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Zygotic Lethal Mutations With Maternal Effect Phenotypes in *Drosophila melanogaster*. II. Loci on the Second and Third Chromosomes Identified by *P*-Element-Induced Mutations

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Manuscript received July 10, 1996
Accepted for publication September 17, 1996

ABSTRACT

Screens for zygotic lethal mutations that are associated with specific maternal effect lethal phenotypes have only been conducted for the *X* chromosome. To identify loci on the autosomes, which represent four-fifths of the Drosophila genome, we have used the autosomal "FLP-DFS" technique to screen a collection of 496 *P* element-induced mutations established by the Berkeley Drosophila Genome Project. We have identified 64 new loci whose gene products are required for proper egg formation or normal embryonic development.

THE genetic approach to identify the mechanisms underlying embryonic pattern formation in *Drosophila melanogaster* has led to a comprehensive view of the various steps involved in the establishment of the body plan (see reviews by INGHAM 1988; ST. JOHNSTON and NUSSLEIN-VOLHARD 1992). These analyses have demonstrated that the egg contains spatial cues that are deposited during oogenesis. Following fertilization these maternal cues regulate and coordinate the expression of a small number of genes that are further involved in controlling subsequent steps of body patterning. The identification of the maternal and zygotic gene products involved in specific patterning events has been the outcome of large genetic screens. Maternal functions have been identified via screens for female sterility, while zygotic genes have been detected via screens for embryonic lethal mutations (GANS *et al.* 1975; MOHLER 1977; NUSSLEIN-VOLHARD and WIESCHAUS 1980; JURGENS *et al.* 1984; NUSSLEIN-VOLHARD *et al.* 1984, 1987; WIESCHAUS *et al.* 1984; PERRIMON *et al.* 1986; SCHUPBACH and WIESCHAUS 1986, 1989). These screens have identified ~40 maternal and 140 zygotic functions that are instrumental in controlling specific embryonic decisions. This is a small number of genes considering that the Drosophila genome has been estimated to potentially code for 10,000–20,000 different transcripts (see JOHN and MIKLOS 1988).

The assumption underlying these screens is that the expression of genes that encode "decision making" functions is tightly restricted to the corresponding developmental stage. Indeed, some of the maternal gene functions could be missed if the gene products were used at multiple times during the development of the animal. For example, mutations in the *torso* gene, which is required for the establishment of the embryonic termini (NUSSLEIN-VOLHARD *et al.* 1987), would not have been isolated from screens for loci associated with female sterility if its product was also necessary zygotically for production of a viable animal. Similarly, some zygotic gene functions important for embryonic patterning can be missed if the gene is also expressed maternally because the maternal product can mask the zygotic requirement.

To determine whether some genes involved in critical patterning events have not been identified because of their developmental pleiotropy, a screen to analyze the maternal effects of *X*-linked zygotic lethal mutations has been conducted (PERRIMON *et al.* 1984, 1989). From this analysis, it has been estimated that gene activity of 75% of the essential loci is required for either the formation of a normal egg or of a wild-type larvae. This represents a significant fraction of the genome because in Drosophila it is estimated that 5000 loci are mutable to a visible phenotype and that 95% of these are essential for viability (see PERRIMON *et al.* 1989). From the *X*-linked studies, a number of zygotic lethal mutations have been identified that were associated with specific maternal effect phenotypes. Analyses of some of them have revealed that they encode components of the signaling machinery required for interpretation of the maternal/zygotic cues. The specificity of the embryonic phenotypes associated with these mutants reflects the selective utilization of the gene products by specific pathways. For example, genes involved in the transduction of the signal received by the Torso receptor tyrosine kinase (*D-raf, corkscrew, D-sor1,* see review by DUFFY and PERRIMON 1994) have been identified. In addition, mutations corresponding to molecules involved in *wingless* signaling (*dishevelled, porcupine* and *zeste-white 3,* see review by PERRIMON 1994) have been characterized. Another outcome of these screens has been the identifica-

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tion of novel regulatory networks. For example, mutations in a Drosophila *JAK* gene has been identified as a component of a system that regulates the expression of pair rule genes (BINARI and PERRIMON 1994).

The identification of zygotic lethal mutations with specific maternal effect phenotypes has only been conducted systematically on the *X* chromosome (PERRIMON *et al.* 1984, 1989), which represents one-fifth of the Drosophila genome. These screens have been possible because of the unique properties of the *X*-linked germline-dependent, dominant female sterile (DFS) mutation ovo^{D1} (BUSSON *et al.* 1983; PERRIMON and GANS 1983; PERRIMON 1984). This mutation allows the easy detection of female germline mosaics. Further, the application of the FLP-recombinase technology to promote chromosomal site-specific exchange (GOLIC 1991) to this system has led to the development of the "FLP-DFS" technique (CHOU and PERRIMON 1992; see accompanying paper) that allows the efficient production of germline mosaics. We have conducted a large screen using these chromosomes and have identified 64 new autosomal loci that encode gene functions involved in various steps of oogenesis and/or embryonic development.

MATERIALS AND METHODS

Production of germline mosaics using the autosomal FLP-DFS technique: Stocks used to generate germline clones (GLCs) are described in CHOU and PERRIMON (see accompanying paper). To generate homozygous GLCs of a specific mutation (*m*), ~15 females of genotype *CyO/FRT m* or *TM3, Sb/FRT m* were crossed with five males of genotype FLP^{12}/Y; $CyO/P[ovo^{D1}]$ *FRT* or FLP^{22}/Y; *TM3*, $Sb/P[ovo^{D1}]$ *FRT*. These males were generated by crossing females from the *y w* FLP^{12}; *CyO/Sco* and *y w* FLP^{22}; *TM3, Sb/CxD* stocks with the appropriate $P[ovo^{D1}]$ *FRT* males (CHOU and PERRIMON, see accompanying paper). Females were allowed to lay eggs for 1 day in glass vials and their progeny heat shocked twice for 2 hr at 37° in a circulating water bath over a period of 2 days when they reached late L2 to L3 larval stages. Subsequently, ~40 females of genotype *w/w FLP; FRT* $m/P[ovo^{D1}]$ *FRT* mated with either *CyO/FRT m* or *TM3, Sb/FRT m* males were analyzed for the presence of GLCs. All of the $P[ovo^{D1}]$ *FRT* recombinant chromosomes are associated with a fully penetrant DFS phenotype such that all eggs laid by these females are derived from germline recombination events. Due to the efficiency of the FLP-DFS technique (CHOU and PERRIMON, accompanying paper), the analysis of 40 females is usually sufficient to allow unambiguous determination of the GLC phenotype of the mutation tested.

