wimp, a dominant maternal-effect mutation, reduces transcription of a specific subset of segmentation genes in *Drosophila*

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***wimp* is a dominant maternal-effect mutation that interacts with a specific subset of early-acting maternal and zygotic *Drosophila* genes. We show that *wimp* is a change-of-function mutation, allelic to mutations of the 140-kD subunit of RNA polymerase, which causes reduced transcription of interacting genes. Loci that do not interact with *wimp* are expressed at normal levels. We discuss these results in terms of specific interactions between transcription factors and RNA polymerase. Embryos from *wimp* mothers show unaltered fate maps and develop normally, despite the reduction of transcript levels at least twofold. We suggest that spatial cues are determined by a balance of segmentation gene products rather than their absolute concentrations. We also demonstrate powerful genetic screens for otherwise undetected loci required for segmentation, sex determination, and other early functions.**

[Key Words: Segmentation gene expression; RNA polymerase; *Drosophila* maternal-effect mutation; pattern formation; transcription; embryonic fate map]

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Several lines of evidence indicate that anterior–posterior patterning in the *Drosophila* embryo is regulated predominantly at the transcriptional level. The early development of the *Drosophila* embryo is marked by its progressive subdivision into increasingly more precise spatial domains, achieved through the actions of a hierarchy of maternal and zygotic segmentation genes (Nüsslein-Volhard and Wieschaus 1980; for review, see Scott and O’Farrell 1986; Akam 1987; Scott and Carroll 1987; Ingham 1988). These genes have been ranked according to their phenotypes and times of expression and can be subdivided further according to their epistatic interactions: Earlier-acting genes control the patterns and expression of later genes. The maternal genes establish coarse positional cues that define the broad domains of gap gene expression. In turn, the gap genes are needed for the pair-rule genes to be expressed in striped domains (Carroll and Scott 1986; Ingham et al. 1986; Frasch and Levine 1987; Pankratz et al. 1989, 1990; Struhl 1989b; Hülkamp et al. 1990). Such patterns evolve over a period of ~30–60 min, arguing that most of the segmentation gene products act immediately. Protein and transcript domains correspond well, indicating the predominance of

transcriptional control, although post-transcriptional mechanisms may also be involved, particularly in the posterior region of the embryo (Macdonald and Struhl 1986; Gaul et al. 1987; Hülkamp et al. 1989; Irish et al. 1989; Struhl 1989a).

Many segmentation genes encode nuclear proteins with homologies to well-characterized transcription factors. These include proteins with defined DNA-binding motifs: Homeo box domains, “zinc-fingers,” “leucine zippers,” and helix–loop–helix (b-HLH) motifs (for review, see Levine and Hoey 1988; Biggin and Tjian 1989; Murre et al. 1989; Struhl 1989; Benezra et al. 1990; Hayashi and Scott 1990).

The most direct evidence for transcriptional regulation of segmentation gene expression comes from studies of *hunchback* (*hb*) regulation by the anterior morphogen, *bicoid* (*bcd*). The *bcd* protein defines different anteroposterior positions in a concentration-dependent manner (Frohnhöfer and Nüsslein-Volhard 1986, 1987; Driever and Nüsslein-Volhard 1988a). Thus, blastoderm positions of anterior Anlagen are sensitive to copy number of maternal *bcd*⁺ genes (Driever and Nüsslein-Volhard 1988b). Footprinting and in vivo transformation assays indicate that binding of *bcd* protein to the *hb* promoter directly controls the domain of *hb* expression (Driever and Nüsslein-Volhard 1989; Driever et al. 1989; Struhl et al. 1989).

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Further evidence for transcriptional regulation comes from studies of *fushi tarazu* (*ftz*) expression using reporter gene fusions. The *ftz* promoter includes transcriptional activator sites required for expression within stripes and other elements that are needed for *ftz* auto-regulation (Hiromi et al. 1985; Hiromi and Gehring 1987; Dearolf et al. 1989). *ftz* transcription is also under negative control, including repression between stripes and at the embryonic poles (Hiromi et al. 1985; Edgar et al. 1986).

The precise mechanisms whereby segmentation genes modulate transcription are unclear. Different homeo domain proteins have been shown to recognize similar DNA target sequences, indicating that these proteins may compete for binding sites in vivo (see Levine and Hoey 1988; Hayashi and Scott 1990). Many homeo domain target sequences overlap with or are adjacent to sequence motifs recognized by non-homeo domain transcription factors, suggesting that homeo domain proteins may also compete with other families of regulatory proteins for DNA-binding sites (Odenwald et al. 1989). Homeo box proteins can enhance or repress transcription, depending on cell type and the particular target gene; this suggests that they interact with other, more generally required components of the transcriptional machinery. The b-HLH motif has been proposed to mediate functionally required heterodimer formation, suggesting that the pair-rule gene *hairy* (*h*) may also act together with other proteins (Murre et al. 1989; Parkhurst et al. 1990).

The above *Drosophila* genes were identified because they are specifically required during segmentation (although most are reutilized later in development). Other factors that participate in the regulation of segmentation gene transcription might remain undetected because they are required generally for transcription and lead to apparently unrelated mutant phenotypes, or because they are functionally or structurally redundant. Such factors could include ubiquitous factors such as the TATA-binding TFIID factor needed for the transcription of most genes and generalized sequence-specific factors that act on a wide range of genes and are expressed in most cells (Ptashne 1988; Mitchell and Tjian 1989; Parker 1989). Indeed, if the segmentation genes act through such factors, their identification would be critical to understanding how spatially restricted gene expression is achieved. Although general or redundant factors might not be mutable to readily identifiable phenotypes, occasional conditional or tissue-specific alleles that affect only segmentation might be recoverable.

This paper describes the characterization of *wimp*, one such mutation. We show that *wimp* is a dominant maternal-effect mutation, allelic to an RNA polymerase II subunit (*RpII140*), which causes reduced transcription of a specific subset of early-acting genes. Nevertheless, embryos from *wimp* mothers develop normally. We discuss the implications of locus-specific transcriptional interactions for mechanisms of transcriptional regulation and also present a "gene balance" model for specifying positional cues in the *Drosophila* embryo. We also demon-

strate a genetic screen for novel mutations affecting early gene function.

