The use of maternally coded gene products in *Drosophila*

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Summary

Both maternal and zygotic expression of many essential genes are required for normal development. For some of these genes, absence of maternal function yields striking embryonic defects. The experiments reported here examine two questions about such genes: (1) Are embryonic effects of maternal deficits a common property of maternally-and-zygotically active genes? and (2) Is use of the maternal products of these genes restricted to early embryogenesis? A comparison of times of lethality of mutant sons of normal and mutant-heterozygous mothers has been made for six mutations in the *zeste-white* region of the *Drosophila X* chromosome. Four of the mutations are defective in single cistrons and two are deficiencies that between them remove thirteen essential loci. All of these mutations had previously been shown to have both maternal and zygotic effects, and all of them had been tested, using homozygous germ-line clones, for the effects of complete maternal defects. For several of them, homozygous germ-line clones cause embryonic defects. Of the six, only one, *Df(1)K95*, shows a shift from larval to embryonic lethality when the mothers are heterozygous, and even in that case lethality occurs at the very end of embryogenesis. These results have two implications: (1) maternally-derived transcripts do not always serve a solely embryonic role; and (2) an embryonic effect of a complete maternal deficit does not by itself demonstrate an embryo-restricted function for the maternal transcript.

1. Introduction

Recent observations on maternal function of essential genes in *Drosophila* raise questions about how the organism uses the products of individual genes made at different stages (reviewed in Mahowald & Hardy, 1985). The most important of these observations is that function of many zygotically essential genes is required maternally as well as during zygotic development. Whether a particular gene has an early zygotic lethal phase or a late one, its function is often required before embryogenesis even begins.

A number of mutants, such as *pole hole, dishevelled, fused* and *caudal*, show prominent embryonic effects of gross maternal insufficiency (reviewed in Mahowald & Hardy, 1985). It is tempting to think of these as members of a special class of genes for which maternal transcripts have embryo-restricted functions. There are, however, two other possibilities. One is that embryonic effects could be a common property of reduced maternal function for all maternally-and-zygotically active genes. The other arises from the fact that an embryonic phenotype caused by maternal insufficiency is a necessary, but not by itself sufficient,

condition for deciding that a maternal transcript has a stage-specific function. That is, an embryonic defect could reflect early absence of a global function rather than an embryo-restricted role for the maternal transcript.

There are two generally applicable tests for maternal effects of zygotic lethals; one particularly suited to genome-wide surveys, the other more suited to detailed analysis. The first tests for effects of severe maternal deficits by examination of homozygous germ-line clones. This test has been applied to large segments of the genome, providing identification of a subset of maternally-and-zygotically required loci, and demonstrating embryonic effects of some (Garcia-Bellido & Robbins, 1983; Perrimon *et al.* 1984*a*, *b*, 1986). It does not, however, yield any information about zygotic effects of maternal insufficiency for the substantial fraction of mutations that are either germ line lethal or that yield eggs that fall to mature. In the other procedure, the effects of maternal heterozygosity are tested, but this generally requires increasing the sensitivity of the zygote to such partial defects by reducing zygotic gene activity. Because of the difficulty of arranging appropriate conditions of diminished

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zygotic gene activity, this test has been applied comprehensively to only a small segment of the genome. Within that segment, however, nearly all of the genes have demonstrable maternal effects (Robbins, 1980, 1983).

Whether most maternally-and-zygotically functioning genes have embryonic maternal effects is not known. The germ-line clone test certainly indicates that some do, but is uninformative for many mutations, rather than telling us whether they have, or lack, embryonic effects. Thus, to ask whether maternal deficits generally cause embryonic defects, we must examine the effects of partial maternal deficits. Such tests also provide the additional criterion needed to decide whether a gene that yields an embryonic phenotype in the germ-line clone test has an embryo-restricted function. If a maternal transcript's role is restricted to embryogenesis, any reduction of maternal gene activity strong enough to affect the offspring should affect the same embryo-specific process. If, in contrast, a gene encodes a multi-stage function, a complete maternal deficit may affect embryos, but the effects of a partial maternal deficit can be delayed.

The effects of partial maternal defects for several mutants, including two deficiencies, in the *zeste-white* region of the X chromosome are reported here. In no case did partial maternal insufficiency cause embryonic lethality, even though this level of activity had previously been shown to be severe enough to affect the offspring. Thus, embryonic effects are not a property of all maternally and zygotically active genes. Germ-line clones of some of these genes do cause embryonic defects (Garcia-Bellido & Robbins, 1983), but the delayed effects of partial maternal deficits implies that their maternal transcripts are important at other stages as well. Thus, not only is an embryonic phenotype not, *a priori*, a sufficient criterion for deciding that a maternal gene product has an embryo-restricted function, there are now some clear counter-examples where any assumption of stage specificity made on that basis would be wrong.

