MUTATIONS IN GENES ENCODING ESSENTIAL MITOTIC FUNCTIONS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Temperature-sensitive mutations at 15 loci that affect the fidelity of mitotic chromosome behavior have been isolated in *Drosophila melanogaster*. These mitotic mutants were detected in a collection of 168 EMS-induced *X*-linked temperature-sensitive (*ts*) lethal and semilethal mutants. Our screen for mutations with mitotic effects was based upon the reasoning that under *semirestrictive* conditions such mutations could cause an elevated frequency of mitotic chromosome misbehavior and that such events would be detectable with somatic cell genetic techniques. Males hemizygous for each *ts* lethal and heterozygous for the recessive autosomal cell marker *mwh* were reared under semirestrictive conditions, and the wings of those individuals surviving to adulthood were examined for an increased frequency of *mwh* clones. Those mutations producing elevated levels of chromosome instability during growth of the wing imaginal disc were also examined for their effects on chromosome behavior in the cell lineages producing the abdominal cuticle. Fifteen mutations affect chromosome behavior in both wing and abdominal cells and thus identify loci generally required for the fidelity of mitotic chromosome transmission. Mapping and complementation tests show that these mutations represent 15 loci. One mutant is an allele of a locus (*mus-101*) previously identified by mutagen-sensitive mutants and a second mutant is an allele of the lethal locus *zw10*.— The 15 mutants were also examined cytologically for their effects on chromosomes in larval neuroblasts. Taken together, the results of our cytological and genetical studies show that these mutants identify loci with wild-type functions necessary for either (1) maintenance of chromosome integrity or (2) regular disjunction of chromosomes or (3) chromosome condensation. Thus, these mutations define a broad spectrum of genes required for the normal execution of the mitotic chromosome cycle.

THE elaborate and precise behavior of chromosomes during mitosis makes the cell cycle an attractive process to study; however, its complexity has made analysis difficult. Indeed, it has been argued that the analysis of chromosome behavior during mitotic division has reached an impasse that requires the adoption of fresh approaches (PICKETT-HEAPS, TIPPET and PORTER 1982).

One such approach, pioneered by HARTWELL and his colleagues (reviewed by HARTWELL 1974; PRINGLE and HARTWELL 1981), employed mutational analysis to identify and analyze specific cell cycle functions in *Saccharomyces cerevisiae*. More than 50 loci have been identified that specify functions essen-

tial for progression through the mitotic cell cycle. The logic used to detect mitotic mutants in *S. cerevisiae* has been successfully adapted to other fungal species as well as mammalian tissue culture systems (LING 1981). The analysis of these mutants has been particularly useful in providing information about the control and integration of the many events that make up cell division.

We have begun a similar genetic dissection of the mitotic cycle in *Drosophila melanogaster* (BAKER *et al.* 1976; BAKER, CARPENTER and RIPOLL 1978; BAKER and SMITH 1979; GATTI 1979; GATTI, PIMPINELLI and BAKER 1980; BAKER, SMITH and GATTI 1982; GATTI, SMITH and BAKER 1983). Our previous studies have shown that loci identified by meiotic mutants and mutagen-sensitive mutants frequently carry out functions in mitotic cells that are necessary for the maintenance of chromosome integrity, *i.e.*, they function in the repair or prevention of spontaneous damage (BAKER, CARPENTER and RIPOLL 1978; BAKER and SMITH 1979; GATTI 1979). To recover mutations in loci controlling a broader array of mitotic functions we have screened lethal mutations for those affecting mitosis. The screen reported here differs in certain features from those that have generally been employed in Saccharomyces (HARTWELL 1974) and mammalian cells (see SIMCHEN 1978). There, the diagnostic phenotype for a cell division cycle mutant is that all cells in a population either arrest the mitotic cycle at some identifiable point or display a unique cellular morphology. The screen we employed was designed to detect elevated frequencies of errors in the transmission of mitotic chromosomes. The rationale for this screen depends upon the recognition that chromosome transmission is a major function of mitosis so that transmission errors would be a common phenotype of mutations in essential mitotic functions.

The consequences of such errors (*e.g.*, breakage, nondisjunction, loss, etc.) during development can be genetically detected in the cuticle of adult Drosophila by the use of somatic cell marker mutations. Since this screen requires that mutation-bearing flies live to adulthood, most mitotic divisions in such individuals must be normal. Thus, such a screen will detect leaky defects in fundamental cell cycle processes and defects in peripheral functions. We sought to maximize our chances of finding mutants in important cell cycle functions by first isolating temperature-sensitive lethal mutations. These were then reared under semirestrictive conditions so that a few individuals survived to adulthood and could be screened for infidelity in mitotic chromosome behavior. Fifteen mutations affecting the fidelity of chromosome transmission were recovered from a collection of 168 temperature-sensitive lethals and semilethals. This report describes the identification and preliminary cytological and genetic characterization of these mutations. In addition to identifying new loci with functions necessary for the maintenance of mitotic chromosome integrity, these mutants identify representatives of two new categories of mitotic functions in Drosophila: those responsible for chromosome condensation (*mus-101*^{ts}, *mit(1)-4*) and chromosome segregation (*l(1)zw10*^{ts}).

MATERIALS AND METHODS

All flies were reared on a standard Drosophila cornmeal-molasses-brewers yeast-agar medium at the temperatures indicated. With the exceptions of the multiply inverted balancer chromosome

FM7 (MERRIAM 1968) and the *ts*-lethal mutants reported here, descriptions of the mutants and chromosomes used in this study can be found in LINDSLEY and GRELL (1968).

Isolation of temperature-sensitive lethals: Males of the constitution *y/y*$^{+}$*Y*; *SM1*; *TM2/T(2;3)S9,bw e*; *spa*^{pol}/*spa*^{pol} were treated with EMS (0.1 ml/100 ml of 1% sucrose) for 24 hr by the procedure of LEWIS and BACHER (1968) and mated to *FM7/FM7* females. Single *y*/FM7*; *SM1*/+*; *TM2*/+*; *+/spa*^{pol*} daughters (where * = EMS-treated chromosome) were picked and mated to *FM7/y*$^{+}$*Y;spa*^{pol}/*spa*^{pol} males. Single *y*/FM7*; *+/+*; *+/+*; *spa*^{pol}/+ daughters from each cross were backcrossed to *FM7/y*$^{+}$*Y*; *spa*^{pol}/*spa*^{pol} males to establish a stock. This mating scheme not only establishes a series of pure stocks, each with an independently mutagenized *X* chromosome, but also, by replacing the mutagenized autosomes, places the *X* chromosomes in a homogenous background genotype. A total of 4925 such stocks were generated from three mutagenesis series (A, B, C). *y*/FM7* females from each of these stocks were mated to *FM7/y*$^{+}$*Y* males at 28° and 18° to determine the relative viability of males carrying the mutagenized *X* chromosome. Potential *ts* lethals were selected by the criterion that at 28° a single female produced no more than ten *y/y*$^{+}$*Y* sons (series A) or no more than 10 *y/y*$^{+}$*Y* sons and more than 20 *B/+* daughters (series B and C). This test gave 296 putative *ts*-lethal stocks which were then retested at 18° and 28°. Upon retest, 124 of these stocks were identified as *ts* lethals by the criterion that, at 28°, <1% of *y*/y*$^{+}$*Y* males survived, whereas >5% survived at 18° (including three cases of 0% survival at 28° but <5% survival at 18°). In addition, 44 *ts* semilethals were identified by the criterion of a greater than 30% difference in survival at the two temperatures. In each of these 44 stocks, survival at 28° was less than 20% of expected, whereas at 18° it was greater than 30%.

The screen for mitotic mutants: *y ts*-lethal/*FM7*; *+/+* virgins from the 168 *ts* stocks described above were crossed to *FM7/Y*; *DP(1:3)sc*^{J4}, *y*$^{+}$ *mwh/TM6* males at 18°, 21°, 25° and 28°. One wing from each of three *y ts*-lethal/*Y*; *y*$^{+}$ *mwh/mwh*$^{+}$ males, recovered at the highest temperature yielding such males, was mounted (BAKER *et al.*, 1978) and examined at ×500 magnification for *mwh* clones. Such clones can arise by a variety of mechanisms (nondisjunction, loss, mutation, breakage, recombination; see Figure 1) which render the recessive *mwh* allele on the *Dp(1:3)sc*^{J4}, *y*$^{+}$ *mwh* chromosome either hemizygous or homozygous. Stocks were identified as carrying putative mutants decreasing the fidelity of chromosome transmission if there were two or more *mwh* clones per wing, as contrasted to less than 0.5 clones per wing in control flies. For each putative mutant thus identified, abdomens from additional males of the above genotype were examined for *y* clones and *y//mwh* twin spots. Abdomens were mounted by the procedure of SZABAD (1978) and scored at ×160–240 magnification. Stocks were retained as mutants if, in addition to ≥2 *mwh* clones per wing, they had ≥0.04 *y* and *y//mwh* clones per hemitergite (control levels 0.02; Table 2).

