localization changed, such as S2 egg chambers with remaining nurse cell expression, S6 egg chambers with continued posterior localization and two S9 egg chambers with a diffuse oocyte cortical pattern (data not shown).

In egg chambers produced by rhi^1 and rhi^2 hemizygous females, we observed two major defects in Grk expression. Virtually all germaria ($n = 34$) and one-third of S2–S4 rhi^1/Df egg chambers (32%, $n = 37$) lacked detectable α -Grk staining (Figure 7D). By S5, however, almost all egg chambers produced normal levels of Grk protein (Figure 7D). Thus, rhi egg chambers exhibit a delay in Grk protein accumulation. rhi^2/Df egg chambers exhibited a weaker phenotype; half the germaria and S2–S4 egg chambers produced normal levels of Grk protein (52%, $n = 54$; Figure 7G). The remaining samples lacked detectable expression (37%) or exhibited only weak staining (11%; data not shown). These results suggest that rhi mutations affect a process required early in oogenesis for the translation or stability of Grk protein.

The second defect apparent in rhi mutant egg chambers was the presence of large, Grk-containing vesicles in S6–S10 egg chambers (Figure 7, E, F, and H). Rhodamine-phalloidin staining demonstrated that actin colocalized with these vesicles, giving the appearance of a cage surrounding Grk protein (yellow overlap in Figure 7, E, F, and H). These structures were found in 54.3% ($n = 70$) of S6–S10 rhi^1/Df egg chambers and 29.6% ($n = 88$) of S6–S10 rhi^2/Df egg chambers. We never observed such vesicles in wild type ($n = 68$). We speculate that rhi mutants are defective in Grk translation or processing such that Grk protein is delayed in the endoplasmic reticulum, yielding large vesicles. Although a delay may exist in Grk synthesis, some product is secreted and taken up by the overlying follicle cells (Figure 7, F, H, and I). Our results suggest that Rhi is necessary for the synthesis or maturation of Grk protein. Lack of Rhi function leads to a delay in Grk production early in oogenesis and the accumulation of Grk protein

in vesicles in S6–S10 egg chambers. Taken together, our results suggest that Rhi is not acting to repress *grk* transcription nor is it required specifically to coordinate S5 nurse cell chromosome reorganization with axis-determining events. The effects on eggshell patterning may involve Rhi function as a transcriptional regulator or as a mediator of chromosome structural reorganization, but at least one role must occur before the obvious S6–S10 chromosomal and D/V polarity defects observed in rhi mutant egg chambers.

DISCUSSION

We describe the genetic and molecular characterization of a new *Drosophila* female-sterile gene, *rhi*, which encodes a protein molecularly similar to the HP1-like subfamily of chromo-domain proteins (for review see CAVALLI and PARO 1998). *rhi* is required for proper chromosome conformation in the nurse cells during oogenesis and for patterning events that are reflected in eggshell structures. We generated two independent *P*-element alleles and many excision lines, all of which disrupt the major ovarian transcript of 1.6 kb, encoding a protein of ~ 46 kD. *rhi* expression is not detectable in males but is found at low levels in somatic tissue in adult females and at higher levels in the germline; thus, *rhi* likely plays a specific role in oogenesis. Here we discuss Rhi's homology to a subfamily of chromo-domain proteins. We then consider the chromosome, eggshell, mRNA-mislocalization, and Grk-protein defects in *rhi* mutants and present hypotheses for *rhi* function during oogenesis.

Rhino is a member of the chromo-domain protein family: The chromo domain was first recognized as a conserved protein motif through homologies shared by *Drosophila* proteins Heterochromatin-associated Protein 1 (HP1) and Polycomb (Pc) (PARO and HOGNESS 1991). HP1 was identified genetically as a suppressor of position-effect variegation, an epigenetic phenomenon in which euchromatic genes are silenced when they are next to heterochromatin (EISENBERG *et al.* 1990, reviewed in WEILER and WAKIMOTO 1995). Pc was first described as a repressor of homeotic genes and is now known to be part of a large protein complex that binds ~ 100 sites at specific times during embryogenesis and maintains transcriptional quiescence (LEWIS 1978; PARO and HARTE 1996). As new members of the chromo-domain family were isolated through their homology to HP1 and Pc, it became clear that two distinct classes exist within the family. The HP1-like proteins contain a carboxyl-terminal motif, the chromo-shadow domain, that shares homology with the amino-terminal chromo domain (AASLAND and STEWART 1995). Rhi belongs to the HP1-like class of proteins containing the chromo-shadow domain. Although this class of proteins is believed to function in heterochromatin formation, repressing transcription of euchromatic genes, disrupting

these proteins may also activate transcription (HEARN *et al.* 1991; GORMAN *et al.* 1995).