2. Experimental design

The basic experimental idea is straightforward, though its execution is more complex: choose a group of mutants in genes known to have required maternal as well as zygotic function, devise a level of reduced (but not germ-line lethal) maternal activity that can be shown to affect the offspring, and arrange crosses to yield lethal genotypes derived from either normal eggs or from eggs with reduced levels of those gene products. If the maternal gene product only affects the early embryo, the lethal progeny of heterozygous mothers should die as embryos even if the death of corresponding progeny of normal mothers is delayed until later stages. There are several matters, however,

that must be considered in the design of the actual experiments:

(1) What criteria can be used to choose the mutants? Genes falling into the maternally-and-zygotically needed class have been detected in several ways. Some have been detected because of embryonic effects of maternal insufficiency (e.g. *almondex* Shannon, 1972, 1973; for other examples, see Mahowald & Hardy, 1985). Some are known because of lethality of homozygous germ-line clones, or because of defective development of eggs from such clones (Garcia-Bellido & Robbins, 1983; Perrimon *et al.* 1984*a*). Yet others have been found because of a lethal interaction of partial maternal and partial zygotic deficiencies neither of which alone is lethal (Robbins, 1980, 1983; Simpson, 1983). The first group, having been selected on the basis of an embryonic effect, are not an unbiased sample. For most genes identified by germ-line clone effects, we do not know if partial maternal deficits have any effect at all (see item (2) below). That leaves the third class, those defined by a lethal interaction of partial maternal and partial zygotic defects.

(2) How can a level of maternal defect be established that affects zygotic development, but that is not so severe as to create non-functional ova? In the experiments that demonstrated an interaction between partial maternal and partial zygotic defects of *zeste-white* region mutants, the mothers were heterozygous for the mutants. For those genes, that level of maternal deficit has little effect on normal embryos, but can have a pronounced effect on embryos that are partially defective because of position effect variegation of the same gene(s). Maternal mutant heterozygosity, then, provides a lesion extreme enough to affect zygotic development, while still yielding functional eggs. The *zeste-white* mutations have also been tested as germ-line clones (Garcia-Bellido & Robbins, 1983). Although the thirteen essential genes in the *zeste-white* region are only a small sample of the genome, they are an unbiased sample. They also appear to be a representative sample since our previous findings for those genes (Robbins, 1980, 1983; Garcia-Bellido & Robbins, 1983) have been confirmed in larger germ-line clone surveys (Perrimon *et al.* 1984*a, b*, 1986).

(3) To look for maternal effects on the time of lethality of mutant zygotes, crosses must be used in which mutant zygotes are produced by normal mothers or by mutant-heterozygous mothers. The crosses used were:

Normal mothers:

$mutant, y^+$ or $y^2/y; Dp(1;4)mg/spa^{pol}$
$$\times y/Y; spa^{pol}/spa^{pol},$$

Heterozygous mothers:

$mutant, y^+$ or $y^2/y; spa^{pol}/spa^{pol} \times y/Y; spa^{pol}/spa^{pol},$

where $Dp(1;4)mg$ carries wild-type (though slightly

variegating) alleles of the entire *zeste-white* region. Although these crosses do not yield the same sets of offspring genotypes, *mutant/Y; spa^{pol}/spa^{pol}* sons are common to both crosses and are completely inviable whether the mothers are of one type or the other.

(4) It must be determined how much of the observed lethality results from death of *mutant/Y; spa^{pol}/spa^{pol}* zygotes, how much results from death of the other genotypes generated in the crosses, and how much might be independent of genotype. It is also important to assure that early lethals are not confused with unfertilized eggs. Adult survivals were used to partition the observed lethality, and both visible-light microscopy and Hoechst-stained preparations were used to verify classification of fertilized versus unfertilized eggs. In several cases, two additional crosses that generate mutant offspring from defective or normal mothers were done to check the reliability of the conclusions. Those crosses were:

Normal mothers:

mutant, y^+ or *y^2/y; Dp(1;4)mg/spa^{pol}*
$$\times attached\text{-}XY, y B/0; spa^{pol}/spa^{pol},$$

Heterozygous mothers:

mutant, y^+ or *y^2/y; spa^{pol}/spa^{pol}*
$$\times attached\text{-}XY, y B/0; Dp(1;4)mg/spa^{pol}$$

These two crosses, unlike those preceding, are reciprocal and generate identical offspring genotypes. However the viability of one class, *mutant/0; Dp(1;4)mg/spa^{pol}* (Robbins, 1980, 1983), is quite dependent on maternal genotype; a problem avoided in the first series although at the cost of using non-reciprocal crosses.

3. Materials and methods

Two deficiencies and four single-cistron mutations in the *zeste-white* region of the *Drosophila X* chromosome were examined. The two deficiencies, *Df(1)w^{258-45}* and *Df(1)K95*, which between them delete the entire *zeste-white* region, are described in Kaufman *et al.* (1975). The single-cistron mutations are described in Shannon *et al.* (1972). The maternal-zygotic interactions of these mutations are considered in Robbins (1980, 1983) and the time of transition from maternal dependence to reliance on zygotic gene activity is considered in Robbins (1984). The effects of homozygous germ-line clones are reported in Garcia-Bellido & Robbins (1983). The mutations chosen include some for which homozygous germ-line clones cause embryonic problems, as well as some for which maternal effects of germ-line clones are either absent or are un-testable because of lethality of the clones.