Results

wimp is both a conditional maternal-effect and a recessive lethal mutation

The *wimp* allele was identified in a screen for zygotic segmentation genes (Jürgens et al. 1984) and originally designated an allele of the *hairy* (*h*) locus on the basis of complementation tests and its homozygous lethal phenotype (see below). However, subsequent experiments showed that this noncomplementation is due to a maternal effect that causes lethality of flies heterozygous for *h* when derived from a *wimp*/+ mother (P. Ingham, unpubl.). Figure 1A illustrates the dominant maternal effect: when *wimp*/+ females are mated to *h*/+ males, both classes of *h* heterozygous progeny (*h*/+ and *h*/*wimp*) die. In the reciprocal cross using *h*/+ females, heterozygous *h* embryos survive (Fig. 1B). Although this paper will concentrate on the maternal interaction, we first provide a brief introduction to the *wimp* locus and its recessive lethality.

The initial classification of *wimp* as a segmentation gene was based on its homozygous pair-rule phenotype. The phenotype is somewhat variable: The most common phenotype is pair-wise fusions of adjacent denticle belts (Fig. 1C), but some embryos show more severe fusions (Fig. 1D) and, rarely, weaker phenotypes (not shown). However, the pattern defects in homozygous *wimp* embryos are likely to result from cellularization defects as well as alterations in segmentation gene expression. When staining with antibodies against segmentation proteins, we noticed regions of altered nuclear density and holes in the cell monolayer (Fig. 2A,B). Detailed histological analysis shows that nuclear organization is severely disrupted in homozygous *wimp* embryos and that their cortical cytoplasm is irregular (cf. Fig. 2C with Fig. 2D–H). Nuclei are often of different sizes, indicative of asynchronous nuclear divisions that often accompany perturbations of nuclear density (Foe and Alberts 1983; Sullivan 1987; Wieschaus and Sweeton 1988).

For the rest of this paper, we use the term "*wimp* embryos" to refer to maternally affected embryos, that is, embryos from a "*wimp*" mother (i.e., *wimp*/+). Such +/+ or *wimp*/+ embryos are wild type for all other segmentation genes and develop normally unless fertilized with sperm mutant for an interacting locus.

The *wimp* maternal effect interacts with many genes

The *wimp* maternal-effect interaction is not limited to the *h* locus: flies heterozygous for *Krüppel* (*Kr*) or *even-skipped* (*eve*) are also lethal if derived from *wimp* mothers (P. Ingham, unpubl.). We have extended this analysis and find that *wimp* shows genetic interactions with a wide range of genes (summarized in Table 1; discussed in more detail below). These include both zygotic and maternal genes, segmentation genes, and genes not affecting

Figure 1. The *wimp* maternal-effect and recessive lethality. (A,B) Genetic crosses illustrating the interaction between the dominant *wimp* maternal effect and the zygotically required pair-rule gene *h*. Heterozygous *h* progeny are embryonic lethal when derived from a *wimp*/+ mother (A). The same two classes of *h* heterozygous progeny survive if the genotypes of the parents are reversed (B). (C,D) Ventral cuticle phenotype of homozygous *wimp* larvae showing weak pair-wise fusions (C) and more severe fusions (D). Anterior is to the left.

pattern (Table 1). The broad spectrum of interacting loci is consistent with a general/basic cellular function being affected by the *wimp* mutation.

Affected embryos show phenotypes characteristic of intermediate alleles of the interacting loci. For example, *h*/+ embryos from *wimp*/+ mothers resemble pair-rule *h*/*h* embryos, lacking alternate metamereres (Fig. 3A). Similarly, *Kr*/+ embryos from *wimp* mothers show a characteristic gap phenotype (Fig. 3B). This suggests that *wimp* acts to reduce gene expression.

Most classes of gene shown in Table 1 include both *wimp*-sensitive and *wimp*-insensitive loci. For example, the gap genes *hb* and *tailless* are insensitive to *wimp*, while other gap genes, *Kr* and *knirps* (*kni*), are sensitive. Genes that act after blastoderm formation, for example, the segment-polarity genes, appear not to interact with *wimp*. However, genes that are sensitive or insensitive to *wimp* are not distinguished merely by dosage sensitivity, as *wimp* appears not to interact with the extremely haploinsufficient gene *decapentaplagic* (*dpp*) (Irish and Gelbart 1987). Nor is there an obvious correlation between time of expression and *wimp* sensitivity; effects on gene expression can persist past the blastoderm stage (see below and Discussion).

Maternal-effect genes *wimp* fails to complement several maternal mutations affecting pattern (Fig. 3D; Table 1). For example, females heterozygous for both *wimp* and *bcd* (+ *wimp*/*bcd* + females) produce embryos with an intermediate *bcd* phenotype in which

head and thoracic structures are largely lacking (Fig. 3C). *wimp* also interacts with the *dorsal* (*dl*) gene, which encodes a nuclear protein that acts as the dorsoventral morphogen (Steward 1987; Steward et al. 1988). Interestingly, *wimp* does not interact with posterior genes [e.g., *nanos* (*nos*)] that appear to act in a very different manner from *bcd* and *dl* (Nüsslein-Volhard et al. 1987; Lehmann 1988; Hülskamp et al. 1989; Irish et al. 1989; Struhl 1989a).

wimp also interacts with genes required for sex determination. Female embryos from homozygous *daughterless* (*da*) mothers die because they fail to activate the *Sex-lethal* (*Sxl*) gene (Cline 1980). Similarly, most female progeny die from *da*/+; *wimp*/+ mothers: 86% (567/656) of adult progeny are male. The (presumed) female embryos die, some with poorly developed cuticles resembling those of female embryos mutant for *Sxl*.

Zygotic genes The *wimp* maternal effect interacts with members of both the gap and pair-rule zygotic segmentation gene classes (Table 1). The lethal embryos display cuticular phenotypes characteristic of homozygous segmentation mutant embryos (e.g., Fig. 3A,B). Embryos heterozygous for the gap gene *kni* and the pair-rule genes *paired* (*prd*) and *odd-paired* (*opa*) are not completely lethal. Of the expected heterozygous progeny, 80% (*kni*), 5% (*prd*), and 52% (*opa*) eclose as adults, although survivors have defective or missing structures such as fused/missing segments or legs (see footnote to Table 1).

The dorsoventral genes, *twist* (*twi*) and *snail* (*sna*) also interact with the *wimp* maternal effect, although with reduced penetrance. Approximately 40% and 55% of such embryos show *twi*- or *sna*-like cuticular phenotype, respectively (not shown). The remaining heterozygous progeny hatch and develop into apparently normal adults. Either these embryos retain sufficient *twi* and *sna* activity to allow survival or the lethality is an indirect consequence of reduced *dl* expression (see below). Both *twi* and *sna* have been shown to be sensitive to reduced *dl* dosage (Simpson 1983; Nüsslein-Volhard and Roth 1989; Roth et al. 1989).

wimp also interacts with *Sxl* and two zygotic genes implicated in *Sxl* initiation, *sisterless-a* and *sisterless-b* (Cline 1986, 1988), causing female-specific lethality of heterozygous female progeny. *wimp* interferes with a process that is used in sex determination as well as segmentation.