Insight into how chromo-domain proteins can function as transcriptional repressors has been provided by the three-dimensional structure of the chromo domain from the HP1-like MoMOD1 (BALL *et al.* 1997) and by structural and biochemical studies of the chromo-shadow domains from fission yeast Swi6 and *Drosophila* HP1a, HP1b, and HP1c (COWIESON *et al.* 2000; DELATRE *et al.* 2000; SMOTHERS and HENIKOFF 2000, 2001). These authors postulate that the chromo-domain and chromo-shadow domain are modules that facilitate interaction with proteins at target sites designated for compaction. This work provides a mechanism to explain the localization of HP1-like proteins (PLATERO *et al.* 1995; COWELL and AUSTIN 1997), since few specific DNA sequences bound by HP1-like molecules are known (SUGIMOTO *et al.* 1996). Indeed, the common feature of the HP1 subfamily of chromo-domain proteins is their association with heterochromatin found near centromeres. Thus, loss-of-function mutations not only affect transcription and position-effect variegation but chromosome segregation as well. For example, the *Schizosaccharomyces pombe* chromo-domain-containing Swi6 protein is required for silencing at the silent-mating-type loci and is also an essential centromere component (EKWALL *et al.* 1995; ALLSHIRE 1996). *swi6* mutants have a high rate of chromosome loss, as do *HP1* *Drosophila* mutants (EKWALL *et al.* 1995; KELLUM *et al.* 1995). These phenotypes provide an insight into the potential functions of Rhi in oogenesis. Other recent evidence suggests more diverse roles for chromo-domain proteins and may help explain some other features seen in *rhi* mutants (see below).

Rhino may regulate chromosome condensation at a pseudo S-M transition: Rhi is a novel chromo-domain protein in that it is female specific. Expression of the gene beginning in region I of the germarium suggests a function early in oogenesis while the large increase in transcript levels at S10 could indicate a requirement for Rhi in remodeling nurse cell chromosomes at the end of oogenesis. In addition, since these transcripts are loaded into the embryo, Rhi may function during the rapid cell cycles of the early embryo. *rhi* mutations reveal roles for this novel chromo-domain protein in two distinct processes, nurse cell chromosome reorganization at S5 and Grk synthesis in the oocyte. Less frequent defects include a decrease in the number of germline cysts, egg chambers that contain too many or too few nurse cells, and a failure to transfer the cytoplasmic contents of the nurse cells into the oocyte.

How does Rhi function? We hypothesize that Rhi regulates chromosome condensation at the onset of a mitotic-like phase that occurs during endocycle 5 of nurse cell chromosome duplication. Due to the highly polyploid nature of the nurse cell chromosomes, defects in this process are readily visible in *rhi* mutants. The nurse

cells follow a highly coordinated and specific pattern of endoreplication during egg chamber development involving three distinct types of endoreplication cycles (SPRADLING 1993; REED and ORR-WEAVER 1997; DEJ and SPRADLING 1999). During the first four rounds of replication, DNA synthesis is complete and is followed immediately by a growth phase in which the chromosomes remain associated in a polytene conformation. No M phase occurs. For most nurse cell nuclei, the second type of endocycle takes place during S5. A weakening of homolog pairing accompanies early DNA replication, creating the five-blob morphology of S5 nurse cell nuclei. Late DNA replication is then truncated by induction of a unique mitotic-like state in which the 64C chromosomes dissociate into 32 pairs. It is this process that creates the dispersed chromosomes visible in later-stage egg chambers. The third endocycle type occurs during the remaining approximately six rounds of synthesis. Each of the 32 pairs of chromosomes undergoes incomplete DNA replication to generate a subpolytene structure, alternating between growth and DNA synthesis phases (DEJ and SPRADLING 1999).

We propose that Rhi facilitates chromosome condensation at the onset of the mitotic-like phase at the end of the unique endocycle 5. Lack of Rhi results in a failure to complete the mitotic process of separating homologs and sister chromatids. As a result, nurse cell chromosomes remain in the five-blob configuration that developed during the DNA replication phase of endocycle 5. In addition, the chromosomes remain attached to the nuclear envelope, leading to the distinctive donut hole visible in DAPI-stained nurse cell nuclei at later stages in *rhi* mutants. It is possible that Rhi acts indirectly in this process, for example, by competing with putative HP1 binding at the nuclear envelope (*e.g.*, YE and WORMAN 1996; YE *et al.* 1997) or by inhibiting origin recognition complex activity at late origins (PAK *et al.* 1997). Nevertheless, the HP1-like protein encoded by *rhi* is needed to reconfigure the large amounts of chromosomal material present in nurse cell nuclei.

