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Identification of ORD, a *Drosophila* protein essential for sister chromatid cohesion

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Attachment between the sister chromatids is required for proper chromosome segregation in meiosis and mitosis, but its molecular basis is not understood. Mutations in the *Drosophila ord* gene result in premature sister chromatid separation in meiosis, indicating that the product of this gene is necessary for sister chromatid cohesion. We isolated the *ord* gene and found that it encodes a novel 55 kDa protein. Some of the *ord* mutations exhibit unusual complementation properties, termed negative complementation, in which particular alleles poison the activity of another allele. Negative complementation predicts that protein–protein interactions are critical for ORD function. The position and nature of these unusual *ord* mutations demonstrate that the C-terminal half of ORD is essential for sister chromatid cohesion and suggest that it mediates protein binding.

Keywords: chromosome segregation/*Drosophila*/meiosis/ORD/sister chromatid cohesion

Introduction

Sister chromatids separate and segregate from each other in anaphase of mitosis and anaphase II of meiosis. For proper orientation and segregation in mitosis, the sister chromatids must remain attached until the metaphase/anaphase transition. In meiosis, the sister chromatids must remain associated throughout meiosis I as the homologs segregate and retain these associations until the metaphase II/anaphase II transition. Although mechanisms that ensure sister chromatid cohesion have been postulated, few candidate genes have been identified (for a review, see Miyazaki and Orr-Weaver, 1994). There are likely to be structural proteins that hold sister chromatids together, as well as regulatory functions that time the release of cohesion until the metaphase/anaphase transition. Identifying proteins necessary for sister chromatid cohesion is critical for an understanding of segregation.

The *Drosophila ord* (*orientation disruptor*) gene is required for sister chromatid cohesion in meiosis (Mason, 1976; Goldstein, 1980; Miyazaki and Orr-Weaver, 1992). Mutations in the *ord* gene cause chromosome missegregation during female and male meiosis that is consistent with precocious sister chromatid separation early in meiosis I, followed by random segregation of the sister chromatids during the two meiotic divisions. This interpretation was confirmed cytologically in mutant males; sister chromatids prematurely disjoin as early as prometaphase I (Mason, 1976; Goldstein, 1980; Miyazaki and Orr-Weaver, 1992). Strong *ord* mutations cause missegregation in the mitotic germline divisions in the male, indicating that the *ord* gene product may also promote sister chromatid cohesion during mitosis (Lin and Church, 1982; Miyazaki and Orr-Weaver, 1992). However, any mitotic requirement for *ord* is likely to be restricted to the germline, because *ord* mutations do not affect mitosis in the somatic tissues (J.Wu, W.Miyazaki and T.Orr-Weaver, unpublished results).

In addition to causing premature sister chromatid separation in meiosis, most *ord* mutations reduce recombination in females (Mason, 1976; Miyazaki and Orr-Weaver, 1992). Normally, recombination occurs in *Drosophila* female meiosis, but not in male meiosis (for a review, see Orr-Weaver, 1995). It is possible that the *ord* gene primarily controls cohesion between sister chromatids and without cohesion the homologs are unable to undergo recombination with normal efficiency, perhaps because they cannot be brought into proper alignment. Alternatively, the *ord* gene could have multiple regulatory roles in meiosis and independently affect recombination and sister chromatid cohesion. The non-disjunction observed during female meiosis is not due solely to a failure in exchange, however, because both exchange and non-exchange chromosomes segregate improperly in *ord* mutants (Mason, 1976).

The genetic properties of mutations can provide insight into the function of the gene product. For example, intragenic complementation is diagnostic of multiple functional domains within a protein. Another intragenic effect has been termed negative complementation (Fincham, 1966). In negative complementation, the residual activity of one allele can be poisoned by another. Negative complementation occurs when two alleles of a locus *in trans* to one another exhibit a more severe phenotype than homozygotes of either allele. Another case of negative complementation is when two alleles *in trans* to each other have a stronger phenotype than one or both *in trans* to a deficiency. A subset of negative complementing interactions is exhibited by mutations known as antimorphs (Muller, 1932), also referred to as dominant negatives (Herskowitz, 1987). Antimorphs are special cases because instead of poisoning the residual activity of a mutant allele, they can antagonize the function of the wild-type allele, thus producing a dominant phenotype.

Here we show that some of the mutations in *ord* exhibit negative complementation. We cloned the gene, identified the ORD protein and mapped the changes that the *ord* mutations cause in the protein. The combination of phenotypic and molecular analysis provides critical insight into ORD protein function.

Results

Identification of the ORD protein

We determined the cytological position of *ord* by testing the ability of several deficiencies to complement the *ord* mutation (Table I). *ord* maps proximal to the *brown* (*bw*) locus at 59E. Since the gene was uncovered by *Df(2R)bw-S46*, but not by *Df(2R)bw-HB132*, it must lie in the interval 59D5–11 on the right arm of chromosome 2. To localize *ord* further, we generated additional deficiencies in region 59 by irradiating a stock containing a *white*$^+$ P-element insertion at 59C in addition to the normal *bw*$^+$ allele (Levis *et al.*, 1985). This allowed us to score for deficiencies in the region by loss of either visible marker. Although these deficiencies did not refine the location of *ord* further at a cytological level, they were critical in delineating *ord* molecularly. Two important deletions were *Df(2R)bw-WI366*, which removes the *bw* locus, and *Df(2R)WI370*, which removes the *w*$^+$ P element but retains the *bw* gene (Table I). Both of these deficiencies have breakpoints in 59D5–11, but *Df(2R)bw-WI366* is phenotypically ord$^+$, while *Df(2R)WI370* is ord$^-$.

We carried out a molecular walk to clone the genomic region containing *ord*. By quantitative Southern analysis, the proximal *Df(2R)bw-S46* breakpoint mapped within the most distal phage (G21) of the *twist* walk (Thisse *et al.*, 1987). We walked distally from the *twist* walk using a genomic library in lambda phage. Lambda clones from each step were hybridized to genomic DNA from deficiency strains to map the breakpoints. Crossing the proximal breakpoint of *Df(2R)bw-WI366* defined the minimal interval containing *ord* to ~25 kb (Figure 1).

To identify the *ord* gene within the chromosomal walk, DNA from this region was transformed into *Drosophila* to test for rescue of *ord* mutants. Inserts from overlapping lambda clones were subcloned into a P-element transformation vector containing a *white*$^+$ selectable marker (Pirrotta, 1988). Transformed lines were generated for three transposons, crossed into *ord*^{1}/*ord*^{3} transheterozygotes and tested for sex chromosome missegregation (Figure 1). Transposon *P{D39}* restored normal chromosome segregation to *ord* mutant flies (data not shown). Restriction fragments from transposon *P{D39}* were transformed into *Drosophila* and tested for complementation (Figure 1). The 6.3 kb *Bam*HI fragment in *P{6.3BB}* fully rescues the *ord* missegregation phenotype in both male and female meiosis (Table II). Moreover, recombination levels are restored to normal levels in *ord*^{1}/*ord*^{3} females carrying the *P{6.3BB}* transposon (Table II).

We identified the *ORD* transcription unit by isolating testes cDNAs that hybridized to *P{6.3BB}*, because we knew that the gene is required for proper meiotic chromosome segregation. The cDNAs fell into two groups by cross-hybridization experiments. Representative cDNAs from each transcription unit as well as one strand of the genomic DNA were sequenced. This placed one transcription unit completely within *P{3.6RR}*, making it unlikely that it encoded ORD, because this transposon did not complement *ord* mutations. To confirm that the other transcription unit was *ORD*, we generated a 136 bp deletion that only disrupted the second transcription unit (*P{7.3BPΔ}*, Figure 1). When transformed into flies, *P{7.3BPΔ}* failed to rescue *ord* mutants, indicating that this transcription unit is *ORD*.

