Genetic Analysis of the *brahma* Gene of *Drosophila melanogaster* and Polytene Chromosome Subdivisions 72AB

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ABSTRACT

The *brahma* gene is required for activation of the homeotic genes of the Antennapedia and bithorax complexes in Drosophila. We have isolated and characterized 21 mutations in *brahma*. We show that both maternal and zygotic functions of *brahma* are required during embryogenesis. In addition, the severe abnormalities caused by loss of maternal *brahma* expression show that the homeotic genes are not the only targets for *brahma* activation. The complex pattern of interallelic complementation for the 21 *brahma* alleles suggests that *brahma* may act as a multimer. In addition to mutations in *brahma*, we have isolated mutations in four other essential genes within polytene chromosome subdivisions 72AB. Based on a compilation of similar studies that include about 24% of the genome, we estimate that about 3600 genes in Drosophila can mutate to cause recessive lethality, with fewer than 900 additional genes essential only for gametogenesis. We have identified three times more transcripts than lethal complementation groups in 72AB. One transcript in 72AB is the product of the essential *arflike* gene and encodes a member of the ARF subfamily of small GTP-binding proteins. Two other transcripts are probably the products of a single gene whose protein products are similar to the catalytic subunits of cAMP-dependent protein kinases.

THE determination of body segment identity in Drosophila requires the correct patterns of expression of the homeotic genes of the Antennapedia complex (ANTC) and the bithorax complex (BXC). Misexpression of the ANTC and BXC genes causes changes in body segment identity. Mutations that cause such changes in body segment identity have identified two groups of *trans*-acting factors, the Polycomb group and the trithorax group (reviewed in PARO 1990; KENNISON 1993). Polycomb group mutations cause changes in segment identities by derepressing transcription of ANTC and BXC genes, suggesting that the Polycomb group proteins are transcriptional repressors. The phenotypes of Polycomb group mutants can be suppressed by mutations in the second group of genes, the trithorax group. The trithorax group proteins are positive factors required for activation or function of ANTC and BXC genes.

Among the trithorax group genes isolated in screens for dominant suppressors of Polycomb group mutants is the *brahma* (*brm*) gene (KENNISON and TAMKUN 1988). The molecular characterization of *brm* showed that the brm protein is strikingly similar to the yeast transcriptional activator SNF2/SWI2 (TAMKUN *et al.* 1992). SNF2/SWI2 encodes a putative helicase that assists a wide variety of DNA-binding regulatory proteins (including GAL4 and SWI5) to activate the transcription of their target genes (HAPPEL *et al.* 1991; LAURENT and CARLSON 1992; PETERSON and HERSKOWITZ 1992; LAURENT *et al.* 1993; YOSHINAGA *et al.* 1992). *snf2/swi2* mutations cause alterations in chromatin structure *in vivo* (HIRSCHHORN *et al.* 1992) and are suppressed by mutations in nucleosomal histones (PETERSON and HERSKOWITZ 1992; WINSTON and CARLSON 1992), suggesting that SNF2/SWI2 counteracts the repressive effects of chromatin components on transcription. The structural similarities between brm and SNF2/SWI2 suggest that they play related, though not necessarily identical, roles in transcriptional activation.

The existing *brm* alleles were isolated because of their dominant effects on other homeotic mutations that alter adult cuticle. Because these alleles were selected for a specific phenotype, we could not be certain that they would lack all *brm* functions. As part of our study of the function of the *brm* gene, we wanted to isolate additional alleles. We also wanted to determine whether the homeotic genes are the only genes that require *brm* function. To isolate *brm* alleles with less likelihood of a functional bias we screened for *brm* alleles that are lethal when heterozygous to a *brm* deficiency. The *brm* transcription unit is completely deleted by deficiencies that remove lettered subdivisions 72A and 72B (hereafter referred to together as 72AB) of the left arm of the third chromosome. We have attempted to identify both the number of essential genes and the number of different transcripts encoded within this small region of the Drosophila genome.

MATERIALS AND METHODS

Stocks: Flies were raised on a cornmeal-molasses-yeast-agar-Tegosept medium at 25°. Unless otherwise noted, all

TABLE 1

Chromosome aberrations

Aberration	Breakpoints	Discoverer
Deficiencies		
Df(3L)brm11	71F3-5;72D1-5	J. A. KENNISON
Df(3L)th102	71F3-5;72D12	F. SMITH
Df(3L)st-f13	72C1-2;73A3-4	J. BELOTE and M. MCKEOWN
Translocations and transpositions		
T(Y;3)L131	75D4-5;YS	D. L. LINDSLEY
Tp(3;Y)L131-D3[a]	72A;75D4-5 inserted in YS	J. A. KENNISON
T(Y;3)ST1	In(3L)62B;74A + T(Y;3)70-71	S. Y. K. TIONG

[a] *T(Y;3)72A;1A* [*Y* breakpoint distal to y^+ on $B^S Yy^+$] superimposed on *T(Y;3)L131*.

mutations and chromosome aberrations are described in LINDSLEY and ZIMM (1992). *Dp(3;Y)ST1* is the aneuploid segregant from *T(Y;3)ST1* [In(3L)62B;74A + T(Y;3)70-71] and is duplicated for polytene chromosome subdivisions 61A to 62B and 70-71 to 74A. The duplication carries the wild-type alleles of *brm*, *th*, *st* and *tra*, but not *dev* [cytological location 70D1-2]. *Df(3L)brm11* was recovered as a dominant suppressor of Pc^4 from a hybrid-dysgenic background. Table 1 lists the chromosome rearrangements and their cytological breakpoints.

The isolation of brm^{1-6} and brm^{8-10} have been previously described (KENNISON and TAMKUN 1988; TAMKUN *et al.* 1992). brm^7 was recovered from a chromosome marked with *st* hh^3 e^{11} [$hh^3 = hh^{6N16}$]. We initially observed that flies heterozygous for the hh^3 chromosome and the dominant mutation *Moonrat* did not express the *Moonrat* phenotype. We mapped a strong dominant suppressor of *Moonrat* on this chromosome to brm^7, which was probably induced simultaneously with hh^3 (JÜRGENS *et al.* 1984). brm^{19} was isolated as a dominant suppressor of *Moonrat* after ethyl methanesulfonate mutagenesis.