Protein levels are reduced in *wimp* embryos

The dominant lethality and lethal phenotypes caused by the *wimp* maternal effect are most simply explained if *wimp* causes reduced expression of susceptible loci. Figure 4, A–D, shows that the levels of *ftz* and *Kr* proteins are indeed reduced in *wimp* embryos, although their patterns are unchanged (see also *eve* in Fig. 7D–F, below). A *ftz-lacZ* reporter gene (Hiromi et al. 1985) is similarly affected (Fig. 5A–D). Lowered expression persists during germ-band extension when continued transcription is under autoregulatory control (Fig. 5C,D). *h* expression is similarly affected in *wimp* embryos (not shown). (For discussion of the effects on *bcd*, see below.)

Protein levels are further reduced in embryos heterozygous for interacting mutations, indicating that their lethality is due to insufficient expression. *ftz* expression in *wimp* embryos that contain only one wild-type *ftz* gene is either undetectable (Fig. 4E) or weak and disorganized (Fig. 4F). The extreme reduction in *ftz* expression leads to disruption or lack of the *ftz*-requiring even-numbered *engrailed* (*en*) stripes (Fig. 4G,H). Although *en* pat-

Figure 2. The homozygous *wimp* embryonic phenotypes. Dorsal surface of *wimp/wimp* embryos stained with anti-*ftz* (A) or anti-*en* (B) show that the pattern of staining is disrupted due to large regions devoid of nuclei. Tissue sections of wild-type (C) and homozygous *wimp* (D–H) embryos are shown at the blastoderm stage. The uniform layer of nuclei and cytoplasm appears very uneven in homozygous *wimp* embryos, including regions without nuclei and regions without cytoplasm. Although the first nuclear divisions in *Drosophila* embryos are synchronous (Foe and Alberts 1983), the nuclei are larger in the anterior half of the homozygous *wimp* embryo shown in D and at higher magnification in F (arrowhead marks border), suggesting that the posterior half has undergone an extra round of nuclear division. Nuclei appear to migrate into the central yolk region leaving holes in the periphery of the blastoderm cortex (arrow in E; and the same embryo at higher magnification in G and H). For whole embryo sections, anterior is to the left and dorsal is upmost.

Table 1. Summary of interactions with the wimp maternal effect

Class	Interacting	Noninteracting	Reference
Maternal			
anterior	<i>bicoid</i> ^{E1} , <i>Df(3R)LIN</i> <i>exuperantia</i> ^{PJ42}	<i>Bicaudal</i> <i>D</i> ^{III E} <i>swallow</i> ¹⁴ <i>oskar</i> ¹⁶⁶ <i>pumilio</i> ⁶⁸⁰ <i>nanos</i> ^{L7} <i>vasa</i> ^{PD23} <i>valois</i> ^{RB71} <i>staufer</i> ^{HL54} <i>tudor</i> ^{WC8} <i>torso</i> ^{WK34}	1, 2 3, 4 5 6 7 3 3 3 8 3, 3
posterior		<i>easter</i> ¹ <i>snake</i> ⁰⁷³ <i>pipe</i> ⁶⁶⁴ <i>tube</i> ^{Z38} <i>Toll</i> ^{QRE} <i>cappuccino</i> ^{HK3,G7} <i>spire</i> ^{RP48}	9, 10 10 10 10 11 12 12
terminal	<i>trunk</i> ^{RA41}		3
dorsal	<i>dorsal</i> ¹	<i>Polycomb</i> ³	13, 23
ventral	<i>cactus</i> ^{A2}		3
others	<i>daughterless</i> ²		13, 23
Zygotic			
dorsal/	<i>twist</i> ^{IID96 a}	<i>zerknüllt</i> ^{W36}	14, 15
ventral	<i>snail</i> ^{IIC:05 a}	<i>decapentaplegic</i> ²⁷	14, 14
gap/	<i>Krüppel</i> ^{1,2}		
cardinal		<i>hunchback</i> ^{PXT15} <i>tailless</i> ^{L10} <i>huckebein</i> ²	14, 16 17, 16 17, 18
	<i>giant</i> ^{YA} <i>unpaired</i> ^{YC43,YM55} <i>knirps</i> ^{IID48} <i>hairy</i> ²² <i>even-skipped</i> ^{1 27,3,77 17} <i>runt</i> ^{III2} <i>fushi tarazu</i> ^{Df(Scb),W20} <i>paired</i> ^{IIN27,2 45,17 b} <i>odd-paired</i> ^{SH97 b}	<i>sloppypaired</i> ⁷⁶⁴⁸ <i>odd-skipped</i> ^{IIID36}	16 16, 14 14, 14 17 16 14 16
segment		<i>engrailed</i> ^B <i>wingless</i> ^{CX4} <i>patched</i> ^{IIN108} <i>smooth</i> ^{Q14} <i>naked</i> ^{7E89} <i>gooseberry</i> ^{IIX62}	14 14 14 14 16 14
polarity		<i>Ultrabithorax</i> ¹ <i>Polycomb</i> ³ <i>BX-C (Df(3R)P9)</i>	23 23 23
homoeotics		<i>empty spiracles</i> ^{7ID99,10A37} <i>buttonhead</i> ^{IIIA} <i>string</i> <i>branch</i> ^{IOE113} <i>RpII215</i> ^{Ubl} <i>UPHZ50T</i> ^c <i>tailup</i> ^{IIIB29} <i>u-shaped</i> ^{IIA102}	17, 16 19, 17 20, 16 20, 16 22, 21 22 22
others	<i>orthodenticle</i> ^{YH} <i>Sex-lethal</i> ^{7B0} <i>sisterless-a</i> ^c <i>sisterless-b</i> (<i>sc</i> ³⁻¹) ^c <i>ftz-lacC/FG2</i> ^d		

^aOnly ~40% and 55% of the expected embryos are embryonic lethal showing a *twist* or *snail* cuticular phenotype, respectively.

^bThe gap gene *knirps* and the pair-rule genes *paired* and *odd-paired* do not lead to complete embryonic lethality: 80% (94/118), 5% (5/102), 32% (14/44), and 52% (41/79) for *kni*^{IID48}, *prd*^{2,45,15}, *prd*^{IIN27}, and *opa*^{SH97}, respectively survive to adulthood.

^cFor *sisterless-a* and *sisterless-b*, 39% (24/62) and 84% (46/55) of the progeny are embryonic lethal, respectively. Of the surviving progeny, 85% (197/232) and 67% (330/491) are male, respectively.

