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The *claret* locus in *Drosophila* encodes products required for eyecolor and for meiotic chromosome segregation

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The *claret* (*ca*) locus in *Drosophila* encodes products that are needed both for wild-type eyecolor and for correct meiotic chromosome segregation. Mutants described previously provide evidence that two mutationally independent coding regions are present at *ca*. We have recovered six new P element-induced and one spontaneous *ca* mutant. Four of these new mutants affect both eyecolor and chromosome segregation. The high frequency of co-mutation of these two functions suggests that the corresponding genes are closely adjacent to one another. We recovered genomic DNA sequences corresponding to the *ca* locus by chromosome walking, and showed using revertant analysis that the cloned region encodes ca^+. Transformation experiments demonstrate that the mutant effect resulting in meiotic chromosome non-disjunction (nd) and loss is fully rescued by DNA from the cloned region. Two RNAs of 7.4 and 2.2 kb have been identified by Northern blot analysis as the putative eyecolor and segregational products. Expression of the RNAs with respect to males and females, and their presence or absence in *ca* and *nd* mutants indicate that the 7.4 kb RNA corresponds to the product needed for wild-type eyecolor and the 2.2 kb RNA is the product required for normal chromosome segregation. These RNAs are transcribed in opposite directions to one another. Alleles that affect both eyecolor and chromosome segregation are deletion mutants that affect both transcripts. Thus, the putative eyecolor and segregational products are encoded by separate genes. Mutants that affect both eyecolor and chromosome segregation apparently do so because they delete essential regions of both genes.

Key words: claret locus/*Drosophila*/meiotic chromosome segregation

Introduction

The locus encoding the *claret* (*ca*) eyecolor gene has received special attention since an unusual mutant allele was discovered in a stock of *Drosophila simulans* (Sturtevant, 1929). This allele was unlike the previously isolated *ca* mutations of *D.melanogaster* in that homozygous *ca D. simulans* females not only had claret eyes, but also showed a high frequency of meiotic chromosome non-disjunction and loss. In addition, chromosomes transmitted by *ca D.simulans* females showed frequent loss in early embryonic cleavages, indicating a maternal effect of the mutation on mitotic chromosome segregation in the zygote. Although the original *ca* of *D.simulans* has been lost, a corresponding *ca* allele was found in *D.melanogaster* after X-irradiation (Lewis and Gencarella, 1952). This allele was called *claret non-disjunctional* (ca^{nd}). Like the *ca* of *D.simulans*, ca^{nd} results in claret eyecolor, frequent non-disjunction of chromosomes in meiosis I and early mitotic loss of maternally transmitted chromosomes.

The basis for the coincident mutation by ca^{nd} of two apparently unrelated phenotypes, eyecolor and chromosome segregation, has been suggested to be the presence of two separate, but closely linked genes (Chan and Davis, 1970). This view is supported by the existence of *ca* alleles that affect eyecolor but not chromosome segregation, and by the discovery of *non-claret disjunctional* (*ncd*), an allele of ca^{nd} that affects chromosome segregation but not eyecolor. These mutant alleles are evidence that the effects on eyecolor and chromosome segregation can be mutationally separated from one another. Because *ncd* complements the claret eyecolor phenotype, but fails to complement the non-disjunctional effect of ca^{nd}, O'Tousa and Szauter (1980) concluded that ca^{nd} was a double mutant in *ca* and *ncd*. We have now recovered several independent *ca* mutants that affect both eyecolor and chromosome segregation. The recovery of these mutants suggests that a single molecular change may cause both phenotypes, and supports the idea that the sequences at *ca* needed for normal eyecolor and chromosome segregation are very closely apposed to one another.

Because of the unusual structural features of the *ca* locus and its involvement in meiotic chromosome segregation, we have undertaken a genetic and molecular analysis of the *ca* region in *D.melanogaster*. Here we report the recovery and characterization of additional *ca* alleles and the molecular cloning of the DNA sequences responsible for claret eyecolor, meiotic chromosome non-disjunction and loss, and mitotic chromosome loss. We show that the molecular change in ca^{nd} and four other mutant alleles is a deletion that affects two RNAs of 7.4 and 2.2 kb. These two transcripts are identified as the putative ca^+ and nd^+ products by their expression patterns in wild-type and mutant males and females. The 7.4 kb RNA is present in both males and females, is absent or reduced in mutants that affect eyecolor, and present in a mutant that affects segregation but not eyecolor. It is the putative ca^+ transcript. The 2.2 kb RNA is present predominantly in ovaries in females, is absent in non-disjunctional deletion mutants and is probably fully encoded by a DNA fragment that rescues chromosome non-disjunction. It is is the putative nd^+ product. The putative ca^+ and nd^+ RNAs are transcribed in

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opposite directions to one another, and are thus encoded by separate genes.

Results

Recovery and characterization of additional ca alleles

The previously described alleles of *ca* include *ca*, ca^2 and ca^3, which affect eyecolor but not chromosome segregation, *ncd*, which affects chromosome segregation but not eyecolor, and ca^{nd}, which affects both chromosome segregation and eyecolor.

ca^{Cm} is a spontaneous mutation that causes claret eyecolor and high frequencies of chromosome non-disjunction and loss. ca^{Cm} is allelic to *ca*, ca^{nd} and *ncd*, but has an additional phenotype in that it causes reversion of *bobbed* alleles in rDNA-non-deficient flies. The detection, recovery and characterization of ca^{Cm} will be reported separately.

Four P element-induced mutants were recovered by screening offspring of hybrid dysgenic females for claret eyecolor. Of these mutants, denoted ca^{P1}, ca^{P2}, ca^{P3} and ca^{P6}, only ca^{P6} showed instability, reverting spontaneously to ca^+ at a frequency of 1–5%. Instability of claret eyecolor indicated that ca^{P6} was likely to carry a P element at *ca*. Two additional mutants, $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$, were recovered after mutagenesis by the Jumpstarter system developed by Cooley *et al.* (1988) in a screen for chromosome 3 mutations that affect meiotic chromosome segregation.

The four ca^P mutants, $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ were tested for their ability to complement the claret eyecolor of *ca* and ca^{nd}, and chromosome non-disjunction and loss shown by ca^{nd} and *ncd*. The mutant eyecolor is illustrated in Figure 1. Claret is ruby red compared with the brick red of wild-type eyecolor. Table I shows that the four ca^P mutants, $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ fail to complement *ca* and ca^{nd} for eyecolor. Tests for chromosome segregation were carried out by determining the frequencies of offspring that were exceptional or mosaic for the X chromosome or chromosome 4, produced by females that were wild-type, homozygous or heterozygous for *ca* mutant alleles. Non-disjunctional offspring are recognized by the presence (X/X/Y females) or absence (X/0 males) of a dominantly marked Y chromosome, or by phenotypic characteristics including slow development, small body size and short, thin bristles (*haplo-4*s or *Minutes*) (Figure 2). X/0 males and *Minutes* may arise from meiotic chromosome non-disjunction or meiotic chromosome loss. Gynandromorphs, which are mosaics of X/X female and X/0 male tissue (Figure 2), and *haplo-4* mosaics arise from early zygotic chromosome loss. The frequencies of X and 4th chromosome non-disjunctional exceptions and X chromosome mosaics among offspring of females heterozygous for ca^{P3}, $ca^{3\text{-}1}$ or $ca^{22\text{-}8}$, and ca^{nd} or *ncd* ranged from 25 to 60%, compared with frequences for *non-disjunctional*$^+$ alleles of ≤0.6%. ca^{P3} and $ca^{22\text{-}8}$ fail to complement ca^{nd} and *ncd*, and $ca^{3\text{-}1}$ fails to complement ca^{nd} for chromosome non-disjunction and loss. ca^{P3}, $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ also fail to complement ca^{Cm} or each other for chromosome non-disjunction and loss. They show a recessive mutant effect on chromosome segregation. Thus, ca^{P1}, ca^{P2} and ca^{P6} have a mutant eyecolor gene but show normal chromosome segregation. ca^{P3}, $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ are mutant both in eyecolor and in meiotic and early mitotic chromosome segregation. ca^{P3}, $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ are allelic to ca^{nd} ca^{Cm} and to each other.