The *ORD* message is of low abundance, and on Northern blots we detected *ORD* transcripts only in adult ovary and

Table I. Deficiencies that define the *ord* interval

Deficiency	Cytological breakpoints	*ord* phenotype[a]
Df(2R)bw-S46	59D5–11; 60A7	–
Df(2R)bw-HB132	59D5–11; 59F6–8	+
Df(2R)bw-WI366	59D5–11; 60B1–3	+
Df(2R)WI370	59B3; 59D5–11	–

[a]'–' indicates that the deficiency uncovers *ord* and is mutant for the locus. '+' indicates that the deficiency does not delete the *ord* gene.

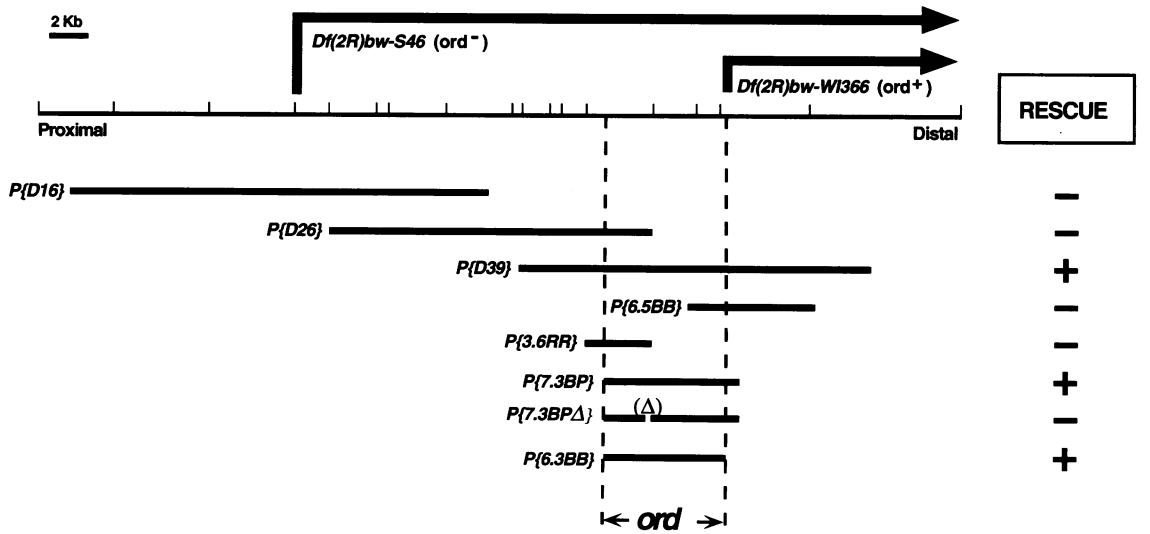

Fig. 1. Identification of the *ord* gene. An *Eco*RI restriction map is shown for the genomic interval containing the *ord* gene. The DNA missing in the deficiencies *Df(2R)bw-S46* and *Df(2R)bw-WI366* is indicated by thick lines and arrows. Because *Df(2R)bw-WI366* is ord$^+$, its breakpoint defines the distal border of the region containing the *ord* gene. The DNA fragments transformed into *Drosophila* are shown relative to the genomic map. A '+' indicates that the fragment rescued the chromosome missegregation phenotype of *ord* mutants; a '–' indicates that it did not. These experiments defined a 6.3 kb region containing the *ord* gene, shown by dashed lines.

Table II. Transposon rescue of *ord* phenotypes

(A) Male segregation tests

Genotype[a]	Regular gametes		Exceptional gametes			
	X	*Y*	O	*XX*	*XY*	*XXY*
ord^1/ord^3	45	65	58	15	26	1
ord^1/ord^3 ; *P{6.3BB}*	285	225	0	0	3	0

(B) Female segregation tests

Genotype[b]	Regular gametes	Exceptional gametes	
	X	O	*XX*
ord^1/ord^3	48	7	11
ord^1/ord^3 ; *P{6.3BB}*	75	0	0

(C) Recombination map distances

Genotype[c]	*y*–*cv* (cM)	*cv*–*f* (cM)	Progeny scored
+/+[d]	10.4	38.7	1536
ord^1/ord^3	2.1	2.5	591
ord^1/ord^3 ; *P{6.3BB}*	7.6	36.5	978

[a] y/y^+Y males with the genotype indicated were crossed to attached-*X*, y^2 *su*(w^a) w^a females. Numbers indicate progeny scored.
[b] *y w* females with the genotype indicated were crossed to attached-*XY*, *v f B males*. Numbers indicate progeny scored.
[c] *y w*/*w cv v f car* females with the genotype indicated were crossed to *y w* males.
[d] Data taken from Miyazaki and Orr-Weaver (1992).

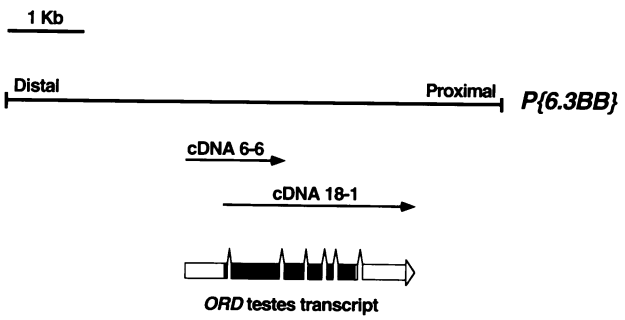

Fig. 2. The *ORD* transcription unit. The position of testis cDNAs corresponding to *ord* are shown relative to the 6.3 kb rescuing fragment. The direction of transcription is indicated by arrows. Sequencing of the cDNAs and genomic DNA demonstrated that the *ORD* transcription unit contains six introns. The open reading frame is shown in black.

testis and in no other tissue or developmental stage (data not shown). In testes and ovary poly(A)$^+$ RNA, the predominant message is ~2.7 kb. This agrees with the predicted size of an *ORD* transcript derived from aligning overlapping testes cDNAs (Figure 2). In addition, there are larger transcripts of even lower abundance that differ in size between ovary and testis.

The direction of *ORD* transcription is distal to proximal with respect to the chromosome, and the predominant 2.7 kb transcript contains six introns (Figure 2). The introns are small, ranging in size from 53 to 67 bp. The *ORD* transcription unit has an open reading frame encoding 479 amino acids, predicted to be a 55 kDa protein (Figure 3). There is a 5′ untranslated region of 502 bp and a 771 bp 3′ untranslated region which includes the last intron. No sequences with significant regions of homology

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MYGETTLNKN HVIKFIKLKI NNCLGCDEVE INFSKADNVH IIIYSLVTDM   50

AKDLPAVTPV AQAILLLCSL TYPDSDSLET IPQLKIGKGS VSMSFKVYPV  100

NKEEETEPES ESDLDEGPST SKQALERMVQ RAERKAKEAS TRNVHSKGIY  150

VNVERRFDMY FALDTVSYYI NGGKRQSCPL PEFHAKFFVR PQHSINLLRQ  200
                                                ord5
                                                 •
LHEKCSGNWL KVIQSDGDGD AFKKFKDPDS PFETFVKLFE SNPIKPNDMM  250
                                                ord6
                                                 R
GKLAKTCLHV NEAVRLTERE FILEVFNQVR HIFEYITAQE YTVWFLVPCL  300
        ord3
         ▼
GDKDQLRSKT LEDFDLTKVR TSIRRAGDTS NIWWDHTDHN IKDILLVAFQ  350
                                ord2
                                 T
LDLATHVNQS VLVISHLETL AEFSTMQYVT AFFMNDFYAK KNTEPKWICH  400
ord1                    ord4
 H                       V
RYLERIIDVA LFLGVIVIIE YPSAFTLLQE GRHLIKCFQK ENAESSRTSQ  450

WEIFEDVVKE NESDLEFLKE AVGKVQQNV.
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Fig. 3. The ORD protein. The 479 amino acid sequence of the ORD protein encoded by the testis transcription unit is shown together with the position and nature of the sequenced *ord* mutations.

were identified in the databases using several search programs (Smith and Waterman, 1981; Lipman and Pearson, 1985; Altschul *et al.*, 1990).