^d*ftz-lacC* and *FG2* are germ-line transformants carrying a fusion gene with the *ftz* promoter driving the expression of β -galactosidase in a *ftz* pattern (Hiromi et al. 1985; Hiromi and Gehring 1987).

^e*UPHZ50T* is a germ-line transformant with the *ftz* upstream enhancer sequences placed in reverse orientation 5' to a *hsp70*- β -gal reporter construct (Hiromi and Gehring 1987).

References: (1) Frohnhofer and Nüsslein-Volhard (1986); (2) Mohler and Wieschaus (1986); (3) Schüpbach and Wieschaus (1986); (4) Frohnhofer and Nüsslein-Volhard (1987); (5) Lehmann and Nüsslein-Volhard (1986); (6) Lehmann and Nüsslein-Volhard (1987); (7) Lehmann (1988); (8) Boswell and Mahowald (1985); (9) Nüsslein-Volhard (1979); (10) Anderson and Nüsslein-Volhard (1986); (11) Anderson et al. (1985); (12) Manseau and Schüpbach (1989); (13) Cline (1980); (14) Nüsslein-Volhard et al. (1984); (15) Wakimoto et al. (1984); (16) Jürgens et al. (1984); (17) Wieschaus et al. (1984); (18) Wiegand et al. (1990); (19) Cline (1986); (20) Cline (1988); (21) Mortin and Lefevre (1981); (22) Hiromi et al. (1985)/Hiromi and Gehring (1987); (23) Lewis (1978).

Figure 3. Interaction of the *wimp* maternal effect with maternally and zygotically active genes. (A) Cuticle phenotype of a heterozygous *h* larva derived from a *wimp*/+ mother showing pair-wise segmental fusions characteristic of intermediate homozygous *h* larvae. (B) Cuticle phenotype of a heterozygous *Kr* embryo derived from a *wimp*/+ mother showing segmental deletions characteristic of homozygous *Kr* larvae. (C,D) Interaction of the maternal gene *bcd* with the *wimp* maternal effect. All embryos derived from mothers transheterozygous for the maternal gene *bcd* and *wimp* do not survive (D) and show defects in the anterior pattern characteristic of intermediate *bcd* phenotypes (C). Anterior is uppermost.

tern is affected, the level of expression is not affected (cf. Figs. 4G,H with 5M,N).

wimp affects the transcription of interacting genes

That *wimp* affects the transcription of interacting genes is confirmed by in situ analysis of *ftz* transcription, comparing *wimp* embryos with *oskar* (*osk*) embryos that can be recognized by their lack of pole cells (see Materials and methods, Lehmann and Nüsslein-Volhard 1986; Driever and Nüsslein-Volhard 1988b). [The *ftz* pattern, but not level, is altered in *osk* embryos (Lehmann 1988).] Figure 5, E–J, shows that levels of *ftz* transcripts are clearly reduced in *wimp* embryos, indicating that the reduced protein levels result from a primary defect in transcription. *h* transcripts are similarly lowered in *wimp* embryos (not shown). As *wimp* affects both maternal and zygotic genes, reduced transcript levels seem unlikely to be due to lowered mRNA stability. Rather, it appears that *wimp* may diminish the efficiency of transcription.

Transcription of noninteracting genes is unaffected in *wimp* embryos

There are two alternative explanations for selective interactions with the *wimp* maternal effect. First, the dis-

tingtion could reflect different threshold requirements for development: All transcript levels are reduced in *wimp* embryos, but there would be no apparent developmental consequences for noninteracting genes. Alternatively, noninteracting loci are *wimp* independent and retain normal transcript levels. We have confirmed the latter explanation by showing that expression levels of several noninteracting genes are the same in *wimp* and wild-type embryos. Figure 5, K–P, shows that *hb*, *zerknüllt* (*zen*), and *en* protein levels do not differ among embryos of *wimp* heterozygous mothers and *osk* females. Indeed, Figure 4, G and H, shows that *en* levels (though not their pattern) are normal in *wimp/ftz* embryos in which *ftz* expression is reduced to essentially undetectable levels. Even-numbered *en* stripes correspond to the anterior margins of *ftz* expression (Lawrence et al. 1987; Carroll et al. 1988), so individual cells can express reduced *ftz* and normal *en* levels (Figs. 4B and 5N). We also find that transcript levels of *torso* (*tor*) and *UPHZ50T* (see Table 1) are unaffected (not shown). Thus, transcription of many, if not all, noninteracting loci is *wimp* insensitive.

wimp is allelic to an RNA polymerase subunit

wimp maps to position 54 cM on chromosome 3R, within the region duplicated in *Dp(3R)kar⁵¹* (86D–90F)

Figure 4. Protein levels are reduced in *wimp* embryos. *ftz* protein expression is reduced in embryos from *wimp*/+ mothers (B) compared to wild-type (A) embryos. Similarly, the *Kr* protein levels are reduced in *wimp* (D) compared with wild-type (C) embryos. To standardize the staining, these embryos have been processed in parallel and stained and photographed identically. (E–F) Heterozygous *ftz* progeny from a *wimp*/+ mother stained with anti-*ftz* antibodies. The level of protein expression is undetectable for most embryos (E), consistent with their cuticle patterns. Occasional embryos show very weak and disorganized *ftz* protein expression (F). (The staining intensity of this embryo has been photographically enhanced to show the disorganized pattern.) (G,H) Reduced *ftz* expression in homozygous *ftz* embryos is associated with the lack or disruption of the even-numbered *en* stripes (Howard and Ingham 1986). As expected, the heterozygous *ftz* progeny from a *wimp*/+ mother stained with anti-*en* antibodies shows an altered *en* pattern. Such embryos show a broadened fourth stripe (arrowhead) and reduction or absence of other even-numbered stripes (arrows). For all embryos, anterior is to the left and dorsal is uppermost.

(see Materials and methods; P. Ingham, unpubl.). More precise mapping shows that the recessive lethality and the maternal effect colocalize, indicating that both are due to the same mutation (Fig. 6A; Materials and methods). The *wimp* locus is deleted in *Df(3R)red^{P1}* but retained in *Df(3R)red^{P93}*, mapping it to 88B1.2.

Chromosomal deficiencies uncovering *wimp* do not interact with segmentation gene mutations (see Materials and methods), suggesting that *wimp* encodes an altered function. Moreover, homozygous *wimp*[−] deficiencies lack the segment fusions and cellularization defects

characteristic of *wimp/wimp* embryos (Fig. 6C). To examine a null *wimp* allele, we isolated a revertant, *wimp-RQ*, that no longer shows maternal interactions (see Materials and methods). This mutation fails to complement the zygotic lethality of the original *wimp* allele and maps to the same chromosomal region as *wimp* but no longer interacts with *h* and other segmentation genes. Cellularization and segmentation are normal in homozygous *wimpRQ* embryos (Fig. 6D) and are not affected in *wimpRQ/wimp* embryos unless derived from *wimp*/+ mothers (see Materials and methods).