One potential consequence of this failure to reorganize nurse cell chromosomes in *rhi* mutants may be an inability to create the nucleolar network that spans the inner membrane of the nuclear envelope beginning at S5 (HAMMOND and LAIRD 1985; SPRADLING 1993). This structural defect could inhibit ribosome biogenesis, leading indirectly to a decrease in the amount of Grk protein and, as in *sop* mutants, to the eventual degeneration of S6–S10 egg chambers.

Models for Rhino function in egg polarity and eggshell patterning: If Rhi regulates chromosome condensation at the onset of the unique “mitosis” in endocycle 5, how does it affect the eggshell? Expression levels of *grk*, *osk*, *bcd*, and *dpp* were not grossly altered by defects in nurse cell chromosome structure, demonstrating that Rhi does not act as a general modifier of transcription nor as a specific regulator of these patterning genes.

Rather, defects in Grk protein accumulation early in oogenesis coupled with aberrant mRNA localization indicate that axis determination was defective prior to the reorganization of nurse cell chromosomes at S5/S6. These results suggest either that Rhi plays two distinct roles in oogenesis or that the link between germ cell chromosome structure and Grk protein production is subtle or indirect.

We envision three potential mechanisms to explain *rhi* polarity defects. First, Rhi could regulate nurse cell chromosome condensation at S5 and independently regulate transcription of a specific locus that controls early patterning processes via Grk translation. For example, Rhi might normally repress transcription of the *arrest* gene, which encodes the translational repressor Bruno (WEBSTER *et al.* 1997). Bruno binds specific sequences, Bruno response elements, in the 3' untranslated region of *osk* mRNA and inhibits translation of *osk* until the messages are correctly localized at the posterior of the oocyte (KIM-HA *et al.* 1995). These same sequences reside in the 3'-UTR of *grk* mRNA and may control translation of this message (KIM-HA *et al.* 1995; WEBSTER *et al.* 1997). Loss of Rhi function would lead to overexpression of Bruno, thus delaying Grk translation. Alternatively, Rhi might activate transcription of *cornichon*, *vasa*, *orb*, or other genes that mediate Grk synthesis (reviewed by COOPERSTOCK and LIPSHITZ 2001). This hypothesis is supported by the observation that *grk* and *osk* mRNA localization defects occur in S1–S5 egg chambers. Failure to translate Grk correlates with mislocalization of *grk* mRNA early (CLEGG *et al.* 1997) and late (TINKER *et al.* 1998) in oogenesis. In addition, delayed Grk signaling early in oogenesis could alter the dynamics of posterior follicle-cell patterning and affect mRNA localization in later stages. Leaky regulation of Grk translation could lead to the accumulation of Grk protein in unusual endoplasmic reticulum-like vesicles. Together, these defects would eventually lead to the final rhino eggshell phenotypes. Independently, Rhi would facilitate nurse cell chromosome reorganization at the end of endocycle 5. This hypothesis is analogous to the dual role of Swi6 in mating-type locus repression and chromosome segregation (EKWALL *et al.* 1995; ALLSHIRE 1996).

A second, highly speculative hypothesis argues that Rhi could effect chromosome structural transitions in nurse cells at the end of endoreplication cycle 5 and in the oocyte during meiotic I prophase. The progress of these changes would be monitored by “egg chamber maturation checkpoint proteins” that coordinate germ cell nuclear events with microtubule functions. Mechanistically, Rhi could bind directly to chromosomes to alter structure or regulate expression of a gene that lies upstream in a pathway controlling both chromosomal and microtubule reorganizations, *e.g.*, *cdk1* (reviewed by NIGG 1995). Defects in chromosome condensation would trigger a checkpoint that inhibits Grk translation,

disrupting the S3–S5 Grk signaling pathway that establishes posterior follicle-cell fate (GHABRIAL *et al.* 1998; GHABRIAL and SCHÜPBACH 1999; reviewed by NILSON and SCHÜPBACH 1999). Improper posterior follicle-cell patterning and/or additional defects in microtubule reorganization at S6–S7 would disrupt localization of axis-determining mRNAs.

