The zygotic control of Drosophila pair-rule gene expression

I. A search for new pair-rule regulatory loci

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Summary

The examination of pair-rule gene expression in wild-type and segmentation mutant embryos has identified many, but not necessarily all, of the elements of the regulatory system that establish their periodic patterns. Here we have conducted a new type of search for previously unknown regulators of these genes by examining pair-rule gene expression in blastoderm embryos lacking parts of or entire chromosomes. This method has the advantage of direct inspection of abnormal pair-rule gene patterns without relying upon mutagenesis or interpretation of larval phenotypes for the identification of segmentation genes. From these experiments we conclude that: (i) most zygotically required regulators of the fushi tarazu (ftz), even-skipped (eve) and hairy (h) pair-rule genes have been identified, except for one or more loci we have uncovered on chromosome arm 2L; (ii) the repression of the ftz and eve genes in the anterior third of the embryo is under maternal, not zygotic control; and (iii) there are no general zygotically required activators of pair-rule gene expression. The results suggest that the molecular basis of pair-rule gene regulation can be pursued with greater confidence now that most key trans-acting factors are already in hand.

Key words: pair-rule genes, Drosophila embryogenesis, spatial gene regulation.

Introduction

Segmentation of the Drosophila larva is achieved through the action of several sets of regulatory genes during early embryogenesis. Some of these genes are expressed maternally and their mRNA or protein products persist through oogenesis into embryogenesis, while others are products of the zygotic genome that becomes activated during the last few nuclear division cycles of blastoderm formation (for reviews see Scott and Carroll, 1987; Akam, 1987; Ingham, 1988). Inspection of segmentation patterns in mutant larvae has led to the classification of segmentation genes into several types based upon the defects observed. These types include the maternally expressed embryonic polarity or coordinate genes that roughly establish the basic anteroposterior polarity of the embryo (for a review see Nüsslein-Volhard et al. 1987); the zygotically expressed gap genes that control the development of large mostly contiguous blocks of segments; the pair-rule genes that affect pattern elements at double-segment intervals; and the segment polarity genes that specify the compartments within, and the orientation of, each segment (Nüsslein-Volhard and Wieschaus, 1980).

We have focused on the pair-rule genes because their expression is the first sign of metameric organization of the embryo. Eight pair-rule genes have been identified (for review see Gergen et al. 1988) and most have been cloned (reviewed in Scott and Carroll, 1987; Ingham, 1988). Each pair-rule gene is expressed in a series of transverse stripes encircling the embryo at double-segment intervals (Hafen et al. 1984; Ingham et al. 1985; Harding et al. 1986; MacDonald et al. 1986; Gergen and Butler, 1988). These patterns depend upon the proper action of maternal products (Carroll et al. 1986) and the zygotic gap genes and, for each individual pair-rule gene, a unique set of other pair-rule genes (Howard and Ingham, 1986; Carroll and Scott, 1986; Frasch and Levine, 1987; Ingham and Gergen, 1988). Determining which interactions among these genes are direct has not been a simple task for two reasons. First, observed changes in pair-rule gene patterns in various mutants may be caused by other, intermediary genes (see accompanying paper). Second, it is possible that all of the genes necessary for proper pair-rule gene expression have not yet been identified.

There are certain general questions that can be studied at the formal genetic level to help solve some of these issues. They include: are there any previously unidentified genes that could be part of the regulatory network? And, which genes are most likely to directly regulate another? Does this regulation involve gene activation or repression? By analyzing pair-rule gene expression in embryos deficient for an entire chromosome or chromosome arm, we effectively screened the entire genome for any zygotically required pair-rule
regulatory genes, which may have been missed in previous mutagenesis screens. While the known pair-rule regulatory loci account for most of our observations, we have identified at least one new locus involved in the spatial regulation of pair-rule gene expression. In the accompanying paper, we address the more specific questions of which genes regulate others and in what manner.

Materials and methods

Antibodies and mutant strains

We have examined pair-rule gene expression in whole-mount cellular blastoderm embryos by filtered fluorescence imaging (Karr and Kornberg, 1989; Carroll et al. 1988) after immunoperoxidase staining with polyclonal antibodies specific for the ftz (Carroll and Scott, 1985) and eve (Frasch et al. 1987; antibody gift of M. Frasch and M. Levine) proteins. This technique gives sharp images of protein localization and was also used to double-label embryos to examine relative expression patterns or to unambiguously identify the genotype of an individual embryo derived from crosses that yield a variety of mutant progeny.