Figure 5. *wimp* affects transcription of interacting but not noninteracting genes. β -gal protein expression is reduced for a *ftz-lacZ* hybrid gene in blastoderm or germ band-extended embryos derived from *wimp* (B,D) compared with wild-type mothers (A,C), suggesting that *wimp* acts at the level of transcription. In situ hybridization has confirmed that *ftz* transcription is substantially reduced in similarly staged embryos derived from heterozygous *wimp* (F,H,I) compared with homozygous *osk* (E,G,I) females. Embryos derived from homozygous *osk* females have wild-type levels but altered patterns of *ftz* expression (Lehmann and Nüsslein-Volhard 1986; Driever and Nüsslein-Volhard 1988b). (E,F) Bright field; (G,H) dark field. Higher magnification of the ventral cortex surrounding the stripe 1 region of the sections in E and F shows that the embryos are of similar staging. The level of protein expression does not differ between wild-type (control) and *wimp* embryos stained for protein from the noninteracting genes *hb* (K,L), *en* (M,N), and *zen* (O,P), respectively. The control embryos for the *hb* and *zen* staining were from homozygous *osk* mothers and were mixed with *wimp* embryos during fixation and staining. For all embryos, anterior is to the left and dorsal is uppermost.

wimp maps to a region previously cloned in a phage/cosmid "walk" (R.L. Kelley, pers. comm.; S.M. Parkhurst, unpubl.). Despite *wimp*RQ being a γ -ray-induced revertant, we were unable to detect differences in chromosomal organization between *wimp*, *wimp*RQ and the

parental stock using genomic Southern blots (data not shown). We therefore localized the *wimp* gene by cosmid rescue (see Materials and methods). Two cosmids, 15 and 92, rescue flies homozygous for *wimp* and *wimp*RQ (Fig. 6A). These cosmids also rescue the maternal effect, pre-

Figure 6. Genetic and molecular localization of *wimp* and *wimpRQ*. (A) Diagram of the 87–89 region of chromosome 3. The horizontal solid lines indicate the genetic material deleted in each of the chromosomal deficiencies shown. The molecular extent of the small genetic region containing *wimp* and *wimpRQ* defined by complementation analysis with the deficiencies is shown, indicating the four overlapping cosmids that were used in the rescue experiments and the region of ~25-kb overlap that rescues this locus. The end points of the deficiencies have been mapped to particular restriction fragments, and their positions on the molecular map are approximate. The cuticular phenotype of wild-type (B); a deficiency (*Df(3R)red^{P1}/Df(3R)red^{3l}*) for the *wimp* locus (C); and the homozygous phenotype of the *wimpRQ* revertant (D) are shown. The deficiency for the region has normal denticle patterns with the exception of defects in the mouth parts and a transformation of the denticles in the eighth abdominal segment characteristic of the *trithorax* mutation contained within the deleted region (Ingham 1983). The *wimpRQ* revertant phenotype (D) resembles the deficiency phenotype (C) with normal denticle patterns except for defects in the mouth parts rather than the homozygous *wimp* phenotype (Fig. 1B,C). Anterior is uppermost in all cases.

sumably by providing an extra wild-type gene copy. Thus, *wimp* must map within the ~25-kb region of overlap between the two cosmids (Fig. 6A).

This interval includes two lethal complementation groups defined by saturation mutagenesis (M. A. Mortin, pers. comm.). Crosses to alleles of these loci show that *wimp* and *wimpRQ* fail to complement the zygotic lethality of mutations A5, Z3, and Z24, alleles of the second largest subunit of RNA polymerase II, *RpII140* (Mortin 1990; B. Hamilton, M.A. Mortin and A. Greenleaf, in prep.). The effects of *wimp* on transcription appear to be due to an altered RNA polymerase II subunit, and the precise nomenclature for the original mutation and its revertant should be *l(3)RpII140^{wimp}* and *l(3)RpII140^{wimpRQ}*.

Altered bcd levels in wimp embryos do not affect the fate map

Driever and Nüsslein-Volhard (1988a,b) have shown that *bcd* behaves as an anterior morphogen, with different levels of *bcd* protein defining different anteroposterior positions. Surprisingly, we noticed that *wimp* reduces

bcd levels without appearing to alter the embryonic fate map. We defined the extent of *bcd* reduction by comparing *wimp* embryos to those with one or two maternal *bcd*⁺ copies (see Materials and methods).

Figure 7, A–C, shows that the intensity of *bcd* staining in *wimp* embryos is comparable to that of embryos from *bcd* heterozygous mothers, that is, ~50% of wild type (Driever and Nüsslein-Volhard 1988b). Nevertheless, there is no corresponding shift in the blastoderm fate map, as judged by the positions of *eve* (Fig. 7D–G) and *ftz* stripes 1 (not shown). Reducing maternal *bcd* dosage by 50% shifts the anterior margin of *eve* stripe 1 by 6% of egg length (Table 2; cf. Driever and Nüsslein-Volhard 1988b), while stripe positions are unchanged in *wimp* embryos. We suggest that the effects of reduced *bcd* levels in *wimp* embryos are modulated by equivalent reductions in other factor(s) (see Discussion).

A powerful genetic screen for novel loci affecting early development

Although the majority of loci that cause cuticular pattern defects have been identified in previous screens

Figure 7. *bcd* levels and the embryonic fate map in *wimp* embryos. (A,B) *bcd* protein expression in embryos from females with one or two copies of *bcd*⁺, respectively. These two classes have been processed together to control for the fixing/staining artifacts, the latter class being distinguished by the lack of pole cells due to the homozygous maternal mutation *osk*. (C) *bcd* protein expression in embryos (from *wimp*/+ females) contain two wild-type copies of *bcd*⁺. Despite the presence of two wild-type copies of *bcd*⁺, the intensity of anti-*bcd* staining is reduced >50%, comparable to embryos with one wild-type copy of *bcd*⁺ (Driever and Nüsslein-Volhard 1988b). These embryos were also processed with embryos derived from homozygous *osk* mothers for an internal control. All embryos were photographed and printed under the same conditions. (D,E) Expression pattern of *eve* in embryos from females with one or two copies of *bcd*⁺, respectively. (F) *eve* expression in embryos from *wimp*/+ females that contain two wild-type copies of *bcd*⁺. (G) Comparison of the ventral surface of the embryos shown in D–F showing the anterior shift in embryos with one copy of *bcd*⁺ (upper). The arrowheads mark the position of *eve* stripe 1 in wild-type (two copies of *bcd*⁺) embryos (middle). Reduced *bcd* expression in *wimp* embryos does not shift the position of *eve* stripe 1 (lower). For all embryos, anterior is to the left and dorsal is uppermost.