Recombination of the *P*-element mutations on the *FRT* chromosomes: The collection of *P* element-induced mutations characterized by SPRADLING *et al.* (1995) as part of the Berkeley Drosophila Genome Project was obtained from the Bloomington Stock Center. These mutations are kept with either the *CyO* or *TM3, Sb* balancer chromosomes. The *P* elements carry either the *rosy* or *white* genes as markers. *P*-element insertions were recombined onto the *FRT* chromosomes. To facilitate the recombination events, we constructed different stocks that contain the FRT elements and dominant visible mutations. These are as follows: *Tft* $FRT^{2L\text{-}40A}/CyO$, *Sco* $FRT^{2L\text{-}40A}/CyO$, $FRT^{2R\text{-}G13}$ *L/CyO*, *Gl* $FRT^{3L\text{-}2A}/TM3$, *Sb*, *D* $FRT^{3L\text{-}2A}/TM3$, *Sb* and $FRT^{3R\text{-}82B}$ *Sb/TM6, Ubx*. The dominant markers we use are described in LINDSLEY and ZIMM (1992).

For *P*-element insertions on *2*L, males were collected from each of the *P*-element lines and crossed to *Tft* $FRT^{2L\text{-}40A}/CyO$ females. From this cross eight *Tft* $FRT^{2L\text{-}40A}/m$ were collected and crossed to *w; CyO/Sco* males, in G418 treated vials (0.25 g geneticin/40 ml dH_2O). *Cy*, Tft^+ recombinant males that survived the G418 selection were collected and mated in single pairs to *w; CyO/Sco* females. For *P*-element lines that were proximal to the *Tft* marker, six to 10 males that have lost the dominant marker were collected. *Tft* maps at 37A3-6. For *P*-element lines that were distal to the dominant marker, one to three males without the dominant marker were collected. To establish lines, *m* $FRT^{2L\text{-}40A}/CyO$ virgins and males from each of the potential recombinants were collected and backcrossed. The presence of the original *P*-element mutation on the *FRT* chromosome was determined by checking for the absence of homozygous animals in the stock. We also used a *Sco* $FRT^{2L\text{-}40A}$ chromosome for recombination, but realized that this chromosome carried a lethal mutation outside of the *Sco* region; therefore it was not used further.

For *P*-element insertions on *2*R, males were collected from each of the *P*-element lines and crossed to *w; L* $FRT^{2R\text{-}G13}/CyO$ females. From this cross eight *w; L* $FRT^{2R\text{-}G13}/m$ females were collected and crossed to *w; CyO/Sco* males. Putative *w; m* $FRT^{2R\text{-}G13}/CyO$ male recombinants with orange eyes from the *FRT* element that had lost the *L* dominant marker were selected and crossed as single pairs to *w; CyO/Sco* females. For *P*-element lines that were proximal to the dominant marker *L*, six to 10 putative *m* $FRT^{2R\text{-}G13}/CyO$ recombinant lines were established. For *P*-element lines that were distal to the dominant marker, one to three lines were established.

Recombinations of the *3*L *P*-element mutations followed the same strategy as described for *2*R recombinants. The stocks used for these experiments were *w; D* $FRT^{3L\text{-}2A}/TM3$, *Sb* and *w; TM3, Sb/Ly*. For *P*-element lines that were proximal to the dominant marker *D* (which is inseparable from *In(3L)69D3-E1; 70C13-D1*), 10–20 individual lines were established since it was found that the rate of recombination was significantly reduced with this chromosome. Otherwise, one to three lines were kept.

Recombinations of the *3*R *P*-element mutations followed the same strategy as described for *2*L recombinants. The stocks used for these experiments were *Sb* $FRT^{3R\text{-}82B}/TM6$, *Ubx* and *w; TM3, Sb/Ly*. For *P*-element lines that were proximal to the dominant marker *Sb* (at 89B), six to 10 males that lost the dominant marker were collected. For *P*-element lines that were distal to the dominant marker, one to three males that lost the dominant marker were collected. The G418 selection used was 1.0 g geneticin/40 ml dH_2O.

It should be noted that we usually analyzed a single *FRT m* recombinant line for each mutation. Thus, if the original mutant chromosome carried an additional mutation, the GLC phenotype would represent the phenotypes of both mutants. We believe that this is not a great concern as many of the *P* elements that we analyzed have been shown to revert to wild type (Berkeley Drosophila Genome Project, personal communication).

Determination of the GLC phenotype of the zygotic lethal mutations: *P* element-induced zygotic lethal mutations were classified into three groups based upon their GLC phenotypes. Group 1 corresponds to those that do not lay eggs. Group 2 corresponds to those where eggs are laid and most hatch, and Group 3 corresponds to those that do not lay eggs but possess developed ovaries, those that lay abnormal eggs, or those where at least 20% of the eggs fail to hatch. Mutations that belong to Group 3 were set up a second time for GLC analyses and the embryonic phenotypes further characterized.

Females carrying homozygous mutant GLCs are crossed with either *CyO/FRT m* or *TM3, Sb/FRT m* fathers. Two classes of embryos are produced: embryos that lack both maternal

and zygotic copies of the wild-type gene, and embryos that lack only the maternal copy. To determine the extent to which the introduction of a wild-type copy of the gene from the father influences the maternal effect phenotype, the embryonic phenotypes of 200 eggs derived from females carrying homozygous GLCs crossed with either *m*/+ or +/+ males were examined. In addition, to distinguish between an embryonic zygotic lethal mutation with no maternal effect and a fully paternally rescuable maternal effect, the stages of lethality of the zygotic lethal mutations were determined. In the case of an embryonic lethal mutation that does not exhibit a maternal effect phenotype, 50% embryonic lethality is observed when females with *m/m* GLCs are crossed with heterozygous +/*m* fathers and 0% when crossed with wild-type +/+ fathers.

