An F_1 Genetic Screen for Maternal-Effect Mutations Affecting Embryonic Pattern Formation in *Drosophila melanogaster*

Stefan Luschnig,[1] Bernard Moussian, Jana Krauss, Isabelle Desjeux,[2] Josip Perkovic[3] and Christiane Nüsslein-Volhard

Max-Planck-Institut für Entwicklungsbiologie, Abteilung Genetik, D-72076 Tübingen, Germany

Manuscript received November 11, 2003
Accepted for publication January 29, 2004

ABSTRACT

Large-scale screens for female-sterile mutations have revealed genes required maternally for establishment of the body axes in the Drosophila embryo. Although it is likely that the majority of components involved in axis formation have been identified by this approach, certain genes have escaped detection. This may be due to (1) incomplete saturation of the screens for female-sterile mutations and (2) genes with essential functions in zygotic development that mutate to lethality, precluding their identification as female-sterile mutations. To overcome these limitations, we performed a genetic mosaic screen aimed at identifying new maternal genes required for early embryonic patterning, including zygotically required ones. Using the Flp-FRT technique and a visible germline clone marker, we developed a system that allows efficient screening for maternal-effect phenotypes after only one generation of breeding, rather than after the three generations required for classic female-sterile screens. We identified 232 mutants showing various defects in embryonic pattern or morphogenesis. The mutants were ordered into 10 different phenotypic classes. A total of 174 mutants were assigned to 86 complementation groups with two alleles on average. Mutations in 45 complementation groups represent most previously known maternal genes, while 41 complementation groups represent new loci, including several involved in dorsoventral, anterior-posterior, and terminal patterning.

THE establishment of the major body axes of the Drosophila embryo is governed by maternal gene activity. Four groups of genes act in a largely independent manner to specify the anterior-posterior and the dorsoventral axes, as well as the terminal regions of the embryo. Localized, maternally derived RNAs provide the source for anterior and posterior determinants, while local activation of transmembrane receptors and subsequent signal transduction pathways define the dorsoventral axis, as well as cell fates at the embryonic termini. About 35 genes whose products are required maternally for embryonic pattern formation have been identified by classic screens for recessive female-sterile mutations (Gans *et al.* 1975; Mohler 1977; Perrimon *et al.* 1986; Schüpbach and Wieschaus 1986, 1989, 1991; reviewed in St. Johnston and Nüsslein-Volhard 1992). Analysis of these genes has revealed a framework of the axis-forming systems in the Drosophila embryo. However, taking into account all the known components involved in axis determination, we still do not understand several aspects. For instance, How are anterior and posterior determinants localized to opposite poles of the oocyte? How are spatially restricted ventral and terminal signals generated and maintained in the perivitelline space? Growing knowledge about the molecular properties of the pathways involved in axis formation also allows us to postulate the existence of missing factors, such as a missing link in the dorsoventral signaling cascade between the proteins Pelle and Cactus (Grosshans *et al.* 1999) or a missing transcriptional activator downstream from the Torso pathway (reviewed in Furriols and Casanova 2003). For reasons explained below, it is likely that several components of these pathways have not been identified by the classic screens.

A large number of maternal gene products are deposited in the Drosophila egg during oogenesis (Garcia-Bellido and Robbins 1983; Perrimon *et al.* 1984; Schüpbach and Wieschaus 1991; reviewed in Wilkins 1986). Screens for female-sterile mutations led to the isolation of the majority of "strictly" maternal genes, which are required for the normal development of the egg and/or the embryo, but whose functions are dispensable for adult viability (Perrimon *et al.* 1986; Schüpbach and Wieschaus 1986, 1989, 1991). A limiting factor in these screens has been the large effort associated with generating enough lines to saturate the

[1]*Corresponding author:* Department of Biochemistry/HHMI, 279 Campus Dr., Beckman Center, Rm. B453, Stanford University School of Medicine, Stanford, CA 94305-5307.
E-mail: luschnig@stanford.edu

[2] *Present address:* Temasek Life Sciences Laboratory (TLL), National University of Singapore, Singapore 117604.

[3]*Present address:* Max-Planck-Institut für Entwicklungsbiologie, Abteilung Molekulare Biologie, D-72076 Tübingen, Germany.

genome for maternal-effect mutations. Moreover, identification of a gene by means of its mutant phenotype can be obscured by functional redundancy of different genes on the one hand or by multiple functions of a single gene during development on the other hand. While functional redundancy poses a general problem for forward genetic approaches, it is of less concern for Drosophila as compared to vertebrate genomes. In many cases, Drosophila possesses a single or few homologs of a corresponding multigene family in vertebrates, and often the Drosophila gene has been shown to mutate to a distinct phenotype (Adams *et al.* 2000; Rubin *et al.* 2000). However, multiple requirements of a gene product at different developmental stages are rather common. For example, components involved in signal transduction [*e.g.*, the RAS-mitogen-activated protein kinase (MAPK) pathway] or in intracellular transport (of RNA or proteins) are used repeatedly in different contexts during development. Genes encoding such multiply required components are likely to mutate to zygotic lethality, thus precluding their identification in genetic screens for female-sterile mutations. In Drosophila, it is possible to overcome the requirement of a gene for viability by studying mosaic animals that carry clones of homozygous mutant cells in an otherwise heterozygous animal. Such mosaics can be generated by mitotic recombination between homologous chromosomes, induced by X-rays (Patterson 1928; Stern 1936), or, more recently, by a heterologous site-specific recombination system from yeast, the *Fli*pase-*F*lipase *r*ecombinase *t*arget (Flp-FRT) system (Golic and Lindquist 1989; Golic 1991). The Flp-FRT system can induce clones in germline and somatic tissues with high efficiency, and combining FRT chromosomes with the dominant female-sterile ovo^D mutation has allowed positive selection for recombination events in the female germline ("Flp-DFS"—dominant female sterile—technique; Chou *et al.* 1993; Chou and Perrimon 1996). Systematic screens have been carried out using germline clonal analysis to identify genes involved in embryonic patterning. A screen of X-linked lethal mutations and screens of *P*-element-induced lethal mutations on the autosomes have revealed specific maternal functions of several zygotic lethal loci for embryonic patterning (Perrimon *et al.* 1989, 1996; Bellotto *et al.* 2002). These screens have also identified embryonic lethal mutations that become phenotypically manifest only in the absence of both the maternal and zygotic contributions of a gene product.

We have carried out a large-scale germline clone (GLC) screen specifically aimed at identifying new genes involved in the four axis-forming systems. Because the early steps of axis formation are controlled by the maternal genome before the onset of zygotic transcription, the paternal contribution to the embryo's genotype is irrelevant for these processes. This situation allowed us to devise an efficient F_1 screening scheme, in which individual females carrying clones homozygous for a mutagenized chromosome are mated with wild-type males. The F_1 scheme allows large numbers of individuals to be scored in a rapid fashion, as the same animal is used to detect a mutant phenotype and to establish a line of mutant carriers. In contrast to F_2 or F_3 screens, stocks of potential mutants are established only after a phenotype has been scored, thus greatly reducing the number of lines to maintain.

In this work, we describe the technique and results of our GLC screen on the five major chromosome arms, comprising most of the euchromatic portion of the Drosophila genome. We identified 232 mutations with distinct phenotypes and ordered the mutants into 10 different phenotypic classes. We were able to assign 174 (75%) of the 232 mutants to complementation groups, with 86 complementation groups in total and an average allele frequency of 2 alleles per complementation group. A total of 41 complementation groups represent previously undescribed loci, while 45 complementation groups represent most of the previously known maternal genes. We found on average a higher number of alleles for previously known genes (2.71 alleles per locus) than for new mutations (1.27 alleles per locus), 47 of which are represented by a single allele.

We found several new loci involved in dorsoventral, anterior-posterior, and terminal patterning, as well as mutants affecting other processes, such as embryonic segmentation, epidermis development, and morphogenesis. Interestingly, a significant fraction of the new loci isolated in this work represent viable mutations, suggesting that these genes had been missed in earlier screens due to incomplete saturation, rather than to a zygotic requirement of these genes.

MATERIALS AND METHODS

Fly stocks: Balancer chromosomes and marker mutations used in this study are listed in Lindsley and Zimm (1992) and in FlyBase (1999). The following FRT and hs-Flp chromosomes were used:

hs-Flp22 (X): P[ry^+, hs-Flp]22 (Chou and Perrimon 1996)
hs-Flp122 (X): P[ry^+, hs-Flp]122 (Struhl and Basler 1993)
FRT9-2 (X): P[>w^+>, FRT]18E (Chou and Perrimon 1996)
FRT40A (2L): P[ry^+, hs-neo, FRT]40A (Xu and Rubin 1993)
FRTG13 (2R): P[>w^+>, FRT]42B (Chou and Perrimon 1996)
FRT2A (3L): P[>w^+>, FRT]79D-F (Chou and Perrimon 1996)
FRT82B (3R): P[ry^+, hs-neo, FRT]82B (Xu and Rubin 1993).

hs-Flp22 was used for the third chromosome screens; hs-Flp122 was used for the first and second chromosome screens. hs-Flp122 showed considerably stronger Flipase activity than hs-Flp22 when we tested for the frequency of germline clones or flip-out clones in the eye (data not shown). The Flp-DFS technique and FRT chromosomes containing ovo^D mutations are described in Chou and Perrimon (1996). Conditional dominant temperature-sensitive balancer chromosomes (*CyO, P[hs-hid, w^+]* and *TM3, P[hs-hid, w^+]*) have been described

(Grether *et al.* 1995; Moore *et al.* 1998). The marked FRT chromosomes listed in Table 1 were constructed by recombining proximal FRT sites (Xu and Rubin 1993; Chou and Perrimon 1996) with distal visible markers or with P[ubi-nlsGFP, w^+] insertions (Davis *et al.* 1995). All recombinant FRT chromosomes generated in this work are available from the Bloomington Stock Center (http://flystocks.bio.indiana.edu/).

Generation of P[ubi-nlsGFP, w^+] insertions: To generate P[ubi-nlsGFP, w^+] insertions on all major chromosome arms, we first combined two insertions of this construct on the third chromosome (P[ubi-nlsGFP, w^+]34N, P[ubi-nlsGFP, w^+]34A3; Davis *et al.* 1995) to make a "jumpstarter." We then mobilized the *P*-element construct by crossing these flies to a *Delta2-3* transposase source on the *TM2* balancer chromosome. New P[ubi-nlsGFP, w^+] insertions were outcrossed and analyzed for segregation of the insertion with the second or the X chromosome. New third-chromosomal insertions were generated separately in a similar scheme. Several homozygous viable and strongly expressing insertions were mapped genetically to a chromosome arm (visible genetic markers indicated in Figure 3 were used for mapping). A single or two different insertions were recombined to proximal FRT sites to make the stocks listed in Table 1.

Screening procedure: The crossing scheme, exemplified for chromosome arm 2R, is illustrated in Figure 1. Strains that were used to screen the other chromosome arms in the same fashion are listed in Table 1. The crossing scheme for the X chromosome differed from the scheme for the autosomes in that isogenic lines were started from single F_2 females (rather than males) balanced with *FM7c*.

Mutagenesis: We carried out several rounds of mutagenesis for each chromosome arm. For one experiment, ~600 males homozygous for a marked FRT chromosome were starved on water-saturated Kleenex paper for 4 hr before they were fed for 14 hr with ethyl methanesulfonate (EMS; Sigma, St. Louis) in 1% sucrose according to standard methods (Lewis and Bacher 1968). We used different EMS concentrations in the range between 25 and 45 mM, the chosen dose depending on how well a particular strain tolerated the EMS treatment. We noticed that males carrying a *y w hs-Flp* X chromosome showed greatly increased sensitivity to EMS as compared to males carrying a *w* chromosome in an otherwise genetically identical background. Per 200-ml food bottle, ~50 mutagenized males and 80 females were mated and cultured at 25°. Male parents were removed after 3 days and females were transferred to fresh medium every 2 days. To estimate the efficiency of the EMS treatment for inducing lethal mutations, we determined the fraction of lethal chromosomes among a sample of randomly picked F_1 males from mutagenized fathers. In a test for the screen on chromosome arm 3L, we found that 82% (90/110) of mutagenized (30 mM EMS) *ru h th st FRT2A* chromosomes were lethal. Assuming a Poisson distribution for the frequency of lethal hits, this corresponds to an average of 1.7 lethal hits per chromosome. Similar lethality rates were observed for other chromosome arms tested (data not shown).