Generation of the iso-1 strain: To aid in the molecular analysis of *brm*, we generated a strain isogenic for all four chromosomes (the iso-1 strain). We have constructed an embryonic cDNA library (TAMKUN *et al.* 1991) and a genomic library in a cosmid vector (TAMKUN *et al.* 1992) from the iso-1 strain. This strain was also used to construct the P1 genomic library (SMOLLER *et al.* 1991) that is being sequenced by the Drosophila Genome Center at Berkeley. Figure 1 shows the origin of the iso-1 strain. The *X* chromosome in the iso-1 strain carries the mutation *y* and the second chromosome carries the mutations *cn*, *bw* and *sp*. To further reduce any variation in our iso-1 strain that might have been generated by double exchanges with the balancers, we used only single pairs of flies to maintain the iso-1 strain for the first two generations after its construction.

Chromosome rearrangements: *T(Y;3)L131* is a reciprocal translocation between an unmarked third chromosome and the marked *Y* chromosome, $B^S Yy^+$. We isolated *Tp(3;Y)L131-D3* as a γ-ray-induced derivative of *T(Y;3)L131* that reverts the dominant male sterility associated with combinations of reciprocal *Y* autosome translocations and the *Df(1)bb158* chromosome (LYTTLE 1984). The original translocation had breakpoints at 75D4-5 and in YS; the derivative has additional breakpoints at 72A and in *YS* distal to the appended y^+ marker. The resulting complex rearrangement [*Tp(3;Y)L131-D3*] is a reciprocal transposition that inserts 72A-75D4 into the short arm of the *Y* chromosome and the y^+ marker from YS into the third chromosome at the site of the 72A-75D4 deficiency. The aneuploid segregant *Dp(3;Y)L131-D3* is a *Y* chromosome that carries all of the *Y* chromosome male fertility genes, the dominant marker B^S, and a duplication for 72A-75D4. This duplication includes the wild-type alleles of all essential genes in 72AB deleted by *Df(3L)th102*; males of the genotype *Df(3L)th102/Df(3L)brm11; Dp(3;Y)L131-D3* survive. Although *Df(3L)th102* was originally described as deficient for 72B1 to 72D12 (ASHBURNER *et al.* 1980), we believe from our own cytological analyses that it is deficient for all of 72A as well.

Mutagenesis: Adult males that carried the unmarked third chromosome from the iso-1 strain were fed ethyl methanesulfonate (EMS) following the methods of LEWIS and BACHER (1968). After treatment, the males were mated to virgin females heterozygous for *In(3LR)DcxF, ru h D Sb* e^s. The treated males were discarded after 4 days to ensure that only germ cells treated post-meiotically were sampled. Sons that carried a mutagenized third chromosome and *In(3LR)DcxF* were mated individually to three to five virgin females that carried *Df(3L)th102, h ri Sb* ca^2 and either *In(3LR)TM2* or *In(3LR)TM6B*. For each male tested, the phenotypes of progeny with the mutagenized third chromosome heterozygous to *Df(3L)th102* were examined.

Nomenclature for genes and alleles in 72AB: We have followed the revised nomenclature in LINDSLEY and ZIMM (1992) for naming the genes in 72AB. Each gene is designated by *l(3)72A* [*lethal on the third chromosome in salivary chromosome subdivision 72A*] and a different arbitrary lowercase letter for each gene (*a–e*). Two of the genes have synonyms based on their mutant phenotypes or putative functions. Thus, *l(3)72Aa* corresponds to the *brm* gene and *l(3)72Ae* corresponds to *arl*.

Meiotic mapping: To map *brm* with respect to *th* and *st*, females heterozygous for a *brm* mutation and a multiply marked third chromosome (*ru h th st cu* sr_0 e^s *ca*) were mated to males of the genotype *In(3LR)TM3/*brm^7 *st*. The only surviving non-scarlet, non-TM3 progeny come from recombination between *brm* and *st*. Each recombinant was subsequently tested to determine if the recombination event occurred distal or proximal to *th*. Because the frequencies of meiotic recombination often vary among strains, we determined the meiotic map position of *brm* relative to the standard map distance of 0.8 map unit between *th* and *st* (LINDSLEY and ZIMM 1992). The dominant suppression of homeotic mutations was also mapped for nine different *brm* alleles (*1–7*, *19* and *20*) by crossing females heterozygous for a *brm* allele and the markers *ru h th st cu sr* e^s *ca* to males heterozygous for a third chromosome carrying *ru h th st cu sr* e^s *Pr ca*. Single recombinant males were selected and the recombinant chromosomes balanced and tested for suppression of the dominant homeotic mutations $Antp^{Ns}$, Pc^4 and *Mrt*.

RNA analysis: Drosophila RNA samples from different stages were isolated as described by LAUGHON *et al.* (1986). Five micrograms of poly(A)$^+$ RNA from each stage were fractionated on a 1% agarose 4-morpholinepropanesulfonic acid/formaldehyde gel and transferred to a Zetabind nylon membrane (AMF Cuno). RNA blots were probed with DNA fragments labeled by the random-primer method (FEINBERG and VOGELSTEIN 1983) and washed under conditions of high stringency (0.1 × SSC, 0.1% sodium dodecyl sulfate, 65°).

Generation of transgenic strains: Transgenic strains were constructed as described in TAMKUN *et al.* (1991).

Lethal phases and phenotypes for maternal and zygotic requirements for brm: To determine the lethality at various developmental stages, females and males were allowed to mate for several days and then allowed to lay eggs for 12–24 hr. Unhatched eggs were counted 36 hr after the parents were removed to determine embryonic lethality. The number of pupae in each vial and the number of adults that eclosed were