Lethal phase determination: To determine the stages of lethality (*i.e.*, lethal phases) of the mutations associated with zygotic lethality, a minimum of 200 eggs from parents of genotype +/*m* are lined up on an agar plate and allowed to develop at 25°. Since embryos that are homozygous for the *CyO* and *TM3, Sb* balancer chromosomes are associated with embryonic lethality, we first outcrossed each individual stock with a wild-type strain to eliminate the balancer chromosome. After a period of 24 hr, the number of unhatched eggs are counted. The number of unhatched and unfertilized eggs was determined after dechorionation with a 50% bleach solution (PERRIMON *et al.* 1989). If 25% of the developed eggs fail to hatch, it indicates that the mutation is associated with embryonic lethality. The zygotic phenotype of the mutation was examined by cuticle preparation of the eggs. The development of hatched larvae is followed at 25° and examined every 2 days. During this time the number of dead larvae are scored as well as the number of pupae that form. After 11 days of development, emerging adults are counted and the number of dead pupae scored.

Examination of embryos: Larval cuticles were prepared in Hoyers' mountant as described by VAN DER MEER (1977). Cuticles were examined using dark-field and phase illumination.

RESULTS

Analysis of *P*-element mutations: To identify autosomal zygotic lethal mutations (*m*) associated with novel maternal effect lethal phenotypes, we analyzed the collection of *P*-element mutations, established by SPRADLING *et al.* (1995) as part of the Berkeley Drosophila Genome Project. We recombined each *P*-element mutation with the *FRT* element located on the same chromosomal arm by meiotic recombination (see MATERIALS AND METHODS). Then, we prepared GLCs for each of the *m FRT* chromosomes (see MATERIALS AND METHODS for protocols) and analyzed the phenotypes of eggs derived from the clones. The 496 mutations examined were classified into three groups (Table 1) based on their GLCs phenotypes.

Group 1 corresponds to those mutations that do not lay eggs and do not possess obvious developed egg chambers. This group corresponds to gene functions that are required for germ cell viability or early oogenesis. We found that 154 mutations (31%) fell into this class.

Group 2 corresponds to those mutations that lay eggs where most of the eggs hatch. This group corresponds to genes that are either not expressed maternally or genes whose expression during oogenesis is not critical to embryonic development. One hundred ninety-nine of the mutations (40%) examined fell into this class.

Group 3 corresponds to those mutations that do not lay eggs but possess developed ovaries as well as those where a substantial fraction of the eggs were either abnormal or failed to hatch. We placed 143 mutations (29%) in this group. A number of additional tests were done with these mutants to further characterize the phenotype of the clones. These include the determination of the stages at which the mutations cause zygotic lethality. This test is especially important because it allows us to distinguish between embryonic lethal mutations with no maternal effect and zygotic lethal mutants associated with a fully paternally, rescuable maternal effect phenotype (see MATERIALS AND METHODS). In addition, we carefully determined the extent of the paternal contributions to the maternal effect phenotypes. This was done by examining eggs derived from females that carry homozygous GLCs crossed to either wild-type males or males heterozygous for the mutation tested.

The 143 mutations in this group were set up for GLC analysis at least one additional time and the specificity and penetrance of the phenotypes examined carefully. From the original collection of 143, 78 were associated with distinctive phenotypes (Table 2, Figure 1) and were thus retained. Results from the screen are shown in detail in Tables 1, 2 and Figure 1. Among the 143 Group 3 mutants, 65 had very variable phenotypes and were discarded, 18 were associated with oogenesis defects (AO), nine were embryonic lethals with no maternal effects (NME) and 51 were associated with maternal effects (ME, PMER, FMER, or WME). The GLC phenotypes associated with several of the zygotic lethal mutations were not fully penetrant (Table 2). Nevertheless, we have included them in this analysis because >30% of unhatched embryos with specific mutant phenotypes are obtained from females with GLCs. The variability of the maternal effects observed may reflect the residual activity associated with the *P*-element mutations analyzed or the simultaneous occurence of both GLCs and presence of follicle cell clones (see DISCUSSION). Regardless, it suggests that the gene function is involved in a specific developmental processs.

Of the 18 loci in which GLC analysis revealed an abnormal oogenesis (AO) phenotype, we identified two previously known genes based on informations available in Flybase. One of these is *squid,* which encodes a putative RNA binding protein involved in the establishment of the dorsoventral axis of the egg chamber (KELLEY 1993). The other is an allele of the *Protein kinase A* catalytic subunit (*Pka*), which plays a role in the reorganization of the microtubule network during mid-oogenesis (LANE and KALDERON 1994; RONGO and LEHMANN 1996). The functions of the remaining 16 genes have not been characterized.