The sequence immediately upstream of the putative N-terminus matches the Cavener consensus sequence for *Drosophila* translation initiation (Cavener, 1987). Moreover, there are stop codons in all three frames within 55 bp upstream of the N-terminal methionine. Therefore, the methionine indicated in Figure 3 most likely represents the N-terminus of the protein encoded by the 2.7 kb predominant testes transcript.

Several results demonstrate that the 55 kDa protein is ORD. The 136 bp deletion described above (P*{7.3BPΔ}*) changes the frame at amino acid 226 and abolishes *ord* rescuing activity. In addition, we showed by DNA sequence analysis that all *ord* mutations characterized thus far result in single nucleotide changes within the open reading frame (see below). In one of the strongest alleles, ord^5, amino acid 245 is mutated to a stop codon, truncating the protein approximately halfway through the open reading frame.

The ORD protein has several notable features. An interval enriched in hydrophobic amino acids (14 out of 20) lies between residues 409 and 428. Overall, the protein is fairly acidic, with a predicted pI of 5.8. The region spanning amino acids 103–116 is quite acidic with nine out of 14 residues bearing a negative charge. This acidic region lies within a highly significant PEST sequence between amino acids 102 and 122. PEST regions have been found in proteins known to undergo rapid degradation (Rechsteiner, 1988; Chevaillier, 1993), and it has been proposed that they signal proteolysis by a mechanism distinct from a ubiquitin-dependent system (Rogers *et al.*, 1986). Three out of 14 potential casein kinase II phosphorylation sites within ORD reside in this PEST interval, perhaps increasing the negative charge of the region.

Table III. Allele-specific interactions in females

Female genotype[a]	Exceptional gametes		Adjusted total[b]	% missegregation
	XX	O		
ord^4/ord^4	0.7[c] (7)[d]	0.4 (4)	1956	**1.1**
Df^e/ord^4	1.1 (41)	1.1 (40)	7389	**2.2**
ord^1/ord^4	10.5 (209)	9.7 (192)	3971	**20.2**
ord^2/ord^4	3.5 (33)	2.8 (26)	1868	**6.3**
ord^3/ord^4	0.5 (5)	0.3 (3)	2076	**0.8**[f]
ord^5/ord^4	1.0 (16)	0.5 (8)	3173	**1.5**[g]
ord^6/ord^4	1.5 (15)	1.3 (13)	2030	**2.8**[h]

[a]Females were crossed to attached-*XY, v f B males.*
[b]The progeny total is adjusted to correct for recovery of only half of the exceptional progeny.
[c]Percentage of gametes in each class.
[d]Numbers in parentheses are progeny scored.
[e]*Df(2R)WI370.*
[f]The level of missegregation in ord^3/ord^4 females is less than in Df/ord^4 transheterozygotes ($0.001 < P < 0.01$), but not different from that observed in ord^4 homozygotes ($0.30 < P < 0.50$) by χ^2 contingency analysis (see Materials and methods).
[g]Missegregation levels in ord^5/ord^4 and Df/ord^4 females are not statistically different ($0.10 < P < 0.20$).
[h]Missegregation levels in ord^6/ord^4 and Df/ord^4 females are not statistically different ($0.20 < P < 0.30$).

Table IV. Allele-specific interactions in males

Male genotype[a]	Exceptional gametes			Total progeny scored	% missegregation
	XY, XXY	*XX*	O		
ord^4/ord^4	0.12[b] (4)[c]	0.03 (1)	0.41 (14)	3400	**0.6**
Df^d/ord^4	0.05 (3)	0.10 (6)	0.4 (22)	6132	**0.6**
ord^1/ord^4	2.1 (70)	1.1 (34)	4.4 (144)	3271	**7.6**
ord^2/ord^4	0.5 (3)	0.2 (1)	0.5 (3)	636	**1.2**[e]
ord^3/ord^4	0.3 (2)	0.0 (0)	0.3 (2)	639	**0.6**
ord^5/ord^4	0.4 (3)	0.0 (0)	0.3 (2)	688	**0.7**
ord^6/ord^4	1.5 (10)	0.3 (2)	1.2 (8)	660	**3.0**[f]

[a]y/y^+Y males were crossed to attached-X, y^2 *su(w^a) w^a* females.
[b]Percentage of gametes in each class.
[c]Numbers in parentheses are progeny scored.
[d]*Df(2R)WI370.*
[e]Missegregation levels in ord^2/ord^4 and Df/ord^4 males are not statistically different ($0.05 < P < 0.10$).
[f]Missegregation levels in ord^6/ord^4 and Df/ord^4 males are statistically different ($P < 0.001$).

Unusual genetic properties of ord alleles suggest a requirement for protein–protein interactions

Since ORD is a pioneer protein, the position and nature of mutations within the protein can be useful in identifying important functional domains. This is particularly true for ORD because some of the alleles exhibit unusual genetic properties. Previously, five alleles of *ord* were characterized extensively and shown to be of varying strengths (Mason, 1976; Miyazaki and Orr-Weaver, 1992). In analyzing a sixth weak allele, ord^4, we found that it has striking complementation behavior with some of the other alleles that provides insight into ORD function.

The ord^4 mutation is unusual because it has a large amount of residual activity. However, this ability to function is poisoned by some other *ord* mutations. The levels of chromosome missegregation in homozygous ord^4 females were very low (1.1%) (Table III), compared with 60.2% seen in homozygous ord^3 mutants and 0.03% observed in wild-type controls (Miyazaki and Orr-Weaver, 1992). Interestingly, missegregation increased only slightly when ord^4 was placed *in trans* to a deficiency for the *ord* gene (Table III), in contrast to what would have been predicted for a leaky hypomorphic allele. Strikingly, missegregation increased markedly to 20.2% when ord^4 was placed *in trans* to ord^1 (Table III). Levels of missegregation were also significant in ord^4/ord^2 transheterozygotes compared with ord^4/Df (Table III).

The ord^1 and ord^2 mutations are recessive for meiotic chromosome segregation. $ord^1/+$ and $ord^2/+$ females gave 0.0 and 0.2% missegregation, respectively, out of ~2500 progeny scored. Thus, these alleles do not interfere with wild-type function, only the impaired ord^4 function. The intermediate level of aberrant segregation in ord^4/ord^1 mutants compared with that in homozygous ord^4 (Table III) and ord^1 mutants (55.3%) (Miyazaki and Orr-Weaver, 1992) cannot be caused by dominant activity of ord^1.

Mutations in the *ord* gene also exhibit an additional phenotype in female meiosis of reducing recombination (Mason, 1976; Miyazaki and Orr-Weaver, 1992). The frequency of exchange on the X chromosome was lower in ord^4/ord^1 or ord^4/ord^2 transheterozygotes than in ord^4 homozygotes or ord^4/Df females (data not shown). Therefore, ord^1 and ord^2 impair the residual activity of ord^4 both for chromosome segregation and recombination.

Not all *ord* mutations interfere with ord^4 function. In females, ord^3, ord^5 and ord^6 in combination with ord^4 displayed levels of missegregation very similar to that observed in ord^4/Df flies (Table III). In addition, no correlation exists between allele strength and ability to interfere with ord^4 function. ord^6 is a moderate allele and did not interact (Miyazaki and Orr-Weaver, 1992). Certain strong alleles, like ord^1 and ord^2, interacted with ord^4, whereas others, ord^3 and ord^5, did not.