All markers and chromosomes used, except for *Dp(1;4)mg*, are described in Lindsley & Grell (1968). *Dp(1;4)mg* (Robbins, 1977, 1980) carries wild-type

alleles of all of the *zeste-white* region lethals, though their expression is reduced, particularly in *XO* males, because of position effect variegation.

(i) *Culture conditions*

Flies were reared on cornmeal, molasses, brewer's yeast medium. Temperature was maintained at $25\pm$ 0·5 °C except for brief periods at room temperature when various manipulations were made. Since groups of parents were used, it was absolutely necessary that non-virgin parents be avoided – the females were held several days in vials and any that laid eggs were eliminated. Groups of 50 to 60 pairs of parents were mated and held on food supplemented with a paste of live yeast for 2–3 days. For egg collections, they were transferred to non-yeasted food in bottles split so that the medium-containing bottoms could be removed. Eggs were collected for 7 h, counted, and transferred to fresh medium. Between collections, the parents were placed on fresh, yeast-paste supplemented medium. Parallel, contemporaneous, single-pair matings in vials were also made to provide larger samples for determination of adult survivals.

(ii) *Lethal-phase observations*

Forty-two hours after the end of the collection period, unhatched eggs and first-instar larvae were counted and a sample of unhatched eggs was removed for microscopic examination. Pupae were counted 8 days later, adults were collected and scored for markers until all had eclosed, and any non-eclosed pupae were examined. Routine microscopic examination of unhatched eggs were done using mixed incident and transmitted light in a stereomicroscope at 30–100 ×. For some crosses, a sample of those eggs that appeared to be unfertilized were further examined using the abbreviated form of the Hoechst 33258 staining procedure devised by Foe & Alberts (1983). Since small numbers of eggs were examined, the entire procedure was carried out in well slides rather than tubes. Examination by epi-fluorescence in either a Zeiss or Olympus microscope with the appropriate filters unambiguously distinguished unfertilized eggs from those that had completed any nuclear divisions.

4. Results

(i) *Calculations*

The observations and calculated parameters of these crosses are presented in Tables 1–3. Before discussing all of the results, it is useful to follow one in detail, especially to make clear which numbers are observed and which are derived. The analysis of the cross:

$$l(1)zw3^{b12}, y^+/y; Dp(1;4)mg/spa^{pol} \times y/Y; spa^{pol}/spa^{pol}$$

is detailed in Fig. 1.

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STAGE DATA:

Eggs laid	Unhatched	Hatched	Pupae	Dead pupae white	Dead pupae pharate	Lost	Adults
[1] 1318	250	1068	925	18	8	4	895

UNHATCHED EGGS:

Method	Number examined	No development	Multi-nucleate	Dis-organized	Segments	Gut & mouth	Pre-hatch
[2] Unstained	242	185	---	2	31	17	7
[3] Hoechst	169	156	13				

CALCULATION OF NUMBER OF UNFERTILIZED EGGS:

FRACTION SHOWING NO DEVELOPMENT THAT WERE UNFERTILIZED
= (HOECHST NO DEVELOPMENT)/(HOECHST TOTAL)
= 0.92

FRACTION OF UNSTAINED SAMPLE THAT WERE UNFERTILIZED
= (0.92 x UNSTAINED NO DEVELOPMENT)/(UNSTAINED TOTAL)
= 0.71

TOTAL UNFERTILIZED = 0.71 x (UNHATCHED EGGS) = 176

DATA WITH UNFERTILIZED EGGS REMOVED:

Fertile	Hatched	Pupae	Dead pupae white	Dead pupae pharate	Lost	Adults
[4] 1142	1068	925	18	8	4	895

LETHALS:

	Total	Egg	Larva	Pupa	Adult
[5] Number	247	74	143	26	4
[6] Fraction	0.22	0.30	0.58	0.10	0.02

SURVIVING ADULTS:

Sample	FEMALES wt;Dp	wt;pol	mut;Dp	mut;pol	MALES wt;Dp	wt;pol	mut;Dp	Total
[7] Eggs	108	141	143	123	120	131	129	895
[8] Vials	277	270	242	312	214	260	237	1812
Sum	385	411	385	435	334	391	366	2707

PARTITIONING OF LETHALITY:

EXPECTED ADULTS[a] = 4 x (LARGEST FEMALE + LARGEST MALE CLASSES)
= 4 x (435 + 391) = 3304

GENOTYPIC LETHALITY = (EXPECTED ADULTS - TOTAL)/EXPECTED ADULTS
= (3304 - 2707)/3304 = 0.18

NON-GENOTYPIC LETHALITY = TOTAL LETHALITY - GENOTYPIC LETHALITY
= (0.22 - 0.18) = 0.04

LETHALITY FROM DEATH OF *mutant* MALES = (LARGEST MALE CLASS)/EXPECTED ADULTS
= 391/3304 = 0.12

LETHALITY FROM DEATH OF *mutant;Dp* MALES[b]
= (LARGEST MALE CLASS - *mutant;Dp* MALES)/EXPECTED ADULTS
= (391 - 366)/3304 = 0.01

Fig. 1. Calculation of the frequency of unfertilized eggs, stage specific lethality and the sources of lethality are illustrated using data from the cross:

$l(1)zw3^{b12}, y^{+}/y; Dp(1;4)mg/spa^{pol} \times y/Y; spa^{pol}/spa^{pol}$

[a] For crosses without $Dp(1;4)mg$, expected adults = 2 x (largest female + largest male classes).
[b] For crosses without $Dp(1;4)mg$ the lethality from this class is 0.