One factor detracting from this hypothesis is a lack of obvious defects in chromosome structure within the germinal vesicle. This result could be due to the small size of the oocyte chromosomes; differences that are readily apparent in highly polyploid nurse cell nuclei may not be detectable in the compact oocyte nucleus. Visible defects in oocyte chromosomes are apparent in other mutants in which the meiotic checkpoint is triggered, possibly because these genes are required for DNA repair; loss of function leads to breakdown of DNA (GHABRIAL and SCHÜPBACH 1999). We expect that *rhi* mutations would lead to an arrest during chromosome compaction and may produce only subtle differences in chromosome structure. Similarly, loss-of-function mutations in *HP1* and *swi6* produce defects in chromosome segregation but no obvious structural changes in chromosome morphology. In the ovary, mutations that disrupt Dp, a subunit of the E2F transcription factor that regulates the G1-S transition, also produce no obvious defects in chromosome structure but yield phenotypes strikingly similar to *rhi* mutants (MYSTER *et al.* 2000). Ventralized eggshells, nurse cell dumping defects, and reduced Grk protein present in actin-caged vesicles suggest a common pathway regulated by E2F and Rhi.

Finally, we cannot rule out the possibility that *rhi* encodes a cytoplasmic protein with cytoskeletal or transport functions. For example, Rhi could shuttle between the nuclear envelope and the endoplasmic reticulum, regulating the distribution of RNAs and proteins associated with these structures. Loss-of-function mutations in *rhi* would then indirectly lead to defects in nurse cell chromosome conformation and Grk synthesis. Alternatively, Rhi could regulate the synthesis or activity of molecules that perform these functions. The most likely candidates would be products of the *cup*, *otu*, and *fs(2)B* loci, which affect germ cell chromosome conformation and overall egg chamber maturation (KEYES and SPRADLING 1997). Given the homology between Rhi and HP1 family members and the distinct differences in phenotypes of *rhi* vs. *cup*, *otu*, and *fs(2)B* mutants, we favor the two previous hypotheses.

Significance of Rhino function in other systems: *rhi* is the first chromo box gene that functions specifically in oogenesis. Rhi shares significant homology with many chromo-domain-containing proteins, demonstrating its membership in the HP1 family. Specific Rhi homologs, however, which share significant homology outside the chromo box or chromo-shadow domain, have not been identified. One other chromo-domain protein is germline specific. LAHN and PAGE (1997, 1999) describe a

male-specific chromo-domain protein encoded on the nonrecombinant region of the human Y chromosome. This protein is thought to be testis specific; no functional studies, however, have been described. Although thus far Rhi is unique to *Drosophila*, we predict that other species with similar endoreplication cycles may also have adapted an HP1 family member to create a Rhi-like regulatory mechanism that mediates chromosome conformational changes. Similarly, Rhi-like proteins may be required to couple advancement through the cell cycle with the establishment of egg polarity or coordinate other processes whose temporal regulation is important for the ordered progression of development.

In conclusion, we have genetically and molecularly characterized a novel gene that affects nurse cell chromosome conformation and egg polarity during *Drosophila* oogenesis. The molecular nature of Rhi suggests that it may act with other proteins, in a manner analogous to the HP1 subfamily of chromo-domain proteins, to control chromosome condensation at the onset of mitosis. *rhi* mutations also disrupt Grk synthesis, processing, or stability and thereby affect the subsequent establishment of egg polarity. This regulation may involve transcriptional repression of genes that control Grk protein production or it may occur through induction of a checkpoint control in prophase of meiosis I. Rhino is the first chromo-domain protein with a unique developmental role in oogenesis.