Fig. 1. Claret mutant eyecolor. The flies on the left show claret eyecolor. The Oregon R flies on the right have wild-type eyecolor. The claret eyecolor is ruby red compared with the brick red of wild-type.

Table I. Complementation by ca^P mutants of *ca* eyecolor and meiotic chromosome non-disjunction and loss

	Complementation for eyecolor		Complementation for non-disjunction	
	ca	ca^{nd}	ca^{nd}	*ncd*
ca^{P1}	–	–	+	+
ca^{P2}	–	–	+	+
ca^{P3}	–	–	–	–
ca^{P6}	–	–	+	+
$ca^{3\text{-}1}$	–	–	–	NT
$ca^{22\text{-}8}$	–	–	–	–

Failure to complement is indicated by a minus sign, while complementation is indicated by a plus. NT, not tested.

Chromosomes from the four ca^P mutants and $ca^{22\text{-}8}$ were screened by *in situ* hybridization using a cloned P element, pπ25.1 (O'Hare and Rubin, 1983), as probe. Chromosomes from only one of the mutants, ca^{P6}, showed hybridization to the 99B region, to which the *ca* locus has been mapped by deficiency analysis (Frisardi and MacIntyre, 1984). $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ showed little or no homology upon Southern blot analysis with a P element probe, either the 0.84 kb *Hin*dIII fragment from p6.1 (Rubin *et al.*, 1982) or pUChsneo (Steller and Pirrotta, 1985). Since ca^{P1}, ca^{P2}, ca^{P3}, $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ arose under hybrid dysgenic conditions but do not carry P elements at *ca*, it is likely that they arose by insertion, followed by deletion, of a P element or another moveable element. Figure 3 shows the tip of polytene chromosome 3R from ca^{P6} hybridized with a biotin-labeled P element probe. The site of hybridization in the *ca* region is at the 99B/C junction (indicated with an arrow), and probably corresponds to 99B11 or 99C1. This is just distal to 99B5-9, the map position for *ca* determined by Frisardi and MacIntyre (1984).

Chromosome walk

The *ca* region was cloned molecularly from both Oregon R and ca^{P6} DNA by walking to the P element at *ca* from

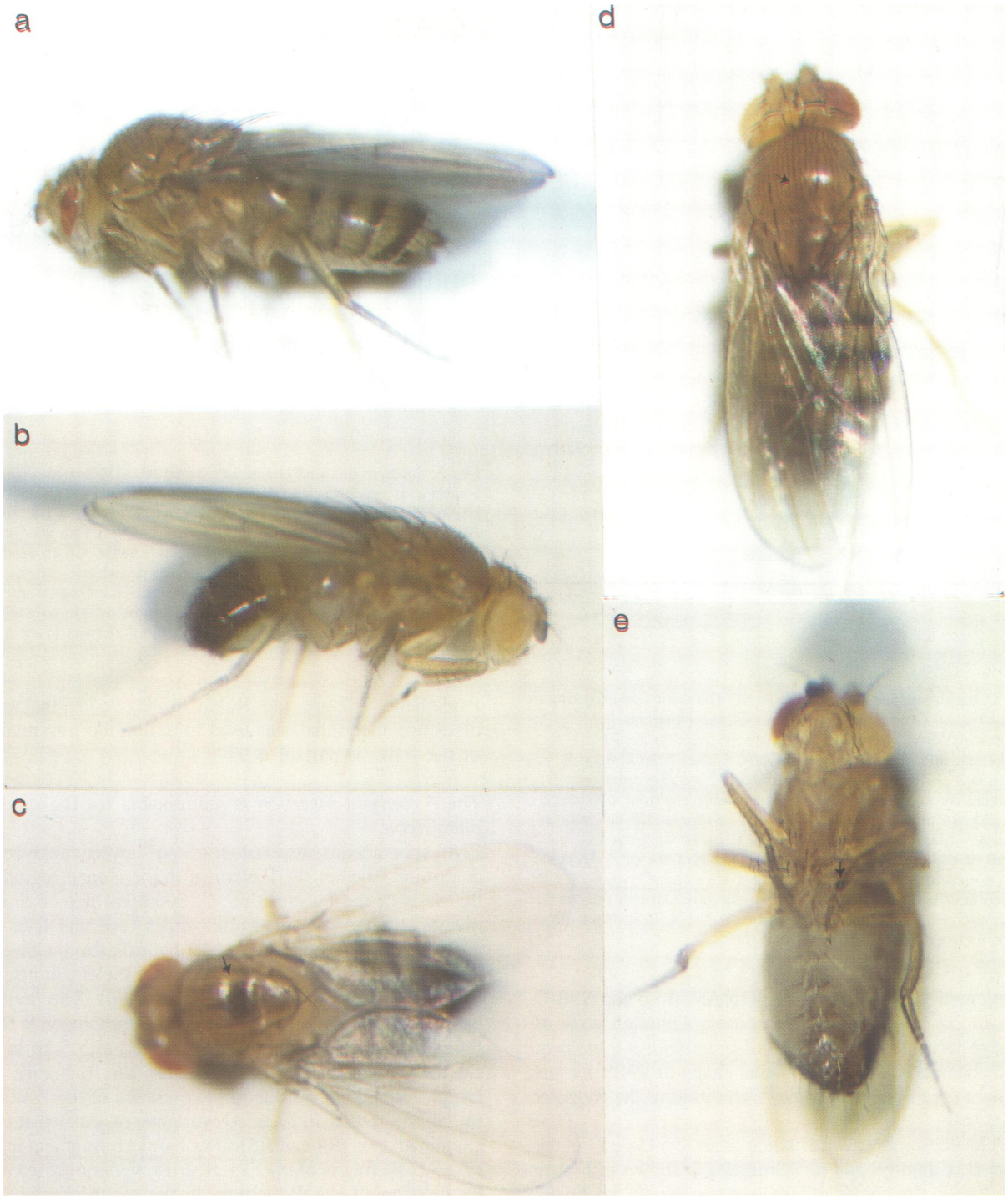

Fig. 2. Non-disjunctional exceptions and abnormal offspring due to early zygotic chromosome loss. (**a**) X/X/Y females are recognized by the dominant marker, B^S, on the Y chromosome, which results in narrow eyes. (**b**) X/0 males are recognized by the absence of the dominant B^S marker on the Y chromosome, resulting in round eyes. (**c**) Haplo-4 or *Minute* males or females are delayed in development, small in body size, and have short, thin bristles and a trident pattern on the thorax (arrow). (**d**) Gynandromorphs are mosaics of X/X and X/0 tissue that arise from chromosome loss during mitotic divisions in the early zygote. The X/X female tissue in this gynandromorph is wild-type, while the X/0 male tissue is yellow (y^2) and white. The thorax shows a line (arrow) marking the boundary between yellow and + tissue. (**e**) This ventral view shows the sex comb on the male foreleg (arrow) and asymmetry of the genitalia.

a nearby sequence, λ559 (Levy *et al.*, 1982), at 99C6-8. Recombinant DNAs homologous to λ559 were recovered from an Oregon R or Canton S library, and the cloned region was extended in both directions using the terminal fragments from the recombinants as probes. Restriction enzyme maps of new recombinants were determined and *in situ* hybridization to polytene chromosomes was carried out in order to ascertain the direction of the walk. When the proximal direction of the walk was determined, the walk was extended in that direction in both the Oregon R and ca^{P6} libraries.