The first line of Fig. 1 gives the numbers of individuals observed at each stage. Two of these bear comment: the unhatched eggs are not necessarily all lethal eggs – they include some unfertilized eggs as well; and those in the column headed 'Lost' are counted in later calculations as dead adults. Discriminating between unfertilized and dead eggs was accomplished as described in Materials and methods and is shown in the next two lines, and shown in Table 2 for all of the crosses. Of the 250 unhatched eggs, 242 were examined in visible light. Of these, 185 showed no indication of development, and are presumably unfertilized, while the remainder had developed to the indicated stages. Fertilized eggs that completed only a part of the nuclear cleavage cycle, however, might not have been detected reliably and 169 of the eggs that showed no signs of development were further examined with the chromatin-specific fluorescent stain

Table 1. *Survival and lethality at various stages*

Mother	Father	Eggs laid	Fertile[a]	Hatched	Pupae	Dead pupae white	Dead pupae pharate	Dead pupae Lost	Adult	Total lethality	Eggs	Larvae	Pupae	Adults
Crosses to y/Y males:														
Dp/pol *pol/pol*	*pol/pol* *pol/pol*	2872 / 2304	2478 / 1918	2337 / 1820	1803 / 1258	35 / 13	*Df(1)w^{258−45}, y^2 w^−/y* 54 / 38	5 / 4	1709 / 1203	0·31 / 0·37	0·18 / 0·14	0·69 / 0·79	0·12 / 0·07	0·01 / 0·01
Dp/pol *pol/pol*	*pol/pol* *pol/pol*	2101 / 1912	1698 / 1736	1579 / 1302	1212 / 1190	30 / 45	*Df(1)K95, y^2/y* 49 / 147	12 / 22	1121 / 976	0·34 / 0·44	0·21 / 0·57	0·64 / 0·15	0·14 / 0·25	0·02 / 0·03
Dp/pol *pol/pol*	*pol/pol* *pol/pol*	1257 / 1146	951 / 923	882 / 876	687 / 625	5 / 1	*l(1)zw1^{d13}, y^+/y* 7 / 2	6 / 3	669 / 619	0·30 / 0·33	0·25 / 0·16	0·69 / 0·83	0·04 / 0·01	0·02 / 0·01
Dp/pol *pol/pol*	*pol/pol* *pol/pol*	1318 / 1437	1142 / 1363	1068 / 1347	925 / 987	18 / 7	*l(1)zw3^{b12}, y^+/y* 8 / 1	4 / 2	895 / 977	0·22 / 0·28	0·30 / 0·04	0·58 / 0·93	0·11 / 0·02	0·02 / 0·01
Dp/pol *pol/pol*	*pol/pol* *pol/pol*	1312 / 1022	1165 / 973	1150 / 960	939 / 744	7 / 2	*l(1)zw6^{b23}, y^+/y* 9 / 2	1 / 5	922 / 735	0·21 / 0·25	0·06 / 0·06	0·87 / 0·91	0·07 / 0·02	< 0·01 / 0·02
Dp/pol *pol/pol*	*pol/pol* *pol/pol*	1374 / 1321	1341 / 1295	1315 / 1272	1120 / 896	3 / 6	*l(1)zw7^{20}, y^+/y* 6 / 2	6 / 5	1105 / 883	0·18 / 0·32	0·11 / 0·06	0·83 / 0·91	0·04 / 0·02	0·03 / 0·01
Crosses to attached-XY, y B/O males:														
Dp/pol *pol/pol*	*pol/pol* *Dp/pol*	1438 / 1381	1345 / 1210	1295 / 1195	1165 / 1005	94 / 61	*l(1)zw3^{b12}, y^+/y* 12 / 61	0 / 6	1059 / 877	0·21 / 0·28	0·17 / 0·05	0·45 / 0·57	0·37 / 0·37	0·00 / 0·02
Dp/pol *pol/pol*	*pol/pol* *Dp/pol*	1132 / 1288	1120 / 1221	1109 / 1193	935 / 1001	3 / 19	*l(1)zw6^{b23}, y^+/y* 12 / 45	4 / 2	916 / 935	0·18 / 0·23	0·05 / 0·10	0·85 / 0·67	0·07 / 0·22	0·02 / 0·01
Dp/pol *pol/pol*	*pol/pol* *Dp/pol*	1379 / 1380	1321 / 1338	1296 / 1318	1080 / 976	3 / 2	*l(1)zw7^{20}, y^+/y* 17 / 6	2 / 3	1058 / 965	0·20 / 0·28	0·10 / 0·05	0·82 / 0·92	0·08 / 0·02	0·01 / 0·01

Note: columns under "Fraction of lethals dying as:" are Eggs, Larvae, Pupae, Adults.