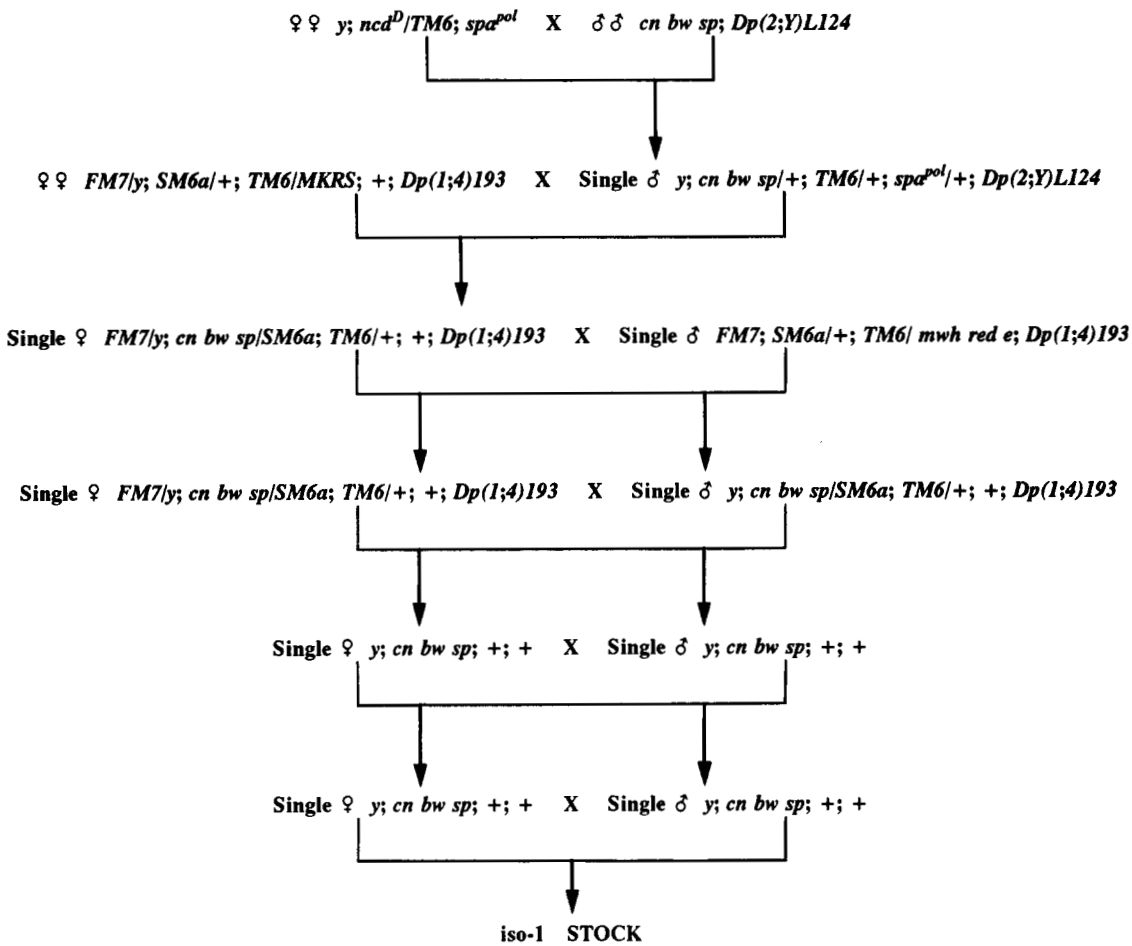

FIGURE 1.—Crossing scheme followed for the generation of the iso-1 strain, a strain marked with the mutations *y*; *cn bw sp* and isogenic for all four chromosomes. *Dp(1;4)193* carries the markers y^+ and spa^{pol}. *FM7*, *SM6a*, *TM6* and *MKRS* refer to the multiply inverted chromosomes *In(1)FM7a*, *In(2LR)SM6a*, *In(3LR)TM6* and *Tp(3;3)MKRS*, respectively (LINDSLEY and ZIMM 1992).

also determined. Embryos were fixed and stained with antibodies by the methods of CARROLL and SCOTT (1985) using either *engrailed* monoclonal antisera 4D9D4 (PATEL *et al.* 1989) or *Ubx* monoclonal antisera FP3.38 (WHITE and WILCOX 1984). Germ-line mosaics were produced using the methods of CHOU *et al.* (1993). Females heterozygous for P[w^+, ovo^{D1}]^{3L} and either a wild-type third chromosome or a *brm* mutant chromosome [*mwh* brm^1 *st cu sr* e^s *ca*] were irradiated at the first larval instar. Females homozygous for the marked brm^1 chromosome are viable and fertile if they also carry the brm^+ transposon, P[w^+, BR14.4].

RESULTS

Essential genes in 72AB: Following mutagenesis with EMS, third chromosomes from 3350 fertile F_1 sons were tested in combination with *Df(3L)th102*. Twenty-six third chromosomes were recovered that failed to complement both *Df(3L)th102* and *Df(3L)brm11* for viability, but complemented *Df(3L)st-f13*. These 26 chromosomes define five essential complementation groups in 72AB. Two of these five genes (*brm* and *arl*) have been previously described (TAMKUN *et al.* 1991, 1992). The five complementation groups and the numbers of alleles recovered for each are listed in Table 2.

TABLE 2

Complementation groups in 72AB and mutations recovered after EMS mutagenesis

Complementation group	No. of alleles	Synonym
l(3)72Aa	10	*brm*
l(3)72Ab	6	
l(3)72Ac	8	
l(3)72Ad	1	
l(3)72Ae	1	*arl*

Alleles of each gene are numbered consecutively with the exception of the *brm* alleles (*12* through *18* and *20* through *22*).

Each mutation was tested in combination with every member of the same complementation group and with at least one member of each of the other four complementation groups in the 72AB subdivisions. Flies of the genotype $l(3)72Ab^3/l(3)72Ab^4$ survived poorly and had rough eyes and held-out wings. No adult survivors were observed for any other *trans*-heterozygous combinations of *l(3)72Ab* alleles or for any combination of *l(3)72Ac* alleles. The interallelic complementation of *brm* alleles will be described below.