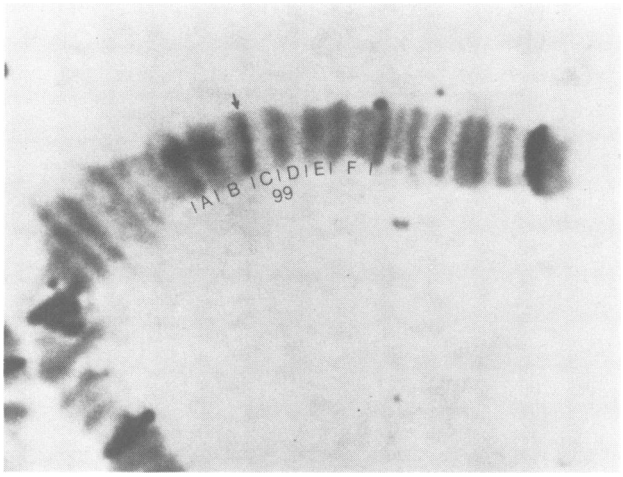

Fig. 3. Salivary gland chromosomes from a ca^{P6} third-instar larva showing hybridization with a biotin-labeled P element probe (pπ25.1; arrow) at the *ca* locus.

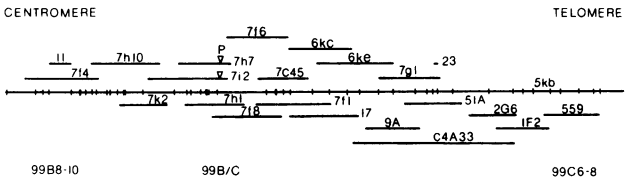

Fig. 4. Chromosome walk from λ559 to *ca*. The walk was carried out both in ca^{P6} and Oregon R. The continuous line represents chromosomal DNA. The vertical slashes correspond to *Eco*RI restriction enzyme sites in Oregon R DNA. Clones from Oregon R and Canton S are below, and clones from ca^{P6} are above the line representing chromosomal DNA. ϕ51A and ϕ9A are from a Canton S library and c4A33 is from a cosmid library. ϕ11 and ϕ23 are from a microsurgery library prepared from ca^{P6}. The walk comprises ~130 kb of DNA from Oregon R, and ~115 kb from ca^{P6}. The total region covered is ~160 kb. The P element at *ca* in ca^{P6} was recovered in ϕ7h7 and ϕ7i2. Cytological map positions and directions of the centromere and telomere are indicated. The scale is denoted by the 5 kb fragment near the distal end of the walk.

A microsurgery library was prepared from the 99B/C region of ca^{P6} polytene chromosomes. Although none of the phage were positive by hybridization with a P element probe, one recombinant, ϕ11, that maps to 99B8-10 just proximal to the P element in ca^{P6}, was used as the endpoint for the walk.

The chromosome walk extending from λ559 to ϕ11 in the Oregon R, Canton S and ca^{P6} libraries is shown in Figure 4. The line in the center of Figure 4 represents the *Eco*RI map for Oregon R DNA. Recombinants from ca^{P6} are shown above the line, while those from Oregon R or Canton S are shown below the line. DNA fragments of 500 bp or less may have been missed; in some instances they were detected and mapped by analysis of partial digestion products. Overlaps between recombinants, and between recombinants and genomic DNA fragments were confirmed by Southern blots of phage and genomic DNA. The chromosome walk represents ~160 kb of DNA, and covers the interval from 99B8-10 to 99C6-8 on the polytene chromosome map. The P element insertion was found in two of the recombinants from the ca^{P6} library, ϕ7h7 and ϕ7i2. The size of the insertion, 0.9 kb, indicates that it is a deleted

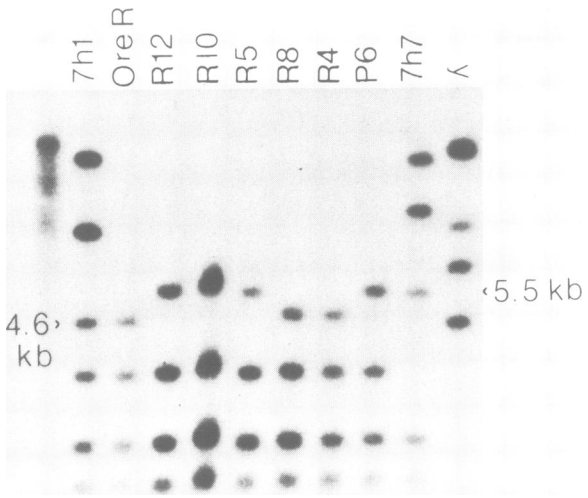

Fig. 5. Southern blot analysis of ca^{P6} revertants. DNA from homozygous revertants was digested with *Bam*HI and hybridized with [^{32}P]ϕ7h1. Marker DNAs of *Bam*HI-digested ϕ7h1 and ϕ7h7, and *Hin*dIII-digested λ DNA are in the side lanes. The 5.5 kb band in P6 (ca^{P6}) contains the P element and corresponds to the 4.6 kb band in Oregon R. The 5.5 kb band is present in the partial or pseudorevertants, R12, R10 and R5, while the 4.6 kb band is present in revertants R8 and R4. Phenotypic reversion of ca^{P6} to ca^{+} in R8 and R4 is therefore correlated with loss of the P element in the cloned region.

P element (O'Hare and Rubin, 1983). ϕ11, the microdissection clone that was used to define the proximal end of the walk, is part of ϕ7f4.

The P element insertion is responsible for the ca phenotype

Five spontaneous revertants of ca^{P6} were analyzed to determine whether the P element in the cloned region was the basis of the *ca* mutation. A Southern blot of *Bam*HI-digested DNA from the homozygous revertant lines ca^{P6} and Oregon R was examined after hybridization with ϕ7h1, the recombinant that includes the site of P element insertion in ca^{P6} but lacks the P element (Figure 4). The Southern blot (Figure 5) shows four labeled bands for Oregon R DNA. One of these, the 4.6 kb band, is shifted to 5.5 kb in ca^{P6} DNA. The 0.9 kb difference between the fragments from Oregon R and ca^{P6} is due to the presence of the P element in ca^{P6}. Of the five ca^{+} revertants of ca^{P6} that were examined, two (R4 and R8) show a 4.6 kb fragment instead of a 5.5 kb fragment, as expected if reversion were caused by deletion of the P element in the cloned region. The polytene chromosomes of R4 and R8 also show loss of the P element at *ca* by *in situ* hybridization, and ca^{+} in R8 maps by recombination analysis to the region between *Drop* (99.2) and *brevis* (102.7), which includes *ca* (100.7). Thus, reversion to ca^{+} in R4 and R8 is accompanied by loss of the P element at *ca*. The remaining ca^{+} revertants, R12, R10 and R5, retain the 5.5 kb fragment. R10 and R5 show a variable *ca* phenotype, and R12 shows a *ca* phenotype with some *ca* tester alleles. R10 and R5 are therefore partial revertants, and R12 is a pseudorevertant. The molecular basis for the variable phenotypes of R10 and R5 is not known, although rearrangements within the 5.5 kb fragment have not been ruled out. Both simple and complex rearrangements

have been reported for revertants of a P element mutant of *Sxl* (Salz *et al.*, 1987).