The effects of these mutants on mitotic disjunction were examined in *y ts*-lethal/*Y*; *Dp(1;3)sc*^{J4}, *y*$^{+}$ *Sb/Sb*$^{+}$ males.

Mapping: Temperature-sensitive lethals affecting the fidelity of mitotic chromosome transmission were mapped relative to the markers *y*^{2} *cv v f car* on the basis of their lethal phenotype at the restrictive temperature (29°). Several semilethal mutants were too leaky to be mapped on the basis of their lethality at 29°. Two of these, *mit(1)13* and *mit(1)14*, were mapped by crossing *y ts*-lethal/*y*^{2} *cv v f car* females to *+/Y*; *Dp(1;3)sc*^{J4} *mwh/TM2* males at 29° and scoring sons for the *X*-linked markers and the frequency of *mwh* clones. A third mutant, *mit(1)11*, was mapped by a temperature-sensitive morphological phenotype at 29° (wings held up and out). After mapping, all mutations were tested for allelism with previously identified *X*-linked mutants in their vicinity that affect mitotic chromosome stability (*mei*-9, mei-41, mus-101, mus-102, mus-105 and *mus-109*). It has not been determined whether any of the mutations are allelic to each other.

Mitotic cytology: Aceto-orcein squashes of larval neuroblasts were prepared by the procedure of GATTI, TANZARELLA and OLIVIERI (1974). For controls, larvae were taken from cultures kept continuously at 18°, and all subsequent treatments were done at 21°. Temperature effects were studied in larvae from bottles that had been upshifted from 18° to 29° for a period of time (48 h unless otherwise noted). For metaphase preparations following upshifts ganglia were incubated in colchicine for 1.5 hr at 29°.

RESULTS

Screen for mutants affecting mitosis: The first step in the identification of genes encoding functions required for the normal progression of the mitotic chro-

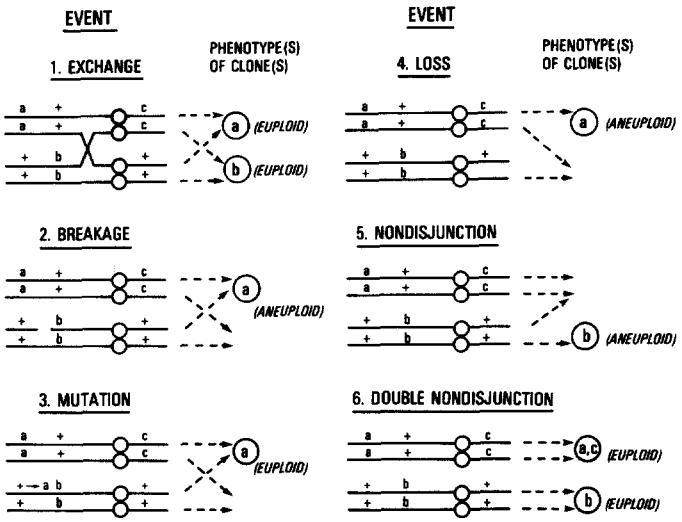

FIGURE 1.—Examples of mitotic events generating cells homozygous or hemizygous for one or more cell markers in heterozygous genotypes.

mosome cycle was the isolation of 124 *X*-linked *ts*-lethal and 44 *ts*-semilethal mutations (see MATERIALS AND METHODS). These *ts* mutations were screened at semirestrictive temperatures for those producing elevated levels of mitotic chromosome instability. If a *ts* lethal decreases the fidelity of mitotic chromosome transmission it should lead to an elevated frequency of somatic spots as a consequence of an increased rate of crossing over, nondisjunction, mutation, chromosome breakage or chromosome loss. Any of the latter events in a fly heterozygous for the recessive cell marker *mwh* can cause a cell to become homozygous or hemizygous for *mwh* and hence lead to that cell or its descendants expressing a *mwh* phenotype (Figure 1). For a detailed discussion of the types of clone-producing events and the properties of the resulting clones see BAKER, CARPENTER and RIPOLL (1978).

In the initial screen, 36 of the *ts* lethals were picked as putative mitotic mutants by the criterion that they had an average of two or more *mwh* clones per wing. Of these, 12 proved to be producing mimics of the *mwh* phenotype: males of the genotype *y ts*-lethal/*Y*; mwh^{+}/mwh^{+} had as many *mwh*-like clones as their *y ts*-lethal/*Y*; mwh/mwh^{+} brothers. Mimicry has also been seen in earlier studies (BAKER *et al.* 1978; BAKER and SMITH 1979); its basis is unknown. The next step in the preliminary screen was to inquire whether presumptive mutants also affected the fidelity of chromosome transmission in other parts of the fly. Fifteen of the mutations that caused clones in wings and were free of mimicry also produced elevated frequencies of *y* clones and *y//mwh* twin spots in abdomens (see below). These 15 mutations thus affect the fidelity of mitotic chromosome behavior in cells of at least two tissues and provide the basis of this report. Thirteen of the mutants define new loci and have been designated mitotic of chromosome *1* (abbreviated *mit(1)2* through *mit(1)14*). [The symbol *mit(1)1* has been left for the mutant *mit* (GELBART 1974).] The other two

proved to be alleles of previously known loci *mus-101* (BOYD, *et al.* 1976; SMITH 1976) and *l(1)zw10* (JUDD, SHEN and KAUFMAN 1972).

Effects of ts *lethals in wing imaginal discs*: Data on the frequencies and sizes of *mwh* clones in wings of males bearing each of these 15 mutants are given in Table 1. The mutants differ both in frequencies of clones per wing and in the size of clones produced. The size distribution of clones is of particular interest since it provides information about the nature of the clone-producing event(s). On the one hand, a mutant that increases mitotic crossing over will produce euploid clones. If the probability of crossing over per cell division is constant throughout development, then such a mutant will generate a geometric distribution of clones of different size (twice as many one-cell as two-cell clones, etc.) since euploid clones have normal growth dynamics. On the other hand, a mutant producing aneuploid cells will show an excess of small clones since aneuploid cells rarely divide (GATTI 1979). A mutant yielding both euploid and aneuploid clones will have a bipartite clone size distribution (BAKER, CARPENTER and RIPOLL 1978; BAKER and SMITH 1979).

Such an analysis of the data in Table 1 allows these mutants to be divided into three categories. Two mutants, *mit(1)10* and *mit(1)4*, produce almost exclusively euploid clones; these data are plotted in Figure 2A together with the theoretical curve for a geometric (euploid) distribution. The remaining mutants all appear to cause both euploid and aneuploid clones, although in very different proportions. They can be divided into those with relatively frequently large clones (>7% of clones consist of three or more cells; $zw10^{ts}$, *mit(1)-5*, *7*, *8*, *12* and *13*) and those in which large clones are less frequent (<2.5% of clones consist of three or more cells; *mit(1)-2*, *3*, *6*, *9*, *11*, *14* and $mus\text{-}101^{ts}$). Figure 2B shows data for $zw10^{ts}$, which has a high proportion of euploid clones, and data for $mus\text{-}101^{ts}$, which has few euploid clones. The deduction that these mutants are producing clones by more than one mechanism suggests that the wild-type functions of these genes carry out processes that have important consequences for different aspects of chromosome biology. A similar variety of clone size distributions has been found for meiotic and mutagen-sensitive mutants studied previously (BAKER, CARPENTER and RIPOLL 1978; BAKER and SMITH 1979).