[a] Derived from Table 2, see text.

Hoechst 33258. Classification of unstained eggs had evidently been reasonably reliable since the Hoechst examination revealed only a small minority of multinucleate eggs. These observations were then used, in the manner indicated, to calculate the number of unfertilized eggs in the total sample, giving the numbers shown in line 4. The same procedure was followed to determine the numbers of fertilized eggs in each of the crosses as shown in Table 1. Calculation of the numbers dying at each stage, the total lethal fraction and the fraction of these dying at each stage yields the numbers shown in lines 5 and 6, with the lethal fractions for all the crosses shown in Table 1.

Although it is the lethality of *mutant*/*Y*; spa^{pol}/spa^{pol} that we are interested in, that is not the only genotype generated in these crosses, and if the total lethality greatly exceeded that due to this genotype the lethality figures would not be very meaningful. To partition the sources of lethality, the genotypes of surviving adults from the egg sample, and from a contemporaneous sample of single-pair matings, were scored. Those results are shown in lines 7 and 8 as well as in Table 3. The total adults indicated here in some instances differs from that shown in Table 1 as the few non-disjunctional offspring and the occasional fly that escaped en route from bottle to anaesthetizer were counted as survivors but were not used in the calculations.

Lethality was partitioned into several classes: that resulting from survivals lower than that of the most fit female and male classes (listed as genotypic lethality), that which, while possibly resulting from genetic effects, is not specific to the marked genotypes (non-genotypic lethality), lethality of the diagnostic males and lethality of the other genotype that might also have some deficit in zygotic function – the *mutant*; *Dp* class. Since segregation in attached-*XY* males is non-Mendelian, the partitioning of lethality must be done in a way that does not assume 1:1 sex ratios. Moreover, recovery of *mutant*; *Dp* males must be estimated from adult survivals. The necessary calculations are explicitly shown for this cross in the remainder of Fig. 1 with the results, expressed as the fraction of lethality in each class, shown for all of the crosses in Table 3.

(ii) *Crosses to* y/Y; spa^{pol}/spa^{pol} *males*

For each mutant, the initial comparison to be made is of the stage of lethality indicated by the lethal frequencies shown in Table 1. Table 2 indicates when any embryonic lethality occurs, and Table 3 provides a check on the source of the lethal zygotes. When maternal heterozygosity is covered by *Dp(1;4)mg*, each of the mutants has a larval lethal phase. Of the six mutants examined, only one, *Df(1)K95*, yields a shift to embryonic lethality when the mothers are heterozygous. The data in Table 2, however, indicate that even this shift is not to early lethality. The *Df(1)K95* zygotes that die as embryos when their mothers are heterozygous do not die until immediately prior to hatching – they appear to be well-formed larvae and are motile within the unhatched egg.

Is this shift in lethal phase actually a maternal effect on *Df(1)K95* sons? As the results in Table 3 show, there is substantial lethality in addition to that of the *Df(1)K95* males, and it is necessary to check whether those other sources of lethality could account for the differences between the two crosses. Two other sources of lethality can be separated: lethality of non-*Df(1)K95* bearing genotypes ('genotypic' lethality), and lethality that is not ascribable to any of the genotypic differences followed in the crosses ('non-genotypic' lethality). Additional lethality of non-mutant offspring when the mothers carry *Dp(1;4)mg* is a common feature of the crosses of all of the mutants (see last column of Table 3). These data are in accord with the earlier observation (Robbins, 1980) that *Dp(1;4)mg* hyperploids are somewhat inviable. The 'non-genotypic' lethality in the *Df(1)K95* crosses is apparently common to all of the genotypes in these two crosses and the much lower frequencies of non-genotypic lethality in most of the other crosses suggests that the source of this lethality in the *Df(1)K95* crosses is genetic rather than environmental.

Whatever the causes of these other deaths, whether because of hyperploidy or because of environment or background genotype, the question remains whether they could account for the shift toward embryonic lethality among the offspring of *Df(1)K95*-heterozygous females. The inviability of hyperploids cannot do so. A bias that could be mis-interpreted as earlier death in the non-duplication sample would require that *Dp(1;4)mg* hyperploids die late. Clearly that is not the case. If anything, the crosses of the other mutants suggest that the hyperploids die earlier than euploid or deficient offspring since three of the five other mutants gave more embryonic lethality in the duplication cross.

That leaves non-genotype-specific lethality as a possible artifactual source of the apparent maternal effect on *Df(1)K95* lethal phase. Since the other crosses did not have as large a non-genotypic lethal component, and since their genetic backgrounds differ as well, they are not useful in deciding whether this affected the *Df(1)K95* result. We can, however, ask whether the numbers of non-specific deaths are sufficient to account for the result. There are two possibilities to be considered. The first is that those dying for non-genotype-specific reasons are themselves contributing to the embryonic lethal class. This can not be the case since there is a smaller proportion of non-genotypic deaths in the cross that has more embryonic lethality.