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LITERATURE CITED

- AASLAND, R., and A. F. STEWART, 1995 The chromo shadow domain, a second chromo domain in heterochromatin-binding protein 1, HP1. *Nucleic Acids Res.* **23**: 3168–3173.
- ALLSHIRE, R. C., 1996 Transcriptional silencing in the fission yeast: a manifestation of higher order chromosome structure and function, pp. 443–466 in *Epigenetic Mechanisms of Gene Regulation*, edited by V. E. A. RUSSO, R. A. MARTIENSSSEN and A. R. RIGGS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- ALTSCHUL, S. W., W. GISH, E. MILLER, E. MEYERS and D. LIPMAN, 1990 Basic local alignment search tool. *J. Mol. Biol.* **215**: 403–410.
- ASHBURNER, M., 1989 *Drosophila: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- BALL, L. J., N. V. MURZINA, R. W. BROADHURST, A. R. RAINE, S. J. ARCHER *et al.*, 1997 Structure of the chromatin binding (chromo) domain from mouse modifier protein 1. *EMBO J.* **16**: 2473–2481.
- BERG, C. A., and A. C. SPRADLING, 1991 Studies on the rate and site-specificity of *P* element transposition. *Genetics* **127**: 515–524.
- CAVALLI, G., and R. PARO, 1998 Chromo-domain proteins: linking chromatin structure to epigenetic regulation. *Curr. Opin. Cell Biol.* **10**: 354–360.
- CLEGG, N. J., D. M. FROST, M. K. LARKIN, L. SUBRAHMANYAN, Z. BRYANT *et al.*, 1997 *maelstrom* is required for an early step in the establishment of *Drosophila* oocyte polarity: posterior localization of *grk* mRNA. *Development* **124**: 4661–4671.
- COOLEY, L., and W. E. THEURKAUF, 1994 Cytoskeletal functions during *Drosophila* oogenesis. *Science* **266**: 590–596.
- COOPERSTOCK, R. L., and H. D. LIPSHITZ, 2001 RNA localization and translational regulation during axis specification in the *Drosophila* oocyte. *Int. Rev. Cytol.* **203**: 541–566.
- COWELL, I. G., and C. A. AUSTIN, 1997 Self-association of chromo domain peptides. *Biochim. Biophys. Acta* **1337**: 198–206.
- COWIESON, N. P., J. F. PARTRIDGE, R. C. ALLSHIRE and P. J. McLAUGHLIN, 2000 Dimerisation of a chromo shadow domain and distinctions from the chromodomain as revealed by structural analysis. *Curr. Biol.* **10**: 517–525.
- CRAMTON, S. E., and F. A. LASKI, 1994 *string of pearls* encodes a *Drosophila* ribosomal protein S2, has Minute-like characteristics, and is required during oogenesis. *Genetics* **137**: 1039–1048.
- DEJ, K. J., and A. C. SPRADLING, 1999 The endocycle controls nurse cell polytene chromosome structure during *Drosophila* oogenesis. *Development* **126**: 293–303.
- DELATTRE, M., A. SPIERER, C. H. TONKA and P. SPIERER, 2000 The genomic silencing of position-effect variegation in *Drosophila melanogaster*: interaction between the heterochromatin-associated proteins Su(var)3–7 and HP1. *J. Cell Sci.* **113**: 4253–4261.
- DENG, W. M., and M. BOWNES, 1997 Two signalling pathways specify localised expression of the *Broad-Complex* in *Drosophila* eggshell patterning and morphogenesis. *Development* **124**: 4639–4647.
- EISSENBERG, J. C., and S. C. R. ELGIN, 2000 The HP1 protein family: getting a grip on chromatin. *Curr. Opin. Genet. Dev.* **10**: 204–210.
- EISSENBERG, J. C., T. C. JAMES, D. M. FOSTER-HARTNETT, T. HARTNETT, V. NGAN and S. C. R. ELGIN, 1990 Mutation in a heterochromatin-specific chromosomal protein is associated with the suppression of position-effect variegation in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **87**: 9923–9927.
- EKWALL, K., J. P. JAVERZAT, A. LORENTZ, H. SCHMIDT, G. CRANSTON *et al.*, 1995 The chromodomain protein Swi6: a key component at fission yeast centromeres. *Science* **8**: 1429–1431.
- ENGELS, W. R., D. M. JOHNSON-SCHLITZ, W. B. EGGLESTON and J. SVED, 1990 High-frequency *P* element loss in *Drosophila* is homolog dependent. *Cell* **62**: 515–525.
- EPHRUSSI, A., L. K. DICKINSON and R. LEHMANN, 1991 Oskar organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* **66**: 37–50.
- FEINBERG, A. P., and B. VOGELSTEIN, 1983 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**: 6–13.
- GHABRIAL, A., and T. SCHÜPBACH, 1999 Activation of a meiotic checkpoint regulates translation of Gurken during *Drosophila* oogenesis. *Nat. Cell Biol.* **1**: 354–357.
- GHABRIAL, A., R. P. RAY and T. SCHÜPBACH, 1998 *okra* and *spindle-B* encode components of the *RAD52* DNA repair pathway and affect meiosis and patterning in *Drosophila* oogenesis. *Genes Dev.* **12**: 2711–2723.
- GILLESPIE, D. E., and C. A. BERG, 1995 *homeless* is required for RNA localization in *Drosophila* oogenesis and encodes a new member of the DE-H family of RNA-dependent ATPases. *Genes Dev.* **9**: 2495–2508.
- GORMAN, M., A. FRANKE and B. S. BAKER, 1995 Molecular characterization of the *male-specific lethal-3* gene and investigations of the regulation of dosage compensation in *Drosophila*. *Development* **121**: 463–475.
- HAMMOND, M. P., and C. D. LAIRD, 1985 Chromosome structure and DNA replication in nurse and follicle cells of *Drosophila melanogaster*. *Chromosoma* **91**: 267–278.
- HEARN, M. G., A. HEDRICK, T. A. GRIGLIATTI and B. T. WAKIMOTO, 1991 The effect of modifiers of position-effect variegation on the variegation of heterochromatic genes of *Drosophila melanogaster*. *Genetics* **128**: 785–797.
- HEINO, T. I., 1989 Polytene chromosomes from ovarian pseudonurse cells of the *Drosophila melanogaster otu* mutant. I. Photographic map of chromosome 3. *Chromosoma* **97**: 363–373.