One of the revertants, R8, has a stable ca^+ phenotype that maps by recombination analysis close to, or at *ca*. R4 and R8 both show loss of the P element at *ca* by *in situ* hybridization and Southern blot analysis. The correlation between phenotypic reversion of ca^{P6} to ca^+, the mapping of this effect to *ca*, and the loss of the P element in the cloned region provide strong evidence that the cloned region corresponds to the *ca* locus. We conclude that the P element at *ca* in ca^{P6} is the likely cause of the mutant eyecolor. It thus provides a molecular marker for the DNA sequences encoding the *ca* gene product.

Rescue of the segregational defect at ca by transformation

We carried out *Drosophila* transformation experiments in order to determine whether we had recovered the DNA sequences at *ca* required for correct eyecolor and meiotic chromosome segregation. DNA fragments from ϕ7h1 were cloned into a P element vector, pUChsneo (Steller and Pirrotta, 1985), and injected, together with phsπ helper, into ca^{Cm} embryos. The ca^{Cm} stock used in these experiments carries a recessive, 3rd chromosome, second-site suppressor of the segregational defect of ca^{Cm}, reducing the frequency of abnormal offspring to 2−3%. Because the suppressor is recessive, females heterozygous for ca^{Cm} from this stock and ca^{nd}, which is *suppressor*$^-$, produce high frequencies of non-disjunctional and mosaic offspring. The use of the suppressor-bearing ca^{Cm} stock as a host strain allowed us to recover regular segregants as transformants, and then to test them for rescue of the segregational defect associated with the *ca* locus by constructing and testing ca^{Cm}/ca^{nd} females. Transformants were selected by growth on food containing G418.

Figure 6 shows the two DNA fragments from ϕ7h1 that were used in transformation experiments. Three transformants carrying insertions of the ϕ7h1 6.9 kb *Eco*RI fragment at different sites on the X chromosome were recovered among offspring produced by 29 injected adults. None of the three transformants showed rescue of either the mutant eyecolor or the segregational defect associated with the *ca* locus.

Two transformants carrying the ϕ7h1 11.5 kb *Xho*I fragment were recovered among offspring produced by 50 injected adults. The two transformants probably arose from the same germline event, since they were produced by a single female. The low frequency of transformation was attributed to the use of the *Xho*I site in pUChsneo for cloning the ϕ7h1 fragment (see Materials and methods). The site of insertion in both transformants carrying the ϕ7h1 11.5 kb *Xho*I fragment was mapped by *in situ* hybridization to the proximal third of chromosome 3R. Claret eyecolor was not rescued by either transformant. Rescue of the segregational defect was tested by mating one of the two transformants to obtain females heterozygous for the transgene and for ca^{Cm} and ca^{nd}. These females were mated to males carrying a recessively marked (y^2 w^{bf}) X chromosome and a dominantly marked (B^S) Y chromosome. Offspring were scored for regular segregants (+ females and B^S males), non-disjunctional exceptions (B^S females, y w males and Minutes), gynandromorphs, and Minute mosaics (Table II). Controls were females heterozygous for ca^{Cm} and ca^{nd}, but not carrying the transgene, and females heterozygous for ca^{Cm} and *ca*.

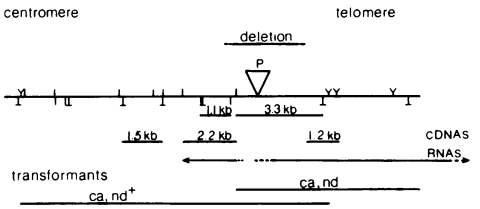

Fig. 6. The *ca* locus in *D.melanogaster*. A restriction enzyme map of ϕ7h1 is shown, together with the site of P element insertion in ca^{P6}, the approximate locations of three cDNA clones, and the approximate map positions and direction of transcription for two poly(A)$^+$ RNAs. cDNAs were mapped roughly by hybridization and possible introns were not localized. The 1.5 kb, 2.2 kb and 1.2 kb cDNAs correspond to ϕ8j9, ϕ8e2 and ϕ7j3 respectively. The approximate position of the 2.6 kb deletion in ca^{nd} and ca^{Cm} is indicated. The same 2.6 kb deletion is present in ca^{P3}, ca^{3-1} and ca^{22-8}. The restriction enzyme fragments that were transformed in *Drosophila* embryos are indicated at the bottom. The upper line is the 6.9 kb *Eco*RI fragment; transformants carrying this fragment were claret in eyecolor and showed abnormal chromosome segregation. The lower line represents the 11.5 kb *Xho*I fragment; transformants with this fragment showed complementation for chromosome non-disjunction and loss, but had mutant eyecolor. The 1.1 kb *Hin*dIII and 3.3 kb *Eco*RI−*Bam*HI fragments used to synthesize RNA probes for Northern blots are indicated. Restriction enzyme sites are indicated by ⊥ (*Eco*RI), ⊤ (*Hin*dIII), ⌶ (*Bam*HI), ⋎ (*Xho*I).

Table II (line 1) shows that females heterozygous for ca^{Cm} and ca^{nd} produce a high frequency (27.7%) of non-disjunctional exceptions and mosaics, including gynandromorphs, among their offspring. Non-disjunction of the X chromosome and chromosome 4 has been shown to occur in meiosis I in ca^{nd} females (Davis, 1969); this is also true for ca^{Cm} females (D.J.Komma and S.A.Endow, unpublished). Loss of maternal X chromosomes occurs in meiosis, accounting for the excess of y w males that carry an X chromosome from their father, or in the early zygote, giving rise to gynandromorphs that comprise wild-type female (X/X) and y w male (X/0) tissue. Females heterozygous for ca^{Cm} and *ca* (line 2) show low levels of abnormal offspring (0.26%). This is expected since *ca* complements ca^{Cm} for chromosome non-disjunction and loss. Females heterozygous for the 11.5 kb transgene, and for ca^{Cm} and ca^{nd} (line 3) produce 0.34% abnormal offspring, indicating that the transgene rescues the segregational defect shown by ca^{nd}/ca^{Cm} females.

The 11.5 kb *Xho*I fragment of ϕ7h1 that rescues the segregational defect associated with the *ca* locus includes the site of P element insertion in ca^{P6} (Figure 6). Transformants carrying the ϕ7h1 11.5 kb *Xho*I transgene have a claret phenotype and are non-disjunctional$^+$, while those carrying the ϕ7h1 6.9 kb *Eco*RI fragment show both claret eyecolor and chromosome non-disjunction and loss.