For the AO phenotype, females with GLCs develop

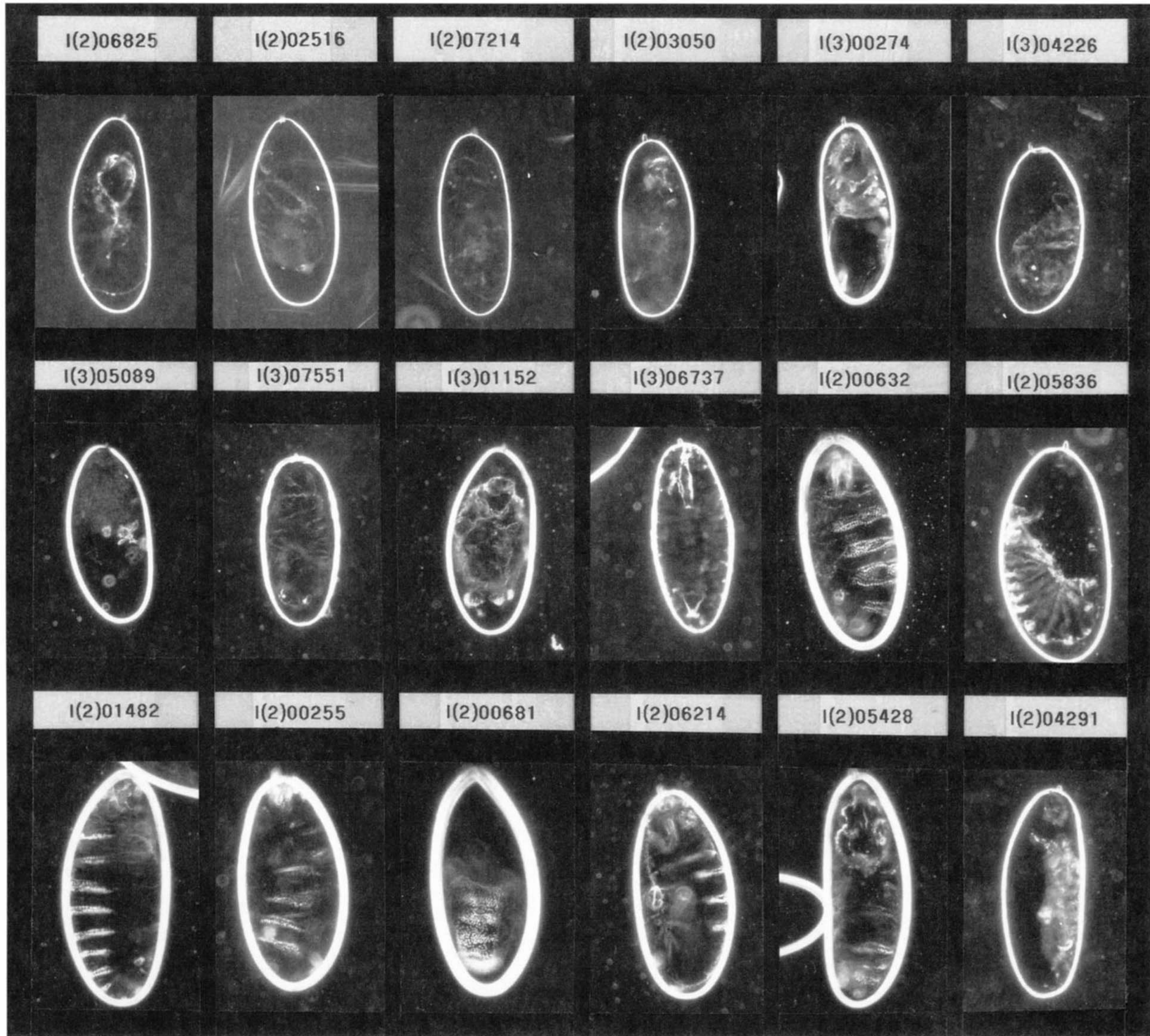

FIGURE 1.—Embryonic phenotypes. Dark field micrographs of the embryonic phenotypes associated with the mutations in loci identified in the screen. Not shown in this figure are the phenotypes of the mutants identified in previous screens. Anterior is up in all panels.

vitellogenic oocytes but lay either few eggs, no eggs, collapsed eggs, small eggs, or eggs with defective appendages. Based on the diversity of these phenotypes, these genes likely function in a variety of developmental processes. They could identify functions that operate specifically during vitellogenesis, oocyte/follicle cell interactions, nurse cell/oocyte cytoskeletal organization and/or dumping of nurse cell contents into the oocyte. It should be emphasized that, because we did not systematically examine the females that laid no eggs for the presence of vitellogenic oocytes, it is likely that a small number of mutants that have been classified to Group 1 (*i.e.*, required for germ cell viability) belong to the "Group 3 AO class".

Females bearing GLCs of mutations in eight loci were identified where normal eggs are laid, however, no or few signs of cuticle development are subsequently observed. These mutants may identify gene functions essential for fertilization and/or completion of meiosis. In addition, we expect that mutations that perturb molecules that participate in early cell division, blastoderm formation and/or early gastrulation would lead to the absence of cuticle formation. Three of the 8 mutants identified in this group (*l(2)09373*, *l(3)04629* and *l(3)01207*) were not fully penetrant.

Females bearing GLCs of mutations in 10 loci were identified where normal eggs are laid but poor or very disorganized cuticle development are subsequently observed. We have distinguished this group of mutants from the next category (patterning mutants) as they may identify gene functions more specifically involved in morphogenesis events such as the formation and maintenance of epithelia and cell junctions. Interestingly, three out of the 10 mutations identified in this