Temperature sensitivity of clone production: It is implicit in our isolation scheme that the independently measured phenotypes of temperature-sensitive lethality (at the level of the organism) and chromosome instability (at the level of single cells) result from the same mutation. Evidence that this is the case was obtained by raising flies bearing each mutant chromosome at a second, less restrictive temperature and scoring the frequencies and sizes of *mwh* clones produced (Table 1). In each genotype, there are fewer clones per wing at the lower temperature, whereas the *pattern* of clones is similar to that observed at the higher temperature. (In some cases the frequency and pattern of clones at the lower temperature are indistinguishable from wild-type controls.) Since *ts* mutants are induced relatively infrequently (3% of treated chromosomes in this study carried *ts* lethals), the finding that both lethality and *mwh* clone produc-

TABLE 1

Number and size of mwh *clones in wings of* y/Y; Dp(1;3)sc^{J4},y^{+}mwh/+ *males carrying the indicated temperature-sensitive lethals*

Genotype	Temperature	% survival[a]	No. clones of indicated size						Total clones	No. wings	Clones/wing	Frequency[c]
			1 cell	2	3–4	5–8	9–16	≥17				
+[b]	25°		47	13	3	0	0	2	65	88	0.7	1.0
zw10^{ts}	18°	67	218	44	11	3	1	4	281	6	46.8	66.9
	22°	23	624	175	52	7	6	6	870	10	87.0	124.3
mus-101^{ts}	18°	76	49	9	2	1	0	0	61	12	5.0	7.3
	22°	45	693	140	22	2	1	1	859	47	18.4	26.3
mit(1)2	18°	59	4	1	1	0	0	0	6	18	0.3	0.4
	22°	48	128	12	2	0	0	0	142	71	2.0	2.9
mit(1)3	18°	36	3	0	0	0	0	0	3	6	0.5	0.7
	22°	26	83	9	1	1	0	0	94	20	4.7	6.7
mit(1)4	18°	75	6	0	0	0	0	0	6	6	1.0	1.4
	22°	61	57	18	6	2	0	0	83	43	1.9	2.8
mit(1)5	22°	84	2	2	0	1	2	1	8	12	0.7	1.0
	25°	67	185	26	13	5	3	1	233	35	6.6	9.5
mit(1)6	25°	72	10	2	0	0	2	0	14	10	1.4	2.0
	29°	18	72	10	1	0	1	1	85	29	2.9	4.2
mit(1)7	22°	66	24	6	0	1	0	0	31	12	2.6	3.7
	25°	53	67	10	4	1	2	1	85	23	3.7	5.3
mit(1)8	25°	74	26	3	2	1	1	0	33	12	2.8	3.9
	29°	53	969	145	76	17	3	1	1211	30	39.4	56.3
mit(1)9	22°	90	3	0	1	0	0	0	4	6	0.7	1.0
	25°	82	197	23	5	0	1	1	227	35	6.5	9.3
mit(1)10	18°	72	20	10	1	1	1	0	33	12	2.8	4.0
	22°	43	37	14	6	2	0	1	60	18	3.3	4.7
mit(1)11	22°	53	6	1	0	0	1	0	8	6	1.3	1.9
	29°	32	221	20	4	0	0	0	245	27	9.1	13.0
mit(1)12	25°	49	5	4	0	1	0	1	11	12	0.9	1.3
	29°	17	134	16	11	5	0	1	167	24	7.0	10.0
mit(1)13	25°	54	35	11	1	0	2	0	49	12	4.1	5.8
	29°	16	279	31	15	9	1	1	336	26	12.9	18.5
mit(1)14	25°	60	244	21	1	0	0	0	266	21	12.7	18.1
	29°	45	510	57	8	3	0	0	578	22	26.3	37.5

[a] Relative to *y ts*-lethal/*FM7* sibs.
[b] Data from BAKER, CARPENTER and RIPOLL (1978).
[c] Relative to control.

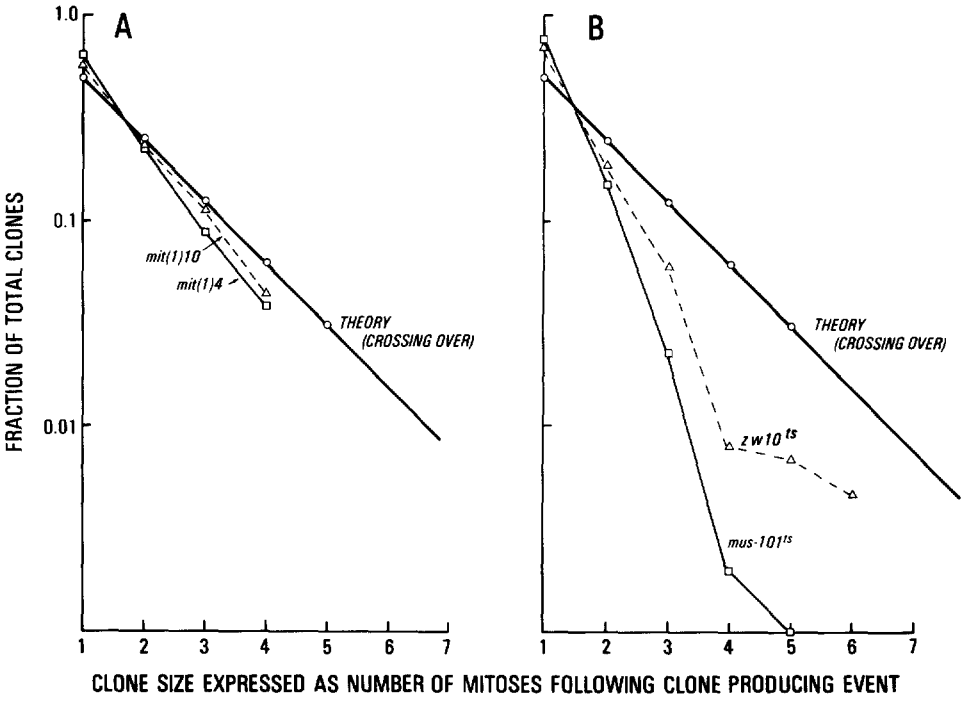

FIGURE 2.—Size distributions of *mwh* clones in wings of *y ts*-lethal/*Y*; $Dp(1;3)sc^{J4}$, y^{+} *mwh*/+ males. The data plotted for each mutant are those from the highest temperature given in Table 1.

tion are temperature sensitive indicates that both phenotypes result from the same mutation.

Effects of ts *lethals in abdominal histoblasts*: Mutations in general mitotic functions would be expected to affect chromosome behavior in all tissues, and we, therefore, required that mutants produce clones in the cuticular derivatives of abdominal histoblasts as well as wing imaginal discs. The effects of these mutants in the cell lineages producing the abdomen were assayed by scoring *y* clones and *y*//*mwh* twin clones in abdomens from *y ts*-lethal/*Y*; $Dp(1;3)sc^{J4}$, y^{+}*mwh*/+ males. The results (Table 2) established that 15 of the mutants decreased the fidelity of chromosome behavior in the abdomen as well as in the wing and are, therefore, likely to identify functions important to all mitotically dividing cells.

Since the cell markers *y* and *mwh* are both scorable in abdomens, *y*//*mwh* twin spots, in addition to single *y* and *mwh* clones, can be detected. The ability to distinguish different kinds of clones in abdomens allows us to delimit the mechanisms by which individual mutations produce chromosome instability. For instance, mitotic crossing over (STERN 1936) or simultaneous nondisjunction of both homologues ["double nondisjunction," in which the two sister chromatids of one homologue segregate to one pole, whereas both sisters of the other homologue segregate to the opposite pole, (Figure 1)] can generate twinspots in these heterozygotes. Similarly, single instances of mutation, chro-

TABLE 2

Numbers and types of clones in abdomens of y ts-*lethal*/Y; Dp(1;3)sc^{J4},y^{+}mwh/+

Genotype (*ts* lethal)	Temperature	*y* clones			*y//mwh* twin spots			No. abdomens	Frequency[a] of:		Twin spots	
		1[b]	≥2	Total[a]	1	≥2	Total[a]		*y*	*y//mwh*	Observed	Expected[c]
+[d]	25°	81	12	93	10	16	26	521	0.02	0.006	26	2.8
zw10^{ts}	22°	33	18	42	48	46	94	29	0.18	0.23	94	79.7
mus-101^{ts}	22°	60	17	70	9	3	12	45	0.19	0.03	12	18.7
mit(1)2	22°	31	12	38	3	3	6	67	0.07	0.01	6	3.6
mit(1)3	22°	17	8	23	3	4	7	68	0.04	0.01	7	1.7
mit(1)4	22°	25	4	28	15	18	33	48	0.07	0.09	33	9.7
mit(1)5	25°	10	0	10	1	9	10	52	0.02	0.02	10	1.0
mit(1)6	29°	6	2	8	1	3	4	25	0.04	0.02	4	0.7
mit(1)7	25°	27	12	37	0	2	2	50	0.09	0.01	2	3.8
mit(1)8	25°	13	3	16	0	1	1	46	0.04	0.003	1	0.8
mit(1)9	25°	18	8	16	1	2	3	70	0.03	0.01	3	0.6
mit(1)10	22°	13	9	19	1	2	3	45	0.05	0.01	3	1.3
mit(1)11	29°	18	3	16	2	2	4	58	0.03	0.01	4	0.9
mit(1)12	29°	25	7	25	3	3	6	36	0.09	0.02	6	3.3
mit(1)13	29°	19	17	30	9	19	28	45	0.08	0.08	28	9.3
mit(1)14	29°	54	18	58	19	9	28	53	0.14	0.07	28	17.4

[a] Omitting clones in the sixth tergite where *mwh* was not scored.
[b] Number of bristles per clone.
[c] Computation explained in text.
[d] Data from BAKER *et al.*, 1978.

mosome breakage, chromosome loss or nondisjunction will generate only single spots.