These data show that if ord^4 was the only form of the protein present (i.e. in ord^4 homozygotes or ord^4/Df transheterozygotes), it functioned fairly well. In addition, certain mutant alleles *in trans* to ord^4 resulted in levels of missegregation similar to ord^4/Df. However, what was striking was that ord^1 and ord^2 *in trans* to ord^4 had a more severe phenotype than ord^4/Df. This behavior has previously been termed negative complementation (Fincham, 1966).

Negative complementation of *ord* alleles was also observed in male meiosis (Table IV). As in females, ord^4 homozygotes displayed low levels of missegregation (0.6%) compared with the strong allele ord^3 (51%) and wild-type flies (0.25%). Missegregation did not increase when ord^4 was placed over a deficiency, but again ord^1

interfered with ord^4 activity (Table IV). There is negative complementation between ord^6 and ord^4 in males that is statistically significant (Table IV). In contrast to its effect in females, ord^2 did not poison ord^4 function in males (Table IV).

These data demonstrate that the near wild-type activity of ord^4 can be significantly compromised in both males and females by the presence of ord^1. Therefore, ord^4 responds quite differently than ord^+ in its sensitivity to the presence of ord^1, since $ord^1/+$ heterozygotes display wild-type levels of chromosome segregation. ord^2 also has the ability to interfere with ord^4 function, but to a lesser extent and only in females. ord^6 weakly poisons ord^4 in males. However, the ability to interfere with ord^4 activity is highly allele specific; ord^3 and ord^5 display no negative complementation effects in combination with the ord^4 mutation. The observation that certain mutant alleles of *ord* act in a more destructive fashion than a deficiency for the locus suggests that protein interactions are essential for *ord* function.

The C-terminal half of ORD is essential for function and mediates negative complementation

The phenomenon of negative complementation such as we observed with ord^4 occurs infrequently, and previously identified examples tend to involve multidomain proteins. In all of the *ord* mutants, with the exception of ord^3, the levels of message on a testis Northern blot were comparable (data not shown); therefore, the mutations were likely to affect the coding sequence. Determining the position and nature of *ord* mutations could indicate possible distinct functional domains within the ORD protein.

To sequence the *ord* mutations, we amplified genomic DNA by polymerase chain reaction (PCR), performing duplicate parallel amplifications and subcloning manipulations to ensure that we would detect any mutations induced during the PCR amplification. The sequence encompassing the open reading frame in each PCR product was compared with the sequence of the isogenic chromosome (*cn bw sp*) on which *ord* mutations 2–6 were generated (Miyazaki and Orr-Weaver, 1992). Each *ord* mutation could be attributed to a single base pair mutation and, surprisingly, all six mutations were located within the C-terminal half of the protein (Figure 3).

The ord^4 mutation lies 55 amino acids from the C-terminus, within the highly hydrophobic interval, and it changes Ala^{424} to Val. Such a conservative change is consistent with the near normal function of the ORD^4 protein. However, it is clear that the C-terminal half of ORD is essential for function in both males and females, because one of the strongest alleles, ord^5, mutates Lys^{245} to a stop codon so that the last 234 amino acids of the protein are missing. In addition, sequence analysis of ord^3 revealed a G to A change that mutates the donor splice site of the third intron. If unspliced and translated, the third intron contains an in-frame stop codon that terminates the open reading frame. Therefore ORD^3, like ORD^5, is predicted to be missing the C-terminus. Moreover, it is striking that ord^3 and ord^5 behave like a deficiency in their interaction with ord^4, indicating that the negative complementation observed between ord^4 and other alleles must be mediated through the C-terminal half of the protein (Figure 4).

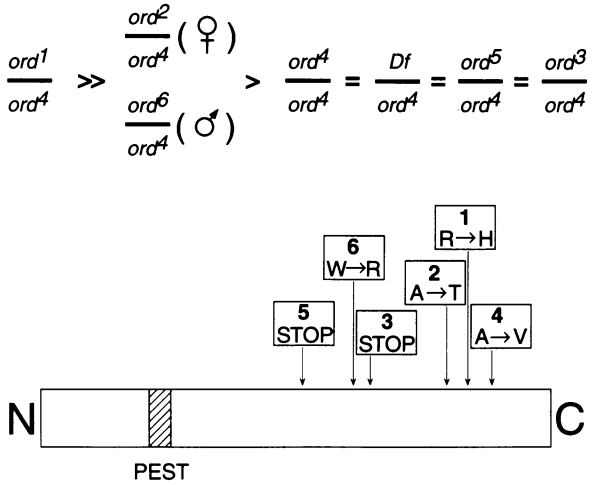

Fig. 4. Negative complementation of *ord* mutations. The levels of aberrant chromosome segregation for the *ord* mutant combinations are summarized on the top. The ord^1 mutation poisons the ability of ord^4 to function. ord^1/ord^4 flies exhibit higher levels of missegregation than ord^4 homozygotes or ord^4 *in trans* to either a deficiency, ord^5, or ord^3. In females, ord^2 shows negative complementation with ord^4, although not as strongly as does ord^1. In males, ord^6 shows weak negative complementation with ord^4. The nature and positions of the *ord* mutations are shown relative to a schematic of the protein. The ord^5 and ord^3 mutations cause stop codons that remove the C-terminal half of the protein, implicating this domain in negative complementation. The other mutations, all of which negatively interact, result in missense changes in the C-terminal domain.

Sequence analysis of ord^1, ord^2 and ord^6 confirmed that negative complementation was a consequence of amino acid changes in the C-terminal half of ORD (Figures 3 and 4). ord^1 results in an arginine to histidine change at position 401. ord^2, which interferes less strongly with ord^4 and only in females, changes Ala^{381} to Thr. ord^6, a third mutation that poisons ord^4 in males, is a tryptophan to arginine substitution at amino acid 294, placing it between the ord^5 and ord^3 truncation mutations (Figures 3 and 4).

Discussion

In this paper, we identify the ORD protein which is known to be essential for sister chromatid cohesion during *Drosophila* meiosis. Cloning *ord* provides an opportunity to understand the molecular basis of cohesion. Our analysis of several *ord* mutations demonstrates that the C-terminal half of ORD is essential for normal function of the protein. In addition, we describe unusual genetic interactions between specific *ord* alleles that implicate the C-terminal part of the molecule in protein–protein interactions.

The interference between specific *ord* alleles belongs to a unique genetic class termed negative complementation. Such effects are rare, allele specific and often involve multidomain proteins known to participate in protein–protein interactions. Certain recessive viable *Abruptex* alleles of the *Notch* locus in *Drosophila* combine to result in lethality (Foster, 1975; Portin, 1975), and specific alleles of the *flb* locus demonstrate negative complementation (Raz *et al.*, 1991). Both *Notch* and *flb* encode transmembrane proteins containing motifs in the extracellular domain that are thought to mediate homotypic and heterotypic interactions (Muskavitch and Hoffmann, 1990; Ullrich and Schlessinger, 1990). The *flb* gene encodes