Compound chromosome stocks C(1)RM w cv; C(2L)RM.C(2R)RM, C(3L)RM.C(3R)RM, C(3)EN cu Ca e; and C(4)RM were all provided by the Bowling Green Stock Center. The C(2)EN cn bw stock was a gift from Dr Barry Ganetzky.

Results

Aneuploid screen for pair-rule regulatory loci

Several outstanding issues remain concerning the whole set of genes that may regulate pair-rule gene patterns including: have all of the fundamental pair-rule regulatory loci been identified? And, what genes are responsible for pair-rule gene activation? Also, how is pair-rule expression, especially the ftz and eve genes, excluded from the anterior third of the embryo? To address these questions, we have searched for additional pair-rule regulatory loci that may have been missed by previous mutagenesis screens either by their failure to be mutagenized or to be recognized as possessing a segmentation phenotype.

Our approach is based upon the strategy used by Merrill et al. (1988) and Wieschaus and Sweeton (1988) to search for zygotic regulators of other early events in embryogenesis. The technique employs compound chromosomes (Novitski et al. 1981) to generate embryos lacking all or part of a chromosome. By staining embryos with pair-rule protein-specific antibodies at the blastoderm stage, before development collapses in these grossly aneuploid animals, we can assess whether individual pair-rule protein patterns are what would be expected from our knowledge of where known pair-rule regulatory loci are located and how these genes interact to affect pair-rule gene patterns. In our screen using pair-rule probes, we were fortunate that no more than two pair-rule regulatory loci (considering just the pair-rule genes hairy, eve, and ftz) were known to be on any one chromosome or chromosome arm (see Fig. 1). In fact, certain chromosomes (Chr. 4) or chromosome arms (the left arm of the 2nd chromosome 2L) contained no known regulators of hairy, eve, or ftz. We also point out that each of these three pair-rule genes are on different arms (Fig. 1). This allowed us to compare at least two patterns for any one aneuploid embryo genotype and to verify the genotype of certain embryos by the presence or absence of staining with antibodies that recognize gene products derived from different chromosome arms. Most of our descriptions focus on eve and ftz. The wild-type ftz and eve protein patterns at the cellular blastoderm stage of embryogenesis consist of seven transverse stripes encircling the embryo in alternating domains (Fig. 2A, B). We chose these two proteins to assay because very few genes are known to affect eve expression at the blastoderm stage, while ftz is subordinate to several more genes. We believed that this would allow us to detect genes at the top and towards the middle of the pair-rule regulatory hierarchy.

Pair-rule gene activation and head repression are not dependent upon strict zygotic genes

The compound chromosome aneuploid screen yielded information pertinent to the overall control of the expression and localization of pair-rule gene products, and uncovered at least one more gene essential for wild-type pair-rule expression.

Our first general observation was that these grossly aneuploid embryos were able to develop to the blastoderm stage and express periodic protein patterns. Many of the mutant embryos were deficient for genes or groups of genes necessary for early functions such as cellularization and gastrulation (Wieschaus and Sweeton, 1988; Merrill et al. 1988). Even without some of
Strict zygotic regulators of pair-rule genes

Fig. 2. eve and ftz protein expression in nullo-X embryos. (A) The wild-type eve protein pattern consists of seven transverse stripes encircling the blastoderm stage embryo in parasegments 1, 3, 5, 7, 9, 11, and 13. (B) The wild-type ftz protein pattern also consists of seven transverse stripes comprising parasegments 2, 4, 6, 8, 10, 12, and 14. (C) Deviations in the eve pattern occur in regions affected by loss of giant+ (fusion of stripes one and two) and runt+ (missing fifth stripe) function. (D) The ftz protein pattern resembles that seen in a runt mutant, compare with Fig. 1E in accompanying paper.

these early functions, interpretable eve and ftz protein patterns developed and persisted at least through the late blastoderm stage. From this, we concluded that no zygotically active genes are required to turn on the eve or ftz genes since every aneuploid embryo (except, of course, for those lacking either the eve or ftz gene) expressed these genes in some pattern.

A second general observation was that pair-rule protein expression was still repressed in the anterior (head) region of the embryo in all mutants studied. If the anterior pair-rule repression was due simply to a zygotically active gene or group of genes on the same chromosome arm, embryos deficient for that arm would exhibit ftz or eve expression in this region. We conclude that head repression is not dependent upon zygotically active genes and thus, probably involves one or more maternal factors. Because the anterior limit of eve (Driever and Nüsslein-Volhard, 1988) and ftz (Frohnhofer and Nüsslein-Volhard, 1987) expression is shifted by alterations in bicoid+ (bed) function, we propose that the anterior maternal regulator of ftz and eve is the bed+ gene product.