Analysis of ca^{nd} and ca^{Cm}

The molecular changes at *ca* in ca^{nd} and ca^{Cm} were determined by cloning the *ca* region from homozygous mutant DNA. Approximately 15 kb of *ca* region DNA was recovered from ca^{nd} in overlapping phage clones and 30 kb from ca^{Cm}. Restriction enzyme sites in the cloned DNA were mapped by analysis of partial digestion products. Homology with ϕ7h1 was determined on Southern blots hybridized with ϕ7h1 restriction enzyme fragments. The

Table II. Complementation by a transformant carrying the ϕ7h1 11.5 kb *Xho*I fragment of the segregational defect associated with ca^{nd}

Female parent	Offspring									
	+ ♀	B^S ♀	M ♀	B^S ♂	M ♂	y w ♂	Gyn	Other	Total	Exceptions (%)
1. ca^{nd}/ca^{Cm}	659	12	120	611	93	141	116	4	1756	27.7
2. ca/ca^{Cm}	1868	1	3	1521	0	1	0	4	3398	0.26
3. $ca^{nd}/G418^R$ ca^{Cm}	2109	1	1	1978	5	2	0	5	4101	0.34

Offspring of crosses to y^2w^{bf}/B^SY males. Regular offspring are + females and B^S males. Non-disjunctional exceptions are B^S females, y w males, and Minutes (M). Gyn, gynandromorphs, M, includes mosaics. Other includes X/0 males carrying a maternal X chromosome and an X/X female homozygous for w^{bf}. Lines 1 and 2 are controls; line 3 shows the test cross. The transgene is denoted by $G418^R$.

major change in both ca^{nd} and ca^{Cm} is a 2.6 kb deficiency that overlaps the site of P element insertion in ca^{P6}. Several restriction enzyme site polymorphisms were also observed. Figure 6 shows the approximate position of the deficiency. One end of the deficiency includes the right-hand end of the ϕ7h1 1.1 kb *Hin*dIII fragment since this fragment is shifted to 6 kb in ca^{nd} and ca^{Cm}. The amount of hybridization of the 1.1 kb fragment to the 6 kb fragment in the mutants suggests that only a small region of the 1.1 kb fragment is deleted. The deficiency extends into the adjacent 7.6 kb *Hin*dIII fragment, which overlaps the ϕ7h1 7.2 kb *Hin*dIII right end fragment, fusing it to the 1.1 kb fragment and deleting ~2.6 kb of DNA sequence. The deficency appears to be the same in ca^{nd} and ca^{Cm} by gel analysis of cloned DNA. Southern blots of genomic DNA from ca^{nd} and ca^{Cm} confirm the presence of the deficiency and show that ca^{P3}, ca^{3-1} and ca^{22-8} appear to have the same deficiency.

cDNAs homologous to the ca region

Three cDNA recombinants homologous to the *ca* region were recovered by probing a first and second larval instar (Poole *et al.*, 1985) or an ovary library with restriction fragments from ϕ7h1. The locations of these cDNAs are shown in Figure 6. ϕ8j9 and ϕ8e2 are the largest of four or six homologous cDNA recombinants respectively, recovered with two different probes from an ovary library. ϕ8j9 is 1.5 kb and ϕ8e2 is 2.2 kb. ϕ7j3 is a 1.2 kb cDNA that was found in a first and second larval instar library.

RNAs homologous to the ca region

Northern blot analysis was carried out to characterize RNAs corresponding to cDNAs in the *ca* region. Hybridization was with single-stranded RNA probes transcribed from subcloned fragments of ϕ7h1. The approximate positions and direction of transcription of mRNAs are shown in Figure 6.

Figure 7(a) shows hybridization to approximately equivalent amounts of poly(A)$^+$ RNA from 3-day Oregon R females and males. The ϕ7h1 1.1 kb *Hin*dIII fragment (Figure 6) is homologous to a 2.2 kb poly(A)$^+$ RNA (arrow) that is present in 3-day adult females (F). The 2.2 kb transcript is present in much lower amounts in 3-day males (M). Bands of hybridization can be observed in RNA from males after 7 times longer exposure. The identity of the higher mol. wt band of 4.7 kb is not known. It is unlikely to be a processing intermediate of the 2.2 kb RNA since it is present in deletion mutants (see Figure 8). The lower band of 1.1 kb may be a degradation product. Figure 7(b) shows hybridization to poly(A)$^+$ RNA from 3-day Oregon R females (F), 3-day females minus ovaries (−ov), and 3-day ovaries (ov). The approximate amounts of RNA loaded are from six adult females, 20 females minus ovaries, and six

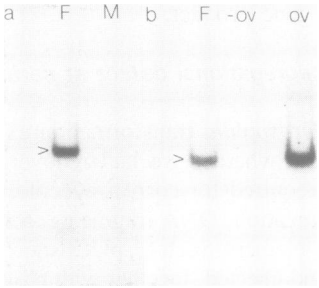

Fig. 7. Northern blot of RNA from wild-type females and males. (**a**) Approximately equivalent amounts of poly(A)$^+$ RNA from 3-day Oregon R females (F) and males (M) was separated on a 1.2% agarose−formaldehyde gel. Hybridization was with an RNA probe made from the ϕ7h1 1.1 kb *Hin*dIII fragment (Figure 6). The major band of hybridization (arrow) is ~2.2 kb in length. The identity of the faint band of high mol. wt (4.7 kb) is unknown. The faint band of lower mol. wt (1.1 kb) may be a degradation product. (**b**) Poly(A)$^+$ RNA from approximately six adult females (F), 20 females minus ovaries (−ov) and six ovary pairs (ov) was loaded in adjacent lanes. Hybridization was with RNA synthesized from the ϕ7h1 1.1 kb *Hin*dII fragment. The 2.2 kb RNA (arrow) is present in RNA from females and from ovaries, but not in RNA from females minus ovaries.

ovary pairs. The 2.2 kb RNA (arrow) is present in females and in ovaries, but is barely detectable after a 5-fold longer exposure in females minus ovaries. The transcript thus is likely to be ovary-specific in females.

Poly(A)$^+$ RNA from *ca* mutant alleles was examined for the presence of the 2.2 kb transcript. Figure 8 shows that the 2.2 kb RNA (arrow) is present in poly(A)$^+$ RNA from 3-day Oregon R, *ca* and *ncd* females, but not in RNA from ca^{nd} or ca^{Cm} females. The absence of the 2.2 kb transcript in RNA from ca^{nd} and ca^{Cm} females is consistent with the presence of a deficiency in these mutants that truncates the 5′ end of the transcript. *ncd* is an EMS-induced allele of ca^{nd}, and may contain a point mutation in the transcribed region. The predominance of the 2.2 kb transcript in the ovary, and its presence in *ca* mutants that affect eyecolor [*ca*, ca^{P6} (not shown)] but not in *ca* mutants that affect chromosome segregation (ca^{nd}, ca^{Cm}) indicate that it is likely to encode the nd^+ product. This is supported by the fact that it appears to be completely encoded by the ϕ7h1 11.5 kb *Xho*I fragment that rescues chromosome non-disjunction and loss of ca^{nd}.