The second possibility is that a higher frequency of non-genotype-specific death in the duplication cross would yield an apparent shift in the calculated time of lethality in the other cross if those deaths were late.

Table 2. *Developmental stage of unhatched eggs*

Mother	Father	Number	No development	Fract. unfertilized[a]	Multi-nucleate	Disorganized	Segments	Gut & mouth	Pre-hatch
Crosses to y/Y males:									
				$Df(1)w^{258-45}, y^2\ w^-/y$					
Dp/pol	pol/pol	388	286	0·74	—	10	40	46	6
pol/pol	pol/pol	350	279	0·80	—	14	13	31	13
				$Df(1)K95, y^2/y$					
Dp/pol	pol/pol	392	303	0·77	—	18	22	38	11
pol/pol	pol/pol	367	106	0·29	—	2	11	64	184
				$l(1)zw1^{d13}, y^+/y$					
Dp/pol	pol/pol	249	203	0·82	—	9	5	25	7
pol/pol	pol/pol	232	192	0·83	—	7	20	11	2
				$l(1)zw3^{b12}, y^+/y$					
Dp/pol	pol/pol	242	185	—	—	2	31	17	7
Hoechst stain		169	156	0·71	13				
pol/pol	pol/pol	79	65	—	—	0	6	3	5
Hoechst stain		60	60	0·82	0				
				$l(1)zw6^{b23}, y^+/y$					
Dp/pol	pol/pol	154	142	—	—	3	0	8	1
Hoechst stain		126	124	0·91	2				
pol/pol	pol/pol	51	40	0·78	—	0	0	10	1
				$l(1)zw7^{g20}, y^+/y$					
Dp/pol	pol/pol	59	42	—	—	4	1	6	6
Hoechst stain		34	27	0·57	7				
pol/pol	pol/pol	49	30	—	—	2	2	11	4
Hoechst stain		30	26	0·53	4				
Crosses to attached-XY/O males:									
				$l(1)zw3^{b12}, y^+/y$					
Dp/pol	pol/pol	139	96	—	—	0	26	10	7
Hoechst stain		88	83	0·65	5				
pol/pol	Dp/pol	158	149	—	—	0	2	5	2
Hoechst stain		111	108	0·92	3				
				$l(1)zw6^{b23}, y^+/y$					
Dp/pol	pol/pol	20	10	—	—	2	0	4	4
Hoechst stain		9	9	0·50	0				
pol/pol	Dp/pol	92	71	—	—	0	5	12	4
Hoechst stain		66	60	0·70	6				
				$l(1)zw7^{g20}, y^+/y$					
Dp/pol	pol/pol	79	57	—	—	5	5	8	4
Hoechst stain		57	55	0·70	2				
pol/pol	Dp/pol	59	43	—	—	3	1	6	6
Hoechst stain		41	38	0·68	3				

[a] In the absence of Hoechst stain data, the fraction unfertilized is taken as:
  (number showing no development)/(number examined).
Where Hoechst stain observations were made, that fraction was multiplied by:
(number showing no development in Hoechst sample)/(number in Hoechst sample).

This can be examined as follows. Of the 1698 fertile eggs in the $Df(1)K95/y$; $Dp(1;4)mg/spa^{pol}$ cross, 119 died as embryos and 458 died at later stages (Table 1). If, to choose the worst case, all of the 50% (or 288) non-genotypic deaths occurred post-embryonically, there were $119(= 0·41)$ genotype-specific embryonic deaths and $170(= 0·59)$ genotype-specific post-

embryonic deaths. For the $Df(1)K95/y$; spa^{pol}/spa^{pol} cross, subtracting the 274 non-genotypic deaths leaves $434(= 0·74)$ embryonic and $152(= 0·26)$ post-embryonic deaths ascribable to genotype. Even though a larger proportion of the total lethality was subtracted for the duplication cross, there is still an excess of embryonic lethality when the mothers are $Df(1)K95$