- HEINO, T. I., V. P. LAHTI, M. TIRRONEN and C. ROOS, 1995 Polytene chromosomes show normal gene activity but some mRNAs are abnormally accumulated in the pseudonurse cell nuclei of *Drosophila melanogaster* *otu* mutants. *Chromosoma* **104**: 44–55.
- HOROWITZ, H., and C. A. BERG, 1995 Aberrant splicing and transcription termination caused by *P* element insertion into the intron of a *Drosophila* gene. *Genetics* **139**: 327–335.
- HOROWITZ, H., and C. A. BERG, 1996 The *Drosophila pipsqueak* gene encodes a nuclear BTB-domain-containing protein required early in oogenesis. *Development* **122**: 1859–1871.
- HUANG, D. W., L. FANTI, D. T. PAK, M. R. BOTCHAN, S. PIMPINELLI *et al.*, 1998 Distinct cytoplasmic and nuclear fractions of *Drosophila* Heterochromatin Protein 1: their phosphorylation levels and associations with origin recognition complex proteins. *J. Cell Biol.* **142**: 307–318.
- JAMES, T. C., and S. C. R. ELGIN, 1986 Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene. *Mol. Cell. Biol.* **6**: 3862–3872.
- JOWETT, T., 1986 Preparation of nucleic acids, pp. 275–286 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press, Washington, DC.
- KAFATOS, F. C., C. LOUIS, C. SAVAKIS, D. M. GLOVER, M. ASHBURNER *et al.*, 1991 Integrated maps of the *Drosophila* genome: progress and prospects. *Trends Genet.* **7**: 155–161.
- KARPEN, G., and A. C. SPRADLING, 1992 Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome *Dp1187* by single *P* element insertional mutagenesis. *Genetics* **132**: 737–753.
- KELLUM, R., J. W. RAFF and B. M. ALBERTS, 1995 Heterochromatin Protein 1 distribution during development and during the cell cycle in *Drosophila* embryos. *J. Cell Sci.* **108**: 1407–1418.
- KEYES, L. N., and A. C. SPRADLING, 1997 The *Drosophila* gene *fs(2)cup* interacts with *otu* to define a cytoplasmic pathway required for the structure and function of germ-line chromosomes. *Development* **124**: 1419–1431.
- KIM-HA, J., K. KERR and P. M. MACDONALD, 1995 Translational regulation of *oskar* mRNA by Bruno, an ovarian RNA-binding protein, is essential. *Cell* **81**: 403–412.
- KING, R., 1970 *Ovarian Development in Drosophila melanogaster*. Academic Press, New York.
- KOONIN, E. V., S. ZHOU and J. C. LUCCHESI, 1995 The chromo superfamily: new members, duplication of the chromo domain and possible role in delivering transcription regulators to chromatin. *Nucleic Acids Res.* **23**: 4229–4233.
- LAHN, B. T., and D. C. PAGE, 1997 Functional coherence of the human Y chromosome. *Science* **278**: 675–680.
- LAHN, B. T., and D. C. PAGE, 1999 Retroposition of autosomal mRNA yielded testis-specific gene family on human Y chromosome. *Nat. Genet.* **21**: 429–433.
- LA THANGUE, N. B., 1994 DP and E2F proteins: components of a heterodimeric transcription factor implicated in cell cycle control. *Curr. Opin. Cell Biol.* **6**: 443–450.
- LE DOUARIN, B., A. L. NIELSEN, J. M. GARNIER, H. ICHINOSE, F. JEANMOUGIN *et al.*, 1996 A possible involvement of TIF1 α and TIF1 β in the epigenetic control of transcription by nuclear receptors. *EMBO J.* **15**: 6701–6715.
- LEHMANN, M., T. SIEGMUND, K. G. LINTERMANN and G. KORGE, 1998 The Pipsqueak protein of *Drosophila melanogaster* binds to GAGA sequences through a novel DNA-binding domain. *J. Biol. Chem.* **273**: 28504–28509.
- LEWIS, E. B., 1978 A gene complex controlling segmentation in *Drosophila*. *Nature* **276**: 565–570.
- MYSTER, D. L., P. C. BONNETTE and R. J. DURONIO, 2000 A role for the DP subunit of the E2F transcription factor in axis determination during *Drosophila* oogenesis. *Development* **127**: 3249–3261.
- NEUMAN-SILBERBERG, F. S., and T. SCHÜPBACH, 1993 The *Drosophila* dorsoventral patterning gene *gurken* produces a dorsally localized RNA and encodes a TGF α -like protein. *Cell* **75**: 165–174.
- NEUMAN-SILBERBERG, F. S., and T. SCHÜPBACH, 1996 The *Drosophila* TGF- α -like protein Gurken: expression and cellular localization during *Drosophila* oogenesis. *Mech. Dev.* **59**: 105–113.
- NIGG, E. A., 1995 Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. *Bioessays* **17**: 471–480.
- NILSON, L. A., and T. SCHÜPBACH, 1999 EGF receptor signaling in *Drosophila* oogenesis. *Curr. Top. Dev. Biol.* **44**: 203–243.
- PAK, D. T., M. PFLUMM, I. CHESNOKOV, D. W. HUANG, R. KELLUM *et al.*, 1997 Association of the origin recognition complex with heterochromatin and HP1 in higher eukaryotes. *Cell* **91**: 311–323.