An RNA corresponding to the ϕ7j3 cDNA was identified using a single-stranded probe corresponding to the ϕ7h1 3.3 kb *Eco*RI−*Bam*HI fragment (Figure 6). The poly(A)$^+$ RNA detected is shown in Figure 9. The filter is the same as in Figure 8, but has been stripped and rehybridized. The major band of hybridization (arrow) is 7.4 kb in length, and is observed in RNA from Oregon R and *ncd* females, but

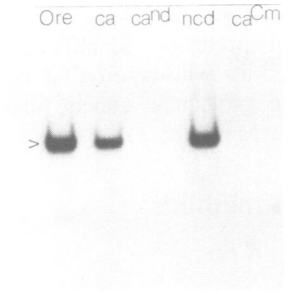

Fig. 8. Northern blot of RNA from *ca* mutants. Approximately equivalent amounts of poly(A)$^+$ RNA from wild-type or mutant females were loaded. Hybridization was with RNA synthesized from the ϕ7h1 1.1 kb *Hin*dIII fragment. The major band of 2.2 kb (arrow) is present in RNA from Oregon R, *ca* and *ncd* females, but absent from RNA of ca^{nd} and ca^{Cm} females. The faint band of 4.7 kb is present in all lanes.

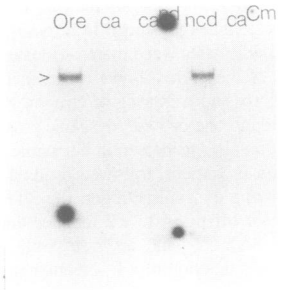

Fig. 9. Northern blot of RNA from *ca* mutants. The filter in Figure 8 was stripped and rehybridized with RNA synthesized from the ϕ7h1 3.3 kb *Eco*RI–*Bam*HI fragment (Figure 6). The major band of hybridization is ~7.4 kb (arrow), present in RNA from Oregon R and *ncd* females. This RNA is either absent or greatly reduced in amount in *ca*, ca^{nd} and ca^{Cm} females. The light bands at the bottom of the autoradiograph are ~1 kb in length. Their identity is not known.

is reduced or absent from RNA of *ca*, ca^{P6}, ca^{nd} and ca^{Cm} females. The 7.4 kb transcript is also present in poly(A)$^+$ RNA from males in amounts approximately half that in females (not shown). The transcript is present in RNA from Oregon R and *ncd* males, but absent or present in greatly reduced amount in RNA from *ca*, ca^{P6}, ca^{nd} and ca^{Cm} males.

The presence of the 7.4 kb RNA in both males and females and wild-type and *ncd* flies and its reduced amount in *ca* mutants [*ca*, ca^{nd}, ca^{Cm}, ca^{P6} (not shown)], identifies it as the putative ca^+ transcript. The approximate map position for the 7.4 kb RNA is indicated in Figure 6. The 7.4 kb transcript is not detected by hybridization with the ϕ7h1 1.1 kb *Hin*dIII probe corresponding to the sense strand for the 2.2 kb RNA. This places the approximate start of the 7.4 kb RNA to the right of the 1.1 kb *Hin*dIII fragment. The P element in ca^{P6} is either in the promoter or coding region for the transcript. The 7.4 kb RNA is shown starting just to the right of the site of P element insertion. The 2.2 and 7.4 kb RNAs are transcribed in opposite directions. It is not known at present whether the genes that encode the two RNAs overlap, although experiments are under way to determine this. The 7.4 kb transcript encoding the putative ca^+ product is not completely encoded by either the ϕ7h1 6.9 kb *Eco*RI or 11.5 kb *Xho*I fragments used in transformation experiments. This explains the failure of both of the fragments to rescue claret eyecolor.

Transcripts homologous to the 1.5 kb cDNA are present in poly(A)$^+$ RNA from 3-day Oregon R females and males, females minus ovaries, ovaries, and *ca*, ca^{nd}, *ncd* and ca^{Cm} females. The presence or absence of the transcripts is not correlated with sex, tissue or mutant *ca* phenotype. The nature of these transcripts is still under study.

Discussion

We have undertaken a molecular and genetic analysis of the *ca* locus of *Drosophila* in order to determine the structural relationship of the genes at *ca*, and the biological role of the product required for meiotic and early zygotic chromosome segregation. Our results indicate that the products at *ca* needed for wild-type eyecolor and normal chromosome segregation are encoded by closely apposed genes that are transcribed in opposite directions.

We recovered and characterized six new P element-induced alleles of *ca*. Three alleles show mutant eyecolor and abnormal chromosome segregation, while three affect only eyecolor. A new spontaneous allele of *ca*, ca^{Cm}, is mutant both in eyecolor and chromosome segregation. Thus, four alleles from a total of seven affect both functions. The high frequency of co-mutation of eyecolor and chromosome segregation supports the idea that the corresponding genes are closely positioned to one another and that a single molecular change may mutate both.

The molecular analysis identifies a transcript that is likely to encode the gene needed for normal chromosome segregation. This transcript is ~2.2 kb in length, is found in high amounts in poly(A)$^+$ RNA from females and in much lower amounts in males, and is found predominantly in ovaries in females. This pattern of expression is that expected for a product that is needed for meiosis I segregation in females, but not in males. In addition, the 2.2 kb transcript is absent from two mutants, ca^{nd} and ca^{Cm}, that show high amounts of chromosome non-disjunction and loss. It is present in mutants (*ca*, ca^{Pb}) that affect only eyecolor. The 2.2 kb RNA is also present in *ncd* females, which show high amounts of non-disjunction and chromosome loss. Since *ncd* was EMS-induced, it may contain a point mutation which is expressed post-transcriptionally. The presence of the transcript in eyecolor mutants and its absence in segregational deletion mutants support the assignment of the 2.2 kb RNA as the nd^+ product. In addition, the transcript is probably completely encoded by the ϕ7h1 11.5 kb *Xho*I fragment that fully rescues the segregational defect in ca^{nd}. We conclude that the 2.2 kb RNA is the likely product of the nd^+ gene at *ca*.

The putative ca^+ product is a transcript of ~7.4 kb in length. The 7.4 kb RNA is present in poly(A)$^+$ RNA of both females and males, as expected for a product that affects eyecolor in both sexes. It is absent or reduced in mutants that affect eyecolor (*ca*, ca^{P6}, ca^{nd}, ca^{Cm}) but is present in *ncd*, which affects chromosome segregation but not eyecolor. The absence of reduction of the 7.4 kb RNA in *ca* mutants, and its presence in a mutant that is abnormal only in chromosome segregation provide evidence that it corresponds to the ca^+ product. The transcript is not completely encoded by either of the two fragments used in transformation experiments, thus explaining their failure to rescue the ca phenotype.

The genes that encode the putative ca^+ and nd^+ products

lie within 1 kb of one another, but are transcribed in opposite directions. The P element in ca^{P6} marks the ca^{+} gene. Transcription starts at some point to the right of the adjacent *Hin*dIII site. The 2.2 kb RNA starts to the right of the *Eco*RI site, since a probe made from the ϕ7h1 3.3 kb *Eco*RI−*Bam*HI fragment shows a small amount of hybridization to the 2.2 kb transcript. The close proximity of the RNAs in the region raises the possibility that their regulatory and/or coding regions may overlap. This is currently under investigation. Possible introns in the coding regions have not yet been mapped.

Co-mutation of the eyecolor and segregational products apparently occurs by deletion of the 5′ flanking and/or coding regions of the two genes. Two mutant alleles, ca^{nd} and ca^{Cm}, have a 2.6 kb deficiency that overlaps the site of P element insertion in ca^{P6} and results in the absence of both the putative ca^{+} and nd^{+} transcripts. Three additional mutant alleles that cause both claret eyecolor and abnormal chromosome segregation also have a 2.6 kb deficiency that appears to be the same by gel analysis as that in ca^{nd} and ca^{Cm}. The finding of five independent mutants with the same deletion, recovered spontaneously, after X-irradiation or after P element mutagenesis, suggests that sequences in the region are prone to intrachromatid recombination. A similar case has been reported for four independent *unc-86* mutations that also show a meiotic non-disjunction (Him) phenotype, in which all four are apparently caused by the same 18 kb deletion (Finney *et al.*, 1988).