As can be seen in Table 2, twin spots are observed in the presence of each of these mutations. Because the progenitors of bristle-forming cells migrate during development of the abdomen, the two halves of a twin do not necessarily remain contiguous (GARCIA-BELLIDO and MERRIAM 1971). Thus, our criterion for twin spots in the abdomen is that cells marked with *y* and *mwh* occur on the same hemitergite. This scoring criterion means that some twin spots must be false twins—the consequence of two independent *y* and *mwh* clones arising during the development of a hemitergite. An estimate of the contribution of such false twins to total twin spots has been made as follows. Since small *mwh* clones are hard to detect in the abdomen, we assume that *y* and *mwh* single clones are produced with equal frequencies and estimate the expected false *y//mwh* twin spot frequencies per hemitergite as the square of the *y* clone frequency. Twin spot data are, on the other hand, relatively accurate since, when a yellow clone is found, its hemitergite is scrutinized especially closely for a *mwh* clone. The observed and expected (=false) numbers of twin spots are presented in the last two columns of Table 2. One mutant (*mus-101*^{ts}) clearly fails to produce twins, whereas six [*mit(1)3*, *zw10*^{ts}, *mit(1)4*, *mit(1)5*, *mit(1)13* and *mit(1)14*] do. In the presence of the remaining mutants, twin spots are increased little if at all.

Taken together, the data on chromosome behavior in the abdomen and the

TABLE 3

Mapping and allelism data for temperature-sensitive mutations affecting mitosis

ts mutation[a]	Map position	Allele of
zw10^{ts}	1.2	*zw10*
mus-101^{ts}	41.6	*mus-101*
mit(1)2	15.3	
mit(1)3	46.1	
mit(1)4	28.0	
mit(1)6	34.9	
mit(1)7	15.7	
mit(1)10	1.4	
mit(1)11	21.4[b]	
mit(1)13	36.0[c]	
mit(1)14	49.0[c]	

Except as noted below, mapping was on the basis of the lethal phenotype.

[a] *mit(1)5*, *8*, *9* and *12* have not been mapped.

[b] Mapped by temperature-sensitive wing phenotype thought to be a consequence of the *mit(1)10* allele.

[c] Mapped by *mwh* clone production phenotype (MATERIALS AND METHODS).

wing allow us to subdivide these mutants into several classes. Of the two mutants producing predominately euploid clones in the wing, one, *mit(1)4*, significantly increases twin spots in the abdomen, suggesting that the euploid clones it produces arise either by mitotic crossing over or double nondisjunction. On the other hand, the second of these mutants, *mit(1)10*, does not increase the frequency of twin spots. This leads us to infer that the clones produced by *mit(1)10* arise by mutation—the only other clone-producing event that can generate exclusively euploid clones. Of the six mutants with a relatively high proportion of euploid clones in the wing, three (*zw10*^{ts}, *mit(1)5*, *mit(1)13*) produce a substantial increase in twin spots in the abdomen, suggesting that the euploid clones they produce arise by mitotic crossing over or double nondisjunction. The remaining mutants in this category [*mit(1)7*, *mit(1)8*, and *mit(1)12*] do not significantly increase twin spots and, therefore, presumably produce euploid clones by an elevated mutation rate. Finally, of the six mutants that produced a relatively low proportion of euploid clones in wings, two, *mit(1)3* and *mit(1)14*, produced a significant increase in twin spots in abdomens, suggesting that the euploid clones they produce are due to mitotic crossing over or double nondisjunction.

Recombination and deficiency mapping of ts *mitotic mutants*: The results of recombinational mapping of these mutants are shown in Table 3 (for details see MATERIALS AND METHODS). Where appropriate, mutants were tested for allelism with previously identified mutants affecting mitotic chromosome stability. One mutant, *mus-101*^{ts}, proved to be an allele at the mutagen-sensitive locus *mus-101* (BOYD *et al.*, 1976; SMITH 1976). The *mus-101* locus as defined by the phenotypes of increased mutagen sensitivity and chromosome instability

is deleted by the deficiency *Df(1)HA92* (BAKER and SMITH 1979). The *ts* lethality of *mus-101^{ts}* also maps to *Df(1)HA92*: *mus-101^{ts}/Df(1)HA92* females do not survive at temperatures greater than 22°. That *mus-101^{ts}* is a *mus-101* allele was shown by its failure to complement known *mus-101* mutations. Thus, *mus-101^{ts}/mus-101^{D2}*; *Dp(1;3)sc^{J4},y^{+}mwh/mwh^{+}* females reared at 29° have an average of 26.0 *mwh* clones per wing compared to 18.4 clones for *mus-101^{ts}* males reared at 22° (Table 1) and 8.4 *mwh* clones per wing in *mus-101^{D2}* males. Furthermore, at 29° *mus-101^{ts}/mus-101^{D2}* females are hypersensitive to killing by MMS and have reduced fertility (data not shown). Reduced fertility is also observed in *mus-101^{D2}/mus-101^{D2}* and *mus-101^{ts}/mus-101^{ts}* females. Finally, *mus-101^{D2}/mus-101^{ts}* females have a pattern of aberrant chromosome condensation in larval ganglion cells like that seen in *mus-101^{ts}* males (see below).

A second mutant, *zw10^{ts}*, mapped near white and was shown by deficiency mapping and complementation tests to be an allele at the *zw10* locus (JUDD, SCHEN and KAUFMAN 1972). Thus, a deficiency, *Df(1)w^{rJ1}* that includes *zw10* uncovers both the clone producing and lethal phenotypes of *zw10^{ts}*. In addition *zw10^{ts}* fails to complement the lethal and morphological phenotypes of *zw10* point mutants. Furthermore, other alleles at *zw10* we have examined (*e.g.*, *l(1)65h10*, *l(1)65i21*) cause patterns of aberrant mitotic chromosome behavior comparable to those produced by *zw10^{ts}* (see below).

Mitotic nondisjunction: The large (euploid) clones produced by some mutants must be generated by mitotic crossing over, mutation or double nondisjunction. Reductional segregation of the two homologues in one anaphase or sequential nondisjunction events in one lineage can be distinguished from mitotic crossing over or mutational events because they produce simultaneous homozygosity of cell markers on opposite sides of a centromere. As a test for nondisjunction, males bearing the *ts* lethals and heterozygous for the centromere-spanning cell markers, *Dp(1:3)sc^{J4}*, *y^{+}* and *Sb*, were reared under semirestrictive conditions and scored for clones (Table 4); the occurrence of *y Sb^{+}* clones is diagnostic for mitotic nondisjunction.

For three mutants [*zw10^{ts}*, *mit(1)13*, *mit(1)14*] the frequencies of *y Sb^{+}* clones are much greater than can be accounted for by assuming that the *y* and *Sb^{+}* phenotypes are produced by two independent events (see footnote to Table 4). Thus, the data provide strong evidence that these loci specify functions important for mitotic chromosome segregation. However, the finding that these mutations also increase the frequencies of clones that are either just *y* or just *Sb^{+}* indicates that the lesions they produce can also lead to elevated rates of chromosome breakage or mutation.

In the remaining mutant strains no *y Sb^{+}* clones were detected, although chromosome instability, as detected by clones that are just *y* or just *Sb^{+}*, is increased to levels consistent with previous observations (Table 2). The instability produced by these mutations must occur by a mechanism other than than mitotic nondisjunction.