(Nüsslein-Volhard and Wieschaus 1980; Jürgens et al. 1984; Nüsslein-Volhard et al. 1984; Wieschaus et al. 1984; Schüpbach and Wieschaus 1986), several classes of mutations would have been missed in such experiments. These would include duplicated loci that encode related products, zygotic loci with significant maternal contributions, and mutations that cause early embryonic lethality before cuticle is laid down. Indeed, *wimp* itself is an example of a general transcription factor whose effects are not specific to segmentation.

Table 2. The embryonic fate map in *wimp* embryos

Maternal genotype	Copies <i>bcd</i> ⁺	<i>ftz</i>		<i>eve</i>	
		stripe 1	stripe 7	stripe 1	stripe 7
<i>bcd</i> ^{EL} /+	1	—	—	76.2 ± 1.4	24.3 ± 2.3
<i>osk/osk</i>	2	66	13	70.4 ± 1.8	21.9 ± 2.9
<i>wimp</i> /+	2	66	12	70.2 ± 1.6	21.0 ± 2.8

Embryos were measured in percent egg length, where 0% is the posterior end. In all cases, 15 stage 14 embryos were measured.

We have identified such novel loci by screening for maternal and zygotic mutations that interact with *wimp*. The strategy for isolating zygotic loci is shown in Figure 8A: Induction of an interacting mutation causes lethality in heterozygous progeny of *wimp* mothers. The zygotic screen also recovers second-site enhancers of the *wimp* mutation that are not dependent on the *wimp* maternal effect (see legend to Fig. 8). A slightly modified screen allows the recovery of maternally interacting mutations: mothers transheterozygous for *wimp* and novel mutations lay eggs whose development is affected (Fig. 8E).

In a preliminary experiment, we recovered seven zygotic and four maternal mutations from 744 and 589 ethylmethane sulfonate (EMS)-mutagenized chromosomes, respectively. All proved to be recessive lethals whose phenotypes (see below) resembled those seen in combination with *wimp*. Thus, *wimp* renders many recessive mutations pseudodominant, greatly simplifying their recovery and analysis. We are currently determining whether any of these mutations represent previously characterized loci.

The newly induced mutations show a variety of phenotypes. Figure 8 presents cuticular preparations of embryos derived from three zygotic (B–D) and three maternal (F–H) mutations. All maternal mutations are recessive zygotic lethals, indicating that they are required both maternally and zygotically. One mutation, *8.14*, shows similar maternal and zygotic phenotypes: *knirps*-like fusions of abdominal segments A1–7 (Fig. 8F–H). The maternal phenotype is variable (cf. Fig. 8H,I), but the homozygous zygotic phenotype is consistent (Fig. 8J). Mutation *8.14* maps to chromosome 2 (unpubl.) and appears to represent a novel segmentation gene. We expect this and future mutations to encode novel factors whose activities are crucial for early development.

Discussion

Specificity of wimp interactions reflects differential interactions with RNA polymerase

Deletion mapping and complementation analysis place *wimp* in the *RpII140* complementation group, the second largest subunit of RNA polymerase II (Fig. 6A; Mortin 1990; B. Hamilton, M.A. Mortin, and A. Greenleaf, in prep.). The strong homozygous *wimp* phenotype and the lack of interaction of chromosomal deletions for the locus indicate that the *wimp* allele encodes an altered *RpII140* protein, rather than a complete loss of subunit function. This is confirmed by the *wimpRQ* revertant that no longer interacts with early genes and that has normal cellularization and segmentation phenotypes.

We have shown that expression of all interacting genes tested is reduced in *wimp* embryos (i.e., embryos from *wimp/+* mothers), apparently due to reduced transcript levels (Figs. 4, 5, and 7). Presumably, the altered RNA polymerase subunit reduces transcription of affected loci so that a single wild-type copy of the interacting gene being tested no longer supports normal development. We suggest that the locus selectivity of *wimp* reflects promoter-specific mechanisms of transcriptional activation. This is consistent with the proposal that different transcription factors recognize different faces of the large multisubunit RNA polymerase II (Mortin 1990). Several zygotic suppressors of mutations in the large subunit of RNA polymerase II are allele-specific, indicating that each compensates for a different perturbation in polymerase function (Mortin 1990). The *wimp* mutation would alter one face of the polymerase, only perturbing interactions with factors recognizing that face. Alternatively, the *RpII140* subunit may not be required for all transcription complexes.

An individual locus will be susceptible to *wimp* if it requires the activity of factor(s) using that subunit or the altered face. Noninteracting genes are not distinguished by dose sensitivity. Indeed, all of the noninteracting genes that we have tested (*hb*, *tor*, *zen*, *en*, *UPHZ50T*) show normal levels of expression in embryos from heterozygous *wimp* mothers, suggesting that they utilize different transcription factors from interacting loci. Of course, there may be *wimp*-sensitive genes that do not

interact phenotypically, genes whose transcription is affected but that retain sufficient expression for normal development.

All of the interacting genes that we have identified act during or before the blastoderm stage. This is not because wild-type polymerase function is supplemented by zygotic *RpII140* protein made during the late blastoderm stage. Depressed *ftz* expression in *wimp* embryos persists throughout gastrulation and into germ-band extension: thus, individual cells can express *en* normally, while *ftz* expression is depressed (Figs. 4B and 5N). In addition, *wimp/wimp* embryos, completely lacking zygotic wild-type *RpII140*, express normal levels of *en*, although the domains are distorted by cellularization defects (Fig. 2B).

Gene-balance and positional cues in the Drosophila embryo

There is considerable evidence that the *bcd* protein gradient acts as an anterior morphogen, defining position on the embryonic fate map by organizing spatial expression of zygotic segmentation genes (Frohnhofer and Nüsslein-Volhard 1986; Driever and Nüsslein-Volhard 1988a,b, 1989; Tautz 1988; Driever et al. 1989, 1990; Struhl et al. 1989). Manipulating gene dosage to reduce *bcd* concentrations shifts anterior Anlagen posteriorly; increasing *bcd* concentrations anteriorizes cell fates (Driever and Nüsslein-Volhard 1988b). Nevertheless, reduced *bcd* expression in *wimp* embryos is not accompanied by an anterior displacement of the fate map (Fig. 7D–G; Table 2). *hb* expression appears unaffected in *wimp* embryos (Fig. 5K,L), despite the strong evidence that *bcd* acts directly in activating anterior *hb* transcription (Driever et al. 1989; Struhl et al. 1989).