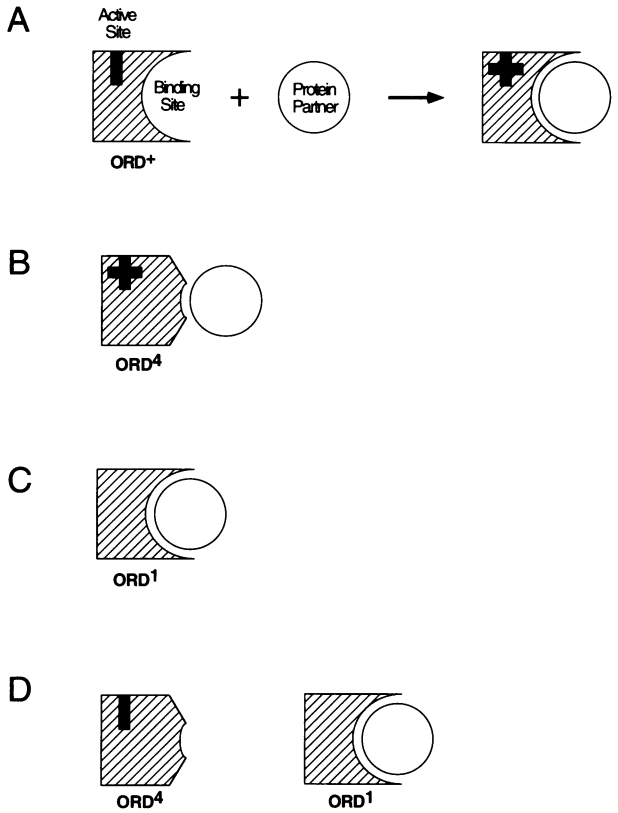

Fig. 5. Model to explain the negative complementation observed with *ord* mutations. The negative complementation is explained most simply by the ORD protein requiring two functions for activity. (**A**) In wild-type ORD protein (stippled), there is a binding site for another protein (or another molecule of ORD) indicated by the open circle. The 'active site', shown in black, promotes sister chromatid cohesion. The binding site and active site can be separably mutated. Binding is essential but not sufficient for cohesion activity. (**B**) The ORD^4 protein binds with reduced affinity, but still functions. If ORD^4 is the only form of the ORD protein present, it can act to maintain cohesion. (**C**) Although the ORD^1 protein binds with normal affinity, the active site is altered, as indicated by the absence of the black box. The *ord^1* mutation would be recessive to wild-type *ord*, but it would poison *ord^4* by competing out the interacting protein, as in (**D**).

the *Drosophila* EGF receptor. Of seven *Abruptex* alleles sequenced, all map within six EGF-like repeats in the extracellular domain of NOTCH (Kelley *et al.*, 1987). In *Caenorhabditis elegans*, the body morphology loci *sqt-1*, *sqt-3* and *rol-8* all display negatively complementing heteroallelic combinations (Kusch and Edgar, 1986). The *sqt-1* locus encodes a collagen molecule (Kramer *et al.*, 1988). Protein–protein interactions are crucial for collagen function because the procollagen polypeptides trimerize to form the collagen fibrils in the *C.elegans* cuticle.

In ORD, the mutations that negatively interact are missense changes in the C-terminus, while the mutations that do not poison ORD^4 (ORD^3 and ORD^5) are missing the C-terminal part of the protein. Thus, the C-terminal domain appears critical for negative complementation. The negative complementation mediated through the C-terminal domain of ORD is most simply explained by a model in which protein interaction is necessary for ORD activity (Figure 5).

We propose that ORD activity depends on two functions: one required for protein binding and one responsible for promoting sister chromatid cohesion (Figure 5). Cohesion requires binding, but binding does not ensure cohesion. Both functions must lie within the C-terminal half of ORD. The phenotypes can be explained by the binding function being somewhat compromised in ORD^4 protein. Although binding would be weaker than that of ORD$^+$, ORD^4 could still interact with its protein partner and promote cohesion, since its cohesion function would be unaffected. The *ord^4* mutation lies in a very hydrophobic region of the protein which is consistent with it being involved in protein interaction.

Unlike *ord^4*, *ord^1* would disrupt the cohesion function, but not the binding affinity of the mutant protein. In an *ord^1/ord^4* transheterozygote, ORD^1 protein would outcompete ORD^4 for binding to the protein partner, but it would form a non-functional complex. This would reduce the amount of functional ORD^4 complex, thereby reducing the ability of ORD^4 to promote cohesion. Since *ord^1* is not a dominant negative mutation for segregation and does not interfere with wild-type *ord*, the binding ability of ORD^1 must not be greater than that of ORD$^+$. The *ord^2* and *ord^6* mutations would, like *ord^1*, affect the cohesion function but still allow binding.

The model we present for ORD protein function relies on interaction between ORD and a protein partner. Such a protein could be another ORD molecule in a scenario whereby dimer or multimer formation is a prerequisite for function at the active site. An ORD^4/ORD^1 heterodimer could form, but it would not be functional. ORD^1 would therefore directly poison ORD^4 by tying it up in a non-functional complex. If this were true, it might be possible to detect dimerization of the ORD protein. We have been unable to detect ORD dimerization using the yeast two-hybrid system (S.Bickel, L.Young and T.Orr-Weaver, preliminary results). Considering the complexity of sister chromatid cohesion, it is likely that ORD binds to another protein, yet to be identified. In transheterozygotes, ORD^1 would titrate out available binding sites and make the formation of a functional ORD^4 complex less likely.

The ORD protein is essential for meiotic sister chromatid cohesion. ORD could maintain cohesion by structurally holding the sister chromatids together or by regulating the signals that trigger sister chromatid separation. Another *Drosophila* protein required for sister chromatid cohesion in meiosis is MEI-S332 (Davis, 1971; Goldstein, 1980; Kerrebrock *et al.*, 1992). However, MEI-S332 differs from ORD in the time and location at which it appears to be necessary. In *mei-S332* mutants, sister chromatid cohesion is unaffected until late in anaphase I, in contrast to the premature sister separation seen in prometaphase I in *ord* mutants. These phenotypes suggest that MEI-S332 acts only at the centromere to promote cohesion, while ORD is required when cohesion must be maintained along the entire chromatid. A MEI-S332-GFP protein has been shown to localize specifically to the centromere region of meiotic chromosomes and to disappear when cohesion is lost at the metaphase II/anaphase II transition (Kerrebrock *et al.*, 1995). The localization of MEI-S332 is consistent with it structurally holding sister chromatids together at the centromere, and its destruction or release being required for separation. We have not detected any significant regions of similarity between ORD and MEI-S332, thus these two proteins may utilize different mechanisms in promoting

cohesion at different times of meiosis and at different locations on the sister chromatids.

ord mutations exhibit several phenotypes in addition to premature sister chromatid separation in meiosis, suggesting that ORD might act as a regulatory protein. Recombination is reduced in *ord* mutant females, and mitotic non-disjunction is observed in the male germline of *ord* mutants (Mason, 1976; Lin and Church, 1982; Miyazaki and Orr-Weaver, 1992). The low abundance of *ORD* message is more consistent with a regulatory rather than a structural role. Moreover, it remains a formal possibility that different forms of the ORD protein may exist, and these could have different regulatory activities. The C-terminal half of ORD must be used for cohesion in both the sexes and for recombination, since all the mutations we have characterized lie within this part of the protein. However, in addition to the predominant transcript, in both ovary and testis there are even rarer larger transcripts. If alternatively spliced, these messages could encode proteins with differing N-termini. The effects of *ord* mutations on both sister chromatid cohesion as well as recombination might be explained by different protein isoforms operating in separate pathways. In addition, a slightly different form of ORD could regulate mitotic cohesion in the germline.

At the sequence level, one of the most striking features of ORD is a very high-scoring PEST sequence in the N-terminal half of the protein, suggesting that ORD may be rapidly degraded. Such a mechanism might provide the abrupt loss of arm cohesion that is necessary to allow separation of recombined homologs at the metaphase I/ anaphase I transition and/or the timely loss of centromere cohesion that allows the sister chromatids to separate in anaphase II. Recently, several laboratories have implicated the ubiquitin pathway as being instrumental in the mitotic metaphase/anaphase transition, possibly by targeting for proteolysis the proteins holding sisters together (Irniger *et al.*, 1995; King *et al.*, 1995; Tugendreich *et al.*, 1995). The destruction box of mitotic cyclins is known to be necessary for ubiquitination and rapid decay of MPF kinase activity (Glotzer *et al.*, 1991). Because the metaphase/ anaphase transition appears to require ubiquitin-mediated proteolysis of as yet unidentified proteins, the protein(s) responsible for sister association might contain a destruction box (Holloway *et al.*, 1993). There is, however, no destruction box matching the cyclin consensus in the ORD open reading frame. It remains to be determined whether ORD is degraded at the metaphase/anaphase transition and the mechanism of proteolysis.