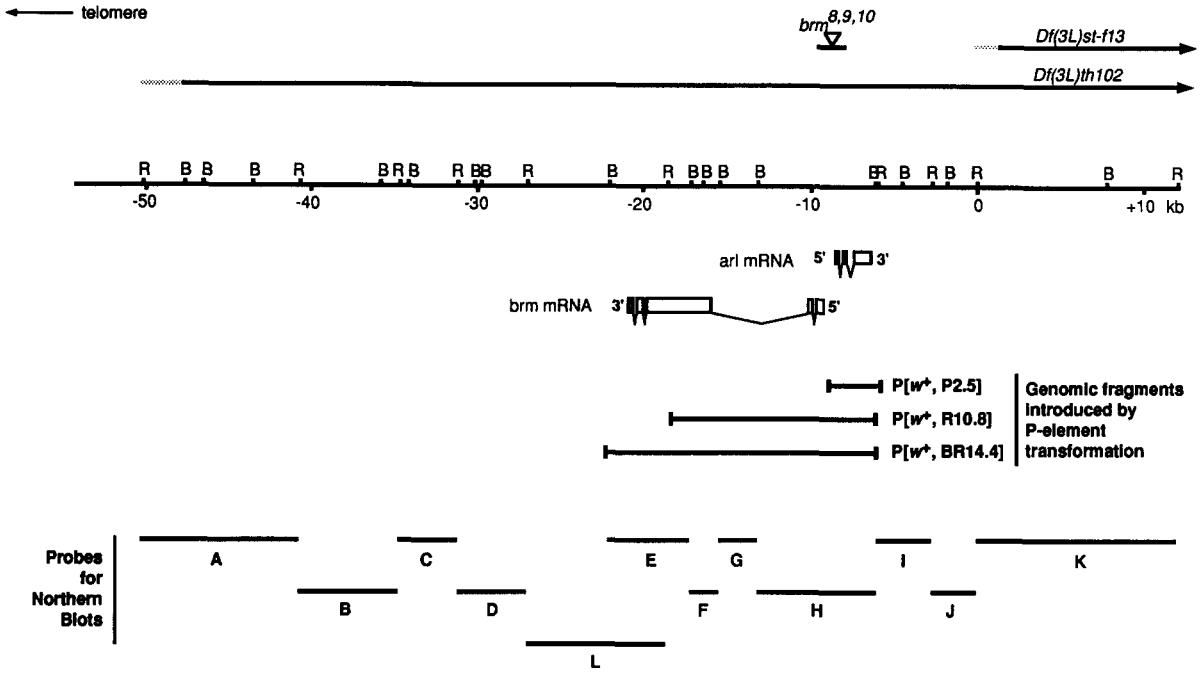

FIGURE 2.—Molecular map of the 72AB region. The two deficiencies that define the 72AB region are shown above the restriction map. B are *Bam*H1 restriction sites and R are *Eco*R1 restriction sites. Exons of the *brm* and *arl* transcripts are shown as boxes. Genomic DNA fragments reintroduced for rescue experiments are shown as solid lines. The genomic DNA fragments for the Northern blot analysis are shown at the bottom (fragments A through L). Fragments E, F, G and H are *Bam*H1 restriction fragments, the remaining fragments are *Eco*RI restriction fragments.

In addition to the lethal mutations described above, about 2% of the mutagenized chromosomes only partially complemented *Df(3L)th102*. Flies heterozygous for one of these mutagenized chromosomes and *Df(3L)th102* had reduced viability, thin bristles and outstretched wings. We mapped the mutations responsible for the interactions with *Df(3L)th102* on several of these mutagenized chromosomes; none mapped near *th*. GAY and CONTAMINE (1993) recently reported similar results with deficiencies that include polytene chromosome subdivision 37D. They found that about 2% of mutagenized second chromosomes caused reduced viability, thin bristles, outstretched wings, and deformed legs when heterozygous to deficiencies that include *l(2)37Dh*. Many of these mutations also mapped outside of the region of the *l(2)37Dh* deficiencies. The similar phenotypes observed in the two experiments led us to examine flies heterozygous for *Df(3L)th102* and a *l(2)37Dh* deficiency. Flies heterozygous for *Df(3L)th102* and *Df(2L)JK12* had thin bristles and weakly outstretched wings, suggesting that the two experiments do identify the same set of interacting genes.

Transcripts in 72AB: We examined RNA transcripts encoded within the 72AB subdivisions by probing Northern blots with genomic DNA fragments spanning the region between the distal breakpoints of *Df(3L)th102* and *Df(3L)st-f13* (Figures 2 and 3). Genomic DNA fragments A through J identified at least 14 different transcripts. These transcripts derive from between 9 and 13 different transcription units. The two most distal probes, A and B, identified three transcripts from two different transcription units. The 2.0- and 2.8-kb transcripts are the overlapping transcripts encoded by the *Pka-C3* gene (the 2.4- and 3.0-kb DC2 transcripts described by KALDERON and RUBIN 1988; D. KALDERON, personal communication), but the 1.1-kb transcript detected on by probe B does not appear to derive from the *Pka-C3* gene. The 1.3- and 1.6-kb transcripts detected by probe C may be overlapping transcripts from a single transcription unit. The 6.9-kb transcript detected by probes D and L derives from the transcription unit just distal to *brm*. We do not know whether the 1.2-kb transcript also detected by probe D shares any exons with the 6.9-kb transcript. The 5.5-kb transcript detected by probes E, F and H in Figure 3 is the product of the *brm* gene (TAMKUN *et al.* 1992). The 1.0-kb transcript detected by probe H in Figure 3 is the product of the *arl* gene (TAMKUN *et al.* 1991). The 1.1- and 1.4-kb transcripts detected by probes G and H, respectively, lie within the large *brm* intron and do not appear to overlap with either the *brm* or *arl* transcripts (TAMKUN *et al.* 1991, 1992). Probe I detects a single 3.6-kb transcript. This may be the same 3.6-kb transcript detected by the next most proximal genomic DNA fragment, probe J. The other two transcripts detected by probe J are puzzling. The genomic probe J is only about 2.7 kb, but detects transcripts of 4.5, 3.6 and 2.8 kb. The 3.6-kb transcript is also detected by an adjacent genomic fragment, but the 4.5- and 2.8-kb transcripts are not detected by any other genomic DNA fragments from the