Mitotic cytology of mutant-bearing larval ganglia: The effects of these *ts*-lethal mutants on chromosome behavior were examined cytologically in aceto-orcein squash preparations of metaphases from third instar larval ganglia (GATTI,

TABLE 4

Numbers and types of clones in abdomens of y/Y; DP(1;3)sc^{J4}, y^{+} Sb/+ *males carrying the indicated temperature-sensitive lethal*

Genotype	Temperature	y phenotype			y Sb^{+} phenotype			Sb^{+} phenotype			No. abdomens	Frequency/abdomen		
												Singles		Doubles
		1[a]	≥2[a]	Σ[b]	1[a]	≥2[a]	Σ[b]	1[a]	≥2[a]	Σ[b]		y	Sb^{+}	y Sb^{+}
+	29°	6	3	9	0	0	0	2	0	2	60	0.15	0.03	
zw10^{ts}	22°	53	16	69	31	23	54[c]	22	9	31	23	3.0	1.35	2.34
mus-101^{ts}	22°	37	4	41	0	0	0	1	1	2	31	1.32	0.06	
mit(1)2	22°	10	2	12	0	0	0	2	2	4	20	0.60	0.20	
mit(1)3	22°	3	3	6	0	0	0	0	1	1	25	0.24	0.04	
mit(1)4	22°	9	1	10	0	0	0	1	0	1	26	0.38	0.04	
mit(1)5	25°	8	2	10	0	0	0	0	0	0	37	0.27		
mit(1)6	29°	21	0	21	0	0	0	1	0	1	57	0.37	0.02	
mit(1)7	25°	20	4	24	0	0	0	1	1	2	12	2.0	0.17	
mit(1)8	29°	9	2	11	0	0	0	0	0	0	26	0.42		
mit(1)9	25°	20	5	25	0	0	0	3	0	3	46	0.54	0.06	
mit(1)10	22°	20	2	22	0	0	0	4	2	6	53	0.42	0.11	
mit(1)11	29°	9	6	15	0	0	0	1	1	2	25	0.60	0.08	
mit(1)12	29°	20	0	20	0	0	0	2	1	3	29	0.69	0.10	
mit(1)13	29°	12	11	23	2	0	2	6	13	19	29	0.79	0.66	0.07
mit(1)14	29°	109	38	147	5	0	5	21	8	29	47	3.13	0.62	0.11

[a] Numbers of clones.

[b] Σ = total clones.

[c] If *y* clones and *Sb*$^{+}$ clones were produced independently, one would have expected $\left(\frac{69 + 54}{6000}\right)\left(\frac{31 + 54}{6000}\right)(6000)$ or about 1.74 *ySb*$^{+}$ clones (there are approximately 6000 bristles in 20 abdomens). The expected numbers of *ySb*$^{+}$ clones for *mit(1)13* and *mit(1)14* are 0.06 and 0.37, respectively.

TABLE 5

Chromosome aberrations in neuroblast metaphases from male larvae

Genotype	No. cells scored	Breaks[a] cd	Breaks[a] iso	Exchanges	Total aberrations	Frequency (%)
Oregon-R[b]	5000	6	10	1	17	0.34
Oregon-R	1000	2	2	0	4	0.40
FM7	1000	1	2	0	3	0.30
mit(1)2	345	4	0	0	4	1.16
mit(1)3	306	2	3	0	5	1.63
mit(1)5	1015	13	12	0	25	2.46
mit(1)6	1033	4	1	0	5	0.48
mit(1)7	950	7	3 (2)	2[c]	14	1.47
mit(1)8	1399	10	12	2	24	1.72
mit(1)9	1507	8	7 (1)	1[d]	17	1.13
mit(1)10	698	2	0	0	2	0.29
mit(1)11	1217	5	6	0	11	0.90
mit(1)12	907	7	2	0	9	0.99
mit(1)13	1366	9	14	0	23	1.68
mit(1)14	903	28	28 (9)	0	65	7.20

[a] cd = chromatid breaks; iso = isochromatid breaks; numbers in parentheses are metaphases with broken chromosomes without the corresponding fragment and metaphases with a normal chromosome complement plus an acentric fragment.

[b] Data from GATTI, TANZARELLA and OLIVIERI (1974). Larvae reared at 25°.

[c] Two clones of more than 20 cells, one carrying a ring autosome and the other a *T(Y;A)*.

[d] Clone of six cells carrying a *T(X;Y)*.

TANZARELLA and OLIVIERI 1974). In all mutants but two [*mit(1)6* and *mit(1)10*] there were cytologically detectable effects on chromatin condensation, chromosome integrity or chromosome segregation (Tables 5–8).

Two of these mutants, *mit(1)4* and *mus-101^{ts}*, have their major effects on chromosome condensation (Figure 3, Table 6). In *mit(1)4* larvae grown at the permissive temperature of 18°, metaphase chromosome morphology is normal. However, after a shift to 29° for 48 hr an abnormal pattern of chromosome condensation can be observed in about 40% of metaphases (Fig. 3g–i). Condensation of both euchromatic and heterochromatic portions of the genome is affected. Chromatids of the *X* and autosomes are swollen and fuzzy, and sister chromatids tend to be closely apposed. In addition, heterochromatin of the *X* and autosomes is slightly elongated and often understained. The *Y* chromosome is also elongated and consistently exhibits a substantial segment that is undercondensed.

In *mus-101^{ts}* larvae reared at 18° metaphase chromosome morphology is normal. When shifted from 18° to 29°, *mus-101^{ts}* affects the condensation of heterochromatin but not euchromatin. After 24 hr at 29° the *Y* chromosome is elongated in nearly all cells and in many cases it has undercondensed areas of variable extent or apparent gaps (Figure 3c). The *X* and autosomal heterochromatin is also affected in about 40% of both male and female cells (Table 6). The phenotype of affected cells ranges from undercondensation of the

TABLE 6

Chromosome aberrations in larval neuroblast metaphases from mus-101^{ts} *and* mit(1)4 *males raised continuously at 18° or given a 29° pulse of the indicated duration*

Genotype	Hr at 29°	No. metaphases scored	Cells with decondensed chromosomes			Cells with chromosome aberrations[a]						% cells affected
			Only *Y* chromosomes affected	*Y* plus at least one *X* or autosome affected	All chromosomes affected	A	B	C	D	E	T	
mus-101^{ts}/Y	0 (18°)	300	0	0	0	0	0	0	0	0	0	0
mus-101^{ts}/Y	24	772	438	170	120	0	0	7	7	0	14	96.1
mus-101^{ts}/mus-101^{ts}	24	885	0	210	111	0	0	10	9	1	20	38.5
mus-101^{D2}/Df(1)HA92	0 (25°)	1059	0	3	1	0	12	15	4	0	31	3.3
mus-101^{D2}/mus-101^{ts}	48	1000	0	5	0	0	7	15	8	0	30	3.5
mit(1)4/Y	0 (18°)	200	0	0	0	0	0	0	0	0	0	0
mit(1)4/Y	48	995	0	0	404	1	1	0	0	1	3	40.9

[a] A, chromatid break; B, isochromatid break; C, isochromatid break with no fragment; D, normal complement plus extra fragment; E, exchanges; T, total number aberrations.

TABLE 7

Frequency and size of mwh *clones in wings of* X/y$^+$Ymwh$^+$; mwh/mwh *males*

X chromosome genotype[a]	Clone size				Total clones	No. wings	Clones/wing
	1	2	3–4	5–8			
FM7	6	1	0	0	7	24	0.29
mus-101^{ts}	1184	275	38	5	1502	10	150.2

[a] The two classes of male were sibs raised at 22°.

heterochromatin of only one or two chromosomes (Figure 3d and e) to the complete failure of condensation of most or all heterochromatic material (Figure 3f). Euchromatic portions of chromosome arms were never observed to be affected. A more detailed cytogenetic study of *mus-101^{ts}* has been recently published (GATTI, SMITH and BAKER 1983).

The *mus-101^{ts}* mutation also affects chromosome integrity: about 2% of cells have either a broken chromosome without the corresponding acentric fragment or a normal chromosome complement plus an extra fragment (Table 6). We infer that these cells result from chromosome breakage during the previous cell cycle when chromosome arms that are connected to their centromeres by decondensed areas are broken by anaphase movement or severed as nuclear membranes reform and daughter cells separate. The acentric fragments thus formed would be either lost or included in a daughter cell with a normal complement.

Some of the effects of *mus-101^{ts}* on chromosome integrity are manifest as what appear to be chromatid and isochromatid breaks. However, in *mus-101^{ts}* most breaks occur in heterochromatic regions and are difficult to distinguish from cases of drastic decondensation. Thus, the frequencies of chromatid and isochromatid breaks presented in Table 6 are subject to substantial error.

The finding that *mus-101^{ts}* is an allele at the *mus-101* locus prompted us to reexamine the cytological effects of the existing viable *mus-101* alleles. A previous cytological examination of metaphases in these mutants had not detected any effect (GATTI 1979). We have now tested *mus-101^{D2}*, one of the viable alleles, under two conditions in which the mutant phenotype should be more extreme. In both cases—*mus-101^{D2}/Df(1)HA92* (grown at 25°) and *mus-101^{D2}/mus-101^{ts}* (grown at 18° and exposed to 29° for 48 hr)—the patterns of chromosome damage (Table 6) are quite similar to those seen in *mus-101^{ts}* homozygous females. The finding that other alleles at *mus-101* also affect heterochromatin condensation shows that the effects of *mus-101^{ts}* are not an allele-specific phenotype. This strengthens the suggestion that the wild-type function of the *mus-101* locus is important for heterochromatin condensation.