Production of germline clones: To induce clones in the F_1 females that were tested in the screen, third instar larvae in 200-ml food bottles were heat-shocked twice (one heat shock per day on two consecutive days) for 2 hr at 37° in a circulating water bath. For retesting mutant candidates, virgin females of a balanced candidate line were crossed to males of the general genotype *hs-Flp/Y; FRT ovo^D/Balancer, P[hs-hid]*. Larval progeny of these crosses were heat-shocked and mosaic females of the genotype *hs-Flp; FRT mutation/FRT ovo^D* were collected in egg-laying blocks and examined for unhatched embryos. A single 1-hr heat shock at the third instar larval stage was sufficient to induce germline clones, as well as, at the same time, to eliminate all unwanted progeny. The only surviving genotype was mosaic *hs-Flp; FRT mutation/FRT ovo^D* flies, thus eliminating the need to manually sort flies from the retest cross (note that in the retest the males are therefore heterozygous for the mutagenized chromosome).

Detection of embryonic phenotypes: We used the block-agar method for large-scale collection of eggs from single females (Nüsslein-Volhard 1977). Techniques for collection and observation of embryos are described in Wieschaus and Nüsslein-Volhard (1998). Undechorionated live embryos, 0 to 18 hr old, on apple juice agar plates were covered with Voltalef 3S oil and sorted using a Leica MZ12 epifluorescence stereomicroscope equipped with a green fluorescent protein (GFP)1 filter set. GFP-negative embryos were picked and transferred to fresh apple juice agar plates, allowed to develop for 24 hr at 25°, and then examined for abnormalities in the embryonic cuticle. Cuticle preparations were done according to standard procedures (Wieschaus and Nüsslein-Volhard 1998).

Mapping of mutations: Autosomal mutations were mapped genetically by testing for noncomplementation of lethality or female sterility, using a set of chromosomal deficiencies that uncovers most of the respective chromosome arm. X-linked mutations were mapped using a set of duplications that covers most of the X chromosome. Deficiency and duplication kits were obtained from the Bloomington Stock Center. In parallel to deficiency or duplication mapping, mutations were mapped by meiotic recombination to verify cytological map positions, as well as to clean the mutagenized chromosome from associated lethal mutations. All mutations were induced on recessively marked FRT chromosomes; for mapping, the marked mutagenized FRT chromosome was allowed to recombine with the corresponding unmarked parental FRT chromosome. Approximately 50 independent recombinants were analyzed for segregation of the mutant phenotype with visible markers. In the case of w^+-marked FRTs (FRT9-2, FRTG13, and FRT2A), the FRT site itself could be used as an additional visible marker (due to our screening procedure, the FRT-flanked w^+ marker on the mutagenized P[>w^+>, FRT] chromosomes is excised by Flipase, resulting in a single remaining FRT site lacking the w^+ marker).

RESULTS

Overview of the screening procedure: To screen on a large scale for maternal-effect lethal mutations, we adapted the Flp-FRT system (Chou and Perrimon 1992; Xu and Rubin 1993) to devise an F_1 screening scheme (see Figure 1). In this scheme, mutant phenotypes are detected among the eggs from single females, which are obtained as F_1 progeny of mutagenized EMS-treated males, thus allowing large numbers of individuals to be scored in a rapid fashion. The use of GFP as a vital marker allowed us to distinguish embryos derived from homozygous germline clones. The polyubiquitin (*ubi*) promoter directs strong maternal GFP expression in eggs, which is detectable up to late stages of embryogenesis (see Figure 2; Davis *et al.* 1995). We constructed a set of *ubi*-GFP-marked FRT chromosomes that allow marking of clones in germline and somatic tissues by the absence of the GFP marker from the clones (see Figure 1 and Figure 2, B and C). A list of these strains is in Table 1.

We carried out a separate screen for each of the five

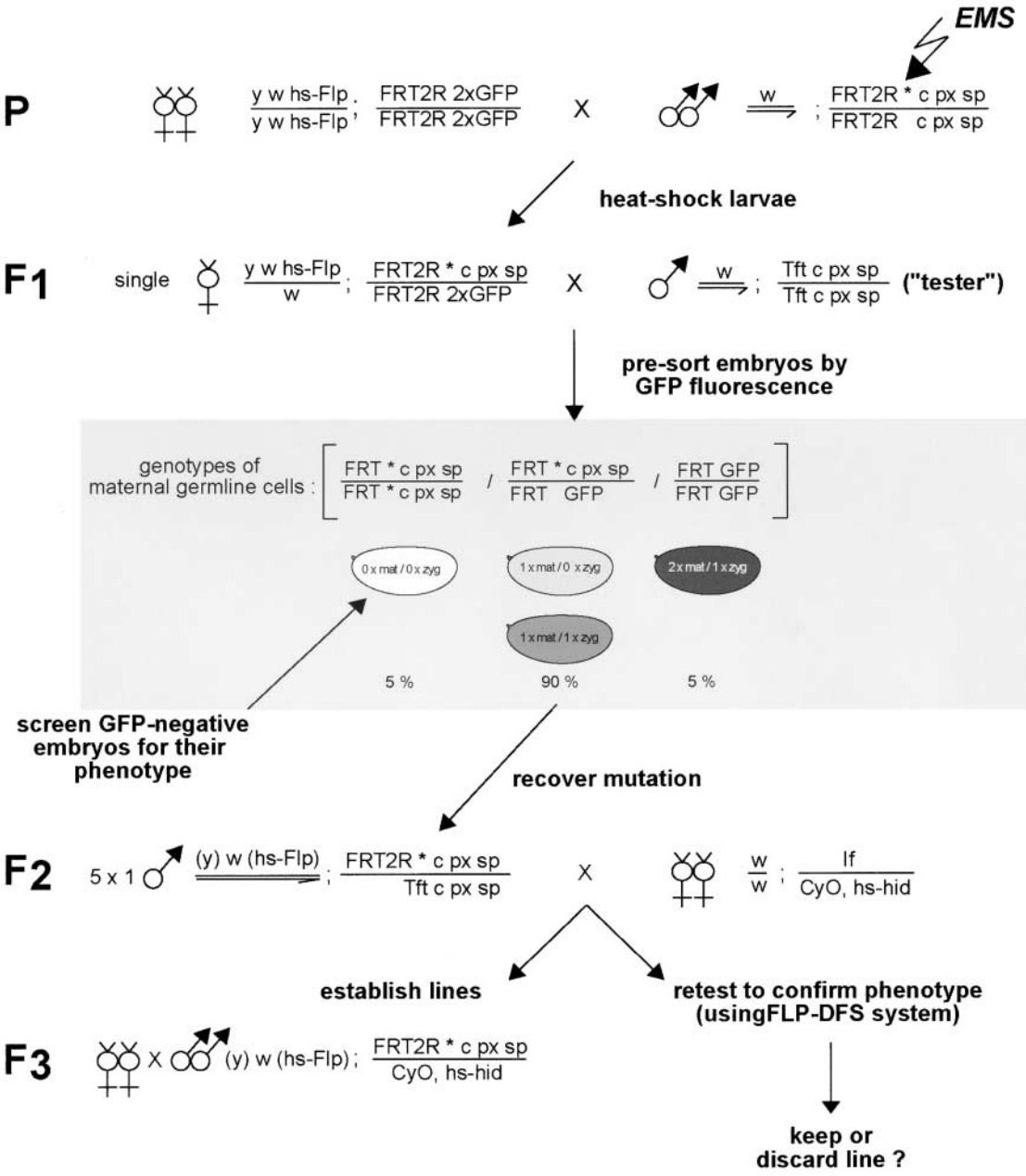

FIGURE 1.—Crossing scheme exemplified for chromosome arm 2R. In the shaded box, the different maternal genotypes of eggs laid by a mosaic female are indicated; the corresponding intensity of GFP fluorescence is shown schematically. The asterisk indicates an EMS-induced mutation. Strains that were used to screen the other arms are listed in Table 1. The scheme for the X chromosome differs from the autosomal schemes in that F_2 females rather than males were used to recover the mutagenized chromosome. The retest procedure is not shown in this scheme (see MATERIALS AND METHODS for details).

major chromosome arms. The crossing scheme, exemplified for chromosome arm 2R, is shown in Figure 1. Corresponding schemes were applied for the other autosome arms and, slightly modified, for the X chromosome (see Table 1). In general, males carrying an isogenized, recessively marked FRT chromosome were treated with EMS and crossed *en masse* to females carrying an *hs-Flp* source and an FRT chromosome with distal P[*ubi*-nlsGFP, w^+] insertions. Larval progeny of this cross were heat-shocked to induce Flp-mediated mitotic recombination. The emerging adults carry clones (in somatic and germline tissues) that are homozygous for the mutagenized FRT-based chromosome arm. F_1 females were crossed individually to males with a marked tester chromosome and embryos from the F_1 females were genotyped on the basis of the presence or absence of maternally contributed GFP: embryos derived from GLCs homozygous for the mutagenized chromosome arm lack GFP, while the remaining embryos derived from heterozygous germline cells or from GFP-homozygous twin

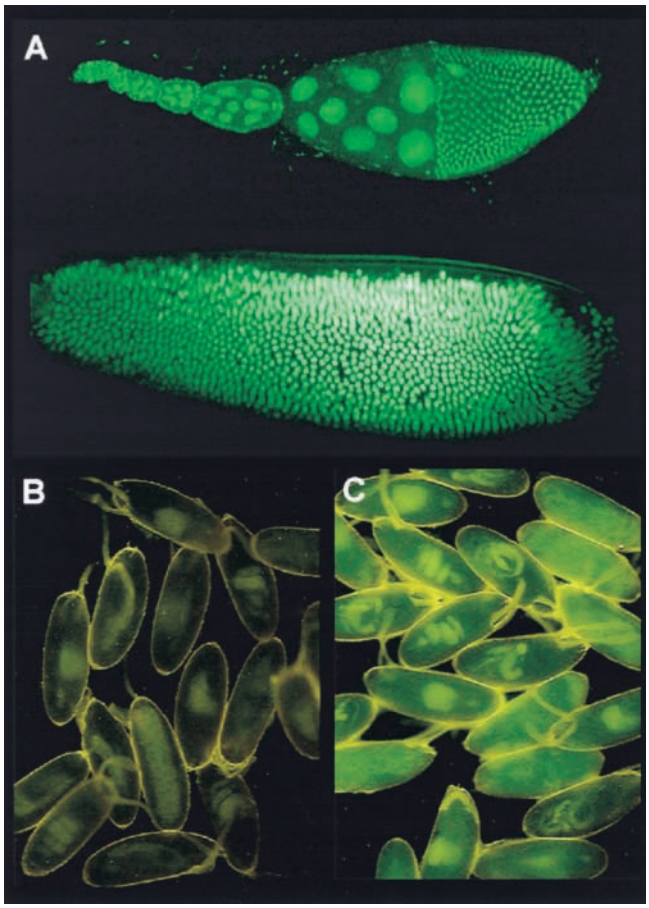

FIGURE 2.—*ubi*-nlsGFP in egg chambers and embryos. (A) Confocal micrographs of an egg chamber (top) and cellular blastoderm embryo (bottom) from *2x GFP FRT2A* females; the father of the embryo is wild type (GFP negative). Anterior is to the left. (B and C) Undechorionated embryos from a cross of mosaic *y w hs-Flp22/w; 2x GFP FRT2A/ru h th st FRT2A* females and wild-type (GFP-negative) males. The photo was taken from embryos on an agar plate covered with Voltalef oil. (B) GFP-negative GLCs. The embryos show weak yellow autofluorescence. (C) GFP-positive siblings from the same egglay.

spots fluoresce green. The intensity of fluorescence depends on the copy number of GFP provided both maternally and zygotically (see Figure 2, B and C).