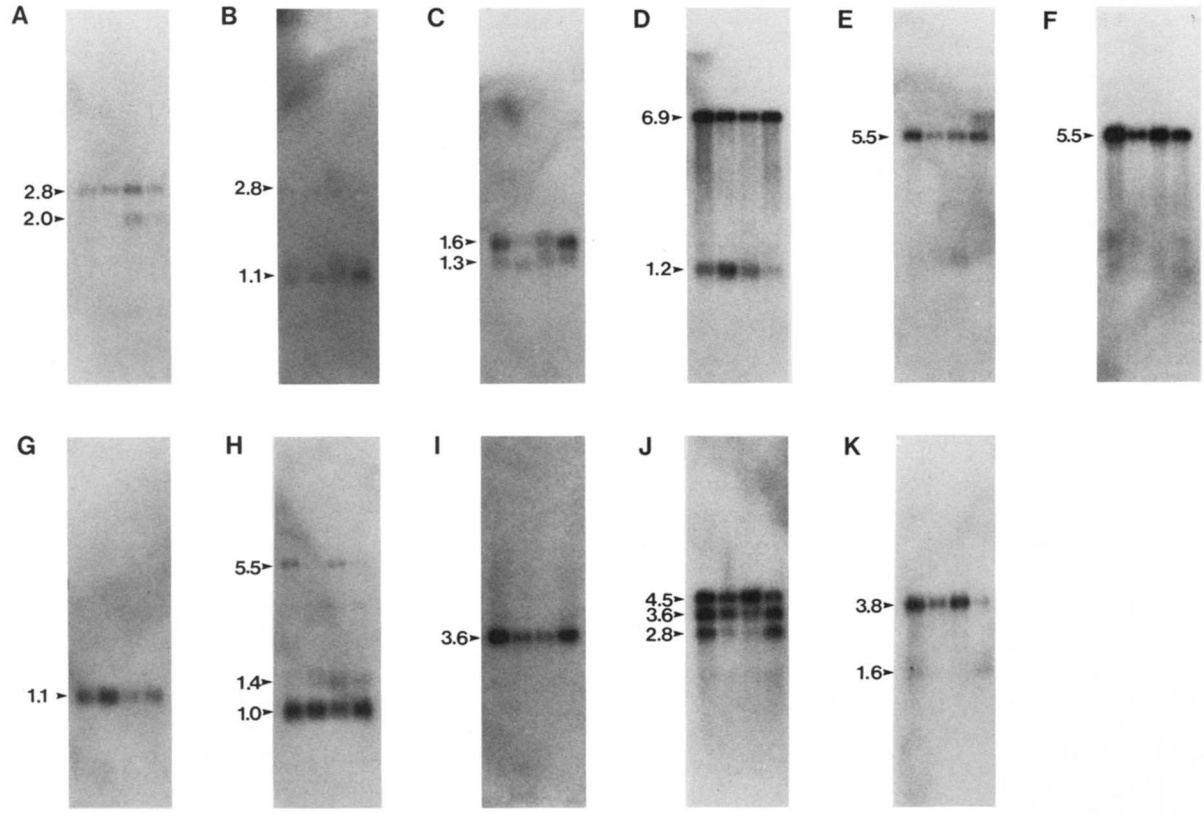

FIGURE 3.—Transcript analysis of the 72AB region. Northern blots containing poly(A)$^+$ RNA were hybridized to DNA probes (genomic DNA fragments A through K shown in Figure 2) spanning the region between the *Df(3L)th102* and *Df(3L)st-f13* breakpoints. For each Northern blot, the four lanes from left to right contain 5 μg of poly(A)$^+$ RNA from embryos, larvae, pupae and adults, respectively. To the left of each blot is the size of the transcripts as determined from log plots.

72AB subdivisions. Since the 4.5-kb transcript is clearly too large to be encoded by the 2.7-kb genomic fragment, some (or all) of its exons must lie outside the cloned region. Two additional transcripts identified by genomic fragment K (the 1.6- and 3.8-kb transcripts in panel K of Figure 3) are probably deleted by *Df(3L)st-f13* and are not included in the 72AB transcript estimates.

Using *P* element-mediated transformation, we reintroduced three genomic DNA fragments (shown in Figure 2) into the germ line (data not shown). The 2.5-kb *Pst*I fragment in P[w^+, P2.5] was previously shown to rescue the *arl* mutation (TAMKUN *et al.*, 1991), but does not rescue alleles of the other five essential genes. The 10.8-kb *Eco*RI fragment in P[w^+, R10.8] also rescues only the *arl* mutation. The larger 14.4-kb *Bam*HI-*Eco*RI fragment in P[w^+, BR14.4] rescues both *arl* and *brm* mutations. Two transcripts (the 1.1- and 1.4-kb transcripts detected by probes G and H in Figure 3) appear to lie within the largest intron of the *brm* gene. We screened an embryonic cDNA library using a genomic DNA fragment from this intron, but did not recover any clones derived from either of these small *brm* intronic transcripts (data not shown). Both P[w^+, R10.8] and P[w^+, BR14.4] contain the large *brm* intron that detects these small transcripts on Northern blots, but do not rescue mutations in the other three essential genes in 72AB [*l(3)72Ab*, *l(3)72Ac* and *l(3)72Ad*]. This suggests that these three essential genes must be either distal or proximal to *brm* and *arl*. We have attempted to map these lethals with respect to *brm* by meiotic recombination, but failed to recover any recombinants among several thousand progeny.

Genetic characterization of *brm*: Six different *brm* alleles (brm^{1-6}) were used to map the recessive lethality. All six alleles mapped just distal to *st* and the data have been pooled. From 2106 progeny, we recovered 3 recombinants between *brm* and *th* and 12 recombinants between *th* and *st*. We have placed *brm* at meiotic map position 3–43.0.

The results of the complementation tests between the 21 *brm* alleles are shown in Figure 4. All possible pairwise combinations of the 21 *brm* alleles were generated. Adult homozygotes were not recovered for any of the alleles. For nine of the alleles (brm^{1-7} and brm^{19-20}), the lethality of the homozygotes was mapped to *brm*. Although the majority of *trans*-heterozygous combinations were completely lethal, some combinations survived. Surviving *trans*-heterozygous adults usually have a characteristic phenotype that includes a reduction in the number of sex comb teeth, held-out wings, loss of humeral bristles, and patches of lightly pigmented cuticle in the fifth and sixth tergites of males. These are all

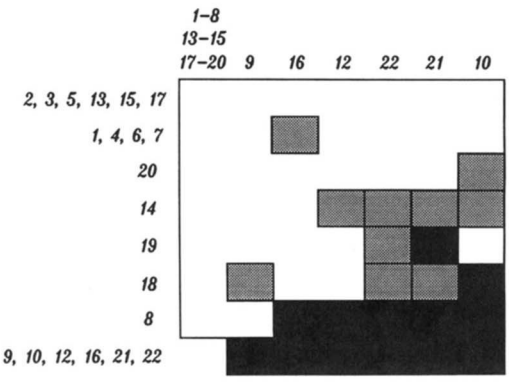

FIGURE 4.—Interallelic complementation between *brm* alleles. *Trans*-heterozygous combinations in the white box fail to complement for viability. *Trans*-heterozygous combinations in the shaded boxes complement, but with less than 25% survival. *Trans*-heterozygous combinations in the black boxes complement well (≥30% survival).

abnormalities observed in loss of function mutations of ANT-C and BX-C genes. The pattern of interallelic complementation observed for *brm* is highly complex and has not been observed for proteins that function as single subunits. However, such patterns of interallelic complementation are observed for genes that encode proteins that function as dimers or higher multimers (FINCHAM and CODDINGTON 1963; CRICK and ORGEL 1964; GELBART *et al.* 1976; PELLICCIA and COOPER 1984).