We conclude that reduced *bcd* levels in *wimp* embryos must be accompanied by compensatory changes in the expression of other genes, that is, that anteroposterior position is sensed through a balance of factors, rather than the absolute *bcd* concentration. The fate of an anterior cell would depend on the balance in concentrations between the *bcd* morphogen and other *wimp*-sensitive factors. The latter may be generalized, probably maternal, transcription factors that themselves need not convey spatial information.

Similar considerations must apply to establishing dorsoventral position and to pair-rule striping. Dorsoventral fate depends on a dorsoventral gradient of nuclear *dl* protein (Steward et al. 1988; Roth et al. 1989; Rushlow et al. 1989; Steward 1989). The phenotypic interaction between *wimp* and *dl* (Table 1) suggests that *dl* protein levels are reduced in *wimp* embryos. There is no evidence of disrupted dorsoventral pattern in *wimp* embryos, despite *dl* being haplo-insufficient (Nüsslein-Volhard et al. 1980). We presume that dorsoventral pattern is measured by comparing levels of *dl* morphogen to that of other factors.

The unaltered fate map in *wimp* embryos also argues against pair-rule striping being regulated by absolute concentrations of gap proteins or by ratios between dif-

Figure 8. (See facing page for legend.)

ferent gap proteins. *ftz* and *eve* stripes are compressed in *Kr/+* embryos (Carroll and Scott 1986; Frasch and Levine 1987), but the reduced *Kr* levels in *wimp* embryos does not affect the positions of pair-rule stripes (Figs. 4C,D and 7E,F). This indicates that reduced expression of other factors compensates for the reduced *Kr* levels in *wimp* embryos.

In retrospect, such gene-balance models are not particularly surprising, given the wide variety of transcription factors and their overlapping binding specificities (see Levine and Hoey 1988; Mitchell and Tjian 1989; Hayashi and Scott 1990). Apparently unrelated factors bind to similar DNA sites, indicating that binding by a particular factor will be modulated by potential competitors. Different homeo box proteins can bind the same DNA sequences, and their effects on transcription depend on other coexpressed competitors (Jaynes and O'Farrell 1988; Han et al. 1989). Similarly, transcription factors that act as heterodimers (e.g., *fos/jun*, *da/AS-CT4*) will be susceptible to competition that affects either member of the protein dimer (Halazonetis et al. 1988; Kouzarides and Ziff 1988; Nakabeppu et al. 1988; Murre et al. 1989; Parkhurst et al. 1990).

wimp and transregulators of early genes

Most mutations in *Drosophila* are recessive, requiring that they be studied in homozygous individuals. *wimp* appears to render many such mutations pseudodominant, greatly simplifying their identification and analysis. Thus, mutational screens for loci that interact with *wimp* are considerably faster and easier than conventional schemes. We have already identified an apparently novel mutation affecting posterior pattern (see Results), and we recovered mutations that are required both maternally and zygotically, which can be missed in conventional screens because of recessive lethality (see Perimon et al. 1984). The genes against which *bcd* levels are measured may fall into this class.

Similarly powerful screens may be necessary to isolate mutations that affect the general transcriptional machinery. The allele specificity shown by second-site suppressors of RNA polymerase II mutations (Mortin 1990) indicates that alternative screens will identify different but overlapping classes of transcriptional regulators. Trioire (1988) has identified five regions on the X chromosome that interact maternally with the segmentation

genes *Kr*, *hb*, or *h*. These also may represent loci encoding polymerase components or other proteins that interact with polymerase.

Null alleles of the *wimp* locus were not recovered in previous screens for segmentation genes, presumably because the maternal *Rp1140* normally deposited into the egg is sufficient to satisfy early transcriptional requirements. Indeed, similar maternal contributions would suppress early zygotic phenotypes from most of the general transcription factors required for early gene expression. Many mammalian transcription factors (e.g., *jun*, CTF) appear to be encoded as families of related factors that would be refractory to conventional genetic screening (Nakabeppu et al. 1988; Santoro et al. 1988). Such factors may be detectable in the maternal *wimp* screen, even if they are genetically or functionally redundant, as it sensitizes the embryo to otherwise minor alterations of gene dosage.

wimp will also be useful in studying other early developmental events, including sex determination and blastoderm nuclear division and cellularization. The wide range of loci that are sensitive to *wimp* means that identifying the affected process will require detailed phenotypic and molecular analysis of mutant embryos. Inducing such mutations by hybrid dysgenesis should allow their immediate molecular cloning and characterization (O'Kane and Gehring 1987; Cooley et al. 1988).

Materials and methods

Fly stocks

Flies were cultured on yeast/maize meal/molasses/malt extract/agar medium at 25°C unless otherwise stated. The allele designation of segmentation genes used is listed in Table 1. *wimp* was originally designated *h^{9N53}*; its full name is now *l(3)Rp1140^{wimp}*. It was induced on a *ruca* (*ru h th st cu sr e^s ca*) chromosome. Most experiments used a recombinant chromosome where these markers have been replaced by the dominant marker *Stubble* (*Sb*). The official designation of the *wimp* revertant is *l(3)Rp1140^{wimp^{RQ}}*. The deficiencies shown in Figure 6 have been described elsewhere (Lindsley and Zimm 1987; Parkhurst et al. 1988).

Embryo analysis

Embryos were prepared and analyzed as described by Wieschaus and Nüsslein-Volhard (1986). Antibody staining was performed essentially as described previously by Macdonald and Struhl

Figure 8. Genetic screens for mutations interacting with the *wimp* maternal effect. The detail shown is for recovering mutations on the third chromosome. (A) Strategy to recover zygotically interacting mutations. Males with genetically marked chromosomes [*scarlet* (*st*)] are mutagenized and outcrossed to balanced (*TM3*) females. Resulting males, carrying a putative mutation (*st*/*TM3*), are then mated to *wimp Sb/TM3* females. For induced mutations that interact with the *wimp* maternal effect, the two classes of progeny marked with single and double daggers do not survive. For mutations that interact with zygotically contributed *wimp* product (i.e., second-site noncomplementing loci), only the class marked with a single dagger dies. (B–D) Homozygous cuticle phenotypes of three zygotically interacting mutations isolated in a test screen. (E) Strategy for recovering maternally interacting mutations. Although similar to the screen described in A, the induced mutation and the *wimp* mutation must both be present in the female (*st*/*wimp Sb*) to see an interaction. (F–H) Cuticle phenotypes of embryos from *wimp*/*maternal mutant* transheterozygous mothers representing three different mutations isolated in a test screen. One mutation, 8.14, shows similar maternal and zygotic phenotypes: The maternal phenotype is variable (*H* and *I*), but the homozygous zygotic phenotype is consistent (*J*). Anterior is *uppermost* in all cases.