Only a few proteins that are candidates for controlling meiotic sister chromatid cohesion have been identified. The *Zea mays* mutants *desynaptic* (*dsy* and *dy*) (Maguire, 1978; Maguire *et al.*, 1991, 1993), the *Sordaria macrospora* mutant *spo76* (Moreau *et al.*, 1985) and mutations in the *Saccharomyces cerevisiae* gene *RED1* (Rockmill and Roeder, 1988, 1990) cause meiotic defects compatible with these genes having a function in cohesion. Premature separation of the sister chromatids is observed during meiosis in *rec8* mutants of *Schizosaccharomyces pombe* (Molnar *et al.*, 1995). To date, only the *rec8* and *RED1* genes have been cloned and sequenced (Thompson and Roeder, 1989; Lin *et al.*, 1992). ORD does not share any significant regions of homology that would indicate that it represents the *Drosophila* homolog of either of these yeast genes.

Other potential cohesion proteins have been identified based on the criterion that they are localized between the sister chromatids, but it is difficult to analyze their function (for a review, see Miyazaki and Orr-Weaver, 1994). One example is the mammalian protein Cor1, a component of the lateral elements of the synaptonemal complex (Dobson *et al.*, 1994). The localization pattern of Cor1 is consistent with it acting to maintain sister chromatid cohesion throughout meiosis. However, no homology exists between Cor1 and ORD.

The *ord* gene is unusual in being one of the few genes known to be essential for sister chromatid cohesion. Therefore, the identification of the ORD protein provides molecular access to a critical player in cohesion. We have identified regions of ORD necessary for function and protein interaction. This will permit the elucidation of the nature and regulation of cohesion through understanding the basis of ORD function and isolation of its interacting partners.

Materials and methods

Stocks

All *Drosophila* stocks and crosses were raised at 25°C on standard cornmeal–brewer's yeast–molasses–agar food. ord^1 was isolated and characterized by Mason (1976). All other *ord* alleles were isolated by their failure to complement ord^1 and have been described previously (Miyazaki and Orr-Weaver, 1992). They exhibit a wide range of strength, and they are recessive with respect to both chromosome segregation and recombination. The decreased fertility of strong alleles varies in intensity, depending on strain background. In addition, the defects in nurse cell morphology described by Miyazaki and Orr-Weaver (1992) also depend on genetic background. The deficiencies *Df(2R)bw-WI366* and *Df(2R)WI370* were isolated in the screen described below. *Df(2R)WI370* was called *Df(2R)3-70* previously (Miyazaki and Orr-Weaver, 1992). *Df(2R)bw-S46* was obtained from R.Lehmann and *Df(2R)bw-HB132* was obtained from R.Nothiger. All other mutations used in these experiments are described in Lindsley and Zimm (1992).

Deficiency screen

We isolated deficiencies in the cytological interval 59 from an X-ray screen. We screened for deletions that removed either bw^+ at 59E or a P-element carrying the $white^+$ gene inserted into 59C ($P\{w^+, ry^+ = A\}3\text{-}1$ transformant, obtained from R.Levis) (Levis *et al.*, 1985). *w/Y; cn* $P\{w^+\}$ 59C males were irradiated with 3000–4000 rads, crossed to *w; cn bw If/CyO* S^2 *cn bw* virgins and their progeny scored for white eyes. Of the 383 000 chromosomes screened, eight deletions were obtained that removed the P element, while 10 deficiencies deleted the bw^+ locus. Newly isolated deficiencies were tested for their ability to complement the chromosome segregation phenotype of *ord* mutants using the non-disjunction tests described in Kerrebrock *et al.* (1992). The deficiency breakpoints were mapped on the genomic walk by quantitative Southern analysis (see below).

Isolation of the ord genomic region

We carried out a chromosomal walk using a λ library (gift from Jennifer Mach, R.Lehmann laboratory) constructed from a partial *Sau*3A digest of genomic DNA from *Df(2R)bw-HB132/+* flies (ord^+). The walk was initiated from the most distal clone of the *twist* walk (G21) (Thisse *et al.*, 1987). Two steps distally towards *bw* were taken.

The positions of deficiency breakpoints were mapped on the genomic walk by quantitative Southern blots. Using hybridization probes from each step, we compared genomic DNA isolated from various *Df/+* heterozygotes with wild-type Canton S DNA. A Fuji BAS2000 Bioimager was used to quantify the signal in each band. A probe containing the *rosy* gene was used as an internal control to standardize for the amount of DNA loaded in each lane. The signal for each deficiency band was then expressed as a fraction of the analogous Canton S band using the following formula: (deficiency band 'X'/deficiency *rosy* band)/(Canton

S band 'X'/Canton S *rosy* band). In such a calculation, a value of 0.5 indicated that the fragment of interest was deleted on the deficiency chromosome. Using this strategy, the minimal interval containing *ord* was restricted to 25 kb (Figure 1).

A chromosome walk was also carried out starting from the most proximal clone of the *bw* walk (Dreesen *et al.*, 1988) and walking proximally. This walk utilized the Tamkun cosmid library (Tamkun *et al.*, 1992). Clones from five overlapping steps (~150 kb) were recovered, but a sixth step proved impossible due to 'holes' in this library as well as in several other cosmid libraries tested. Molecular identification of the breakpoint of *Df(2R)bw-WI366* made it unnecessary to continue walking in this direction.

Transformation rescue

For transformation rescue experiments, *Not*I-excised inserts from three overlapping λ clones spanning the breakpoints of *Df(2R)bw-S46* and *Df(2R)bw-WI366* were subcloned into pCoSpeR (Pirrotta, 1988) to generate the three transposons *P{w^{+mC} ori Amp=D16}*, *P{w^{+mC} ori Amp=D26}* and *P{w^{+mC} ori Amp=D39}*. *Df(1)yw^{67c23}* embryos were injected with plasmid DNA at 1 mg/ml with 0.3 mg/ml pIChsπΔ2-3 helper plasmid, a derivative of wings clipped (Mullins *et al.*, 1989). Multiple, independent lines were established for each construct. Crosses were performed to obtain flies carrying the transposon in an *ord^{1}/ord^{3}* background (both are strong alleles) and sex chromosome missegregation measured in both males and females (Kerrebrock *et al.*, 1992). In addition, simultaneous sibling tests were performed. *ord^{1}/ord^{3}* flies lacking the transposon as well as *ord* heterozygotes with or without the transposon were scored for missegregation frequencies. By testing for rescue of *ord* transheterozygotes, we avoided any phenotypes arising from making other background mutations on the second chromosome homozygous.

Smaller rescue constructs were made as follows. *Bam*HI partial digest fragments from the genomic insert of *P{D39}* were ligated into the *Bam*HI site of pCaSpeR4 (Pirrotta, 1988) to generate the overlapping clones *P{w^{+mC} ori Amp=6.3BB}* and *P{w^{+mC} ori Amp=6.5BB}* (Figure 1). *P{w^{+mC} ori Amp=3.6RR}* has a 3.6 kb *Eco*RI fragment inserted into pCaSpeR4 (Figure 1). The *P{w^{+mC} ori Amp=7.3BP}* transposon was constructed by inserting a 2.1 kb *Bam*HI–*Pst*I fragment into pCaSpeR4, then opening up the clone with *Pst*I and ligating in a 5.2 kb *Pst*I fragment (Figure 1). Correct orientation was determined by diagnostic restriction digests. To generate *P{w^{+mC} ori Amp=7.3BPΔ}*, a 136 bp deletion was introduced into *P{w^{+mC} ori Amp=7.3BP}* by fusing the *Pst*I and *Eco*RI sites, which were made blunt using T4 DNA polymerase. This construct mutates the splice acceptor site of intron 2 which overlaps with the *Pst*I site. If intron 2 is unspliced, a stop codon at the beginning of the intron will terminate the open reading frame. Conversely, if the next consensus acceptor is utilized, the open reading frame changes frames and then truncates prematurely. Transformed lines were generated for these constructs and tested as described above, except that transposon DNA was injected at 0.5 mg/ml and the helper was 0.1 mg/ml.