***brm* zygotic function is required for late embryogenesis:** To determine at which developmental stage zygotes that lack a functional *brm* gene die, we used three different non-complementing alleles: brm^2, brm^3 and brm^5. The results of these experiments are given in Table 3. For all three alleles examined, only one-quarter of the embryos (the *brm* homozygotes) failed to give rise to viable adults. There appears to be no decrease in adult viability of *brm* mutant heterozygotes if the mother carried at least one wild-type *brm* allele. The majority of the lethality observed (about 91%) occurred during the embryogenesis. The embryonic lethality of *brm* homozygotes was late, as most of the mutant embryos secreted cuticle. We were unable to detect any major abnormalities in either the formation or the identity of the trunk segments. The formation of the larval head structures was not completely normal, however, we were not able to determine the precise nature of the defects.

***brm* maternal function is required for early embryogenesis:** *brm* function is required in imaginal tissues for expression of multiple homeotic genes (TAMKUN *et al.* 1992). Although the phenotype of embryos that lack a functional *brm* gene does not suggest a role for *brm* in regulation of homeotic genes during embryogenesis, there is maternal mRNA present in unfertilized eggs (TAMKUN *et al.* 1992) that may provide sufficient *brm* function for the activation of homeotic genes during early embryogenesis. We have used the ability to generate mutant females heterozygous for different *brm* alleles to demonstrate a requirement for *brm* function in the early embryo. Table 4 shows the effect of maternal *brm* genotype on the viability of embryos regardless of zygotic genotype. Females of three different *brm* mutant genotypes (brm^1/brm^{16}, brm^9/brm^{10} and brm^8/brm^{10}) were tested and had greatly reduced fertility. More than 90% of the eggs produced by mutant females failed to give rise to viable adults. That the lethality was due to a maternal effect of *brm* is shown by the reciprocal crosses. Of the embryos from the cross of brm^8/brm^{10} females to wild-type males, only 2% gave rise to viable adults; of the embryos from the cross of wild-type females to brm^8/brm^{10} males, 97% gave rise to viable adults.

The maternal-effect lethality of *brm* mutant mothers does not depend on the zygotic genotype. When the *brm* mutant mothers are crossed to wild-type males, all of the progeny are heterozygous for a *brm* allele. We have shown above that *brm* mutant heterozygotes do not show decreased viability if derived from heterozygous mothers. Only 2% of the heterozygous progeny survived when the mother was mutant for *brm*. Even when the progeny received two wild-type *brm* alleles from the father, the survival was not increased. Males that carry a duplication on the *Y* chromosome that includes the wild-type *brm* gene [*Dp(3;Y)ST1*] produce sons with two paternally inherited wild-type *brm* alleles. As shown in Table 4, these males did not survive any better than their sisters who carried only one paternally inherited *brm* allele. When brm^8/brm^{10} mutant females were mated to $brm^2/+$ males instead of wild-type males, half of the progeny carried no wild-type *brm* allele and only half as many adults were recovered (1% compared to 2%, Table 4), but we were unable to detect any difference in the embryonic lethal phenotype described below.

We have examined the maternal mutant phenotype in the embryos derived from brm^8/brm^{10} females. Very few embryos secreted cuticle and abnormalities were apparent even as early as gastrulation. We initially examined *Ubx* expression in these embryos, but very few had detectable levels of Ubx proteins. In addition, at the stages of embryogenesis in which the Ubx proteins are most abundant in normal embryos, most of the embryos from the *brm* mutant mothers were so abnormal that we could not even determine their developmental stage. Because of the defects early in gastrulation, we decided to examine expression of engrailed proteins. We chose the *engrailed* gene because it is expressed early in embryogenesis in a relatively simple and repetitive pattern (14 stripes from anterior to posterior shortly after gastrulation). The pattern of *engrailed* expression was extremely abnormal in embryos from *brm* mutant mothers, but the phenotype was highly variable. There was no characteristic pattern to the defects, but many cells failed to express engrailed proteins. In some embryos, only a few scattered cells expressed engrailed proteins, while in

TABLE 3

Zygotic expression of *brm* is required for embryonic survival

Genotypes of parents		No. of individuals at each stage				Percent lethal	Lethal phase, percent embryonic
Maternal	Paternal	Eggs	Larvae	Pupae	Adults		
$brm^2/+$	$brm^2/+$	231	177	170	166	28	83
$brm^3/+$	$brm^3/+$	197	149	149	147	25	96
$brm^5/+$	$brm^5/+$	237	183	181	180	24	95
Total		665	509	500	493	26	91

TABLE 4

brm is required maternally for embryonic survival

Genotype of parents		No. of individuals at each stage				Percent lethal	Lethal phase, percent embryonic
Maternal	Paternal	Eggs	Larvae	Pupae	Adults		
brm^1/brm^{16}	+/+	295	40	26	26	91	95
brm^9/brm^{10}	+/+	901	12	6	5	99	99
brm^8/brm^{10}	+/+	1252	50	26	23	98	98
+/+	brm^8/brm^{10}	145	142	142	141	3	
brm^8/brm^{10}	+/+/+[a]	711	22	15	13	98	99
brm^8/brm^{10}	$brm^2/+$	2027	39	24	24	99	99

[a] Males carrying *Dp(3;Y)ST1*, which is duplicated for brm^+.

other embryos, many of the stripes appear normal with only small gaps in one or two stripes.

We have also generated homozygous *brm* mutant germ cells in brm^1 heterozygous females using radiation-induced mitotic recombination. To identify embryos derived from these mutant germ cells, we used a transposon carrying the ovo^{D1} mutation (CHOU *et al.* 1993). The ovo^{D1} mutation is a dominant female-sterile mutation that blocks oogenesis. In P[ovo^{D1}]/brm^1 heterozygous females, mitotic recombination proximal to *brm* removes both the ovo^{D1} transposon and the wild-type *brm* allele. The presence of the brm^1 mutation caused a significant reduction in the frequency of germ-line clones recovered. We recovered no clones among 285 females compared to 7 clones among 230 control females ($0.01 > P > 0.001$ by a chi square test for homogeneity).