We determined the efficiency of GLC induction by scoring the proportion of GLC-derived eggs (marked either by a mutation or by the absence of GFP) among the eggs laid by single females. In control experiments without mutagenesis, nearly 100% of the mosaic females laid GLC-derived eggs; the average proportion of GLCs among the total number of eggs laid by a single female was between 5 and 10%. If the fathers were mutagenized, only between 49 and 63% (depending on the chromosome arm) of the F_1 females laid GLC-derived eggs (see Table 2). The remaining females did not produce GLCs, presumably due to the presence of cell-lethal mutations on the FRT-tagged chromosome arm. The average number of eggs laid by a female over 1 day was 30, and thus the number of GLC-derived eggs that can be collected per day was small (in most cases 1–3 eggs). In the screen we therefore collected eggs from most of the females on two consecutive days. The use of a strongly expressing *hs-Flp* source (*hs-Flp122* instead of *hs-Flp22*; see MATERIALS AND METHODS) increased the proportion of GLCs to >10% on average (data not shown).

We sorted out 0- to 24-hr-old GFP-negative embryos from each single female, transferred these embryos to an agar plate where they were allowed to complete embryonic development, and examined them after 24 hr. The majority of the embryos were phenotypically normal and had hatched by 24 hr; embryos that did not hatch were examined for patterning defects in larval cuticle preparations. To establish a line from a mutant candidate, the female was recovered and allowed to lay eggs in a food vial. Three to six isogenic lines balanced for the mutagenized chromosome were established from single males in the F_2 generation (in the case of the X chromosome screen, lines were established from single F_2 females carrying an X chromosome balancer chromosome). The presence of recessive markers (see Figure 3) on the mutagenized FRT chromosome was used to avoid the isolation of recombinants that may have lost the mutation. Stocks of mutant candidates were retested to confirm the initially scored phenotype. We used the Flp-DFS (ovo^D) technique (CHOU and PERRIMON 1996), aided by conditional temperature-sensitive balancer

TABLE 1

List of chromosomes used for the screen

Arm	Mutagenized chromosome	GFP FRT chromosome	Tester chromosome	Balancer chromosome
X	*w f B FRT9-2 hs-FLP122*	*y w GFP FRT9-2*	*FM7c, y, w^a, sn, B*	*FM7c, y, w^a, sn, B*
2L	*al dp b pr FRT40A*	*y w hs-FLP122; 2x GFP FRT40A*	*al dp Tft/CyO, dp, pr*	*Cyo, [hs-hid], dp, pr*
2R	*FRTG13 c px sp*	*y w hs-FLP122; FRTG13 2x GFP*	*Tft c px sp*	*Cyo, [hs-hid], dp, pr*
3L	*ru h th st FRT2A*	*y w hs-FLP22; 2x GFP FRT2A*	*ru h th st cu sr e^s ca*	*TM3, e, Sb, Ser*
3R	*FRT82B cu sr e^s ca*	*y w hs-FLP22; FRT82B GFP*	*ru th st cu sr e^s Pr ca*	*TM3, e, Sb, Ser*

Males carrying the tester chromosome were crossed to the F_1 females to test for the presence of visible markers on the mutagenized chromosome in the next generation (see MATERIALS AND METHODS for details). All marked chromosomes generated in this work have been made available through the Bloomington Stock Center (http://flystocks.bio.indiana.edu/).

TABLE 2

Overview of the screen

	Chromosome arm					
	X	2L	2R	3L	3R	Total
No. of crosses started	8,400	14,800	17,400	8,600	10,000	59,200
No. of successful crosses (% of total no. of crosses)	5,500 (65.5%)	10,700 (72.3%)	12,200 (70.1%)	4,800 (55.8%)	6,200 (62.0%)	39,400 (66.6%)
GLC collected from F_1 females (% of successful crosses)	3,200 (58.2%)	6,760 (63.2%)	6,400 (52.5%)	2,560 (53.3%)	3,050 (49.2%)	21,970 (55.8%)
No. of potential mutants isolated	160	190	169	90	117	726
Positive retest (% positive of lines isolated)	92 (57.5%)	111 (58.4%)	52 (30.8%)	21 (23.3%)	77 (65.8%)	353 (48.6%)
No. of lines described in this study	49	70	46	11	56	232

Numbers are given for each chromosome arm screen as a separate experiment. The number of successful crosses represents the fraction of initial crosses from which a sufficient number of eggs could be collected; percentage values in parentheses refer to the total number of crosses started for each chromosome arm. The percentage of F_1 females from which we were able to collect GLCs was between 49 and 63% of the number of successful crosses, depending on the chromosome arm. Most females that did not produce GLC-derived eggs presumably carried a cell-lethal mutation (see DISCUSSION). A total of 353 (48.6%) of the 726 potential mutants isolated were found to show a distinct phenotype in the retest. A total of 232 of these 353 lines are described in this work.

chromosomes (see MATERIALS AND METHODS; GRETHER *et al.* 1995; MOORE *et al.* 1998) for the retest, as a higher number of mutant embryos can be obtained by the *ovo*^{D} method than by sorting according to GFP fluorescence. We discarded all lines that produced a high percentage of hatching larvae in the retest. A total of 353 (48.6%) of the 726 potential mutants initially isolated gave embryos that failed to hatch in the retest. These lines were kept for further analysis.

In this work, we describe 232 of these lines, which show distinct and, in most cases, completely penetrant phenotypes. Table 2 summarizes the five screens that were carried out. For each screen, we counted the number of females from which we collected GLCs as the number of screened chromosome arms. On the basis of lethality tests, most of these chromosomes contained more than one lethal mutation (see MATERIALS AND METHODS).

Complementation analysis and mapping: To establish complementation groups, all mutants on a given chromosome arm that showed a similar phenotype were

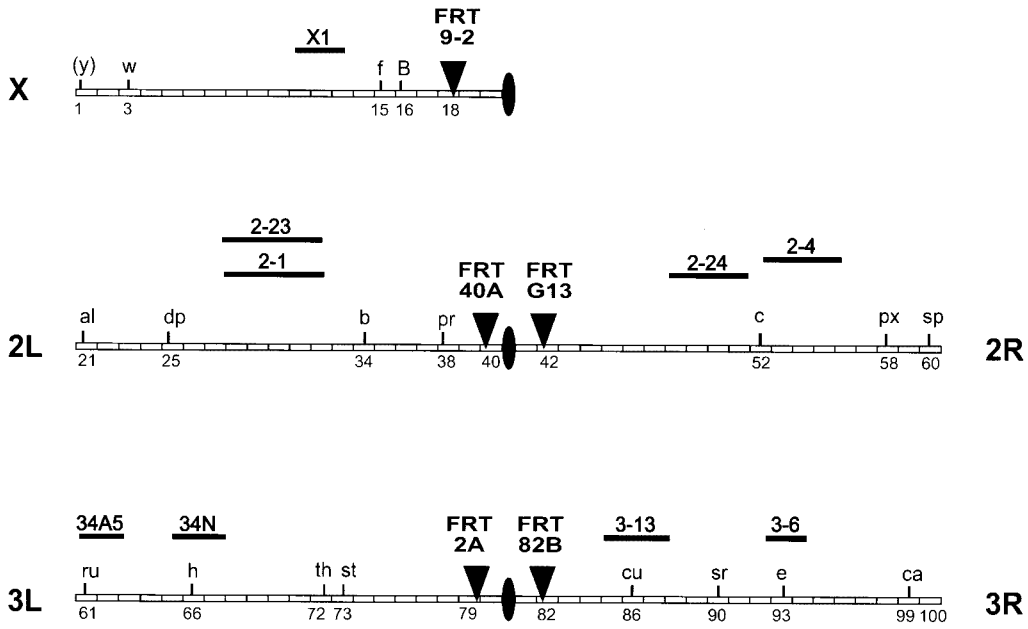

FIGURE 3.—Graphic representation of marked chromosomes used for the screen. Horizontal bars correspond to approximate genetic map positions of P[ubi-nlsGFP, w^+] insertions that were recombined to an FRT site on the respective chromosome arm. Visible markers that were recombined to FRT sites are indicated with their positions (numbered cytological bands).

crossed to each other and to mutants in known candidate genes located on the relevant chromosome arm. We tested for viability and female fertility of the *trans*-heterozygous progeny of these crosses. Mutations that did not fall into any of the previously known complementation groups were subsequently mapped by meiotic recombination and/or complementation tests, using a set of overlapping deficiencies on the relevant chromosome arm. Due to the time-consuming X chromosomal genetics, mapping and complementation of many X chromosomal mutants is still in process and has not been included in this work. The number of newly found alleles at previously known loci was used to estimate the mutation rate achieved using our screening procedure. Overall, we isolated 2.7 alleles on average for previously known loci (122 mutants falling into 45 complementation groups), corresponding to a mutation rate of approximately one hit per locus in every 1000–2000 chromosome arms screened (see Tables 2 and 4).

Phenotypic classification: Mutations were initially grouped into the following phenotypic categories: defects in dorsoventral, anterior, posterior, or terminal patterning; segmentation defects; defects in morphogenetic processes (such as germband retraction or dorsal closure); defects in epidermis development; and alterations of eggshell morphology. Eighteen lines that did not fit any of the above categories were grouped as "other phenotypes." Although our screening scheme was specifically designed to isolate maternal mutations that are not zygotically rescuable, we also found several zygotic embryonic lethal mutations in the X chromosome screen, where 50% of the GLC-derived embryos are hemizygous males. In addition, some autosomal zygotic mutations were identified coincidentally in the retest, where the fathers are heterozygous for the mutagenized chromosome (see MATERIALS AND METHODS). The mode of action of a mutation (maternal effect, paternally rescuable maternal effect, or zygotic embryonic lethal) is indicated in supplemental Table 1 at http://www.genetics.org/supplemental/. We also isolated several mutants that produce unfertilized eggs or show an early arrest in embryonic development. However, we did not systematically screen for these phenotypes and excluded them from description in this work. Supplemental Table 1 shows a list of the 232 mutants that displayed distinct phenotypes. In addition, 121 lines, which showed less clear or incompletely penetrant phenotypes, were kept for further analysis; these lines are not described in this work. In the following, we describe the different phenotypic classes and emphasize new loci that were identified by this study.

Dorsoventral patterning: *Dorsalized embryos:* Twenty-nine mutants falling into nine complementation groups show dorsalized phenotypes. We identified new alleles of all previously known dorsal group genes (*dorsal*, *easter*, *gastrulation defective*, *pelle*, *snake*, *spaetzle*, and *toll*), except for those that map proximal to an FRT site (*tube*) and those whose products act in the somatic follicle cells (*pipe*, *nudel*, and *windbeutel*). Three new loci were discovered, *krapfen*, *seele*, and *weckle*, which define new dorsal group genes. *krapfen* (*kra*, one allele) and *seele* (*sel*, two alleles) mutants show partially dorsalized [D1–D2, according to the terminology in ANDERSON and NÜSSLEIN-VOLHARD (1986)] phenotypes. After cleaning the original mutagenized chromosomes from associated lethal mutations, we found that both *kra* and *sel* mutants are homozygous viable and female sterile. We also showed that the original *kra* allele isolated in the screen is a hypomorphic allele and that subsequently generated null mutations at the *kra* locus show a completely dorsalized phenotype (CHARATSI *et al.* 2003). The third new locus, *weckle* (*wek*, three alleles) gives rise to strongly (D0–D1) dorsalized embryos and corresponds to a zygotically lethal complementation group. *wek* is allelic to *l(2)35Ea*, which encodes a putative zinc-finger transcription factor (ASHBURNER *et al.* 1999). We have identified point mutations in the *l(2)35Ea* gene in all three *wek* alleles, confirming that *wek* is indeed an allele of *l(2)35Ea*. The role of *wek* in establishing the dorsoventral axis is currently under investigation and will be published elsewhere (B. PRIESTER, unpublished results).