- PARO, R., and P. J. HARTE, 1996 The Polycomb group and trithorax group chromatin complexes in the maintenance of determined cell fates, pp. 507–528 in *Epigenetic Mechanisms of Gene Regulation*, edited by V. E. A. RUSSO, R. A. MARTIENSSSEN and A. R. RIGGS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- PARO, R., and D. S. HOGNESS, 1991 The Polycomb protein shares a homologous region with a heterochromatin-associated protein of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **88**: 263–267.
- PARO, R., H. STRUTT and G. CAVALLI, 1998 Heritable chromatin states induced by the *Polycomb* and *trithorax* group genes. *Novartis Found. Symp.* **214**: 51–61.
- PERI, F., and S. ROTH, 2000 Combined activities of Gurken and Decapentaplegic specify dorsal chorion structures of the *Drosophila* egg. *Development* **127**: 841–850.
- PLATERO, J. S., T. HARTNETT and J. C. EISENBERG, 1995 Functional analysis of the chromo domain of HP1. *EMBO J.* **14**: 3977–3986.
- QUEENAN, A. M., G. BARCELO, C. VAN BUSKIRK and T. SCHÜPBACH, 1999 The transmembrane region of Gurken is not required for biological activity, but is necessary for transport to the oocyte membrane in *Drosophila*. *Mech. Dev.* **89**: 35–42.
- REED, B. H., and T. L. ORR-WEAVER, 1997 The *Drosophila* gene *morula* inhibits mitotic functions in the endo cell cycle and the mitotic cell cycle. *Development* **124**: 3543–3553.
- ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. M. JOHNSON-SCHLITZ, W. K. BENZ *et al.*, 1988 A stable genomic source of *P* element transposase in *Drosophila melanogaster*. *Genetics* **118**: 461–470.
- ROTH, S., and T. SCHÜPBACH, 1994 The relationship between ovarian and embryonic dorsoventral patterning in *Drosophila*. *Development* **120**: 2245–2257.
- ROTH, S., F. S. NEUMAN-SILBERBERG, G. BARCELO and T. SCHÜPBACH, 1995 *cornichon* and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *Drosophila*. *Cell* **81**: 967–978.
- SAFFMAN, E. E., S. STYHLER, K. ROTHER, W. LI, S. RICHARD *et al.*, 1998 Premature translation of *oskar* in oocytes lacking the RNA-binding protein Bicaudal-C. *Mol. Cell. Biol.* **18**: 4855–4862.
- SAUNDERS, W. S., C. CHUE, M. GOEBL, C. CRAIG, R. F. CLARK *et al.*, 1993 Molecular cloning of a human homologue of *Drosophila* heterochromatin protein HP1 using anti-centromere autoantibodies with anti-chromo specificity. *J. Cell Sci.* **104**: 573–582.
- SIDEN-KIAMOS, I., R. D. SAUNDERS, L. SPANOS, T. MAJERUS, J. TREANER *et al.*, 1990 Towards a physical map of the *Drosophila melanogaster* genome: mapping of cosmid clones within defined physical divisions. *Nucleic Acids Res.* **18**: 6261–6270.
- SIEGEL, V., T. JONGENS, L. JAN and Y.-N. JAN, 1993 *pipsqueak*, an early acting member of the posterior group of genes, affects *vasa* level and germ cell-somatic cell interaction in the developing egg chamber. *Development* **119**: 1187–1202.
- SINGH, P. B., and N. S. HUSKISSON, 1998 Chromatin complexes as aperiodic microcrystalline arrays that regulate genome organization and expression. *Dev. Genet.* **22**: 85–99.
- SINGH, P. B., J. R. MILLER, J. PEARCE, R. KOTHARY, R. D. BURTON *et al.*, 1991 A sequence motif found in a *Drosophila* heterochromatin protein is conserved in animals and plants. *Nucleic Acids Res.* **19**: 789–794.
- SMOTHERS, J. F., and S. HENIKOFF, 2000 The HP1 chromo shadow domain binds a consensus peptide pentamer. *Curr. Biol.* **10**: 27–30.
- SMOTHERS, J. F., and S. HENIKOFF, 2001 The hinge and chromo shadow domain impart distinct targeting of HP1-like proteins. *Mol. Cell. Biol.* **21**: 2555–2569.
- SPRADLING, A. C., 1993 Developmental genetics of oogenesis, pp. 1–70 in *The Development of Drosophila melanogaster*, edited by M. BATE and A. MARTINEZ-ARIAS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- STEGER, D. J., R. T. UTLEY, P. A. GRANT, S. JOHN, A. EBERHARTER *et al.*, 1998 Regulation of transcription by multisubunit complexes that alter nucleosome structure. *Cold Spring Harbor Symp. Quant. Biol.* **63**: 483–491.
- STEINHAEUER, W. R., R. C. WALSH and L. J. KALFAYAN, 1989 Sequence and structure of the *Drosophila melanogaster* ovarian tumor gene and generation of an antibody specific for the ovarian tumor protein. *Mol. Cell. Biol.* **9**: 5726–5732.
- STEVENS, L., 1998 Twin peaks: Spitz and Argos star in patterning of the *Drosophila* egg. *Cell* **95**: 291–294.
- ST. JOHNSTON, D., W. DRIEVER, T. BERLETH, S. RICHSTEIN and C.