Epistasis between *brm* and *Polycomb*: *brm* mutations were initially isolated as dominant suppressors of *Polycomb* (*Pc*) mutant phenotypes. Do *Pc* mutations also suppress *brm* mutant phenotypes, or is *brm* functioning downstream from *Pc*? The desired method for determining the epistatic relationship between *brm* and *Pc* would be to compare the phenotype of the double mutant that completely lacks both functions to the phenotypes of the single mutants. Because the complete lack of *brm* function appears to be a germ-line lethal, we cannot recover embryos that lack both the maternal and zygotic *brm* functions. As an alternative, we have used the imaginal loss-of-function phenotypes observed in the brm^1/brm^{16} *trans*-heterozygotes and in the Pc^4 heterozygotes. We have previously shown that the mutant phenotype of $Pc^4/+$ flies is suppressed when the number of wild-type copies of *brm* is reduced from two to one (KENNISON and TAMKUN 1988; TAMKUN *et al.* 1992). Here we show that the reciprocal relationship does not hold. The Pc^4 allele is a strong dominant allele that has complete penetrance even as a heterozygote (KENNISON and RUSSELL 1987). We have examined brm^1/brm^{16} *trans*-heterozygotes that were also heterozygous for Pc^4. We recovered 30 brm^1 *st* Pc^4 p^p *cu sr* e^s *ca*/brm^{16} *trans*-heterozygotes compared to an expected 210 (14% survival). In two different control experiments, we recovered 26 brm^1/brm^{16} *trans*-heterozygotes compared to an expected 180 (14% survival) and 26 brm^1 *st cu sr* e^s *ca*/brm^{16} *trans*-heterozygotes compared to an expected 229 (11% survival). In all three samples, we could not detect any effect of Pc^4 on the hypomorphic brm phenotypes (outstretched wings, transformation of fifth abdominal segments to more anterior identities, and female fertility). We believe that the frequency of survivors is a very sensitive indicator of the degree of transformations. There is no significant difference ($0.5 > P > 0.35$ by a chi square test for homogeneity) between the frequencies of surviving brm^1/brm^{16} *trans*-heterozygotes in the two samples with the greatest difference.

DISCUSSION

brm is one of a large number of genes required for proper homeotic gene function in Drosophila (PARO 1990; KENNISON and TAMKUN 1992; KENNISON 1993). We initially identified the *brm* gene in screens for mutations that suppress the ability of *Pc* mutations to cause alterations in the identities of adult segments (KENNISON and TAMKUN 1988). Because *Pc* mutations derepress transcription of ANTC and BXC genes in regions of the fly where they are not normally expressed, *Pc* is believed to encode a transcriptional repressor (reviewed in PARO

1990). The ectopic expression of ANTC and BXC gene products in *Pc* mutants causes cells to alter their segmental identity and make inappropriate structures, such as the formation of legs in place of antennae on the head. The *brm* suppression of the adult homeotic phenotypes of *Pc* mutants is caused by a failure to derepress transcription of ANTC and BXC genes (TAMKUN *et al.* 1992). The molecular analysis of the *brm* gene showed that the brm protein is strikingly similar to the yeast transcriptional activator SNF2/SWI2 (TAMKUN *et al.* 1992), which is required for inducible (but not basal) transcription of several yeast genes (LAURENT and CARLSON 1992; PETERSON and HERSKOWITZ 1992; WINSTON and CARLSON 1992).

The yeast *snf2/swi2* mutants fail to derepress transcription of target genes under inducing conditions. The *sin* mutants were isolated because they restore the ability to induce the target genes even in the absence of SIN2/SWI2 (PETERSON and HERSKOWITZ 1992; WINSTON and CARLSON 1992). The Drosophila *brm* mutants fail to derepress transcription of homeotic genes in *Pc* heterozygotes (KENNISON and TAMKUN 1988). We have shown here, however, that the Pc^4 mutation does not appear to relieve the requirement for *brm* function in the imaginal tissues. In contrast, the failure of homeotic gene activation in the imaginal tissues in *trx* mutants is suppressed by a heterozygous *Pc* mutation (CAPDEVILA *et al.* 1986). In addition, increasing gene dosage for *trx* enhances the *Pc* mutant phenotype, while increasing gene dosage for *brm* has no effect (CAPDEVILA *et al.* 1986; KENNISON and RUSSELL 1987). We believe that the differences between the *brm* and *trx* genetic interactions with *Pc* mutations may reflect a difference in the biochemical functions of brm and trx proteins in transcriptional activation. trx protein may directly compete with Pc protein to determine the active state of a homeotic gene. In contrast, brm protein may only be required for transcription of genes in the active state, but be unable to actively remove bound Pc protein from a repressed gene.

We have isolated and characterized 21 zygotic lethal alleles of *brm*. Homozygotes of the strongest alleles die late in embryogenesis, with little effect on the patterns of the embryonic cuticle. There is also a maternal requirement for *brm* function. Adult females of some heteroallelic *brm* genotypes survive, but are almost completely sterile. Most of their progeny fail to complete embryogenesis, even when they receive two wild-type *brm* alleles from their fathers. The loss of maternal *brm* function leads to earlier and far more drastic defects than the loss of zygotic *brm* function. Since both maternal and zygotic *brm* functions are required for embryonic survival, could these functions be due to different protein products? We believe that this is unlikely, since we have detected only a single 5.5-kb brm transcript at all developmental stages examined, including oocytes and older embryos (TAMKUN *et al.* 1992). Instead, we suspect that (1) zygotic transcription and translation of *brm* is not completed early enough to provide the maternal function and (2) the maternal product is degraded or inactivated before the zygotic requirement has ended.

The pattern of interallelic complementation for the 21 *brm* alleles suggests that brm proteins function in a dimer or higher multimer (FINCHAM and CODDINGTON 1963; CRICK and ORGEL 1964). We believe that brm protein interacts not only with itself, but may also function as part of a multi-protein complex. Genetic and biochemical studies of SNF2/SWI2, the putative yeast homolog of *brm*, suggest that SNF2/SWI2 acts in concert with at least four other proteins (SWI1, SWI3, SNF5 and SNF6) to activate transcription (LAURENT *et al.* 1991; LAURENT and CARLSON 1992; PETERSON and HERSKOWITZ 1992; YOSHINAGA *et al.* 1992). Our genetic studies have identified numerous candidates for brm-associated proteins. We have isolated alleles of *trithorax*, *kohtalo* and *ash1* as dominant enhancers of *brm* (J. KENNISON, unpublished data) and have evidence for allele-specific interactions between *osa* and *brm* mutations (M. VÁZQUEZ and J. KENNISON, unpublished data). These interactions suggest that brm may interact with the products of other trithorax group genes. Future biochemical studies should identify the number and identities of the proteins that interact with brm.