Ventralized embryos: Twenty-six mutations falling into 10 complementation groups show ventralized phenotypes. We isolated new alleles of the previously known genes *cactus* (*cact*, eight alleles), *medea* (*med*, four alleles), *mothers against dpp* (*mad*, four alleles), *saxophone* (*sax*, two alleles), *thick veins* (*tkv*, two alleles), and *Toll* (one ventralizing allele, as well as three dorsalizing alleles). Interestingly, we found that two of the four *medea* alleles give rise to a partially penetrant bicaudal phenotype in addition to ventralization of the embryo, suggesting a potential role of DPP/SMAD signaling in anterior-posterior polarity determination. Five *cact* alleles were identified in the screen on chromosome arm 2R by virtue of the haplo-insufficient dominant phenotype of the *cact* locus, while three *cact* alleles were found on the basis of their recessive phenotype in the 2L screen. Four new loci were identified, which show ventralized phenotypes in homozygous mutant GLCs. *eclair* (*eca*) and *baiser* (*bai*), each defined by a single allele, give rise to weakly ventralized embryonic phenotypes. Both *eca* and *bai* mutants are adult semilethal, and surviving homozygous females do not lay eggs, while only mosaic females produce embryos that show the *eca* or *bai* phenotype. The roles of the *eca* and *bai* genes in dorsoventral patterning will be described elsewhere (S. BARTOSZEWSKI, unpublished results). The third new locus in this group, *croissant* (*cst*; two alleles), corresponds to a viable and female-sterile complementation group. Embryos derived from *cst* GLCs or from homozygous mutant females show a U-shaped phenotype similar to *saxophone* (*sax*) mutants. The fourth new locus in this group, *hoernchen* (*hrn*), is defined by a single X chromosomal mutation, which is

viable and female sterile. Embryos derived from *hrn* GLCs or homozygous mothers show a head-open and U-shaped embryonic phenotype, as well as gastrulation defects characteristic of ventralized mutants (data not shown).

Anterior-posterior patterning: *Anterior defects:* We identified 12 mutants falling into 11 complementation groups that show either distinct anterior (head) defects or a bicaudal phenotype (mirror-symmetric duplications of the abdomen). Five alleles of *staufen (stau*), which show a distinct head defect, as well as posterior abdominal defects, were grouped in the "posterior defects" class. The anterior group mutants include two alleles of *bicoid (bcd).* Two new mutants falling into separate complementation groups, *krake* (*krk*, one allele) and *tintenfisch* (*ttf*, one allele), show strong anterior (thoracic) defects reminiscent of *hunchback* (*hb*) mutants. A third line, *ziehharmonika* (*zih*, one allele), shows a distinct head defect and is homozygous viable. The *zih* mutation was mapped to the cytological interval 43F–44D3-8. Six mutations that display either partially or completely penetrant bicaudal phenotypes were found. One line, *3R-103-30*, shows a partially penetrant bicaudal phenotype and is an allele of *bullwinkle* (*bwk*; Rittenhouse and Berg 1995). A new locus, named *alice* (*ali*, one allele), shows a fully penetrant, recessive bicaudal phenotype (mirror-symmetric embryos with extended Filzkörper at both ends; F. Schnorrer, unpublished data). *gl(2L)179-26* GLCs give rise to short, small eggs, and embryos show a variable bicaudal phenotype. *gl(2L)343-11* embryos either are bicaudal or show head defects. *gl(3R)66-35* is a homozygous viable mutation, and embryos show a partially penetrant bicaudal phenotype and a weakly ventralized eggshell (fused dorsal appendages). *gl(3R)70-22* GLCs show variable head defects, while a small fraction of the embryos are bicaudal. Anterior localization of *bcd* mRNA appeared normal in these embryos (F. Schnorrer, unpublished data).

Posterior defects: Forty-three mutants falling into 16 complementation groups show posterior (abdominal) patterning defects. In addition to alleles of the known genes *cappuccino* (*capu*, four alleles), *oskar* (*osk*, four alleles), *pumilio* (*pum*, three alleles), *spire* (*spir*, seven alleles), *staufen* (*stau*, five alleles), *tudor* (*tud*, three alleles), and *valois* (*vls*, one allele), we identified at least two new loci involved in posterior patterning. An X-linked mutation, which we named *napoleon* (*nap*, one allele), affects formation of the abdomen, but not of the pole cells, suggesting that *nap* acts downstream of *tudor* at the level of *pumilio* and *nanos* in the posterior patterning system (H. Knaut, personal communication). Mutants in a second new locus, which we named *shorty* (*soy*; five alleles), lack pole cells and show defects in abdominal segmentation. *soy* is a lethal complementation group. Another lethal complementation group composed of three alleles (*2L-150-11, 2L-192-9*, and *2L-257-19*) shows variable deletions of abdominal segments and turned out to be allelic to *brain tumor* (*brat*), a factor that has been implicated in the translational control of maternal *hunchback* mRNA (Chagnovich and Lehmann 2001; Sonoda and Wharton 2001). Six mutations [*gl(1)82-33*, *gl(2L)50-27*, *gl(2L)137-8*, *gl(2R)2-11*, *gl(2R)62-37*, and *gl(3R)60-38*] show weak or partially penetrant posterior segmentation defects and complement each other, as well as all known posterior group genes located on the relevant chromosome arms.

Terminal defects: Twenty-one mutants falling into 10 complementation groups show patterning defects at the embryonic termini, the acron and the telson. We identified new alleles of the previously known genes *kinase suppressor of ras* (*ksr*, one allele; Therrien *et al.* 1995), *ras1* (one allele; Simon *et al.* 1991), *l(1)polehole/draf* (three alleles; Ambrosio *et al.* 1989), *son of sevenless* (*sos*, two alleles; Simon *et al.* 1991), *torso* (*tor*, five alleles; Sprenger *et al.* 1989), and *trunk* (*trk*, four alleles; Casanova *et al.* 1995). We also isolated a mutation at the *dshc* locus, whose effects on the Torso and epidermal growth factor receptor signaling pathways we described previously (Luschnig *et al.* 2000). Interestingly, *dshc* mutants are semilethal and homozygous females lay no eggs, while only germline mosaics produce embryos that show the terminal group phenotype. We isolated a new locus involved in terminal patterning, which we termed *rumpf* (*rum*, one allele). *rum* is a viable mutation and embryos derived from GLCs or from homozygous mothers display the amorphic Torso pathway phenotype. Mapping and complementation analysis of three X-linked mutations with terminal group phenotypes is currently in progress. Two of these mutations, *X-145-32* and *X-197-36*, are allelic.

Segmentation defects: The largest number of mutants was grouped in this class. Forty-six mutants show various defects in segmentation, including gap-like, pair-rule-like, and segment polarity phenotypes and homeotic transformations, as well as other, less easily classified deviations from the normal segmental pattern. Due to the variety of different phenotypes, the classification as "segmentation defects" is somewhat artificial. We were able to assign 30 of the mutants in this class to a total of 17 complementation groups, including the previously known genes *eyelid/osa* (*eld*, five alleles; Treisman *et al.* 1997; Vazquez *et al.* 1999), *ftzf1* (four alleles; Guichet *et al.* 1997), *hopscotch* (*hop*, three alleles; Binari and Perrimon 1994), *marelle/stat92E* (*mrl*, two alleles; Hou *et al.* 1996), and *kismet* (*kis*, one allele; Daubresse *et al.* 1999). Two mutations, *3R-92-38* and *3R-112-6*, which show a severe segmentation defect, are allelic to the gene encoding the 140-kD subunit of RNA polymerase II (*rplII140/wimp*). A mutation in this gene has previously been shown to reduce the transcription of a subset of segmentation genes and to cause a specific segmentation defect (Parkhurst and Ish-Horowicz 1991). A lethal complementation group with two alleles (*2L-75-1* and *2L-193-35*) shows a maternal-effect segmentation defect

similar to that seen in amorphic *even-skipped (eve)* mutants. This complementation group turned out to be allelic to a gene that was recently described by three other groups and was named *lilliputian* (*lilli*) on the basis of growth defects seen in *lilli* mutant clones in imaginal tissues (Su *et al.* 2001; Tang *et al.* 2001; Wittwer *et al.* 2001). A new locus with two lethal alleles, named *wollknäuel* (*wol*), shows deletions and fusions of abdominal denticle belts, as well as a short head skeleton and Filzkörper. A locus defined by a single mutation (*3L-22-3*) shows a specific pattern of segmental deletions in the abdomen. We mapped the *3L-22-3* mutation to the cytological region 66C and identified five *P*-element-induced alleles at the locus (see supplemental Table 1). We cloned genomic DNA fragments and cDNAs adjacent to the *P*-element insertion sites (H. Knaut, F. Schnorrer, S. Luschnig and C. Nüsslein-Volhard, unpublished data). The gene affected by the mutations shows homology to the *Caenorhabditis elegans* gene *egl-27* and to the human *atrophin-1/drpla* gene, the locus mutated in patients suffering from dentatorubral pallidoluysian atrophy (DRPLA), a rare hereditary neurodegenerative disorder (Ueno *et al.* 1995). This gene has been recently described as a transcriptional corepressor involved in gap gene regulation in the early embryo and in regulation of *teashirt* expression in imaginal discs, and the gene has been named *Drosophila atrophin* (*atro*) or *grunge* (*gug*; Erkner *et al.* 2002; Zhang *et al.* 2002). Another mutation, *gl(3R)53-6*, shows abdominal segment fusions and head defects. The mutation interacts genetically with certain *eld/osa* alleles: *gl(3R)53-6* is semilethal *in trans* to one *eyelid/osa* allele (*3R-108-31*). Combinations with other *eld/osa* alleles [*eld(308), eld(3R-68-15)*, and *eld(3R-51-26)*] are viable, but adults display wing vein defects or wing blisters (data not shown). Five mutants [*gl(2R)18-14, 2R-183-20, X-100-6, X-63-3*, and *X-97-26*] show segment polarity phenotypes; among those, one line, *2R-183-20*, is a *costal-2* (*cos*) allele. Certain mutations in the segmentation class show a stronger phenotype when embryos lack both the maternal and zygotic contributions of the gene product (these mutants are indicated as "MZ" in supplemental Table 1): mutants in *caudal* (*cad*, one allele; Mlodzik and Gehring 1987), *costal-2* (*cos*, one allele; Grau and Simpson 1987), and *extra sex combs* (*esc*, one allele; Struhl 1981) were isolated on the basis of their weak maternal phenotypes. We also isolated an allele of *even-skipped* (*eve*, one allele; Nüsslein-Volhard and Wieschaus 1980), which has no maternal effect, but was found in the screen because of its partially penetrant dominant segmentation defect.

Morphogenetic defects: Thirteen mutants were grouped on the basis of defects in various morphogenetic processes, such as germband retraction, dorsal closure, and head involution. Mutants affecting these processes are likely to be underrepresented in our collection, as we did not specifically screen for such phenotypes. We genetically characterized two of these lines in more detail. *stocherkahn* (*stoc*, one allele) is a semilethal mutation that affects embryonic dorsal closure and head involution. Interestingly, despite its late phenotypic manifestation, *stoc* acts purely maternally and shows no zygotic rescue. Rare surviving homozygous females produce embryos with the same phenotype as *stoc* GLCs. We mapped the mutation to the cytological region 98E3–99A1/2. A second mutation, *schraube* (*sbe*, one allele), complements *stoc* and gives rise to twisted embryos with dorsal holes. *sbe* is homozygous viable.