In order to recover a *w cv v f car* chromosome with which to test recombination frequencies in flies carrying the transposon *P{6.3BB}*, a recombinant chromosome was isolated from *w^{1118}/cv v f car* females. *X* chromosome recombination frequencies in two intervals (*y–cv* and *cv–f*) were determined in females that were *y w^{67c23}/w^{1118} cv v f car; ord^{1}/ord^{3}* and in identical females carrying one copy of the *P{6.3BB}* transposon on the third chromosome.

Isolation of cDNAs

A testes cDNA library (provided by Dr T.Hazelrigg) was screened with the *ord* genomic insert from the rescuing transposon, *P{6.3BB}*. cDNAs corresponding to two transcription units were isolated from 1.4×10^{6} clones screened. Phage clones were converted to plasmids using the Exassist/SOLR excision system (Stratagene). Of the four unique cDNAs subsequently demonstrated to encode parts of the *ORD* transcription unit, one was determined by sequence analysis to be a hybrid clone and was not characterized further.

DNA sequencing and computer analysis

Both strands of overlapping *ORD* cDNA clones 6-6 and 18-1 were sequenced. Random cDNA fragments generated by sonication of cDNA insert ligated into circles (Bankier *et al.*, 1987) were subcloned into Bluescript KS^{-}. Sequenase 2.0 (Amersham/USB) chain-termination reactions were performed using T3 and T7 primers, and sequences assembled into contigs using SeqMan/DNA Star software. In order to position the cDNA within the rescuing transposon *P{6.3BB}* and to identify intron/exon boundaries, one strand of the 6.3 kb genomic rescue insert was sequenced by generating *Exo*III-nested deletions (Pharmacia) of overlapping subclones.

To identify any sequences with homology to ORD, searches of protein, nucleotide and sequence tag data bases were performed using the programs BLAST, FASTA and BLITZ (Smith and Waterman, 1981; Lipman and Pearson, 1985; Altschul *et al.*, 1990). No significant homologies were uncovered, even when utilizing reduced stringency matrix parameters for BLAST such as BLOSUM30. The PEST sequence in ORD (score = 29.7) was identified using the program PEST-FIND (Rogers *et al.*, 1986) .

PCR amplification and sequence analysis of mutant ord alleles

Genomic DNA was isolated from homozygous mutant females (Ashburner, 1989), digested with *Hin*dIII, precipitated and resuspended in TE. DNA (one fly equivalent) was amplified using primers outside the *ord* coding sequence. The sense strand primer was 5′CGATAAAGCCCCAACGACTACTGG3′ and the antisense strand primer was 5′CGGGCTCTTGGCTTTGCAACTGG3′. The PCR products were restricted with *Nsi*I, gel purified and cloned into *Pst*I-cut Bluescript KS^{-}. To ensure that PCR errors were not mistaken for genuine EMS mutations, clones were generated for each mutation from two independent PCRs and each completely sequenced using *ord*-specific primers. Only one base pair change that occurred in both PCR products was identified for each mutation. In addition, DNA from the isogenic *cn bw sp* strain (ord^{+}) used for the generation of mutants *ord^{2}–ord^{6}* was amplified, sequenced and used for comparison. One polymorphism was uncovered that differed between various wild-type chromosomes, resulting in the conservative substitution of glutamic acid for aspartic acid at position 444. All other polymorphisms identified on different wild-type chromosomes or on the *ord^{1}* mutant chromosome were silent mutations which did not result in amino acid changes.

Analysis of ord^{4} negative complementation

Crosses to analyze the missegregation of sex chromosomes in males and females were performed as described in Kerrebrock *et al.* (1992). By mating mutant *y/y^{+}Y* males to compound-*X* females or mutant females to compound-*XY* males, gametes bearing all normal and most exceptional sex chromosome constitutions were recoverable and distinguishable. In the male non-disjunction tests, exceptional diplo-*Y* sperm were phenotypically indistinguishable from regular mono-*Y* sperm; therefore, 'total missegregation' underestimates the actual level of missegregation. In the female tests, only half of the total number of exceptional gametes were recoverable, but all regular *X* gametes were recovered. To compensate for this, the 'adjusted total' equals the number of progeny in the normal class plus twice the number of progeny in the exceptional classes. Total missegregation was calculated by doubling the number of exceptional progeny and dividing by the 'adjusted total'.

In order to minimize any differences in missegregation frequencies due to genetic background, isogenic *X* and *Y* chromosomes (Kerrebrock *et al.*, 1992) were incorporated into all *ord* stocks including *Df(2R)WI370*. In addition, multiple rounds of recombination had been used previously to cross off any extraneous lethal mutations on mutant *ord* chromosomes that might have been induced during EMS mutagenesis (Miyazaki and Orr-Weaver, 1992). To minimize background differences, all tests were performed with the same *ord^{4}* recombinant chromosome (*ord^{4} bw*). In addition, the *ord* alleles tested *in trans* to *ord^{4}* were from the same round of recombination as *ord^{4} bw*.

A 2×2 (normal and exceptional gametes) χ^{2} contingency analysis (d.f. = 1) (Lindren *et al.*, 1978) was used to determine whether differences in missegregation frequencies were statistically significant when comparing different *ord* transheterozygous combinations.

Accession numbers

The EMBL accession number for the DNA sequence data reported in this paper is X92840.

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References

Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.

Ashburner,M. (1989) *Drosophila. A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bankier,A.T., Weston,K.M. and Barrell,B.G. (1987) Random cloning and sequencing by the M13/dideoxynucleotide chain termination method. *Methods Enzymol.*, **155**, 51–93.

Cavener,D.R. (1987) Comparison of the consensus sequence flanking translational start sites in *Drosophila* and vertebrates. *Nucleic Acids Res.*, **15**, 1353–1360.

Chevaillier,P. (1993) PEST sequences in nuclear proteins. *Int. J. Biochem.*, **25**, 479–482.

Davis,B. (1971) Genetic analysis of a meiotic mutant resulting in precocious sister centromere separation in *Drosophila melanogaster*. *Mol. Gen. Genet.*, **113**, 251–272.

Dobson,M.J., Pearlman,R.E., Karaiskakis,A., Spyropoulos,B. and Moens,P.B. (1994) Synaptonemal complex proteins: occurrence, epitope mapping, and chromosome disjunction. *J. Cell Sci.*, **107**, 2749–2760.

Dreesen,T., Johnson,D. and Henikoff,S. (1988) The *brown* protein of *Drosophila melanogaster* is similar to the *white* protein and to components of active transport complexes. *Mol. Cell. Biol.*, **8**, 5206–5215.

Fincham,J.R.S. (1966) *Genetic Complementation*. Benjamin, New York.

Foster,G.G. (1975) Negative complementation at the *Notch* locus of *Drosophila melanogaster*. *Genetics*, **81**, 99–120.

Glotzer,M., Murray,A.W. and Kirschner,M.W. (1991) Cyclin is degraded by the ubiquitin pathway. *Nature*, **349**, 132–138.