Table 3. *Surviving adults and sources of lethality*

Mother	Father	Sample	Females wt:Dp	Females wt:pol	Females mut:Dp	Females mut:pol	Males wt:Dp	Males wt:pol	Males mut:Dp	Total	Total lethality	Genotypic lethality	Non-genotypic lethality	Lethality of mutant males	Lethality of mutant:Dp males	Lethality of other genotypes
Crosses to y/Y males:																
Df(1)w$^{258-45}$, y^2 w$^-$/y																
Dp/pol	pol/pol	Eggs	258	260	259	297	188	238	206	1706	0·31	0·81	0·19	0·48	0·18	0·35
		Vials	735	865	757	854	424	820	458	4913						
pol/pol	pol/pol	Eggs	0	411	0	415	0	371	0	1197	0·37	0·67	0·33	0·98	0·00	0·03
		Vials	0	955	0	918	0	918	0	2791						
Df(1)K95, y^2/y																
Dp/pol	pol/pol	Eggs	142	165	183	180	135	178	136	1119	0·34	0·50	0·50	0·65	0·10	0·26
		Vials	535	579	544	591	463	562	483	3757						
pol/pol	pol/pol	Eggs	0	330	0	288	0	355	0	973	0·44	0·64	0·36	0·91	0·00	0·09
		Vials	0	1226	0	1099	0	1349	0	3674						
l(1)zw^{r13}, y$^+$/y																
Dp/pol	pol/pol	Eggs	90	94	99	96	91	114	85	669	0·30	0·79	0·21	0·54	0·23	0·23
		Vials	549	623	577	603	453	606	327	3738						
pol/pol	pol/pol	Eggs	0	208	0	213	0	198	0	619	0·33	0·77	0·23	0·98	0·00	0·02
		Vials	0	1027	0	1049	0	1043	0	3119						
l(1)zw^{b12}, y$^+$/y																
Dp/pol	pol/pol	Eggs	108	141	143	123	120	131	129	895	0·22	0·84	0·17	0·66	0·04	0·30
		Vials	277	270	242	312	214	260	237	1812						
pol/pol	pol/pol	Eggs	0	370	0	288	0	319	0	977	0·28	0·92	0·08	0·94	0·00	0·06
		Vials	0	641	0	658	0	637	0	1936						
l(1)zw^{b23}, y$^+$/y																
Dp/pol	pol/pol	Eggs	123	137	147	132	116	145	122	922	0·21	0·89	0·11	0·63	0·06	0·31
		Vials	315	351	360	399	284	327	308	2344						
pol/pol	pol/pol	Eggs	0	234	0	261	0	240	0	735	0·25	0·99	<0·01	0·97	0·00	0·04
		Vials	0	813	0	821	0	723	0	2357						
l(1)zw^{7p20}, y$^+$/y																
Dp/pol	pol/pol	Eggs	166	169	157	158	157	171	127	1105	0·18	1·20	−0·20	0·59	0·16	0·26
		Vials	324	380	379	395	230	366	263	2337						
pol/pol	pol/pol	Eggs	0	317	0	290	0	276	0	883	0·32	0·77	0·23	0·97	0·00	0·03
		Vials	0	755	0	751	0	696	0	2202						
Crosses to attached-XY/O males:																
l(1)zw^{3p12}, y$^+$/y																
Dp/pol	pol/pol	Eggs	138	148	145	139	162	174	151	1057	0·21	0·96	0·04	0·66	0·14	0·20
		Vials	300	321	293	333	289	372	277	2185						
pol/pol	Dp/pol	Eggs	108	120	125	134	175	139	75	876	0·28	0·89	0·11	0·59	0·29	0·12
		Vials	378	377	349	347	423	551	282	2707						
l(1)zw^{b23}, y$^+$/y																
Dp/pol	pol/pol	Eggs	127	132	117	129	143	143	125	916	0·18	1·21	−0·21	0·59	0·18	0·23
		Vials	354	387	347	396	314	429	276	2503						
pol/pol	Dp/pol	Eggs	116	133	119	122	156	162	126	934	0·23	0·95	0·05	0·64	0·20	0·16
		Vials	319	353	337	337	436	484	316	2582						
l(1)zw^{7p20}, y$^+$/y																
Dp/pol	pol/pol	Eggs	138	161	154	143	170	156	136	1058	0·20	0·81	0·19	0·75	0·07	0·18
		Vials	287	317	306	312	280	288	266	2056						
pol/pol	Dp/pol	Eggs	183	153	144	177	165	142	1	965	0·28	1·07	−0·07	0·42	0·42	0·16
		Vials	384	342	344	347	401	399	0	2217						

Column group notes: *Fraction of lethality that is:* Genotypic lethality, Non-genotypic lethality. *Fraction of genotypic lethality resulting from:* Lethality of mutant males, Lethality of mutant:Dp males, Lethality of other genotypes.

heterozygotes. In conclusion, maternal heterozygosity for *Df(1)K95*, unlike maternal heterozygosity for any of the other mutants, does cause an embryonic shift of the lethal phase.

(iii) *Crosses to* attached-XY/0 males

Unlike in the foregoing crosses, these crosses generate both *mutant/0*; non-*Dp* and *mutant/0*; *Dp* sons whether the mothers are simply heterozygous for the mutant or also carry the duplication. Although the offspring genotypes are identical, it should be noted for comparison of these results with the preceding ones that absence of a *Y* chromosome causes more severe position-effect-variegation. Thus, the viability of *mutant/0*; *Dp* sons is reduced and can be dependent on maternal genotype (Robbins, 1983). For *l(1)zw7^{g20}*, and to a lesser extent for *l(1)zw3^{b12}*, an effect of partial maternal insufficiency on the survival of the *mutant/0*; *Dp* sons is evident (Table 3), and all three mutants give generally lower viability of *mutant/0*; *Dp* sons than they did for *mutant/Y*; *Dp* sons.

These crosses yield quite different offspring classes than the crosses to *y/Y* males. Nevertheless, there is again no indication of a shift to earlier lethality when the mothers are partially defective (Table 1).