(1986), using biotinylated secondary antibodies and avidin/biotin/horseradish peroxidase (HRP) complexes (Vector Laboratories, Inc.). The antibodies used in this study were generously provided by the following: H. Krause (rabbit anti-*ftz* antibodies; Krause et al. 1988); W. Driever and C. Nüsslein-Volhard (monoclonal anti-*bcd* antibodies; Driever and Nüsslein-Volhard 1988a); M. Frasch (rabbit anti-*eve* antibodies; Frasch et al. 1987); U. Gaul and H. Jäckle (rabbit anti-*Kr* antibodies; Gaul et al. 1987); D. Tautz (rabbit anti-*hb* antibodies; Tautz 1988); M. Wilcox (monoclonal anti-*en* antibodies; LMB, Cambridge, UK); C. Rushlow and M. Levine (guinea pig anti-*zen* antibodies; Columbia University, NY); S. Roth and C. Nüsslein-Volhard (rabbit anti-*twi* antibodies; Tübingen, Germany); and H. Durbin (monoclonal anti- β -gal antibodies; 4C7; ICRF Lincolns Inn Fields, UK). All secondary antibodies were obtained from Jackson ImmunoResearch Labs (West Grove, PA). Stained embryos were dehydrated in 100% ethanol and mounted under a coverslip in methacrylate mounting medium (JB-4, Polysciences) that was polymerized under CO₂ for 1–2 hr at room temperature.

For accurate comparisons of wild-type and *wimp* staining levels, embryos from *wimp* heterozygous and *osk* homozygous mothers were mixed before fixation and processed together (Lehmann and Nüsslein-Volhard 1986; Driever and Nüsslein-Volhard 1988b). The latter were recognized by their lack of pole cells. Embryos derived from *osk*-homozygous mothers have altered patterns but wild-type levels of segmentation gene protein.

In situ hybridization

Whole-mount immunohistochemical *in situ* hybridization was performed essentially by the protocol of Tautz and Pfieffe (1989). Radiochemical *in situ* hybridization using ³⁵S-labeled RNA probes was performed as described by Ingham et al. (1985).

Isolation of wimp revertants

wimp Sb/TM3, *Ser* males were aged for 3–4 days, γ -irradiated with 6000 rads, and outcrossed to TM1/TM3, *Ser* flies. Resulting *wimp* Sb/TM3 female progeny were mated to Df(3R)Scb/TM3, Sb *Ser* males. In a second screen, *wimp* Sb/TM3, *Ser* females were irradiated and mated directly to Df(3R)Scb/TM3, Sb *Ser* males. In both cases, the only viable class of progeny is *wimp* Sb/*ftz*, and these will die unless the *wimp* chromosome has reverted. From 2550 male and an estimated 7300 female γ -irradiated chromosomes, we recovered three such chromosomes. One of these, *wimp*RQ, also fails to interact with *eve*, *h*, and *Kr*, excluding the possibility that it represents a nonspecific suppressor mutation (e.g., a duplication of the *ftz*⁺ gene). The other two chromosomes are specific for *ftz* but have not been tested further.

Genetic mapping and complementation tests

The original *wimp* allele was mapped by recombination between *cu* and *sr*, at genetic map position 54.0 cM and within Dp(3R) *kar*⁵¹ (P. Ingham, unpubl.). Deletions and duplications for the region were then tested (Fig. 6A). Crosses were done reciprocally to avoid ambiguities caused by the dominant maternal effect of the *wimp* allele. The same approach was used for the complementation crosses involving the *wimp*RQ chromosome.

Interactions with the *wimp* chromosome were performed as shown in Figures 1, A and B, and 3D. Zygotically active genes on the second or third chromosome were tested as *wimp*/+ females crossed to heterozygous mutant males. Zygotically active genes on the X chromosome and maternally active genes were

tested as transheterozygous females outcrossed to wild-type males.

Germ-line transformation

Cosmids *cos15*, *cos92*, *cos50*, and *cos731* were isolated in a chromosomal walk by R.L. Kelley (pers. comm.) and are recombinants in the transformation vector cosP *neo* (Steller and Pirrotta 1985). They were injected (500 μ g/ml) into *bw;st* embryos, together with p π 25.7^{wc} helper plasmid (100 μ g/ml) (Spradling 1986). Hatched G₀ flies were outcrossed to wild type and selected on our standard medium supplemented with Geneticin G418 sulfate (1–1.5 mg/ml, according to batch) (GIBCO-BRL). Putative transformed progeny (G₁) were crossed to *bw;st* and reselected on G418 food, mapping the insert to a specific chromosome. Homozygous or balanced stocks of transformed progeny were then made by conventional means. Transformants were confirmed by polymerase chain reaction (Erlach 1989) using primers specific to the *neo* portion of the transformation vector.

Histology of homozygous wimp embryos

Embryos were fixed as for *in situ* hybridization, devitellinized in MEQH/EGTA, and postfixed in 4% paraformaldehyde/1% glutaraldehyde in PBS. Embryos were then rinsed in PBS before incubating in 2% osmium tetroxide for 30 min at 4°C. Embryos were dehydrated and incubated for 10 min in propylene oxide, followed by 1 hr in propylene oxide/araldite (1 : 1) at room temperature. Embryos were infused with araldite overnight at room temperature and transferred to fresh araldite before curing at 50°C for 24–36 hr. Five-micron sections were mounted on gelatinized slides, stained with toluidine blue (1% in 1% borax), and mounted under a coverslip in Permount mountant (BDH).

Screening for novel interacting mutations

A preliminary screen using 744 zygotically and 589 maternally mutated *bw;st* chromosomes was performed as described in Figure 8(A,E). Males were fed overnight on 25 mM EMS in 1% sucrose, as described previously (Grigliatti 1986). Mutations were identified by screening embryos from individual mutagenized chromosomes on apple juice plates (Wieschaus and Nüsslein-Volhard 1986). Mutations were mapped by segregation of the induced mutant phenotype with the *bw* or *st* markers, and balanced stocks were established. Complementation crosses with *wimp* and *wimp*RQ were performed as described above for the chromosomal deficiencies.

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wimp, a dominant maternal-effect mutation, reduces transcription of a specific subset of segmentation genes in *Drosophila*.

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