Similarities between brm and other proteins suggest that brm contains at least two different functional domains: a DNA-dependent ATPase domain (DAVIS *et al.* 1992; HENIKOFF 1993) and a bromodomain (HAYNES *et al.* 1992; TAMKUN *et al.* 1992). The DNA-dependent ATPase domain is characteristic of a growing family of putative helicases, including the yeast SNF2/SWI2, STH1, MOT1, RAD54 and RAD16 proteins (DAVIS *et al.* 1992; LAURENT *et al.* 1991, 1992); the human hSNF2L, HBRM, BRG1 and ERCC6 proteins (KHAVARI *et al.* 1993; MUCHARDT and YANIV 1993; OKABE *et al.* 1992; TROELSTRA *et al.* 1993); the mouse CHD1, MBRM, BRG1 and ETL proteins (SOININEN *et al.* 1992; DELMAS *et al.* 1993; MUCHARDT and YANIV 1993; RANDAZZO *et al.* 1994); and the Drosophila lodestar and ISWI proteins (GIRDHAM and GLOVER 1991; ELFRING *et al.* 1994). The biochemical function of the bromodomain is not known, however, its presence in a wide variety of eukaryotic regulatory proteins suggests that it is involved in transcriptional activation. Bromodomains have been found in the yeast SNF2/SWI2, GCN5, STH1 and SPT7 proteins (GEORGAKOPOULOS and THIREOS 1992; HAYNES *et al.* 1992; LAURENT *et al.* 1991, 1992), the Drosophila fsh (female-sterile homeotic) and brm proteins (HAYNES *et al.* 1989; TAMKUN *et al.* 1992), the human and Drosophila TAFII250/CCG1 proteins (KOKUBO *et al.* 1993; RUPPERT *et al.* 1993; SEKIGUCHI *et al.* 1991), and the human BRG1 and HBRM proteins (KHAVARI *et al.* 1993; MUCHARDT and YANIV

1993). The DNA-dependent ATPase domain and the bromodomain in brm may be functionally independent, since many of the other proteins listed above contain only one of the two domains. Some of the interallelic complementation that we observed between the 21 *brm* alleles might be due to the ability of mutations in the bromodomain to complement mutations in the DNA-dependent ATPase domain. However, this cannot completely explain the complex pattern of interallelic complementation. It will be interesting to determine which of the *brm* alleles map to these two identified domains.

brm was identified because of its effects on the expression of ANTC and BXC homeotic genes. What other genes may require *brm*? At least some of the segmentation genes appear to require normal levels of brm protein. We have found that expression of the segmentation gene *engrailed* is abnormal in embryos from brm^{8}/brm^{10} mutant mothers. Flies homozygous for the hypomorphic h^{1} allele of the segmentation gene *hairy* survive but have extra macrochaetae. This phenotype is enhanced in *brm* mutant heterozygotes (J. KENNISON, unpublished data). *brm* also appears to affect the segmentation gene *hedgehog* (A. L. FELSENFELD and J. KENNISON, unpublished data). We suspect that the total number of genes whose expression may require *brm* is quite large.

The polytene chromosome subdivisions 72A and 72B include seven bands, five essential genes and at least 14 transcripts. Bridges observed a total of 5059 bands in the polytene chromosomes of the salivary gland. The estimates for the total number of genes in these 5059 bands vary from 2500 to 6400 (MULLER and ALTENBURG 1919; MULLER 1929; GOWEN and GAY 1933; DEMEREC 1934; ALIKHANIAN 1937; LEA 1947; LEFEVRE 1974; LEFEVRE and WATKINS 1986). We have taken advantage of a recent compilation of Drosophila mutants (LINDSLEY and ZIMM 1992) to obtain a more accurate estimate of the number of essential genes. Among the data summarized by LINDSLEY and ZIMM are the results from a large number of experiments to systematically saturate specific chromosome regions for mutations. Regions that include about 1200 polytene bands (about 24% of the euchromatic parts of the genome) have been characterized. Only 854 genes were identified within these regions, suggesting that there are about 3600 lethally mutable genes in *Drosophila melanogaster* (about 7 essential genes for every 10 polytene bands). This estimate only includes essential genes that can mutate to cause recessive zygotic lethality. Other essential genes may only be required during oogenesis or spermatogenesis. The number of essential genes that are required only during gametogenesis can be estimated by comparing the number of sterile mutations to the number of zygotic lethal mutations recovered in the same experiments. SCHÜPBACH and WIESCHAUS (1989) recovered female sterile mutations at about 8% of the frequency of zygotic lethal mutations. The frequency of male sterile mutations is about 15% the frequency of zygotic lethal mutations (LINDSLEY and LIFSCHYTZ 1972). These data suggest that fewer than 4500 genes are essential in either the somatic or germ cells of Drosophila. This estimate is probably too high because it assumes that all of the female sterile and male sterile mutants recovered in these experiments identify genes that are only required for gametogenesis. Many female sterile mutations are alleles of lethally mutable loci and PERRIMON and MAHOWALD (1986) estimate the number of genes essential only for oogenesis at about 50. If some male sterile mutations are also alleles of lethally mutable loci, this would also decrease the estimated number of essential genes.

In contrast to the low estimates of the number of essential genes, it has been estimated that there are between 10,000 and 14,000 different RNA transcripts encoded by the Drosophila genome (TURNER and LAIRD 1973; LEVY and MCCARTHY 1975; LEVY *et al.* 1976; LEVY and MANNING 1981). The difference in the numbers of essential genes and the number of different RNA transcripts also appears in characterizations of specific regions of the genome. In studies of the 87DE subdivisions, 11 essential genes (plus the nonessential *rosy* gene) and 43 transcripts were identified in a region of about 315 kb (HILLIKER *et al.* 1980; HALL *et al.* 1983; BOSSY *et al.* 1984). In the 72AB subdivisions (a region of about 50 kb), we identified five essential genes and 14 transcripts. The two- to fivefold difference between the estimates for the number of essential genes and the number of RNA transcripts in Drosophila suggests that more than half of the genes in Drosophila may be nonessential or redundant.

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