5. Discussion

The time at which gene activity is needed and the time at which reduced gene activity affects development are operationally distinct. The former can be defined by removing a gene, or by reducing its activity, at different times and asking whether the deficit eventually affects the organism. By this criterion, both maternal and zygotic function of most essential genes is required. The latter property, the time at which reduced gene activity has its effects, is less easily defined. It is impossible to be sure of the earliest time of effect since a metabolic or developmental defect at one stage may not be evident to an observer until some later time.

Maternal products of essential genes need to be present only in early development, probably prior to cellularization (Robbins, 1984), but do all maternal deficits only affect early embryos? I have examined this question by comparing when lethal progeny of partially defective mothers and of normal mothers die. Of the six *zeste-white* region mutants examined here, only one, *Df(1)K95*, evinced any shift to an earlier lethal phase when the mothers were heterozygous. Even in that case, however, death did not occur until embryogenesis was nearly completed. The maternal products of all of these genes have been shown to be needed early (Robbins, 1984), and we already know that maternal heterozygosity yields a severe enough deficit to affect the offspring (Robbins, 1980, 1983). It is obvious from these results, however,

that the effects resulting from a partial maternal deficit can be post-embryonic.

These results do appear to differ from those reported earlier by Garcia-Bellido *et al.* (1983) which suggested a shift to early lethality when females were heterozygous for a series of deficiencies. There are three likely sources for this discrepancy. Firstly, Garcia-Bellido and co-workers looked only at embryos and did not follow later development at all. Even the two deficiencies tested here gave mostly post-embryonic lethality. Thus, ignoring all offspring that complete embryogenesis may be grossly misleading. Secondly, they did not measure the frequency of unfertilized eggs. Instead, they assumed that the frequency of unfertilized eggs in all of their deficiency crosses was identical to that of a control. In our experiments, the measured frequency of unfertilized eggs varied from 1 to 24%. Any variation in frequency of unfertilized eggs would hopelessly confound interpretation of Garcia-Bellido and co-workers' data. Thirdly, they examined only deficiencies, generally large deficiencies, and their results may really be similar to those seen here for *Df(1)K95*. At the time of their experiments there was little suspicion that genes active both maternally and zygotically are ubiquitous, and use of deficiencies for a survey made good sense. Such genes are common, however, and the lethal-phase shift seen for deficiencies, including the one reported here for *Df(1)K95*, might indicate occasional genes whose maternal products are in fact used early, or might reflect cumulative effects of deficits for multiple maternal products which are used throughout embryonic and/or larval development. Resolving these possibilities must await testing mutations in each of the genes in a deficiency interval.

Homozygous germ-line clones of most *zeste-white* region mutations do not yield eggs, but complete maternal deficits for several *zeste-white* mutations cause early developmental defects (Garcia-Bellido & Robbins 1983): *zw1* germ-line clones survive, but the eggs are so defective that they are either not fertilized or never develop at all; clones of *zw10* yield few eggs, and the zygotes arrest early in development; *zw3* clones survive, but the embryos die; and eggs from *zw6* clones survive and are rescuable by a sperm-derived *zw6$^+$* allele. All of these loci are absent in either *Df(1)K95* or *Df(1)w$^{258-45}$*. Both deficiencies and three of the individual loci have been tested for embryonic effects of partial maternal deficits. Though maternal heterozygosity for each of these mutations affects zygotes (Robbins, 1983), maternal heterozygosity yields late-embryonic or post-embryonic lethality in every case. The embryonic lethality of zygotes derived from germ-line clones, therefore, does not imply an embryo-restricted role for the maternal transcripts of these genes. Rather, the embryonic lethality merely reflects early absence of what are more general, possibly even housekeeping, functions.

Much recent work (reviewed in Mahowald & Hardy, 1985) has focused on the striking embryonic phenotypes caused by maternal insufficiency of particular zygotic-lethal mutants. There are two ways that these embryonic effects might be viewed. These genes might encode multiple functions, some of which may be specific to a particular developmental step. Alternatively, these genes might be pleiotropic because they encode functions that interfere globally with normal development. An embryonic phenotype alone does not discriminate between these possibilities, and the delayed effects of partial maternal insufficiency of the *zeste-white* region genes underscores the unreliability of any such assumption. Until the stage specificity of a gene has been tested and proven, it is equally plausible to view their effects as markers of how the organism uses general genetic resources, as to view their effects as indices of how genes control development. Perhaps some new insights will arise if we do not enforce a view that every gene with a developmental effect is a developmental determinant.

Are maternally coded products used in the same fashion as later products of the same genes? The experiments reported here only address the simplest likely difference in the use of maternal and zygotic transcripts: an early embryo restriction in the use of maternal information. These experiments do not, however, resolve the broader question of whether maternal and zygotic transcripts generally serve interchangeable roles. To determine whether there is any general difference in the way maternal and zygotic information are used will require a much more detailed look, throughout the fly's life history, at the effects on lethal phenotype of variation of maternal and zygotic gene activity.

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