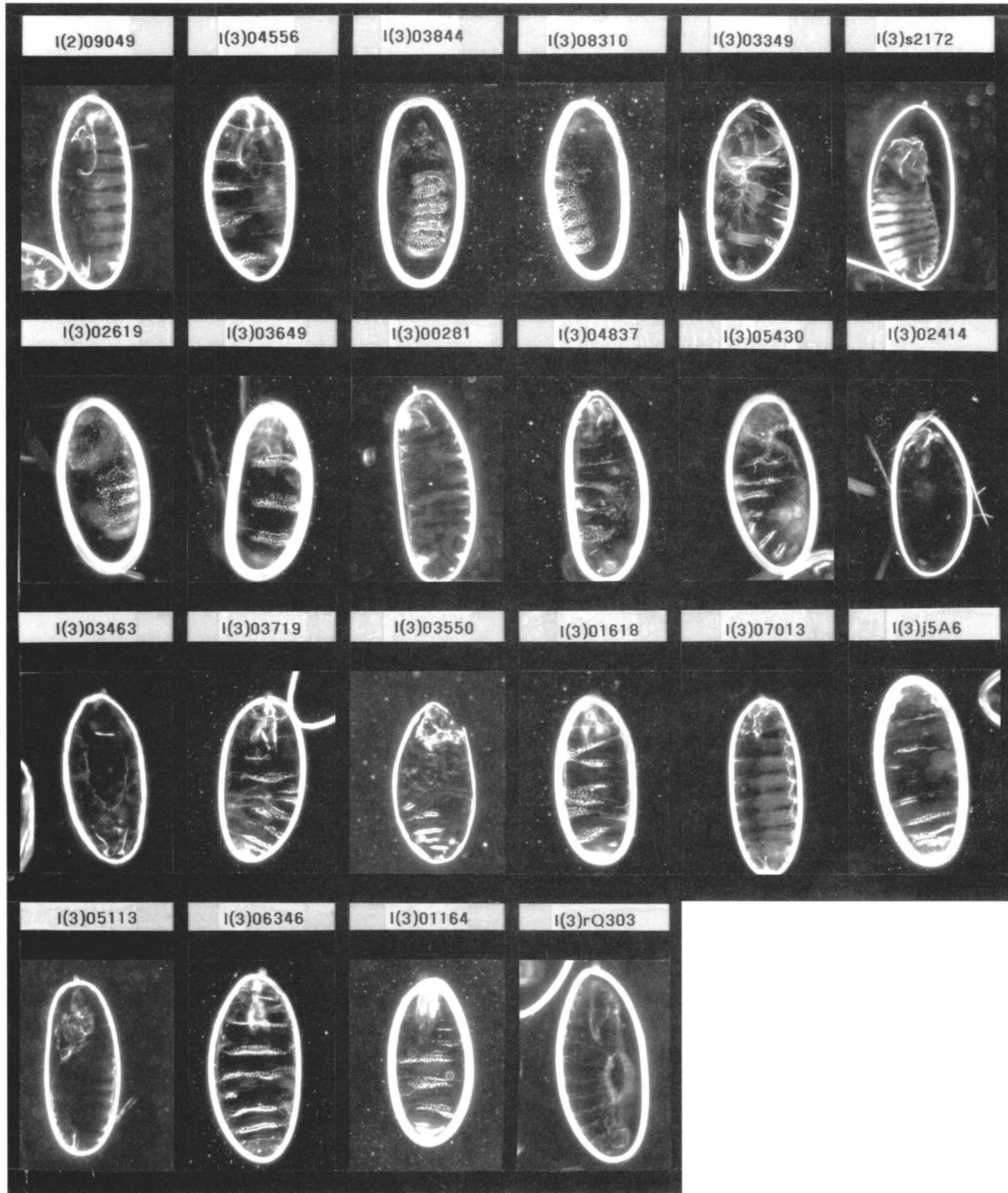

FIGURE 1.—*Continued*

group are embryonic lethals. While two of these produce embryos that do not exhibit obvious cuticular phenotypes (*l(2)01152* and *l(3)06737*), for the third, *l(2)06825*, animals derived from heterozygous mothers die as embryos and exhibit a poorly organized cuticle (data not shown). The embryonic phenotype of unrescued embryos derived from *l(2)06825* GLCs, however, is much more severe (Figure 1).

Mutations in 42 loci were identified that laid normal eggs but produce embryos with specific cuticular patterning defects. Various patterning processes such as segmentation, dorsal closure or head formation are represented in this group of mutants. Among these mutations, we identified nine loci that are associated with embryonic lethality but do not exhibit a maternal effect phenotype. Mutations in these loci (*wingless, gooseberry,*

TABLE 1

Results from the screen

	Total	No. in group 1	No. in group 2	No. in group 3
2L	90	34	34	22
2R	122	42	49	31
3L	116	44	43	29
3R	168	34	73	61
Total	496	154	199	143

The GLC phenotypes of the mutations were classified into three groups. group 1: No eggs were laid and no obvious developed eggs could be found following brief inspection of the adult abdomen. group 2: Most eggs hatch. group 3: Oogenesis defects in which no eggs are laid but vitellogenic oocytes are present, as well as those where a substantial fraction of the eggs were either abnormal or failed to hatch.

hairy, string, schnurri, spitz, trachealess, zipper and *serpent*) have been previously characterized in screens for embryonic lethal mutants (Jurgens *et al.* 1984; Nusslein-Volhard *et al.* 1984).

Among the mutations that exhibit maternal effect phenotypes, we identified two previously known genes, *thickveins* and *punt,* where embryos derived from GLCs exhibit embryonic defects along the dorsoventral axis. These genes have been shown to be involved in signaling by Decapentaplegic (Nellen *et al.* 1994; Letsou *et al.* 1995; Ruberte *et al.* 1995). In addition, we characterized an allele of *Downstream of receptor tyrosine kinase* (*Drk,* Olivier *et al.* 1993; Simon *et al.* 1993) that has been shown to be involved in patterning of the embryonic termini (Hou *et al.* 1995).

Four new loci with segment polarity GLC phenotypes (*l(2)00681, l(3)03844, l(3)08310, l(3)02619*) were recovered. These genes may be implicated in aspects of Wingless or Hedgehog signaling (see Perrimon 1994 for review).

Fifteen loci were associated with deletions of segments. Three mutations exhibited a pair rule-like phenotype (*l(3)03649, l(2)00632, l(3)04556*) while others were associated with variable deletions of segments (*l(2)00255, l(2)06214, l(3)04837, l(3)02414, l(3)03463, l(3)03719, l(3)03550, l(3)01618, l(3)07013, l(3)06346, l(3)j5A6* and *l(3)01164*). Within this group, the only locus that has been previously characterized is *l(3)06346,* which we have shown encodes a Drosophila STAT protein, Marelle, which operates in the Hopscotch/JAK signaling pathway (Hou *et al.* 1996). Characterization of the functions of the other loci may identify maternally stored proteins that act in concert with gap or pair rule zygotic genes to specify proper segmentation.

Various phenotypes are exhibited by the remainer of the loci. These include head and dorsal open phenotypes (*l(2)05836, l(3)00281*), head defects (*l(3)s2172, l(3)05113*), subtle head and tail defects (*l(2)01482*), twisted or tail on dorsal side phenotypes (*l(2)06214, l(3)03349, l(3)05430*), ventral hypoderm absent (*l(2)04291*), necrotic patch in the tail region (*l(2)09049*), and dorsal defects in the amnioserosa (*l(3)rQ303*).

DISCUSSION

In this paper we report the results of a screen to identify the maternal effect of autosomal zygotic lethal mutations. We have analyzed a collection of 496 autosomal *P* element-induced mutations generated by the Berkeley Drosophila Genome Project and identified a number of mutants associated with novel maternal effect phenotypes. Seventy-eight of the mutations were selected for detailed analysis of their GLC phenotypes because they were found to generate reproducible and penetrant phenotypes. Fourteen of them disrupted the gene activity of previously known genes while others appear to identify loci not previously characterized based on the information available from the Berkeley Drosophila Genome Project (see Table 2).

The collection of *P* element-induced mutations was chosen for analysis because it provides a number of advantages over a collection of randomly induced mutations (Spradling *et al.* 1995). First, the *P* element-induced mutations have been mapped cytologically facilitating further genetic characterization of the loci. Second, zygotic lethality has been shown in most cases to be associated with the *P*-element insertion (Berkeley Drosophila Genome Project, personal communication), thus we expect the mutant phenotype identified in GLCs to map at the site of the *P*-element insertion. Third, the availability of *P* insertions will greatly facilitate future molecular characterization of the genes.