One distinctive feature of the cytological phenotype of *mus-101^{ts}* is that the *Y* chromosome shows a far greater frequency and degree of decondensation than do autosomes in the same nuclei (Table 6). Since the effects of *mus-101^{ts}* upon autosomes in males and females are quite similar, the hypersensitivity of

TABLE 8

Chromosome constitutions in larval neuroblast metaphases from males carrying the indicated allele of the zw10 *locus*

Genotype	Treatment		No. metaphases of constitution								
	Temperature	Hr at 29°	Normal XY4A	XY5A	XY6A	XYY4A	XXY4A	XYY5A	XXY5A	Other[a]	Total cells[b]
Oregon-R, zw^+	18° → 29°	48	997	1	0	1	0	0	0	1	1000
FM7, zw^+	18° → 29°	48	999	1	0	0	0	0	0	0	1000
$zw10^{ts}$	18°		140	28	6	5	16	6	2	27	230
	18° → 29°	24	102	31	13	8	16	7	7	31	215
	18° → 29°	42	91	14	12	5	8	3	2	7	142
	18° → 29°	72	123	49	4	9	19	6	8	30	248
l(1)65l21/Y	25°		36	12	4	2	3	1		6	64
l(1)65h10/Y	25°		68	29	8	6	9	5	3	8	136

[a] Includes a wide variety of hyperploid constitutions each of which is individually very rare.
[b] Hypoploid cells not included.

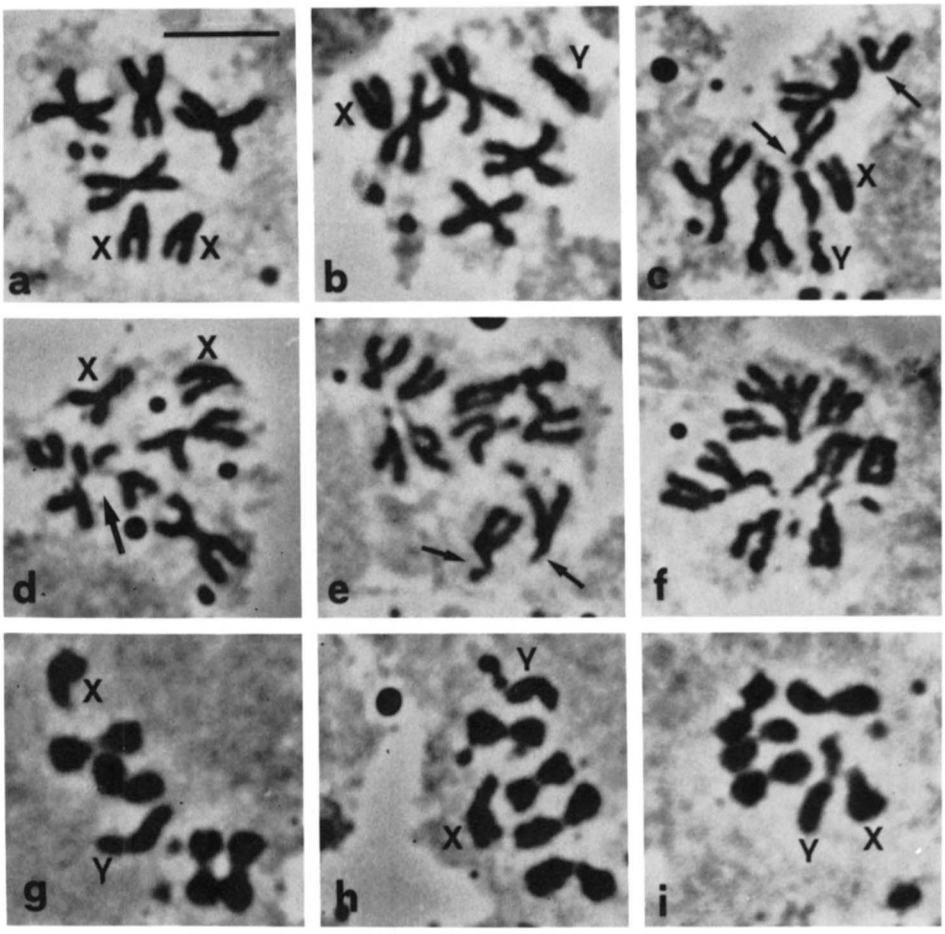

FIGURE 3.—Metaphase nuclei in *mus-101*^{ts} and *mit(1)4* cells. a, Normal female; b, normal male; c, *mus-101*^{ts} male. Arrows indicate separated arms of one autosome. *Y* chromosome is elongated; d–f, *mus-101*^{ts}*/Df(1)HA92* female cells showing various degrees of decondensation of heterochromatin; g–i, cells of *mit(1)4* showing abnormal condensation of all chromatin. Bar = 5 μm.

the *Y* chromosome seems likely to be due to the location or type of chromatin rather than an indirect effect of sexual phenotype.

Genetic evidence that the hypersensitivity of *Y* chromosome heterochromatin to *mus-101*^{ts} results in a relatively high frequency of *Y* chromosome breaks has been obtained in experiments using a *Y* chromosome with the *mwh*$^{+}$ locus attached to the tip of its short arm [the $Y^{P}3^{D}$ element of the reciprocal translocation *T(Y;3)D8* (LINDSLEY *et al.* 1972)]. Males of the constitution *mus-101*^{ts}*/mwh*$^{+}$*Y*; *mwh/mwh*, reared at 22°, have a ten-fold higher frequency of *mwh* clones than *mus-101*^{ts}*/Y*; *mwh*$^{+}$*/mwh* males reared at 22° (Table 7; *cf.* Table 1). These data are most simply explained by the hypothesis that the observed decondensation is causally related to the inferred chromosome breakage.

The third mutation with a dramatic cytological effect is *zw10*^{ts}. In *zw10*^{ts}

larvae at both the permissive and restrictive temperatures about half of all metaphases are hyperploid for one or more chromosomes (Figure 4; hypoploid cells were not scored because of the possibility that such cells could be produced as artifacts during the preparation of squashes). Cells hyperploid for nearly all possible combinations of sex chromosomes and autosomes are found, demonstrating that *zw10*^{ts} affects the segregation of all chromosomes (Table 8, Figure 4). These data strongly indicate that the wild-type function of the *zw10* locus plays a central role in normal mitotic chromosome segregation.

Chromosome breakage is seen in a small fraction of cells (<2%) in *zw10*^{ts} larvae, suggesting that the wild-type function of this locus also plays a role in maintaining chromosome integrity. However, the proportion of cells with broken chromosomes seen cytologically in *zw10*^{ts} is much less than what would be inferred from the analysis of the size distribution of *mwh* clones in wings (Figure 2). The *mwh* clone size data were markedly skewed toward small clones, suggesting that most clones resulted from chromosome breakage. A part of this apparent discrepancy is probably a side effect of the high rate of nondisjunction such that a cell lineage in which nondisjunction has already occurred is likely to suffer additional events in subsequent divisions. Since nondisjunctional events most frequently produce daughter cells that are aneuploid and consequently inviable, the results of sequential nondisjunction events among the descendants of a particular marked cell would be to reduce the size of the clone derived from that cell.

The effects of two other *zw10* alleles [*l(1)65i21*, *l(1)65h10*] on mitotic chromosome behavior were examined. These two alleles also lead to very high levels of aneuploid cells (Table 8), demonstrating that the phenotype of abnormal chromosome segregation is a property of mutants at the *zw10* locus rather than of a particular allele.

Mitotic nondisjunction can arise by several mechanisms including independent anaphase movement of sister chromatids. That the latter is the probable origin of the hyperploid cells in *zw10* mutants is suggested by the finding that sister chromatids frequently undergo precocious separation at prometaphase or metaphase in *zw10*^{ts} nuclei (Figure 4e). Such chromatids are expected to segregate independently. These observations lead to the hypothesis that the *zw10*$^{+}$ product is required for the maintenance of normal preanaphase sister centromere cohesion.

Genetic evidence was presented above (Table 4) that the chromosome instability observed in the presence of *zw10*^{ts} was the consequence of mitotic nondisjunction. We have cytologically confirmed the occurrence of such reductional mitotic segregations. In *zw10*^{ts} males heterozygous for an acrocentric autosome (the multiply inverted second chromosome balancer *SM5*) and a metacentric homologue (a normal second chromosome), one of the possible products of reductional (or sequential) nondisjunction is a diploid chromosome complement consisting of all metacentric autosomes. Such metaphases are observed in larval brains of *zw10*^{ts}; *SM5*/+ males.