- NUSSLEIN-VOLHARD, 1989 Multiple steps in the localization of *bicoid* RNA to the anterior pole of the *Drosophila* oocyte. *Development* **107**(Suppl.): 13–19.
- STROUMBAKIS, N. D., Z. LI and P. P. TOLIAS, 1994 RNA- and single-stranded DNA-binding proteins expressed during *Drosophila melanogaster* oogenesis: a homolog of bacterial and mitochondrial SSBs. *Gene* **143**: 171–177.
- SUGIMOTO, K., T. YAMADA, Y. MURO and M. HIMENO, 1996 Human homolog of *Drosophila* heterochromatin-associated protein 1 (HP1) is a DNA-binding protein which possesses a DNA-binding motif with weak similarity to that of the human centromere protein C (CENP-C). *J. Biochem.* **120**: 153–159.
- TINKER, R., D. SILVER and D. J. MONTELL, 1998 Requirement for the Vasa RNA helicase in *gurken* mRNA localization. *Dev. Biol.* **199**: 1–10.
- TWOMBLY, V., R. K. BLACKMAN, H. JIN, J. M. GRAFF, R. W. PADGETT *et al.*, 1996 The TGF-beta signaling pathway is essential for *Drosophila* oogenesis. *Development* **122**: 1555–1565.
- WEBSTER, P. J., L. LIANG, C. A. BERG, P. LASKO and P. M. MACDONALD, 1997 Translational repressor bruno plays multiple roles in development and is widely conserved. *Genes Dev.* **11**: 2510–2521.
- WEILER, K. S., and B. T. WAKIMOTO, 1995 Heterochromatin and gene expression in *Drosophila*. *Annu. Rev. Genet.* **29**: 577–605.
- XIE, T., and A. C. SPRADLING, 1998 *decapentaplegic* is essential for the maintenance and division of germline stem cells in the *Drosophila* ovary. *Cell* **94**: 251–260.
- YAMADA, T., R. FUKUDA, M. HIMENO and K. SUGIMOTO, 1999 Functional domain structure of human heterochromatin protein HP1 (Hsa): involvement of internal DNA-binding and C-terminal self-association domains in the formation of discrete dots in interphase nuclei. *J. Biochem.* **125**: 832–837.
- YE, Q., and H. J. WORMAN, 1996 Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to *Drosophila* HP1. *J. Biol. Chem.* **271**: 14653–14656.
- YE, Q., I. CALLEBAUT, A. PEZHMAN, J. C. COURVALIN and H. J. WORMAN, 1997 Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. *J. Biol. Chem.* **272**: 14983–14989.

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