The segregational function encoded at *ca* is of interest because it is required for regular chromosome disjunction in meiosis I. ca^{nd} is unique among the female-specific meiotic mutations that have been found in *Drosophila* (reviewed in Baker and Hall, 1976) in that it does not affect recombination and causes non-disjunction of both exchange and non-exchange chromosomes (Davis, 1969). ca^{nd} is therefore independent of the so-called 'distributive pairing' system (Grell, 1962) and may act subsequent to it. ca^{nd} is one of the first *Drosophila* genes to be recovered molecularly with a primary role in meiotic chromosome segregation.

Several meiosis-specific genes from yeast are currently under study. Among them are genes whose products are needed for some aspect of chromosome pairing or recombination, such as *SPO-11* (Klapholz *et al.*, 1985; Giroux *et al.*, 1986), *MER-1* (Engebrecht and Roeder, 1989) and *HOP-1* (Hollingsworth and Byers, 1989). The yeast *RED-1* locus encodes a product apparently needed for meiosis I segregation (Rockmill and Roeder, 1988). The *red1-1* mutant shows only a small decrease in recombination frequency for two intervals that were examined, and causes high spore inviability, which has been attributed to aneuploidy resulting from meiosis I non-disjunction. The phenotype of *red1-1* may therefore be similar in some respects to that of ca^{nd}. No DNA sequence homology between *RED-1* and the *ca* locus is detectable by Southern blot analysis at low stringency (S.A.Endow, unpublished), however, although this does not preclude functional homology.

Further study of the biological function of the wild-type ca^{nd} product is needed to establish its role in meiotic chromosome segregation. *Drosophila* offers advantages over other organisms for the study of meiotic chromosome segregation because of the ease of recovering sex and 4th chromosome non-disjunctions, and the availability of compound chromosomes for study of chromosome 2 and 3 non-disjunctional products. Identification of the DNA sequences needed for wild-type ca^{nd} function provides the first step in the molecular genetic analysis of meiotic chromosome segregation.

Materials and methods

Drosophila stocks

ca and ca^{nd} were in our laboratory collection. *ncd* was obtained from J.Kennison. A stock of *R(3)S1, Df(3R)P47, ca/In(3R)C, Sb cd Tb ca*, referred to as *R(3) ca/In(3R)C, Sb ca*, was from L.Craymer. The $\pi 2$ and $y^2 w^{bf}$ stocks were from W.Engels and L.Searles respectively. The *Jumpstarter* ($Js\ ry^{506}$) (Cooley *et al.*, 1988), $X(neo^r)$ (Steller and Pirrotta, 1985), *mwh red e* and *TM3* ry^{RK} *Sb e* chromosomes were sent to us by L.Cooley. A stock carrying the *TM3 Sb Ser ca e* chromosome was from R.MacIntyre. ca^{g} is a gamma-ray induced and ca^{Cm} is a spontaneous allele of ca^{nd}.

P element-induced mutants

ca^{P1}, ca^{P2}, ca^{P3} and ca^{P6} are new alleles of *ca* recovered after hybrid dysgenesis. Dysgenic females produced by crosses of Oregon R (M′) or $y^2 w^{bf}$ (M) females to $\pi 2$ males were mated to tester *R(3) ca/In(3R)C, Sb ca* males. Offspring of these crosses were screened for *Sb* and *ca*. ca^{P1} was found after backcrossing offspring of Oregon R/$\pi 2$ dysgenic females to *ca* tester stocks through one or more dysgenic crosses. ca^{P1} was unstable in the second generation after its detection but stable thereafter. It probably arose by insertion of a P element, followed by its deletion. ca^{P2} and ca^{P3} were found after crossing dysgenic Oregon R/$\pi 2$ females to tester males, then backcrossing male offspring to $\pi 2$ females and testing offspring of these matings for *ca*. Both ca^{P2} and ca^{P3} are stable and probably arose by insertion and subsequent deletion of a P, or other moveable element. ca^{P6} was recovered after screening 11 460 offspring of $y^2 w^{bf}/\pi 2$ dysgenic females. ca^{P6} is unstable and carries a P element at *ca*. Individuals carrying new *ca* alleles were mated to produce homozygous stocks (ca^{P1}, ca^{P3} and ca^{P6}) or stocks balanced with *In(3R)C, Sb ca* (ca^{P2}).

$ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ are mutants recovered using the Jumpstarter system of P element mutagenesis (Cooley *et al.*, 1988). They arose in a screen for mutations on chromosome 3 that affect meiotic chromosome segregation (D.J.Komma, K.-I.Chung and S.A.Endow, in progress). Dysgenic $X(neo^r)/Y$; $+/Js\ ry^{506}$ males were mated in single pairs to *TM3* ry^{RK} *Sb e/mwh red e* females, and ry^{+} Sb male offspring carrying a potentially mutagenized chromosome 3 were mated in single pairs to *TM3 Sb Ser ca e/*ca^{nd} females. Sb Ser sibling offspring from single vials were mated *inter se*. Sb^{+} Ser^{+} offspring of these sib matings were homozygous for mutagenized third chromosomes, and were tested for effects on meiotic chromosome segregation. The claret eyecolor in $ca^{3\text{-}1}$ arose in the second generation after hybrid dysgenesis. Claret offspring were used to establish the stock, which showed high-frequency meiotic chromosome non-disjunction and loss in combination with ca^{nd} or in homozygous form. $ca^{22\text{-}8}$ showed abnormal chromosome segregation in combination with *TM3 Sb Ser ca e* in the second generation after hybrid dysgenesis (it would not have been detected in the first generation after hybrid dysgenesis since it was recovered in a male). The claret eyecolor arose in the 4th generation after mutagenesis. The *ca* chromosome carried a recessive lethal when first isolated, which was removed by recombination. $ca^{3\text{-}1}$ and $ca^{22\text{-}8}$ were examined by Southern blots and $ca^{22\text{-}8}$ was examined by *in situ* hybridization, and both were found not to contain P elements. They probably arose by insertion, followed by deletion, of a P element or other moveable element.

Reversion of P element mutants

Spontaneous revertants of ca^{P6} were recovered by mating ca^{P6} males to *TM3 Sb Ser ca e/*ca^{nd} or *In(3R)C, Sb ca/*ca^{g} females in single pairs. ca^{+}, Sb ca^{+} or Sb Ser ca^{+} male offspring were mated in single pairs to *TM3 Sb ser ca e/*ca^{nd} females, and Sb Ser ca^{+} offspring of these crosses were mated to each other. ca^{+} offspring of sib matings were homozygous for revertant chromosomes and were mated to each other to establish stocks. ca^{+} in the revertant chromosomes was mapped by recombination with a chromosome 3 carrying *Drop* (99.2), *ca* (100.7) and *brevis* (102.7).

Complementation tests

ca^{P} mutants were tested for stability, and for their ability to complement eyecolor and chromosome non-disjunction and loss of *ca*, ca^{nd} and *ncd*. Tests for chromosome segregation were carried out by mating females carrying *ca* mutant alleles to males carrying a recessively marked X

chromosome and a dominantly marked Y chromosome. Offspring were scored for exceptional X/X/Y females, X/0 males and Minute (haplo-4) females or males, gynandromorphs, and in some experiments haplo-4 mosaics.