It is of interest to compare the results from this screen with results obtained from the previous analysis of *X*-chromosome loci (Perrimon *et al.* 1989). These numbers compare as follows: cell lethal mutations *X*-chromosome screen (*X:* 40%), autosome screen (A: 31%), mutations with no maternal effect (*X:* 31%, A: 40%), abnormal oogenesis (*X:* 8.5%, A: 3.6%), mutations with maternal effects (*X:* 9.5%, A: 10.2%), or with variable maternal effects (*X:* 11%, A: 15.2%). Numbers regarding the *X*-chromosome study are obtained from Table 4 of Perrimon *et al.* (1989) that was derived from the analysis of 211 random *X*-linked lethal mutations. Numbers regarding the autosome screen are derived from the data presented in Tables 1 and 2. The numbers obtained from the different screens are quite similar and differ only for the class of mutations that result in AO phenotypes. This class is underrepresented in the autosomal screen because we did not systematically examine females that lay no eggs for oogenesis defects. Further, the frequency of mutant loci associated with cell lethality *vs.* no maternal effect is higher in the EMS collection. This may reflect the nature of the mutagen used in inducing the mutations. The *X*-chromosome study screen utilized EMS-induced mutations, while we used *P* element-induced mutations in the present analy-

TABLE 2

Description of the mutants

Gene name Location	Mutation tested Lethal phase	Stock number (Bloomington number) Germline clone phenotype	References Classification
		I. Abnormal eggs	
 21F1–2	*l(2)06955* L2	P2330 Misshapened eggs	 AO (100)
*Pka** 30C1–2	*l(2)01272* E–L1	P1030 Eggs collapsed	3 AO (100)
 49D1–3	*l(2)04329* L2–3	P1371 Few tiny collapsed eggs	 AO (100)
 58F1–2	*l(2)rG270* L1–2	P2060 Few collapsed eggs	 AO (100)
 64E08-12	*fs(3)07084* L1–2	P1713 No eggs laid, few vitellogenic oocytes	 AO (100)
 68A04–05	*l(3)j9B4* L3–P	P2082 No eggs laid, vitellogenic oocytes	 AO (100)
 72D01–02	*l(3)03802* Pol	P1607 Tiny eggs, few eggs laid	 AO (100)
 82F8–9	*l(3)09904* E (no defects)	P1740 Collapsed eggs	 AO (100)
*squid** 87F2–3	*l(3)j6E3* L3–P	P2133 Small dorsalized eggs	10 AO (100)
 87F3–4	*l(3)j4B4* L2–P	P2134 Arrest during vitellogenesis	 AO (100)
 88A4–5	*l(3)j1E7* Pol	P2135 Tiny collapsed eggs	 AO (100)
 91F10–11	*l(3)02102* L1–2	P1555 Few eggs laid, defective appendages	 AO (100)
 93B8–11	*l(3)07086* L1–2	P1714 Few abnormal eggs laid	 AO (100)
 93C1–2	*l(3)j2D1* L1–2	P2148 Collapsed eggs	 AO (100)
 93D4–7	*l(3)05241* L3–P	P1654 Few eggs laid, vitellogenic oocytes	 AO (100)
 93E1–2	*l(3)03852* Pol	P1612 Eggs with fused filaments	 AO (100)
 94E3–7	*l(3)03921* E (head defects)	P1614 Few eggs laid, vitellogenic oocytes	 AO (100)
 95F11–12	*l(30)07207* E (ventral hypoderm absent)	P1718 Small collapsed eggs	 AO (100)
		II. Normal eggs with no cuticle development	
 30A3–5	*l(2)01351* L1	P1045 No cuticle	 ME (100/100)
 32E1–2	*l(2)04431* P–A	P1375 No cuticle	 ME (100)
 60B10–11	*l(2)09373* E–L1–2	P2361 84% with no cuticle, others have variable defects	 ME (86/76)
 74B01–02	*l(3)01658* L3	P1542 No cuticle	 ME (100/100)
 86E16–19	*l(3)04629* L1–2	P1634 68% with no cuticle, others have variable defects	 ME (82/100)
	l(3)05203	P1652	

TABLE 2

Continued

Gene name Location	Mutation tested Lethal phase	Stock number (Bloomington number) Germline clone phenotype	References Classification
		II. Normal eggs with no cuticle development	
89B12–13	L1–2	No cuticle	ME (100/100)
	l(3)j2B10	P2142	
90F6–7	No cuticle	ME (100/100)	
	l(3)01207	P1524	
96B10–11	E (abnormal denticle bands)	No cuticle	ME (99/95)
		III. Poor cuticle development	
	l(2)06825	P2324	
29F1–2	E (poor cuticle development)	Very poor cuticle development	FMER (50/5)
	l(2)02516	P1198	
48C1–2	L1–2	Poor cuticle development, U shaped	ME (100/100)
	l(2)07214	P2339	
51B7-10	P	Unrescued poor cuticle development, rescued head and tail defects	PMER (100/80)
	l(2)03050	P1261	
57B13–14	P	Poor cuticle development, paternally rescued embryos show variable cuticle defects	WME (81/63)
	l(3)00274	P1491	
73B01–02	L1–3	Poor cuticle development	PMER (88/87)
	l(3)04226	P1627	
89B6–7	Pol	Poor cuticle development	ME (100/100)
	l(3)05089	P1646	
91A1–2	L2	Poor cuticle development	ME (100/100)
	l(3)07551	P1721	
91B5–6	L2	Poor cuticle development	ME (100/100)
	l(3)01152	P1522	
95B5–6	E (no cuticle defects)	Poor cuticle development	PMER (96/36)
	l(3)06737	P1697	
95E1–2	E (no cuticle defects)	Poorly differentiated cuticle	MER (59/15)
		IV. Patterning defects	
	l(2)00632	P944	
23C1–2	L1–2	20% of embryos with variable pair rule phenotype	WME (83/65)
*thick veins**	*l(2)04415*	P1373	1
25D1–2	L2	Ventralized embryos	PMER
*wingless**	*l(2)02657*	P1205	2
27F1–2	E	Segment polarity mutant, mirror image duplication of denticle bands	NME
	l(2)05836	P1451	
28E1–2	L–P	Head and dorsal open	FMER (56/3)
	l(2)01482	P1062	
29C3–5	E–L1	Subtle head and tail defects	PMER (91/44)
	l(2)00255	P936	
33E7–8	L1–2	Variable segmentation defects	PMER (100/83)
*spitz**	*l(2)s3547*	P2049	2
37F1–2	E	Deletion of median parts of all denticle belts	NME
*schnurri**	*l(2)04738*	P1386	2, 4, 5
47E1–2	E	Dorsal hypoderm absent	NME
*drk**	*l(2)10626*	P2378	6, 7, 8
50A12–14	L	Defects in both head and tail regions	FMER (45/5)
	l(2)00681	P949	