Defects in epidermis development: We isolated 16 mutants that fail to produce a coherent cuticle (Figure 4). Mutant embryos show a characteristic reduction of the embryonic cuticle to small pieces and lack body landmarks such as the head skeleton, Filzkörper, and denticle belts. This phenotype has been associated with defects in apical-basal epithelial cell polarity caused by mutations in the genes *bazooka*, *crumbs*, *stardust*, *scribble*, and others (reviewed in Müller 2000). We identified two new loci with similar phenotypes. The first locus, *pschur* (*psu*, four alleles), shows a strictly maternal requirement, as the phenotype is not rescued by a wild-type allele contributed from the father. Furthermore, the homozygous offspring of heterozygous parents are viable and sterile (three alleles, *2R-69-30, 2R-141-24*, and *2R-265-2*) or die late at the L2 larval stage (one allele, *2R-417-13*). The *psu* mutations are allelic to a *P*-element insertion, *l(2)k06403*, in the *atypical protein kinase C* (*apkc*) gene. aPKC has been shown to be involved in the establishment of cell polarity in epithelial cells (Wodarz *et al.* 2000). The disintegrated cuticle phenotype of the second complementation group, *goldstaub* (*gsu*, two alleles), is unlikely to be caused by a defect in cell polarity, as the localization of the polarity marker α-catenin to the subapical membrane is normal (data not shown). Rather, cellularization appears to be aberrant, as preblastoderm nuclei do not migrate properly to the plasma membrane (data not shown). Four additional mutants with disintegrated cuticle phenotypes were isolated in the 2L screen; each of these four mutants defines a separate complementation group. Three of these were mapped to cytological intervals defined by deficiency breakpoints (see supplemental Table 1).

Other embryonic phenotypes: Sixteen mutants that did not fit any of the phenotypic classes above were grouped in this category. Among those, five X-linked mutations show a neurogenic phenotype similar to *Notch* mutants (*X-115-40, X-210-22, X-31-10, X-75-9*, and *X-87-21*). One of these, *X-31-10*, shows a penetrant maternal-effect phenotype, while the four other lines are either zygotically rescuable maternal-effect or zygotic lethal mutations. Our screening procedure also allowed us to detect alterations in the autofluorescence of embryos. While the yolk of wild-type embryos fluoresces yellow when irradiated with blue light (450–490 nm; GFP excitation filter), three X-linked mutants (*X-164-5, X-167-40*, and *X-187-38*) instead fluoresce bright orange. This orange autofluor-

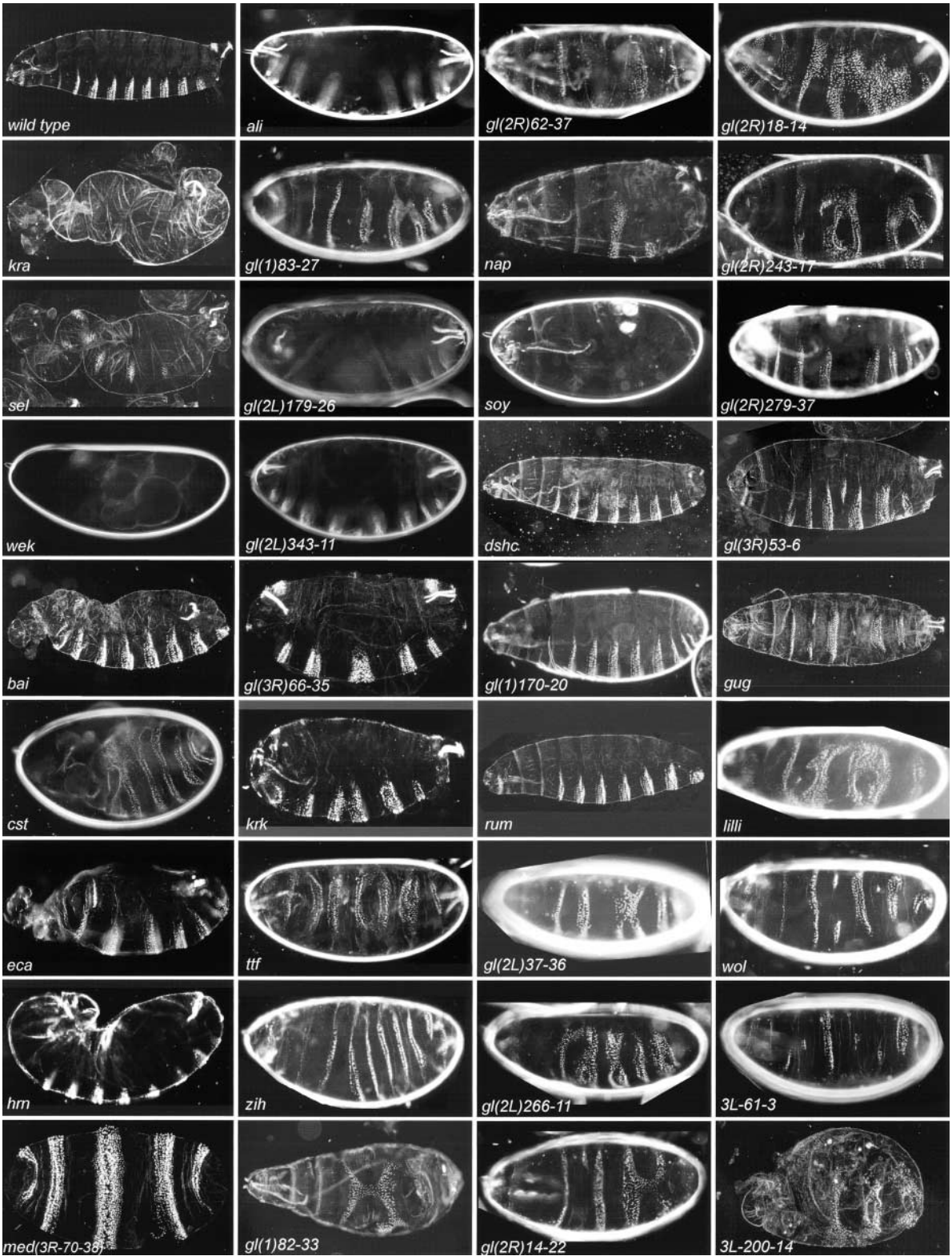

FIGURE 4.—Embryonic phenotypes of the mutants. Cuticle preparations of GLC-derived embryos (or eggs) from mutants of the different phenotypic classes are shown. Anterior is to the left throughout. Mutants shown here are indicated by boldface type in supplemental Table 1.

escence may be due to the accumulation of a metabolic product. Since these embryos hatch and look externally normal, the corresponding mutations are potentially useful as a positive, recessive marker for mitotic clones. The phenotypes of the remaining 10 lines in this group are described in supplemental Table 1.

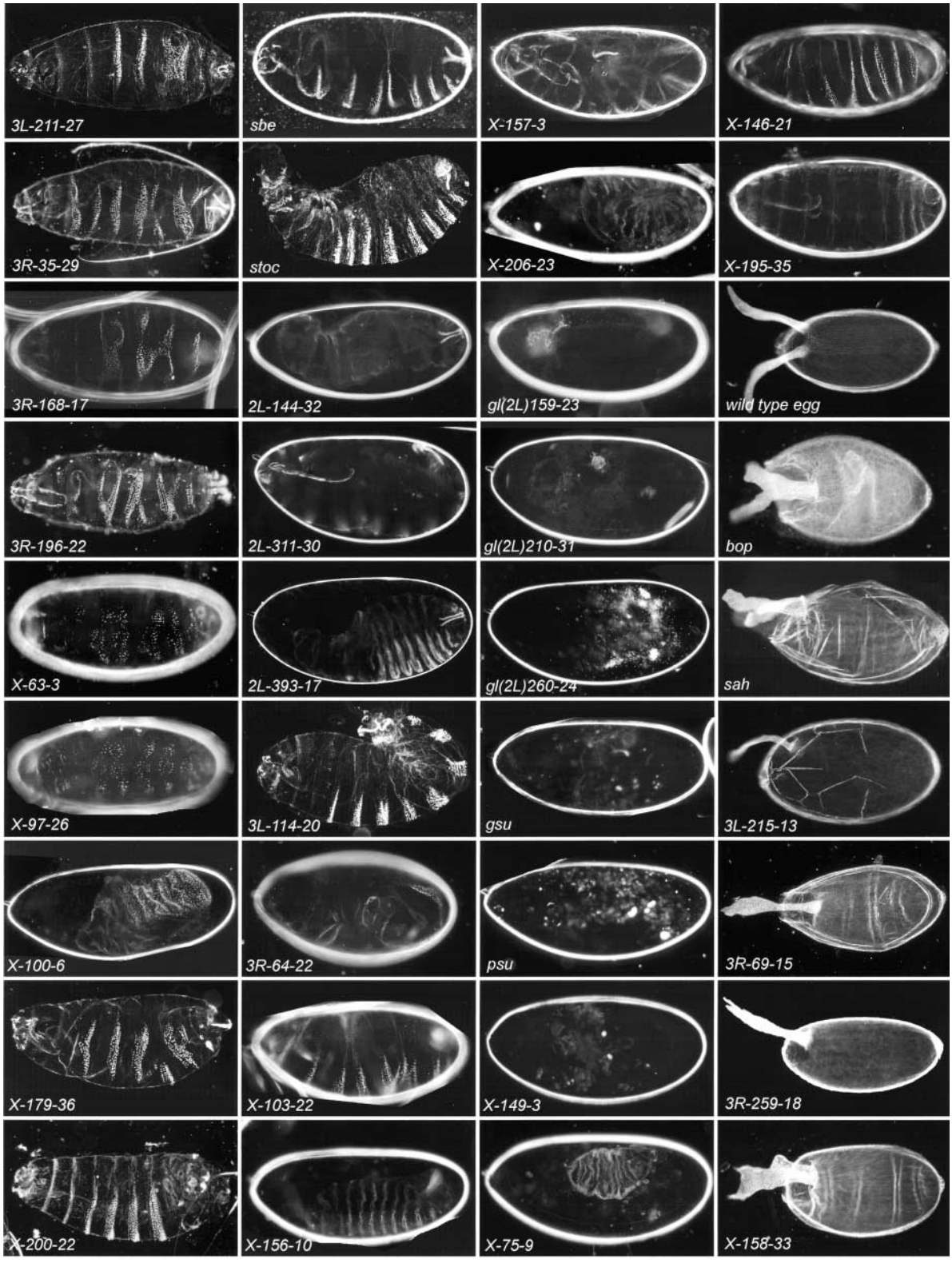

FIGURE 4.—*Continued.*

Defects in eggshell morphology: Ten mutants show altered eggshell morphology. This class includes two alleles of *fs(1)K10*, which give rise to dorsalization of the eggshell and the embryo. A new complementation group, *brontops* (*bop*, two alleles), is viable and homozygous mutant females produce small eggs with fused or branched dorsal

appendages. Mutants at a second new locus, *sahnehäubchen* (*sah*; one allele), produce dorsalized eggs and embryos. Other mutants produce ventralized eggs with fused dorsal appendages (*3L-215-13, 3R-259-18, gl(3R) 66-35*, and *3R-69-15*) or small eggs (*3R-40-7*). *X-158-33* shows small, collapsed eggs with abnormally broad and branched dorsal appendages. We also found several additional X chromosomal mutants that showed this phenotype in GLCs generated using an ovo^{D2} FRT chromosome, but did not show a phenotype when the GLCs were marked by the absence of maternal GFP. This suggests that the phenotype might be due to perdurance of the mutant Ovo^{D2} protein in the GLCs, which may result in a "dumpless" phenotype (Mevel-Ninio *et al.* 1996). These lines were discarded.

DISCUSSION

In this work we present the results of a new screen for genes involved in patterning the early Drosophila embryo, including those genes that had been missed in previous screens due to their essential roles for viability of the adult animal. The screen makes use of chemical mutagenesis and analysis of genetic mosaics as an efficient and unbiased means to isolate mutations. We have developed a system that allows screening for maternal-effect mutations after only one generation of breeding, in contrast to the three generations required for a classic female-sterile screen (Schüpbach and Wieschaus 1989; reviewed in St. Johnston 2002) and the two generations required for a screen using the Flp-DFS (ovo^{D}) system (Chou and Perrimon 1996). Since in our scheme mutation-bearing females are crossed to wild-type males, the paternal contribution may zygotically rescue the lack of a maternal gene product. Hence, autosomal mutations with a zygotically fully rescuable maternal effect could not be found by our screen. Only in the case of the X chromosome were we able to score zygotic mutations in hemizygous male embryos. This explains the significant fraction of zygotic embryonic lethal mutations, which we isolated in the X chromosome screen.