In situ hybridization

Salivary gland chromosomes from third-instar larvae were prepared as described by Pardue and Gall (1975), except that tissue was squashed onto Denhardt's solution-treated slides (Brahic and Haase, 1978). Slides were fixed overnight in absolute ethanol, then incubated in 2 × SSC (SSC = 0.15 M NaCl, 15 mM Na_3 citrate) at 65°C for 30 min prior to denaturation in NaOH. Hybridization was in the presence of 40% formamide for 16–20 h at 37°C with DNA probes labeled with biotin-11-dUTP (BRL) by nick-translation (Langer-Safer *et al.*, 1982) or oligonucleotide priming (Feinberg and Vogelstein, 1984). Slides were washed and incubated with a streptavidin-biotinylated horseradish peroxidase complex (Detek I-hrp, ENZO Biochem, Inc.) and then with diaminobenzidine tetrahydrochloride (DAB) according to the directions supplied by the manufacturer, except that reaction wtih DAB was for 2 h at 37°C.

Chromosome walk

High mol. wt DNA was prepared from Oregon R or $y^2 w^{bf}/B^S Y$; ca^{P6} adult flies, partially digested with *Mbo*I and ligated to *Bam*HI + *Sal*I-digested λEMBL4 DNA. Ligated DNA was packaged *in vitro*, plated onto LE392(P2) host cells and plaques were lifted onto nitrocellulose filters. Filters were pre-hybridized and hybridized in aqueous solution at 65°C. Probes consisted of gel-purified fragments labeled by random priming. Restriction enzyme sites in recombinant DNAs were mapped by overlaps with other recombinants, digestion with a second enzyme, or by analysis of partial digestion products end-labeled by hybridization with ^{32}P-oligonucleotides complementary to the lambda *cos* ends (Rackwitz *et al.*, 1984). Southern blots (Southern, 1975) of recombinant and genomic DNA were carried out to confirm overlaps and fragment map positions.

In the initial stages of the walk several libraries were screened, including a Canton S library from which φ51A and φ9A were recovered. c4A33 was isolated from a cosmid library in pC2RB by colony filter hybridization at 65°C in the presence of 10% sodium dextran sulfate. φ2G6, φI7, φ7f1, φ7f8, φ7h1, φ7k2 and c4A33 (Figure 4) are from P-free Oregon R libraries.

Libraries were constructed from ca^{nd} and ca^{Cm} homozygotes, and screened using the φ7h1 6.9 kb *Eco*RI fragment to recover DNA sequences corresponding to the *ca* locus. Recombinant DNAs were mapped by analysis of partial digestion products, and homology with φ7h1 was determined by probing Southern blots of recombinant DNAs with φ7h1 restriction fragments. Differences between mutant and wild-type recombinant DNAs were confirmed by Southern blots of wild-type ca^{nd} and ca^{Cm} genomic DNAs. DNAs from *ca*, ca^{P3}, ca^{3-1}, ca^{22-8}, ca^{P6} and *ncd* flies were also analyzed on Southern blots after digestion with two or more different restriction enzymes.

Microsurgery library

A microsurgery library was prepared according to methods described by Pirrotta *et al.* (1983) by microdissecting the 99B/C region from salivary gland chromosomes of $y^2 w^{bf}/B^S Y$; ca^{P6} third-instar larvae. DNA was extracted from microdissected chromosome bands, digested with *Eco*RI, ligated to *Eco*RI-digested λ1149 (Murray, 1983) and packaged, and bacteriophage were plated onto POP 13b (hf1). Insertion$^+$ recombinants were mapped by *in situ* hybridization to salivary gland chromosomes.

cDNA libraries

A cDNA library in λgt10 from first- and second-instar larvae (Poole *et al.*, 1985) was obtained from T.Kornberg. Phage were plated onto C600 (hf1) and screened as described above. An ovary cDNA library in λgt11 was from L.Kalfayan. Phage were plated onto Y1088 (pMC9) cells on NZCYM + ampicillin for screening.

Northern blots

RNA was extracted from ovaries of 3-day females dissected in Ringer's solution (Ephrussi and Beadle, 1936), or from 3-day adult males or females by homogenization in phenol and SDS buffer containing 500 μg/ml proteinase K, followed by phenol and chloroform extractions (Spradling and Mahowald, 1979). Poly(A)-containing RNA was prepared by oligo(dT)–cellulose chromatography, and is referred to as poly(A)$^+$ RNA.

Poly(A)$^+$ RNA or total RNA was denatured in formamide/formaldehyde and separated by electrophoresis on 1.2% agarose gels containing formaldehyde. Mol. wt markers were *Hin*dIII-digested λDNA and 18S + 28S rRNA denatured by heating in formamide/formaldehyde prior to loading. RNAs were transferred to nitrocellulose filters and hybridized with single-stranded RNA probes prepared from DNA fragments cloned into Bluescript SK+ (Stratagene), using the T3/T7 promoter system. Hybridization was in the presence of 50% formamide at 60°C. After autoradiography, filters were stripped by boiling for 5 or 10 min in distilled water, then test exposed and rehybridized.

Transformation of Drosophila embryos

The 6.9 kb *Eco*RI or 11.5 kb *Xho*I fragment from φ7h1 was inserted into the *Eco*RI or *Xho*I site of pUChsneo (Steller and Pirrotta, 1985) and injected at a concentration of 300 μg/ml together with 100 μg/ml phsπ into ca^{Cm} *Drosophila* embryos. The stock used as host for injection experiments carries a recessive, chromosome 3, second-site suppressor of the segregational defect of ca^{Cm}, reducing the number of exceptional and abnormal offspring to ~2–3%. Injected adults were mated individually to ca^{Cm} males or females on standard food and transferred to food containing 0.5 mg/ml G418 (Gibco) after first-instar larvae appeared. Matings on food + G418 were heat-shocked 30 min at 37°C every third day. F1 G418^R offspring were mated in single pairs to ca^{Cm} males or females on standard food and then on food + G418, without heat-shock. F2 G418^R sibs were mated *inter se* to establish stocks; thereafter stocks were maintained alternate generations on standard food and food + G418. The unique *Xho*I site in pUChsneo is within the *Xba*I–*Xmn*I *hsp70* promoter fragment, 108 bp from the end of the proposed 'recognition' sequence of the *hsp70* promoter (Ingolia *et al.*, 1980). The φ7h1 11.5 kb *Xho*I fragment was inserted in both orientations into pUChsneo and injected into embryos, with the result that only one of the two constructs produced G418^R adults. This was attributed to transcriptional interference by the φ7h1 fragment of the *hsp70* promoter which drives the neomycin resistance gene.

Transformants were tested for rescue of the mutant segregational effect associated with *ca* alleles by mating single males from transformant stocks to *TM3 Sb ser ca e/ca*nd females on food + G418, or on standard food for G418^R males with X chromosome insertions. ca females from these matings were heterozygous for the transgene, and for ca^{nd} and ca^{Cm}; these females were crossed to y^2 $w^{bf}/B^S Y$ males and offspring were examined for segregational abnormalities as described above. Controls were ca and sb Ser ca females produced by crosses of ca^{Cm} (non-transformant) males to *TM3 Sb Ser ca e/ca*nd females.

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