Of the remaining 12 mutations, ten produce three- to 24-fold increases in the frequency of chromosome aberrations, whereas in two [*mit(1)6* and

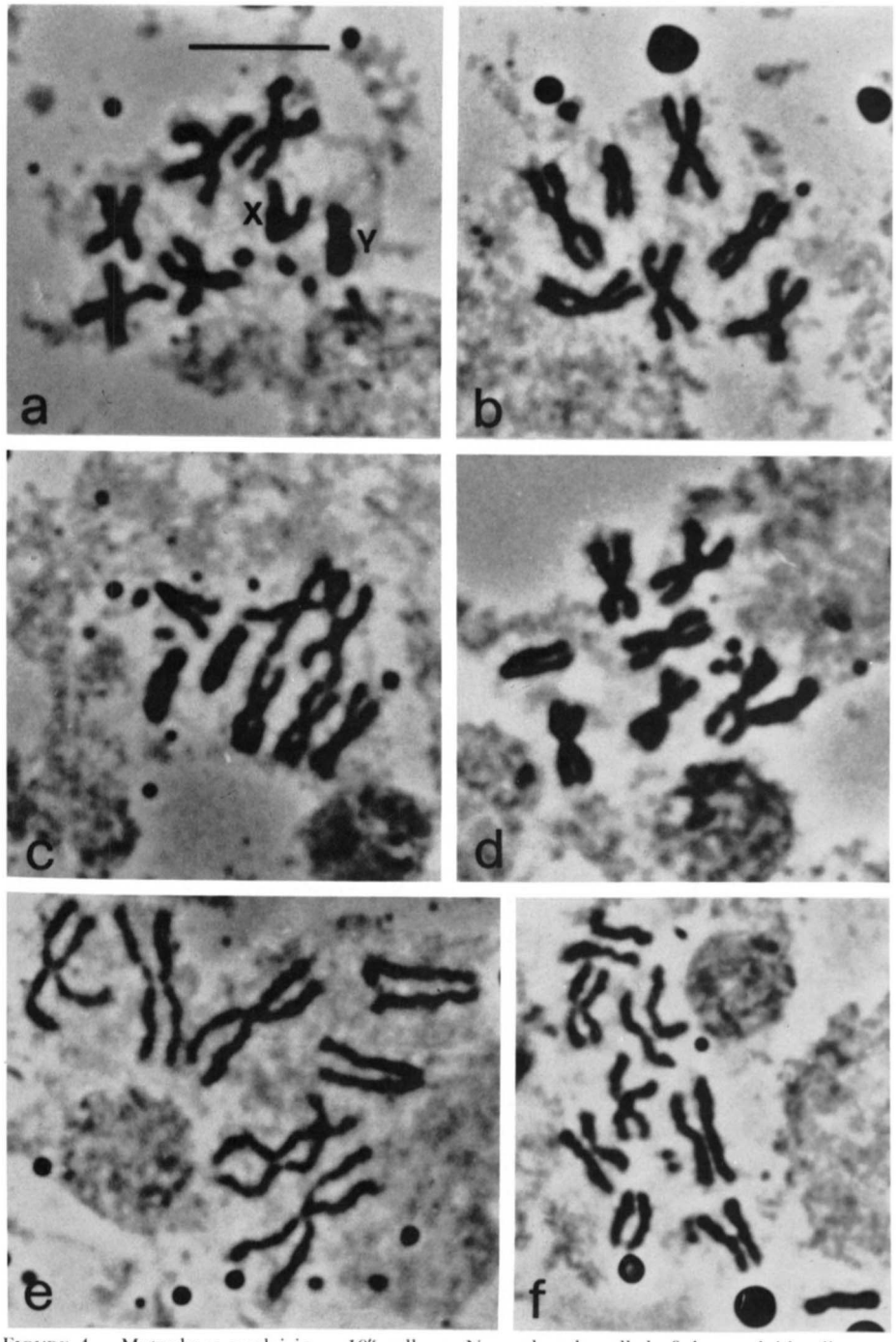

FIGURE 4.—Metaphase nuclei in *zw10*^{6} cells. a, Normal male cell; b–f, hyperploid cells; e and f, metaphase nuclei showing precocious sister chromatid separation. Bar = 5 μm.

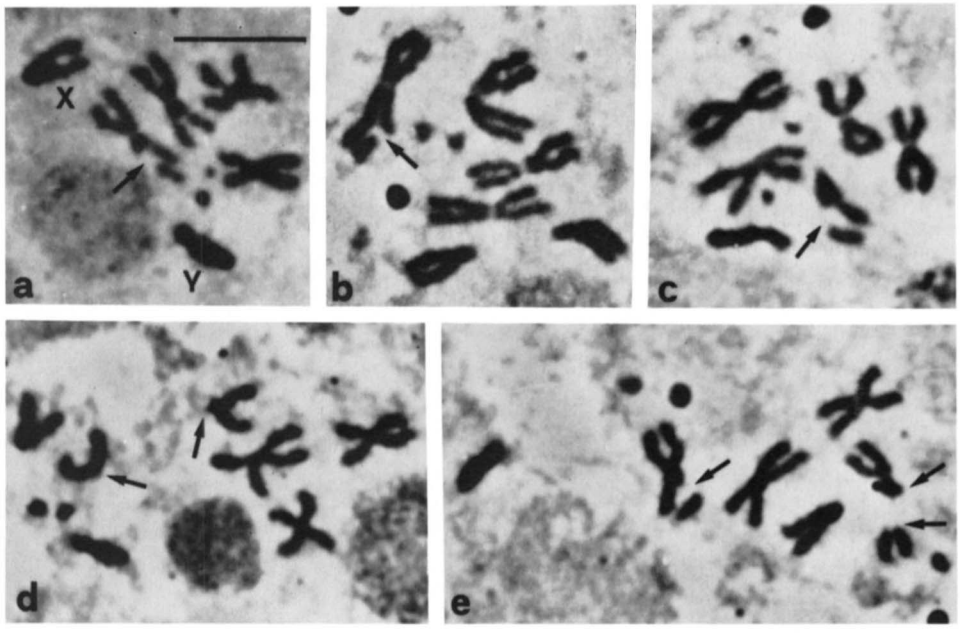

FIGURE 5.—Metaphase nuclei in mitotic mutants. a–c, Chromatid breaks; d, isochromatid break; e, chromatid break (one arrow) and isochromatid break. Bar = 5 μm.

mit(1)10] the frequencies of aberrations are not above control rates (Table 5). Most aberrations caused by these mutations are chromatid and isochromatid deletions (Figure 5; Table 5). In addition, *mit(1)5* and *mit(1)14* exhibit slight but distinctive effects on chromosome morphology. In *mit(1)5* about 5% of the *Y* chromosomes are slightly elongated; however, these chromosomes never exhibit uncondensed or stretched regions. The mutation *mit(1)14* also causes short chromatid gaps and a tendency for chromosomes to be sticky. These effects of *mit(1)14* and *mit(1)5* might be the result of subtle defects in chromosome condensation. Defining the phenotypes of amorphic alleles of these loci should provide a better understanding of their functions.

DISCUSSION

The results of our screen of temperature-sensitive lethal mutations for effects on the fidelity of chromosome transmission show that a significant fraction of essential loci (15/168) are necessary for normal mitotic chromosome behavior. The cytogenetic characterization of these mutations has allowed us to divide them into several categories based on the immediate cause of the chromosome instability they produce and to gain insights into the nature of the primary defects in some mutants.

The chromosome instability produced by the majority (11/15) of the mitotic mutants reported here is, by both genetic and cytological criteria, due primarily to an elevated frequency of chromosome breakage and secondarily to enhanced exchange or mutation. This pattern of instability is the same as that seen with

recombination-defective and mutagen-sensitive mutants in loci with products that function in the maintenance of mitotic chromosome integrity (BAKER, CARPENTER and RIPOLL 1978; GATTI 1979; BAKER and SMITH 1979).

The major effects of mutant alleles at the *mus-101* and *mit(1)4* loci are on chromatin condensation. The *mus-101*$^+$ function is necessary for the condensation of heterochromatin but not euchromatin (GATTI, SMITH and BAKER 1983), whereas *mit(1)4* affects the condensation of all regions, although heterochromatic regions may be preferentially affected. It is not known whether either of these loci plays a direct role in chromatin condensation. Nevertheless, the isolation of *mit(1)4* and *mus-101*^{ts} adds a major new category to the list of mitotic functions that we can identify genetically and suggests that chromatin packaging is susceptible to mutant dissection.