The F_1 scheme is efficient, as it allows rapid screening of a large number of individuals and significantly reduces the efforts of maintaining potentially mutant lines prior to actually screening them. In only few cases (4%) was a potential mutant lost because the respective F_1 female died, was sterile, or produced an insufficient number of progeny. Since the mutation-bearing F_1 females do not carry a balancer chromosome preventing meiotic recombination, a potential mutation could also be lost if an F_1 female produced only recombinant progeny devoid of the mutation. This does not appear to pose a frequent problem, as in most cases we were able to recover the respective mutation when a clear patterning phenotype was scored in the screen. However, in certain cases it was difficult to reliably score a phenotype among a very small number of GLC-derived embryos (often a single embryo), especially for certain phenotypic classes (embryos with holes or a little cuticle or embryos with defective head skeletons). In these cases, the originally observed phenotype was frequently not reproducible in the retest, where many GLC-derived embryos were scored. The rate of positive recovery was different for each chromosome arm screened (*e.g.*, 23% for the 3L screen and 66% for the 3R screen; see Table 2), presumably reflecting differences in the genetic background and our ability to reliably score certain phenotypes. For instance, in the screen on the 3L arm, we frequently saw partially penetrant pair-rule-like segmentation defects, which are likely to be related to the presence of the h^{1} mutation on the FRT chromosome used for mutagenesis (the same phenotype was seen at a low frequency also among embryos from the unmutagenized parental *ru h th st FRT2A* strain); these phenotypes were not reproducible in the retest.

The GFP-FRT chromosomes described in this work also provide a useful set of tools to mark mitotic clones in the germline and in various somatic tissues. In particular, this system can also be used to mark clones carrying mutations on different chromosome arms simultaneously and to induce GLCs in the background of dominant-female-sterile mutations (such as Tor^{4021} or Tl^{10b}), which cannot be combined with the dominant-female-sterile ovo^{D} system (Luschnig *et al.* 2000).

Strategies for mapping chemically induced mutations: Although chemical mutagenesis has proven highly efficient in terms of allowing rapid isolation of large numbers of mutants, genetic mapping of chemically induced mutations has often remained the rate-limiting step in positional cloning projects. This is in part due to the relatively low map resolution of visible genetic markers and chromosomal deficiencies. The recent introduction of single-nucleotide polymorphisms (SNPs) as markers for genetic mapping in Drosophila should provide a valuable tool for facilitating the mapping of chemically induced point mutations (Berger *et al.* 2001; Martin *et al.* 2001; Nairz *et al.* 2002). Fine-scale SNP mapping can be used to narrow down the position of a mutation once an approximate map interval has been defined using classic meiotic and/or deficiency mapping. High-resolution SNP maps have already been generated for some of the strains that were used in this work (Martin *et al.* 2001). In our screen, mutations were induced on FRT chromosomes carrying visible genetic markers distal to the FRT site. Hence, meiotic mapping can be done between the marked FRT chromosome and its unmarked parental version, thus maintaining the presence of the FRT throughout the mapping scheme. Mapping of most of the X chromosomal mutants from the screen is still in progress. To be informative, complementation tests using X chromosomal duplications or transpositions require that lethal mutations on a particular X chromosome can be rescued by a single duplication. Because many of the X chromosomal lines carry

multiple lethal mutations, these chromosomes have to be cleaned by meiotic recombination prior to duplication mapping.

Numbers and saturation: We describe here a collection of 232 mutants that show distinct embryonic phenotypes in homozygous mutant GLCs. The mutants were grouped into 10 different phenotypic classes (supplemental Table 1). By crossing all mutants with similar phenotypes to each other or to mutants in candidate genes, we were able to order 174 mutants into 86 complementation groups, corresponding to an average of two alleles per locus for these 86 groups. Complementation testing of the remaining 58 lines, including most of the X chromosomal mutations, is still in process.

The only criteria for the isolation of these mutants were (1) the inability of the mutant embryos to hatch and (2) a distinct phenotype recognizable in the embryonic cuticle. Using these criteria, we have identified new alleles of most of the previously known maternal-effect lethal loci: 45 of the 86 complementation groups correspond to previously described genes involved in embryonic patterning or morphogenesis. Alleles of most of the previously known maternal genes involved in embryonic patterning were isolated, suggesting a high degree of saturation was achieved in the screen. However, we did not obtain mutants in some previously known genes that we had expected to find, including *cni*, *dos*, *dpar-1*, *drk*, *exu*, *grk*, *nos*, *put*, *vas*, and *swa*. Some of those may have been missed because even amorphic mutations give rise to only subtle embryonic defects and the mutant embryos may be able to hatch (*e.g.*, *dos* and *drk*; Hou *et al.* 1995; Raabe *et al.* 1996; Luschnig *et al.* 2000). In the case of *nos*, it has been shown that most mutant alleles affect an essential function of the gene during oogenesis and result in complete sterility, while only few mutations located in a C-terminal region of the protein affect exclusively embryonic abdomen formation (Arrizabalaga and Lehmann 1999). Similarly, DPP signaling has been shown to be required for germline stem cell maintenance in the ovary (Xie and Spradling 1998), and DPP pathway mutants produce only a small number of germline clone-derived eggs, which may explain our failure to isolate a mutation in the DPP receptor *put*. Correspondingly, for several other genes only certain hypomorphic alleles may result in a visible maternal-effect embryonic phenotype [*e.g.*, *fs(1)Nasrat* and *fs(1)polehole*; Jimenez *et al.* 2002], or, in other cases, a given gene may have phenotypically distinct classes of alleles (*e.g.*, *capicua*, also known as *fettucine*; Goff *et al.* 2001; Roch *et al.* 2002). It is not clear why we failed to isolate *cni*, *grk*, *exu*, and *swa* alleles.

Forty-one of the 86 complementation groups correspond to unknown or previously undescribed loci (on the basis of phenotype, map position, and/or complementation tests). Several of the remaining mutants that are not yet mapped or assigned to a complementation group are likely to represent new loci, as well. We characterized 21 of the 41 new complementation groups at the genetic or molecular level. It is interesting to note that the average mutation rate was lower for the new loci discovered here than for previously known loci: 122 mutants falling into 45 complementation groups (2.7 alleles per gene on average) correspond to previously known genes, while the remaining 52 mutants falling into 41 complementation groups (1.27 alleles per locus on average) represent new loci or possibly unusual alleles of previously known loci (see Table 4). In fact, of the 21 new loci that we studied in more detail, 14 are represented by single alleles. Moreover, in a secondary screen for new alleles of *dshc* and *baiser*, only one new *dshc* allele and no *baiser* allele was isolated from approximately 8000 mutagenized chromosomes (S. Luschnig and S. Bartoszewski, data not shown). These findings underline the notion that certain genes may represent mutational "cold spots" that have been missed in genetic screens and show the advantage of efficient F_1 type screening schemes in picking up rare mutations. However, mutations in certain genes may result in reduced fecundity, as is the case for *bai*, *eca*, *gsu*, and *tkv* germline mosaics. Such mutations have a reduced chance to be picked up by our screen.

To attempt to estimate the degree of saturation that was achieved in the screen, one would have to evaluate each chromosome arm screen as a separate experiment. However, as a first approximation, assuming an approximately equal degree of saturation for each chromosome arm, we can use the obtained allele frequencies as a measure for saturation (see Figure 5, Table 3). The average overall allele frequency was 2.02 alleles per locus (174 mutations falling into 86 complementation groups), counting all mutants that were tested for allelism against various candidates (58 mutants, including many of the X chromosomal mutations, have not yet been tested for complementation and were not included in the calculation of allele frequencies). This number does not change significantly if we exclude the 10 X-linked complementation groups from the calculation and count only the autosomal complementation groups (156 mutants falling into 76 complementation groups; mean allele frequency = 2.05). Assuming a Poisson distribution of allele frequencies with a mean value of 2.02, the zero class would make up 13%, suggesting that we have achieved 87% saturation. However, the observed distribution of allele frequencies deviates from a random Poisson distribution, as single hits are overrepresented in the observed distribution, indicating that the actual degree of saturation is below the theoretical value.

It is of interest to compare our results with previous germline clone screens. Perrimon and coworkers (Perrimon *et al.* 1989, 1996) carried out screens for maternal effects of X chromosomal and autosomal lethal loci. These mutations were induced by EMS (X chromosome screen) or by *P*-element insertions (autosomal screen). The percentage of cell-lethal mutations reported in

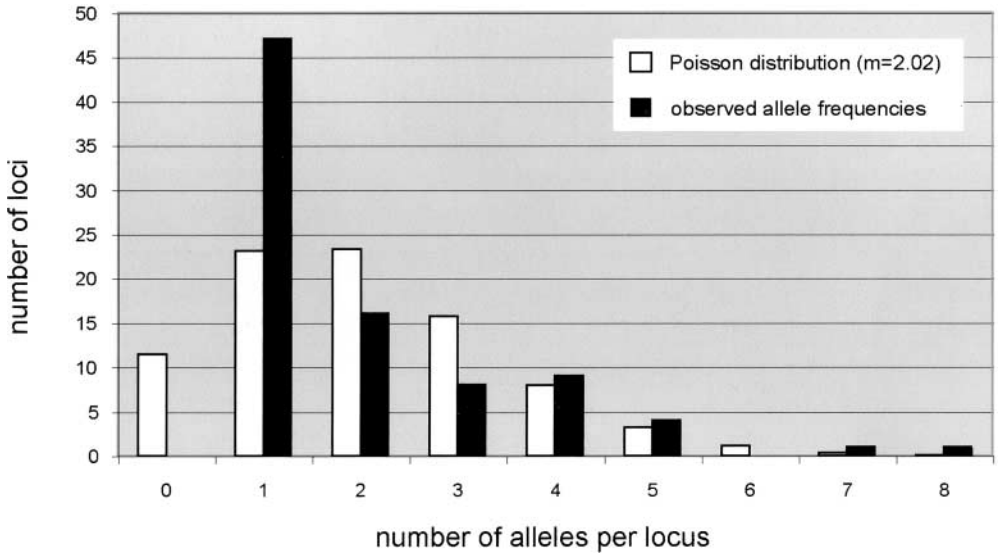

FIGURE 5.—Analysis of allele frequencies. In the graph, the observed allele frequencies are compared to a calculated Poisson distribution for the mean allele frequency. Solid bars indicate the observed distribution of allele frequencies (number of alleles per locus) from the screens on all five chromosome arms. Open bars represent the calculated Poisson distribution for the observed mean allele frequency $m = 2.02$. The *y*-axis represents absolute numbers of loci.

these studies is similar to our results in the case of the X chromosome (40% in their study *vs.* 41.8% in our study; see Table 2), while autosomal cell lethals appear to be more frequent in our screens (31% in their study *vs.* between 36.8 and 50.8% in our study, depending on the autosomal arm screened; see Table 2). This may reflect the different nature of the mutagen used (*P* elements *vs.* EMS), as well as a difference in the screening procedure: Perrimon *et al.* used the DFS (ovo^D) technique to select for GLC-derived eggs, and they scored several females each carrying the same mutation. In our F_1 screen, GLC-derived eggs were not selected for, and only a single female could be scored for GLC-derived eggs. We may therefore overestimate the number of cell-lethal mutations by counting all females in which we fail to detect GLC-derived eggs. For the same reason, it is difficult to compare the number of mutants that show no maternal effect in their study with that in our study. Perrimon *et al.* analyzed a collection of 496 independent *P*-element lethal mutations on the autosomes for maternal effects. In total, they found a maternal effect in 25% of these mutations, and in many of these cases the maternal effect was fully or partially paternally rescuable (PERRIMON *et al.* 1996). A similarly high percentage of lines with paternally rescuable maternal effects was found in an independent screen of *P*-element-induced lethal mutations on the third chromosome (BELLOTTO *et al.* 2002). In our screen, a much smaller fraction of screened individuals showed a maternal effect. This is mostly due to the design of our screen, which was focused on genes acting before the onset of zygotic transcription, and which did not permit the isolation of fully paternally rescuable mutations on the autosomes. It was suggested that several genes involved in embryonic patterning had escaped from detection in the classic female-sterile screens because of their essential functions for viability. This holds true for many components of intracellular signaling pathways (*e.g.*, the RAS-MAPK or JAK-STAT pathway). At least five (*ali*, *gsu*, *soy*, *wek*, and *wol*) of the 21 new loci studied in detail are essential for viability. For four loci (*krk*, *nap*, *sah*, and *ttf*) that are represented by a single allele only, we cannot exclude that lethality may be due to second-site mutations. However, half of the 21 loci characterized in detail are not strictly essential for adult viability: mutants in 11 (*bai*, *cst*, *dshc*, *eca*, *bop*, *sbe*, *kra*, *rum*, *sel*, *stoc*, and *zih*) of the 21 loci are homozygous viable or show only partially reduced viability, although some of these mutations may be hypomorphic alleles of essential

TABLE 3

Allele frequencies (numeric table to Figure 5)

Total no. of mutants	232
Not assigned to a complementation group	58
Assigned to a complementation group	174
No. of complementation groups	86
Average no. of alleles per group	2.02
One allele	47
Two alleles	16
Three alleles	8
Four alleles	9
Five alleles	4
Six alleles	0
Seven alleles	1
Eight alleles	1

The average overall allele frequency (2.02 alleles per locus; 174 mutations falling into 86 complementation groups) was calculated by counting all mutants that were tested for allelism against various candidates. This number does not change significantly if we exclude the 10 X-linked complementation groups from the calculation and count only the autosomal complementation groups (2.05 alleles per locus; 156 mutants falling into 76 complementation groups).