TABLE 2

Continued

Gene name Location	Mutation tested Lethal phase	Stock number (Bloomington number) Germline clone phenotype	References Classification
		IV. Patterning defects	
51B1–5	L–P	Segment polarity mutant, unrescued: mirror image duplication of denticle bands, rescued: wild type	FMER (57/5)
	l(2)06214	P1469	
53C1–2	P	Tail on the dorsal side	WME (66/38)
	L(2)05428	P1407	
53C9–10	L1	Variable segmentation defects, head defects	WME (67/42)
	l(2)04291	P1369	
53F1–2	L1–2	Ventral hypoderm absent	PMER (100/100)
	l(2)09049	P2360	
59F1–2	L2	Small necrotic patch in the tail region	FMER (47/0)
*gooseberry**	*l(2)01155*	P999	2
60F1–3	E	Segment polarity mutant, mirror image duplication of denticle bands	NME
*zipper**	*l(2)02957*	P1215	2
60F1–3	E–L	Dorsal closure defective	NME
*trachaeless**	*l(3)10512*	P1747	9
61C01–02	E	No trachea	NME
	l(3)04556	P1633	
64C01–02	Pol	Variable pair rule segmentation defects	WME (75/50)
	l(30)03844	P1610	
65C1–2	L3–P	Segment polarity mutant, unrescued: mirror image duplication of denticle bands, rescued: wild type	FMER (58/1)
	l(3)08310	P1731	
65D4–5	L1–2	Segment polarity mutant, unrescued: mirror image duplication of denticle bands, rescued: partial fusion of denticle bands or wild type	PMER (85/30)
*hairy**	*l(3)08247*	P1730	9
66D10–12	E	Pair rule segmentation defects	NME
	l(3)03349	P1587	
66E06–07	P	Embryos twisted, variable cuticle differentiation	WME (36/13)
	l(3)s2172	P2089	
71B04–05	L1–2	Head open	WME (38/25)
	l(3)02619	P1572	
74C01–02	L2–3	Segment polarity mutant, unrescued: mirror image duplication of denticle bands, rescued: partial fusion of denticle bands or wildtype	PMER (81/25)
	l(3)03649	P1598	
75D04–05	L2–3	Pair rule segmentation defects	ME (100/100)
	l(3)00281	P1492	
85B8–9	L3–P	Head defects, variable dorsal open	WME (30/16)
	l(3)04837	P1639	
85D5–6	L1–2	Variable segmentation defects	WME (44/38)
	l(3)05430	P1659	
85D8–9	Pol	Embryos twisted, variable cuticle differentiation	WME (56/43)
	l(3)02414	P1568	
85F12–13	Pol	No abdominal segments, poor layer	ME (100/100)
	l(3)03463	P1590	
87D7–9	Pol	Ventral holes, segment fusion	ME (100/100)
*punt**	*l(3)10460*	P1745	9, 11, 12
88C9-10	E (dorsal open)	Ventralized embryos	PMER (100/100)
	l(3)03719	P1605	

TABLE 2

Continued

Gene name Location	Mutation tested Lethal phase	Stock number (Bloomington number) Germline clone phenotype	References Classification
		IV. Patterning defects	
88D1-2	P	Varible segmentation defects	ME (99/100)
	l(3)03550	P1594	
88E8-9	L3-P	Anterior head defects, variable abdominal segmentation defects	WME (38/49)
	l(3)01618	P1539	
89A8-9	L1-2	Variable segmentation defects	MER (53/8)
*serpent**	*l(3)01549*	P1538	9
89B1-3	E	Tail region remains on dorsal side	NME
	l(3)07013	P1711	
91F1-5	L2	Head defects and variable segmentation defects	FMER (49/7)
	l(3)/5A6	P2144	
91F10-11	L3/P	Variable deletions of denticle belts, head defects	ME (61/83)
	l(3)05113	P1647	
92A13-14	E-L1 (no cuticle defects)	Head defects	MER (54/17)
marelle	*l(3)06346*	P1681	20
92E2-4	L	Defects in T2, A5 and A8 mainly	PMER (100/100)
	l(3)01164	P1523	
93B1-2	E (no cuticle defects)	Variable segmentation defects	FMER (58/9)
	l(3)rQ303	P2154	
95D1-2	L1-2	Amnioserosa defects	MER (51/13)
*string**	*l(3)01235*	P1525	9
99A5-6	E	Some denticle rows are missing	NME

Since it is likely that some of these genes are currently under investigation in other laboratories, we have decided not to give descriptive names to the loci at the present time. We feel it is more appropriate that these genes, following further characterization, be renamed in subsequent publications. The numbers in parentheses indicate the specificity of the mutant phenotypes. AO (100) indicates that the abnormal oogenesis phenotype is 100% penetrant. The ratio (A/B) of embryonic lethality observed from females with GLCs crossed with either heterozygous (A) or wild-type (B) males is shown. This ratio allows us to distinguish between the various classes of maternal effects (ME, PMER, FMER, WME or NME). Classification of the GLC phenotypes is as follows: Abnormal Oogenesis (AO), Maternal Effect (ME), Partially (P) or Fully (F) Paternally Rescuable Maternal Effect (MER), Weak Maternal Effect (WME), No Maternal Effect (NME). Lethal phases: Embryonic (E), Larval (L1, L2 and L3) and Pupal (P) stages, Polyphasic (Pol). * Previously known genes. References: Indicated is the original reference that describes the gene identified by *P*-element insertion as well as references on the GLC phenotypes, when available: 1, Nellen *et al.* (1994); 2, Nusslein Volhard *et al.* (1984); 3, Lane and Kalderon (1994); 4, Grieder *et al.* (1995); 5, Arora *et al.* (1995); 6, Olivier *et al.* (1993); 7, Simon *et al.* (1993); 8, Hou *et al.* (1995); 9, Jurgens *et al.* (1984); 10, Kelley (1993); 11, Ruberte *et al.* (1995); 12, Letsou *et al.* (1995).

sis. It is unlikely that these discrepencies reflect the number of mutations present on the chromosome as both the EMS- and *P* element-induced collections of mutants contain few chromosomes with multiple hits (N. Perrimon, unpublished data; Berkeley Drosophila Genome Project, personal communication). Because *P*-element insertions appear to preferentially insert near the 5′ end of transcription units (Spradling *et al.* 1995), one might anticipate that the ratio of genetic null *vs.* hypomorphic mutations to be different.