A third category of mitotic function is identified by alleles of the *zw10* locus. These mutations produce high frequencies of mitotic nondisjunction. They appear to do so by causing premature separation of sister centromeres (Figure 5). This is the first locus we have identified concerned with chromosome segregation. The fact that cytogenetic techniques enabled us to detect and study the resulting hyperploid cells suggests that any cell cycle function in which a mutant produces such aneuploids—whether by aberrant behavior of centromeres, spindles or other components of the mitotic apparatus—can be identified and studied with the methodology employed here.

A fourth category of mitotic function is represented by the mutant *mit(1)10*. We infer that this mutant affects the fidelity of mitotic chromosome transmission by enhancing the frequency of somatic mutation. This conclusion derives from the facts that *mit(1)10* (1) produces almost exclusively euploid clones in wings but (2) in both genetic and cytological tests does not enhance the other possible causes of euploid clones—mitotic recombination and nondisjunction. Thus, although an enhanced mutation rate has not been directly demonstrated, we are led to this hypothesis by elimination.

One general concern with any mutant hunt is whether it has succeeded in identifying the genes of interest—in the current instance those encoding essential mitotic functions. We have utilized *ts* lethals to ensure that we were dealing with essential functions. In addition, we have determined whether they affect mitosis in more than one tissue with the view that this mutant phenotype would be a property of loci with products directly involved in mitosis. Eight of the mutations reported here [*mit(1)3*, *4*, *7*, *12*, *13*, *14*, *zw10*^{ts} and *mus-101*^{ts}] have significant effects in three tissues in that 95% confidence intervals on the frequencies of somatic clones and chromosome aberrations do not overlap those of controls (confidence intervals were computed by the method of CROW and GARDNER 1959). One mutation, *mit(1)10*, has significant effects on clones in wings and abdomens and the properties of these clones suggest that they arose by mutation—a hypothesis consistent with the failure to detect any effect of *mit(1)10* cytologically. The effects of the remaining mutations are statistically significant in two tissues [*mit(1)5*, *8*, *9* and *10*] or just one tissue [*mit(1)2*, *6* and *11*].

The question of the primary roles of the loci identified by these mutants is

more difficult to answer. For only two of these genes (*mus-101* and *zw10*) are the mitotic effects of existing mutant alleles sufficiently frequent and profound that they can readily account for the mutants' lethality. These two loci surely specify major cell cycle functions. For all other mutations the frequencies of cells with detectable chromosomal abnormalities are significantly less than frequencies of chromosome breakage that we know to be compatible with relatively good viability (0.2–0.3 breaks per cell cycle; BAKER, SMITH and GATTI 1982). Thus, it seems unlikely that the lethality of these mutations can be explained by the observed chromosome aberrations. In this context it should be noted that two mutations, *mit(1)4* and *mit(1)14*, in addition to increasing the frequency of chromosome instability also have relatively frequent effects on chromatin condensation. It is conceivable that their effects on chromosome organization are sufficient to account for their lethality through effects on gene expression. We would like to emphasize that this line of reasoning does not imply that any or all of these loci necessarily have minor roles in the chromosome cycle. In previous studies of the mitotic effects of Drosophila mutants we have encountered several loci (*e.g.*, *mus-101*, *mus-105*, *mus-109*) at which the first allele(s) isolated was a leaky mutation and had relatively weak mitotic effects (BAKER and SMITH 1979; GATTI 1979). The subsequent isolation of stronger alleles showed these loci to specify functions with major roles in the chromosome cycle (BAKER, SMITH and GATTI 1982; GATTI, SMITH and BAKER 1983).

Another concern with respect to mutations with relatively weak mitotic effects has been noted by KENNISON and RIPOLL (1981). They encountered a substantial variation in the frequencies of mitotic clones produced by different control stocks and suggested that some weak "mutants" might simply reflect different background rates of instability. We have been cognizant of the fact that such variation can exist (WEAVER 1960; BAKER, CARPENTER and RIPOLL 1978) and have routinely, as described here (see MATERIALS AND METHODS), carried out crosses to make the background genotypes of mutant stocks relatively homogenous with one another. We have found this sufficient to generate reproducible results (*cf.* Tables 2 and 4) and, thus, although we acknowledge the point made by KENNISON and RIPOLL (1981), we do not see that it has relevance to our experiments. More specifically, we have here and in previous work (BAKER, CARPENTER and RIPOLL 1978; BAKER and SMITH 1979) genetically examined the mitotic effects of two or more alleles at nine different loci and have found congruent results with respect to (1) whether alleles at a locus affected mitotic chromosome stability, (2) the relative severity of mutant alleles' effects on mitotic chromosome stability and on another phenotype (*e.g.*, mutagen sensitivity, meiotic recombination or chromosome segregation) and (3) the origins of the chromosome instability produced by alleles (*e.g.*, breakage, nondisjunction, etc.).

There has also been, with but two exceptions, concordance between the results of genetic and cytological examinations of the effects of mutations on mitotic chromosome behavior. One apparent discrepancy is that, for a number of mutations, genetic tests have revealed a significant frequency of euploid

clones, presumably arising from mitotic exchange, whereas cytological examinations have not identified an equivalent class of events. This difference is almost certainly due to the fact that genetic tests are much more sensitive than cytological tests at detecting such events. Genetic tests will reveal exchanges that have occurred at any time during development, whereas cytology only detects those exchanges occurring at the moment the material was fixed. Support for this assertion comes from findings with respect to the *mus-105* locus where alleles with relatively weak mitotic effects produce exactly the above situation [significant mitotic exchange inferred from genetic tests (BAKER and SMITH 1979) but essentially none seen cytologically (GATTI 1979)]. However, more extreme mutant genotypes at the *mus-105* locus produce mitotic exchange sufficiently frequently that it is detectable cytologically as well as genetically (BAKER, SMITH and GATTI 1982).

A potentially more serious discrepancy in our results occurred in the case of *mus-101* alleles in which a low level of chromosome breakage was detected in the initial genetical, but not cytological, tests (BAKER and SMITH 1979; GATTI 1979). The discovery that the initial *mus-101* alleles were leaky and produced more extreme mutant phenotypes when heterozygous with a deficiency for the locus, together with the isolation of a conditional lethal *mus-101* allele, provided more severe mutant genotypes in which effects on chromosome behavior are cytologically as well as genetically detectable.

The diagnostic phenotype that we have employed to detect mitotic mutants among *ts*-lethal mutations—a decreased fidelity in the transmission of chromosomes during division—is atypical among screens for essential cell cycle functions. In other systems in which a genetic dissection of the cell cycle has been pursued, the isolation of mutations in essential cell cycle functions has been based on the premise that such mutations will block the progression of cells through the cycle. Screens and selections based on this reasoning have been highly successful. The analysis of the resulting mutants has led to the view that the cell cycle is comprised of a network of dependent pathways of events; blocking of any one step in a pathway leads to all cells arresting with a unique terminal phenotype.

It would not appear that the screens we have employed to survey *mei-*, *mus-* and *ts*-lethal mutants in Drosophila have detected mutants that produce cell cycle blocks. A possible exception to this conclusion is revealed by a careful consideration of the consequences of chromosome breaks produced by the Drosophila mutants (M. GATTI and B. S. BAKER, unpublished results). Briefly, we have observed that cells in which chromosome breakage occurred prior to metaphase rarely produce daughter cells that reach the following metaphase. This suggests to us that cells possess the capability of monitoring the integrity of their genome and arresting division if they contain broken chromosomes. Moreover, the function that monitors chromosome integrity appears to have completed action by the end of metaphase since cells in which breakage occurs at anaphase produce daughter cells that reach the next metaphase efficiently. However, these cells, like other cells having breaks present prior to metaphase, fail to produce descendants that reach the subsequent metaphase. Although

we do not yet know whether chromosome breakage results in arrest at a unique stage in the cell cycle, all available data are consistent with the hypothesis that an interruption of chromosome integrity leads to a specific cell cycle block analogous to the cell cycle arrests produced by cell division mutants in other organisms.

Despite this one similarity to other systems it is clear that we have not isolated a comprehensive set of cell cycle block mutants from our screens of *mei-*, *mus-* and *ts*-lethal mutations. We have recently begun a search for additional mutations using a cytological screen of mitotic cells from larvae bearing late-acting lethals. We have found that between 30 and 50% of such lethals have substantial mitotic effects including a number that cause cell cycle blocks (B. S. BAKER, M. GATTI and D. A. SMITH, unpublished results; see BAKER, SMITH and GATTI 1982 for rationale).

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