TABLE 4

Classification of mutants assigned to complementation groups

	No. of groups	No. of mutations	Average no. of alleles per group	No. of viable mutants	% of viable mutants
Previously known genes	45	122	2.71	21	46.67
Previously undescribed loci	41	52	1.27	16	39.02

All complementation groups were classified according to whether or not they have been previously described and whether or not the corresponding loci are required for viability (note that for new loci represented by a single allele we cannot exclude the presence of second-site mutations precluding homozygous viability of a mutation).

genes. Overall, the percentage of nonessential loci is similar among the previously known loci (46.6% nonessential for viability; 21 of 45 groups) as compared to the newly identified loci (39% nonessential for viability; 16 of 41 groups; see Table 4). These arguments suggest that for certain genes their low mutation rate, rather than an essential role for viability, has precluded their isolation in earlier genetic screens. Interestingly, homozygous *bai*, *eca*, *dshc*, or *psu* females do not lay eggs, even though at least *bai*, *eca*, and *dshc* homozygotes produce mature oocytes. These genes appear to be required for egg laying due to functions in the somatic follicle cells or in the egg-laying apparatus. Thus, the embryonic phenotype seen in GLCs is "masked" in homozygous females due to earlier somatic functions of those genes, thus precluding their identification in classic screens for maternal-effect embryonic patterning mutants. Multiple requirements of a gene in the soma and the germline might be a rather common phenomenon, although only a few examples have been documented so far (*e.g.*, *cic*; Goff *et al.* 2001). The analysis of *éclair* showed that when the wild-type gene is expressed specifically in the follicle cells of homozygous *éclair* females, the egg-laying defect, but not the embryonic patterning defect, is rescued (S. Bartoszewski, unpublished results). It will be interesting to carry out a systematic mosaic analysis of egg-laying-defective mutants in order to separate somatic from germline functions of the respective genes. Several of the mutants from our screen display somewhat variable phenotypes or different classes of phenotypes. For example, *dshc* is required for patterning events in both the germline and somatic follicle cells, in addition to a later role in zygotic patterning (Luschnig *et al.* 2000). Phenotypic variability seen in embryos from mosaic mothers may be due to variable occurrence (number, size, and position) of mutant follicle cell clones. In other cases, the paternal contribution accounts for phenotypic differences between zygotically mutant and zygotically partially "rescued" embryos, such as in the case of *dshc*, *sos*, *draf*, *ras*, and others.

Morphological screens allow the identification of genes on the basis of their earliest requirement during development. This implies that genes required rather broadly and at early stages, such as genes encoding cytoskeletal components, will mutate to early embryonic lethality, revealing relatively little functional information. Also, it is likely that proteins involved in the localization of *bcd* RNA are generally required for RNA transport; such genes are likely to mutate to cell lethality or to an early arrest phenotype. Examples include *dynein*, *kinesin*, and *ncd* (Endow *et al.* 1994; McGrail and Hays 1997). Using the embryonic cuticle as a readout of earlier patterning events, our screen did not pick up new components involved in *bcd* mRNA localization. However, a secondary screen using an *in situ* hybridization assay was performed to directly examine localization of *bcd* and *osk* mRNAs during oogenesis in mutants that showed early embryonic arrest phenotypes in the original screen (F. Schnorrer, unpublished data). This approach revealed the roles of components of the microtubule cytoskeleton for *bcd* RNA localization during oogenesis (Schnorrer *et al.* 2002). Thus, screening early arrest mutants using a direct visualization assay is an efficient way to identify genes with essential functions for RNA or protein localization.

What is the contribution of this work to the understanding of embryonic pattern formation? The screen led to the isolation of new components involved in the posterior (*nap*, *soy*), dorsoventral (*sel*, *kra*, *wek*, *eca*, *bai*, *cst*, and *hrn*), and terminal patterning (*dshc*, *rum*) systems. The genetic and molecular characterization of these genes is likely to reveal important new insights into all four maternal axial patterning systems.

Three new dorsal group genes, *kra*, *sel*, and *wek*, were identified by this study. We cloned the *krapfen* gene and found that it encodes an adaptor protein homologous to mammalian *myd88*. Kra/dMyd88 is required downstream of the Toll receptor to transmit the signal to the cytoplasmic protein Tube (Charatsi *et al.* 2003). It will be of great interest to characterize the remaining two new dorsal group mutants, *sel* and *wek*, molecularly and to fit them into the dorsoventral signaling cascade. On the basis of genetic epistasis experiments, *seele* is a new component of the Toll pathway required upstream of the Toll receptor, while *krapfen* and *weckle* act downstream of Toll (Charatsi *et al.* 2003 and data not shown). Two genes (*gamma-tubulin37C* and *dgrip75*) not previously known to be involved in localization of *bcd*

RNA were identified in a secondary screen of the mutant collection using an *in situ* hybridization assay (Schnorrer *et al.* 2002). Two new components (*nap*, *soy*) involved in posterior patterning have been isolated, and six others that complement known genes await further characterization. New components of the terminal system will allow studying aspects of receptor tyrosine kinase signaling. We previously reported the identification of mutants in the adaptor protein DSHC and its role in the Torso and EGFR signaling pathways (Luschnig *et al.* 2000). We are currently investigating the role of a new component, *rumpf*, in terminal patterning. On the basis of genetic epistasis experiments, *rum* is required downstream of the Torso receptor (S. Luschnig, unpublished results). Two new genes, *bai* and *eca*, act in the DPP pathway and appear to be required for secretion of TGF-β receptors to the plasma membrane (S. Bartoszewski, unpublished results). These mutants will allow studies of new aspects of the cell biology of TGF-β signaling. Several new loci required for embryonic segmentation were discovered, suggesting new connections between maternal gene activity and gap- or pair-rule gene expression. Analysis of the mutants described in this work will fill in the gaps in current models and stimulate new questions about embryonic patterning and morphogenesis.

It is of interest to note that, while the morphogens and most other upstream components in each of the four patterning systems are encoded by strictly maternal genes, many of the downstream signaling components are encoded by genes with a dual (maternal plus zygotic) mode of action and have multiple functions throughout development (*e.g.*, the RAS-MAPK pathway). We identified only a small number of novel strictly maternal genes that are components of the core axis determination pathways (*kra*, *sel*, and *rum*; Charatsi *et al.* 2003; S. Luschnig, unpublished data); these genes seem to have escaped detection in earlier screens due to their low mutation frequency. This suggests that we are getting close to a complete draft of the maternal control of embryonic development in Drosophila.

We thank Slawek Bartoszewski, Andreas Bergmann, Dirk Beuchle, Jörg Grosshans, Holger Knaut, Iris Koch, Frank Schnorrer, and all other members of the Tübingen fly groups for invaluable help and stimulating discussions during the screen. Holger Knaut and Frank Schnorrer are thanked for collaborating on the characterization of *ali*, *gug*, and *nap*. We thank Amin Ghabrial and Mark Metzstein for comments on the manuscript and Konrad Basler, Celeste Berg, Ilan Davis, Ruth Lehmann, Ernst Hafen, Norbert Perrimon, Trudi Schüpbach, Daniel St. Johnston, Larry Zipursky, and the Bloomington and Szeged Stock Centers for sending numerous fly stocks. S.L. was supported by the Max-Planck Society during the course of this work and presently holds a Human Frontier Science Program long-term fellowship.

LITERATURE CITED

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of Drosophila melanogaster. Science **287:** 2185–2195.

Ambrosio, L., A. P. Mahowald and N. Perrimon, 1989 Requirement of the Drosophila raf homologue for torso function. Nature **342:** 288–291.

Anderson, K. V., and C. Nüsslein-Volhard, 1986 Dorsal-group genes of Drosophila, pp. 177–194 in *Gametogenesis and the Early Embryo*, edited by J. Gall. Alan R. Liss, New York.

Arrizabalaga, G., and R. Lehmann, 1999 A selective screen reveals discrete functional domains in Drosophila Nanos. Genetics **153:** 1825–1838.

Ashburner, M., S. Misra, J. Roote, S. E. Lewis, R. Blazej *et al.*, 1999 An exploration of the sequence of a 2.9-Mb region of the genome of Drosophila melanogaster: the Adh region. Genetics **153:** 179–219.

Bellotto, M., D. Bopp, K. A. Senti, R. Burke, P. Deak *et al.*, 2002 Maternal-effect loci involved in Drosophila oogenesis and embryogenesis: P element-induced mutations on the third chromosome. Int. J. Dev. Biol. **46:** 149–157.

Berger, J., T. Suzuki, K. A. Senti, J. Stubbs, G. Schaffner *et al.*, 2001 Genetic mapping with SNP markers in Drosophila. Nat. Genet. **29:** 475–481.

Binari, R., and N. Perrimon, 1994 Stripe-specific regulation of pair-rule genes by hopscotch, a putative Jak family tyrosine kinase in Drosophila. Genes Dev. **8:** 300–312.

Casanova, J., M. Furriols, C. A. McCormick and G. Struhl, 1995 Similarities between trunk and spatzle, putative extracellular ligands specifying body pattern in Drosophila. Genes Dev. **9:** 2539–2544.

Chagnovich, D., and R. Lehmann, 2001 Poly(A)-independent regulation of maternal hunchback translation in the Drosophila embryo. Proc. Natl. Acad. Sci. USA **98:** 11359–11364.

Charatsi, I., S. Luschnig, S. Bartoszewski, C. Nüsslein-Volhard and B. Moussian, 2003 Krapfen/dMyd88 is required for the establishment of dorsoventral pattern in the Drosophila embryo. Mech. Dev. **120:** 219–226.

Chou, T. B., and N. Perrimon, 1992 Use of a yeast site-specific recombinase to produce female germline chimeras in Drosophila. Genetics **131:** 643–653.

Chou, T. B., and N. Perrimon, 1996 The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. Genetics **144:** 1673–1679.

Chou, T. B., E. Noll and N. Perrimon, 1993 Autosomal P[ovoD1] dominant female-sterile insertions in Drosophila and their use in generating germ-line chimeras. Development **119:** 1359–1369.

Daubresse, G., R. Deuring, L. Moore, O. Papoulas, I. Zakrajsek *et al.*, 1999 The Drosophila kismet gene is related to chromatin-remodeling factors and is required for both segmentation and segment identity. Development **126:** 1175–1187.

Davis, I., C. H. Girdham and P. H. O'Farrell, 1995 A nuclear GFP that marks nuclei in living Drosophila embryos: maternal supply overcomes a delay in the appearance of zygotic fluorescence. Dev. Biol. **170:** 726–729.

Endow, S. A., R. Chandra, D. J. Komma, A. H. Yamamoto and E. D. Salmon, 1994 Mutants of the Drosophila ncd microtubule motor protein cause centrosomal and spindle pole defects in mitosis. J. Cell Sci. **107:** 859–867.