We have included in Table 2 a number of *P* element-induced mutations that exhibit weak maternal effect phenotypes. The nature of the variability associated with these mutations is unclear. Perhaps these *P* element-induced mutations have residual activity raising the possibility that a stronger allele may exhibit a more severe or penetrant GLC phenotype. Alternatively, the variability in penetrance of the mutant phenotype may correspond to the simultaneous occurence of follicle cell clones. A number of signaling pathways involved in embryonic patterning operate between the follicle cells and the oocyte (review by Rongo and Lehmann 1996), thus, one expects to identify a number of mutants that in follicle cell clones would disrupt embryonic patterning. Although it is clear that mutants with fully penetrant GLC phenotypes affect germline specific functions, it remains to be determined whether some of the mutants with variable phenotypes may actually correspond to the simultaneous occurence of clones of mutant follicle cells. Follicle cell clones can be induced following *FLP*-mediated mitotic recombination (Harrison and Perrimon 1993; Xu and Rubin 1993; Margolis and Spradling 1995). However, it remains to be determined how often follicle cell clones are induced

under the heat shock conditions we used (see also HARRISON and PERRIMON 1993 for DISCUSSION). One solution to avoid the occurence of follicle cell clones simultaneously with the GLCs would be to use a *FLP*-recombinase that only expresses in the germline.

In this study we analyzed 496 independent zygotic lethal mutations identified by single *P*-element mutations. It is of interest to estimate the scope of this analysis with regard to the level of saturation achieved. For the entire Drosophila genome, BRIDGES (1938) drew 5059 bands. The counts for each chromosome are as follows: *X* chromosome, 1012 bands; *2*L chromosomal arm, 804 bands; *2*R chromosomal arm, 1136 bands; *3*L chromosomal arm, 1178 bands; *3*R chromosomal arm, 884 bands; and 45 bands are on the fourth chromosome. The number of autosomal loci that mutate to zygotic lethality can be easily estimated from studies on the *X* chromosome. Previously, we (PERRIMON *et al.* 1989) and others (LEFEVRE and WATKINS 1986) estimated that there are ~540 vital loci among the 1012 bands on the *X* chromosome. Extrapolation to the autosomes estimate that 1048 vital loci are linked to the second chromosome and 1114 are linked to the third. In the present study we analyzed 496 independent *P*-element mutations thus representing a 23% level of saturation.

This analysis, in combination with previous studies of *X*-linked mutations that represented an 86% level of saturation (PERRIMON *et al.* 1989), indicates that 36% of loci that mutate to zygotic lethality have been analyzed in GLC analysis. The identification of additional loci will require additional screens. Our laboratory is currently completing a large EMS screen to achieve this goal.

The array of mutant phenotypes we recovered from the screen is diverse and similar to some extent with the previous screen conducted on the *X* chromosome (PERRIMON *et al.* 1989). Of special interest to our laboratory is the recovery of four new segment polarity loci. The recovery of a large number of new mutants associated with this phenotype was predicted from the *X*-chromosome screen as the previous study led to the identification of five *X*-linked segment polarity genes (*armadillo, zeste-white 3, dishevelled, porcupine* and *fused*). Because the autosomal screen is not to saturation, we expect that at least 15 additional loci with segment polarity phenotypes could be identified using the GLC approach.

The GLC approach has led to the identification of many components of signaling pathways that are activated during embryogenesis. The types of molecules encoded by these genes range from kinases [D-raf (AMBROSIO *et al.* 1989), D-sorl (TSUDA *et al.* 1993), Zeste-white 3, a.k.a. Shaggy (BOUROUIS *et al.* 1990; SIEGFRIED *et al.* 1990), Fused (PREAT *et al.* 1990), Hopscotch (BINARI and PERRIMON 1994), Hemiapterous, a.k.a. 7P1 (GLISE *et al.* 1995), Discs large (WOODS and BRYANT 1991), Pka (LANE and KALDERON 1994)], receptors (Punt, Thickveins, see references in Table 1), phosphatases [Corkscrew (PERKINS *et al.* 1992)], adaptor protein (Drk, see references Table 1), cytoskeletal proteins [Armadillo (PEIFER and WIESCHAUS 1990)], RNA binding protein (Squid, see reference in Table 1), transcription factors [Ultraspiracle (ORO *et al.* 1990), Marelle (HOU *et al.* 1996)] and novel proteins [Dishevelled (KLINGENSMITH *et al.* 1994), Porcupine (KADOWAKI and PERRIMON, unpublished data), Brainiac, a.k.a. 6P6 (GOODE *et al.* 1996a), Egghead, a.k.a. Zw4 (GOODE *et al.* 1996b)]. The specificity of the GLC mutant phenotypes reflect the specific utilization of these maternal gene products by signaling pathways activated by a small number of zygotic genes. The mutant phenotypes of the loci we have identified mimic the phenotypes of the zygotic genes because at the time we examine the embryos, the gene products have not been utilized by other pathways. Alternatively, the specificity of the embryonic phenotypes may reflect the strength of the allele, as different signaling pathways may be more or less sensitive to a certain amount of gene product. Interestingly, many of the maternal effects we recovered are fully or partially paternally rescuable. The extent of the paternal rescue reflects a combination of when during embryogenesis the gene product is utilized and the onset of zygotic transcription.

We are indebted to TZE BIN CHOU for developing the "autosomal FLP-DFS technique" that made this work possible. We thank K. MATTHEWS for sending us the collection of *P*-element lethal mutations, LIZ PERKINS and SCOTT GOODE for comments on the manuscript. This work was supported by a grant from the National Science Foundation and the Howard Hughes Medical Institute. N.P. is an Associate Investigator of the Howard Hughes Medical Institute.

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Communicating editor: R. E. DENELL