Goldstein,L.S.B. (1980) Mechanisms of chromosome orientation revealed by two meiotic mutants in *Drosophila melanogaster*. *Chromosoma*, **78**, 79–111.

Herskowitz,I. (1987) Functional inactivation of genes by dominant negative mutations. *Nature*, **329**, 219–222.

Holloway,S.L., Glotzer,M., King,R.W. and Murray,A.W. (1993) Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell*, **73**, 1393–1402.

Irniger,S., Piatti,S., Michaelis,C. and Nasmyth,K. (1995) Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell*, **81**, 269–277.

Kelley,M.R., Kidd,S., Deutsch,W.A. and Young,M.W. (1987) Mutations altering the structure of epidermal growth factor-like coding sequences at the *Drosophila Notch* locus. *Cell*, **51**, 539–548.

Kerrebrock,A.W., Miyazaki,W.Y., Birnby,D. and Orr-Weaver,T.L. (1992) The *Drosophila mei-S332* gene promotes sister chromatid cohesion in meiosis following kinetochore differentiation. *Genetics*, **130**, 827–841.

Kerrebrock,A.W., Moore,D.P., Wu,J.S. and Orr-Weaver,T.L. (1995) Mei-S332, a *Drosophila* protein required for sister chromatid cohesion, can localize to meiotic centromere regions. *Cell*, **83**, 247–256.

King,R.W., Peters,J.-M., Tugendreich,S., Rolfe,M., Hieter,P. and Kirschner,M.W. (1995) A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell*, **81**, 279–288.

Kramer,J.M., Johnson,J.J., Edgar,R.S., Basch,C. and Roberts,S. (1988) The *sqt-1* gene of *C. elegans* encodes a collagen critical for organismal morphogenesis. *Cell*, **55**, 555–565.

Kusch,M. and Edgar,R.S. (1986) Genetic studies of unusual loci that affect body shape of the nematode *Caenorhabditis elegans* and may code for cuticle structural proteins. *Genetics*, **113**, 621–639.

Levis,R., Hazelrigg,T. and Rubin,G. (1985) Effects of genomic position on the expression of transduced copies of the *white* gene of *Drosophila*. *Science*, **229**, 558–561.

Lin,H.P. and Church,K. (1982) Meiosis in *Drosophila melanogaster* III. The effect of orientation disruptor (*ord*) on gonial mitotic and the meiotic divisions in males. *Genetics*, **102**, 751–770.

Lin,Y., Larson,K.L., Dorer,R. and Smith,G.R. (1992) Meiotically induced *rec7* and *rec8* genes of *Schizosaccharomyces pombe*. *Genetics*, **132**, 75–85.

Lindren,B.W., McElrath,G.W. and Berry,D.A. (1978) *Probability and Statistics*. MacMillan, New York.

Lindsley,D. and Zimm,G. (1992) *The Genome of Drosophila melanogaster*. Academic Press, New York.

Lipman,D.J. and Pearson,W.R. (1985) Rapid and sensitive protein similarity searches. *Science*, **227**, 1435–1441.

Maguire,M.P. (1978) Evidence for separate genetic control of crossing over and chiasma maintenance in maize. *Chromosoma*, **65**, 173–183.

Maguire,M.P., Paredes,A.M. and Riess,R.W. (1991) The desynaptic mutant of maize as a combined defect of synaptonemal complex and chiasma maintenance. *Genome*, **34**, 879–887.

Maguire,M.P., Riess,R.W. and Paredes,A.M. (1993) Evidence from a maize desynaptic mutant points to a probable role of synaptonemal complex central region components in provision for subsequent chiasma maintenance. *Genome*, **36**, 797–807.

Mason,J.M. (1976) Orientation disruptor (*ord*): A recombination-defective and disjunction-defective meiotic mutant in *Drosophila melanogaster*. *Genetics*, **84**, 545–572.

Miyazaki,W.Y. and Orr-Weaver,T.L. (1992) Sister chromatid misbehavior in *Drosophila ord* mutants. *Genetics*, **132**, 1047–1061.

Miyazaki,W.Y. and Orr-Weaver,T.L. (1994) Sister chromatid cohesion in mitosis and meiosis. *Annu. Rev. Genet.*, **28**, 167–187.

Molnar,M., Bahler,J., Sipiczki,M. and Kohli,J. (1995) The *rec8* gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister chromatid cohesion during meiosis. *Genetics*, **141**, 61–73.

Moreau,P.J.F., Zickler,D. and Leblon,G. (1985) One class of mutants with disturbed centromere cleavage and chromosome pairing in *Sordaria macrospora*. *Mol. Gen. Genet.*, **198**, 189–197.

Muller,H.J. (1932) Further studies on the nature and causes of gene mutations. *Proc. Sixth Int. Congr. Genet.*, **1**, 213–255.

Mullins,M.C., Rio,D.C. and Rubin,G.M. (1989) *Cis*-acting DNA sequence requirements for P-element transposition. *Genes Dev.*, **3**, 729–738.

Muskavitch,M.A. and Hoffmann,F.M. (1990) Homologs of vertebrate growth factors in *Drosophila melanogaster* and other invertebrates. *Curr. Top. Dev. Biol.*, **24**, 289–328.

Orr-Weaver,T.L. (1995) Meiosis in *Drosophila*: Seeing is believing. *Proc. Natl Acad. Sci. USA*, **92**, 10443–10449.

Pirrotta,V. (1988) Vectors for P mediated transformation in *Drosophila*. In Rodriguez,R.L. and Denhardt,D.T. (eds), *Vectors and their Uses*. Butterworths, Boston, pp. 437–456.

Portin,P. (1975) Allelic negative complementation at the *Abruptex* locus of *Drosophila melanogaster*. *Genetics*, **81**, 121–133.

Raz,E., Schejter,E.D. and Shilo,B.Z. (1991) Interallelic complementation among the DER/*flb* alleles: Implications for the mechanism of signal transduction by receptor-tyrosine kinases. *Genetics*, **129**, 191–201.

Rechsteiner,M. (1988) Regulation of enzyme levels by proteolysis: the role of PEST regions. *Adv. Enzyme Regul.*, **27**, 135–151.

Rockmill,B. and Roeder,G.S. (1988) *RED1*: A yeast gene required for the segregation of chromosomes during the reductional division of meiosis. *Proc. Natl Acad. Sci. USA*, **85**, 6057–6061.

Rockmill,B. and Roeder,G.S. (1990) Meiosis in asynaptic yeast. *Genetics*, **126**, 563–574.

Rogers,S., Wells,R. and Rechsteiner,M. (1986) Amino acid sequences common to rapidly degraded proteins: The PEST hypothesis. *Science*, **234**, 364–368.

Smith,T.F. and Waterman,M.S. (1981) Identification of common molecular subsequences. *J. Mol. Biol.*, **147**, 195–197.

Tamkun,J.W., Deuring,R., Scott,M.P., Kissinger,M., Pattatucci,A.M., Kaufman,T.C. and Kennison,J.A. (1992) *brahma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell*, **68**, 561–572.

Thisse,B., Messal,M.E. and Perrin-Schmitt,F. (1987) The *twist* gene: isolation of a *Drosophila* zygotic gene necessary for the establishment of dorsal-ventral pattern. *Nucleic Acids Res.*, **15**, 3439–3453.

Thompson,E. and Roeder,G.S. (1989) Expression and DNA sequence of *RED1*, a gene required for meiosis I chromosome segregation in yeast. *Mol. Gen. Genet.*, **218**, 293–301.

Tugendreich,S., Tomkiel,J., Earnshaw,W. and Hieter,P. (1995) CDC27Hs colocalizes with CDC16Hs to the centrosome and mitotic spindle and is essential for the metaphase to anaphase transition. *Cell*, **81**, 261–268.

Ullrich,A. and Schlessinger,J. (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell*, **61**, 203–212.

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