Erkner, A., A. Roure, B. Charroux, M. Delaage, N. Holway *et al.*, 2002 Grunge, related to human Atrophin-like proteins, has multiple functions in Drosophila development. Development **129:** 1119–1129.

FlyBase, 1999 The FlyBase database of the Drosophila Genome Projects and community literature (http://flybase.bio.indiana.edu/). Nucleic Acids Res. **27:** 85–88.

Furriols, M., and J. Casanova, 2003 In and out of Torso RTK signalling. EMBO J. **22:** 1947–1952.

Gans, M., C. Audit and M. Masson, 1975 Isolation and characterization of sex-linked female-sterile mutants in *Drosophila melanogaster*. Genetics **81:** 683–704.

Garcia-Bellido, A., and L. G. Robbins, 1983 Viability of female germ-line cells homozygous for zygotic lethals in *Drosophila melanogaster*. Genetics **103:** 235–247.

Goff, D. J., L. A. Nilson and D. Morisato, 2001 Establishment of dorsal-ventral polarity of the Drosophila egg requires capicua action in ovarian follicle cells. Development **128:** 4553–4562.

Golic, K. G., 1991 Site-specific recombination between homologous chromosomes in Drosophila. Science **252:** 958–961.

Golic, K. G., and S. Lindquist, 1989 The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell **59:** 499–509.

Grau, Y., and P. Simpson, 1987 The segment polarity gene costal-2 in Drosophila. I. The organization of both primary and secondary embryonic fields may be affected. Dev. Biol. **122:** 186–200.

Grether, M. E., J. M. Abrams, J. Agapite, K. White and H. Steller, 1995 The head involution defective gene of Drosophila melanogaster functions in programmed cell death. Genes Dev. **9:** 1694–1708.

Grosshans, J., F. Schnorrer and C. Nüsslein-Volhard, 1999 Oligomerisation of Tube and Pelle leads to nuclear localisation of dorsal. Mech. Dev. **81:** 127–138.

Guichet, A., J. W. Copeland, M. Erdelyi, D. Hlousek, P. Zavorszky *et al.*, 1997 The nuclear receptor homologue Ftz-F1 and the homeodomain protein Ftz are mutually dependent cofactors. Nature **385:** 548–552.

Hou, X. S., T. B. Chou, M. B. Melnick and N. Perrimon, 1995 The torso receptor tyrosine kinase can activate Raf in a Ras-independent pathway. Cell **81:** 63–71.

Hou, X. S., M. B. Melnick and N. Perrimon, 1996 Marelle acts downstream of the Drosophila HOP/JAK kinase and encodes a protein similar to the mammalian STATs. Cell **84:** 411–419.

Jimenez, G., A. Gonzalez-Reyes and J. Casanova, 2002 Cell surface proteins Nasrat and Polehole stabilize the Torso-like extracellular determinant in Drosophila oogenesis. Genes Dev. **16:** 913–918.

Lewis, E. B., and F. Bacher, 1968 Methods of feeding ethyl methane sulfonate (EMS) to Drosophila males. Dros. Inf. Serv. **43:** 193.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster.* Academic Press, San Diego.

Luschnig, S., J. Krauss, K. Bohmann, I. Desjeux and C. Nüsslein-Volhard, 2000 The Drosophila SHC adaptor protein is required for signaling by a subset of receptor tyrosine kinases. Mol. Cell **5:** 231–241.

Martin, S. G., K. C. Dobi and D. St. Johnston, 2001 A rapid method to map mutations in Drosophila. Genome Biol. **2:** RESEARCH0036.

McGrail, M., and T. S. Hays, 1997 The microtubule motor cytoplasmic dynein is required for spindle orientation during germline cell divisions and oocyte differentiation in Drosophila. Development **124:** 2409–2419.

Mevel-Ninio, M., E. Fouilloux, I. Guenal and A. Vincent, 1996 The three dominant female-sterile mutations of the Drosophila ovo gene are point mutations that create new translation-initiator AUG codons. Development **122:** 4131–4138.

Mlodzik, M., and W. J. Gehring, 1987 Expression of the caudal gene in the germ line of Drosophila: formation of an RNA and protein gradient during early embryogenesis. Cell **48:** 465–478.

Mohler, J. D., 1977 Developmental genetics of the Drosophila egg. I. Identification of 59 sex-linked cistrons with maternal effects on embryonic development. Genetics **85:** 259–272.

Moore, L. A., H. T. Broihier, M. Van Doren, L. B. Lunsford, R. Lehmann *et al.*, 1998 Identification of genes controlling germ cell migration and embryonic gonad formation in Drosophila. Development **125:** 667–678.

Müller, H. A., 2000 Genetic control of epithelial cell polarity: lessons from Drosophila. Dev. Dyn. **218:** 52–67.

Nairz, K., H. Stocker, B. Schindelholz and E. Hafen, 2002 High-resolution SNP mapping by denaturing HPLC. Proc. Natl. Acad. Sci. USA **99:** 10575–10580.

Nüsslein-Volhard, C., 1977 A rapid method for screening eggs from single Drosophila females. Dros. Inf. Serv. **52:** 166.

Nüsslein-Volhard, C., and E. Wieschaus, 1980 Mutations affecting segment number and polarity in Drosophila. Nature **287:** 795–801.

Parkhurst, S. M., and D. Ish-Horowicz, 1991 wimp, a dominant maternal-effect mutation, reduces transcription of a specific subset of segmentation genes in Drosophila. Genes Dev. **5:** 341–357.

Patterson, J. T., 1928 The effects of X-rays in producing mutations in the somatic cells of Drosophila melanogaster. Science **68:** 41–43.

Perrimon, N., L. Engstrom and A. P. Mahowald, 1984 The effects of zygotic lethal mutations on female germ-line functions in Drosophila. Dev. Biol. **105:** 404–414.

Perrimon, N., D. Mohler, L. Engstrom and A. P. Mahowald, 1986 X-linked female-sterile loci in *Drosophila melanogaster.* Genetics **113:** 695–712.

Perrimon, N., L. Engstrom and A. P. Mahowald, 1989 Zygotic lethals with specific maternal effect phenotypes in *Drosophila melanogaster.* I. Loci on the X chromosome. Genetics **121:** 333–352.

Perrimon, N., A. Lanjuin, C. Arnold and E. Noll, 1996 Zygotic lethal mutations with maternal effect phenotypes in *Drosophila melanogaster.* II. Loci on the second and third chromosomes identified by P-element-induced mutations. Genetics **144:** 1681–1692.

Raabe, T., J. Riesgo-Escovar, X. Liu, B. S. Bausenwein, P. Deak *et al.*, 1996 DOS, a novel pleckstrin homology domain-containing protein required for signal transduction between sevenless and Ras1 in Drosophila. Cell **85:** 911–920.

Rittenhouse, K. R., and C. A. Berg, 1995 Mutations in the Drosophila gene bullwinkle cause the formation of abnormal eggshell structures and bicaudal embryos. Development **121:** 3023–3033.

Roch, F., G. Jimenez and J. Casanova, 2002 EGFR signalling inhibits Capicua-dependent repression during specification of Drosophila wing veins. Development **129:** 993–1002.

Rubin, G. M., M. D. Yandell, J. R. Wortman, G. L. Gabor Miklos, C. R. Nelson *et al.*, 2000 Comparative genomics of the eukaryotes. Science **287:** 2204–2215.

Schnorrer, F., S. Luschnig, I. Koch and C. Nüsslein-Volhard, 2002 Gamma-tubulin37C and gamma-tubulin ring complex protein 75 are essential for bicoid RNA localization during Drosophila oogenesis. Dev. Cell **3:** 685–696.

Schüpbach, T., and E. Wieschaus, 1986 Germline autonomy of maternal-effect mutations altering the embryonic body pattern of Drosophila. Dev. Biol. **113:** 443–448.

Schüpbach, T., and E. Wieschaus, 1989 Female sterile mutations on the second chromosome of *Drosophila melanogaster.* I. Maternal effect mutations. Genetics **121:** 101–117.

Schüpbach, T., and E. Wieschaus, 1991 Female sterile mutations on the second chromosome of *Drosophila melanogaster.* II. Mutations blocking oogenesis or altering egg morphology. Genetics **129:** 1119–1136.

Simon, M. A., D. D. Bowtell, G. S. Dodson, T. R. Laverty and G. M. Rubin, 1991 Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. Cell **67:** 701–716.

Sonoda, J., and R. P. Wharton, 2001 Drosophila Brain Tumor is a translational repressor. Genes Dev. **15:** 762–773.

Sprenger, F., L. M. Stevens and C. Nüsslein-Volhard, 1989 The Drosophila gene torso encodes a putative receptor tyrosine kinase. Nature **338:** 478–483.

Stern, C., 1936 Somatic crossing over and segregation in *Drosophila melanogaster.* Genetics **21:** 625–730.

St. Johnston, D., 2002 The art and design of genetic screens: *Drosophila melanogaster.* Nat. Rev. Genet. **3:** 176–188.

St. Johnston, D., and C. Nüsslein-Volhard, 1992 The origin of pattern and polarity in the Drosophila embryo. Cell **68:** 201–219.

Struhl, G., 1981 A gene product required for correct initiation of segmental determination in Drosophila. Nature **293:** 36–41.

Struhl, G., and K. Basler, 1993 Organizing activity of wingless protein in Drosophila. Cell **72:** 527–540.

Su, M. A., R. G. Wisotzkey and S. J. Newfeld, 2001 A screen for modifiers of decapentaplegic mutant phenotypes identifies lilliputian, the only member of the Fragile-X/Burkitt's Lymphoma family of transcription factors in *Drosophila melanogaster.* Genetics **157:** 717–725.

Tang, A. H., T. P. Neufeld, G. M. Rubin and H. A. Muller, 2001 Transcriptional regulation of cytoskeletal functions and segmentation by a novel maternal pair-rule gene, lilliputian. Development **128:** 801–813.

Therrien, M., H. C. Chang, N. M. Solomon, F. D. Karim, D. A. Wassarman *et al.*, 1995 KSR, a novel protein kinase required for RAS signal transduction. Cell **83:** 879–888.

Treisman, J. E., A. Luk, G. M. Rubin and U. Heberlein, 1997 eyelid antagonizes wingless signaling during Drosophila development and has homology to the Bright family of DNA-binding proteins. Genes Dev. **11:** 1949–1962.

Ueno, S., K. Kondoh, Y. Kotani, O. Komure, S. Kuno *et al.*, 1995 Somatic mosaicism of CAG repeat in dentatorubral-pallidoluysian atrophy (DRPLA). Hum. Mol. Genet. **4:** 663–666.

Vazquez, M., L. Moore and J. A. Kennison, 1999 The trithorax group gene osa encodes an ARID-domain protein that genetically interacts with the brahma chromatin-remodeling factor to regulate transcription. Development **126:** 733–742.

Wieschaus, E., and C. Nüsslein-Volhard, 1998 Looking at embryos, pp. 179–214 in *Drosophila: A Practical Approach*, edited by D. B. Roberts. Oxford University Press, Oxford.

Wilkins, A. S., 1986 *Genetic Analysis of Animal Development*. John Wiley & Sons, New York.

Wittwer, F., A. van der Straten, K. Keleman, B. J. Dickson and E. Hafen, 2001 Lilliputian: an AF4/FMR2-related protein that controls cell identity and cell growth. Development **128:** 791–800.

Wodarz, A., A. Ramrath, A. Grimm and E. Knust, 2000 Drosophila atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. J. Cell Biol. **150:** 1361–1374.

Xie, T., and A. C. Spradling, 1998 decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell **94:** 251–260.

Xu, T., and G. M. Rubin, 1993 Analysis of genetic mosaics in developing and adult Drosophila tissues. Development **117:** 1223–1237.

Zhang, S., L. Xu, J. Lee and T. Xu, 2002 Drosophila atrophin homolog functions as a transcriptional corepressor in multiple developmental processes. Cell **108:** 45–56.

Communicating editor: T. Schüpbach