Zygotically required genes on the first chromosome

The gap gene giant and the pair-rule gene runt are the only known genes on the first chromosome necessary for both normal segmentation and wild-type ftz and eve protein patterns (Carroll and Scott, 1986; Frasch and Levine, 1987). To screen for other zygotically active genes on the first chromosome that affect the ftz and eve patterns, females carrying an attached X-chromosome were mated to normal males generating embryos completely lacking an X-chromosome. The nullo-X embryos (genotype YY) develop normally to cycle 14, the syncytial blastoderm stage, but fail to cellularize properly (Wieschaus and Sweeton, 1988). Deviations from the wild-type eve protein pattern in nullo-X embryos are as follows: the first two stripes are fused, while stripes three and four are normal, as seen in giant mutants (Fig. 2C; Frasch and Levine, 1987). The fifth stripe, which is characteristically weak in runt mutants stained with eve (Frasch and Levine, 1987) is completely absent, while stripes six and seven appear unaffected. The ftz protein pattern in nullo-X embryos resembles the pattern observed in runt mutants (Fig. 2D; Carroll
and Scott, 1986). The first stripe is completely absent, the third stripe is faint and narrow and the fifth and sixth stripes are both missing from the \textit{ftz} pattern. The second, fourth and seventh stripes are relatively unaffected. The strong resemblance of the \textit{ftz} and \textit{eve} patterns in nullo-X embryos to the \textit{gr\textsuperscript{+}} and \textit{run\textsuperscript{+}} patterns suggests that these are the only zygotically required genes on the X-chromosome that affect \textit{ftz} and \textit{eve} expression.

\textbf{Zygotic genes on the second chromosome}

The second chromosome contains two genes necessary for normal segmentation as well as formation of the wild-type \textit{ftz} and \textit{eve} protein patterns (Carroll and Scott, 1986; Frasch and Levine, 1987). Both the \textit{Kruppel} gap gene and the \textit{even-skipped} pair-rule gene are located on the right arm of the second chromosome. The left arm contains no known zygotically active genes that affect either the \textit{ftz} or \textit{eve} pattern.

The second chromosome is metacentric, the left and right arm are roughly the same size and are attached to a single centromere. One of the compound autosome structures used in this screen (as C(2L)RM, C(2R)RM) consisted of both homologous arms of one chromosome attached to a single centromere, that is, both left arms attached to one centromere, and both right arms attached to another (Rasmussen, 1960). In females of this genotype, greater than 95\% of the gametes receive either the left arms or right arms (genotypes 2L,0R or 2R,0L respectively; Grell, 1963, 1970). Males produce four different types of gametes, all at equal frequency. In addition to the 2L,0R and 2R,0L genotypes, males produce gametes carrying both the right and left arms (2R,2L), or neither arm (0R,0L) (Baldwin and Chovnick, 1967; Holm et al. 1967). A cross between compound males and females gives about one-fourth viable progeny (2R,2L), leaving the rest with duplications and/or deficiencies consisting of entire chromosome arms (Fig. 3A).

The genotypes of all embryos from this cross could be readily identified because the \textit{even-skipped} gene lies on the right arm of the second chromosome, only those embryos with at least one copy of the right arm will stain with the \textit{eve} antibody. Phenotypically, three different classes of protein patterns could be distinguished in blastoderm-stage embryos stained with \textit{eve} (possessing at least two copies of 2R). The easiest pattern to identify was the seven-stripe wild-type pattern, found in approximately one quarter of the progeny (2L,2R) from the compound cross (not shown). The second distinct phenotype was also a seven-stripe pattern, very similar to wild-type, but showing slight deviations with respect to stripe width and spacing. Two different genotypes are generated that cause these slight variations, the 4L,2R progeny and the 4R,2L progeny. In addition to the normal chromosomal complement of 2R,2L, each has an additional two copies of either the left arm or the right arm. This difference in gene dosage, which involves either extra copies of \textit{Kr} and \textit{eve} (right arm) or extra copies of the left arm (see below), is responsible for the \textit{eve} pattern deviations (data not shown).

The third distinct expression phenotype observed was not expected. The remaining class of embryos identified by \textit{eve} staining must be of the genotype 4R,0L or 2R,0L. Since no known zygotically acting genes that affect the \textit{eve} pattern lie on the left arm, we expected that a complete deletion of this arm would cause no alterations. However, two novel \textit{eve} patterns were observed in these embryos, one corresponding to each of the remaining genotypes (Fig. 4E,F). One of the \textit{eve} patterns consisted of a diffuse band of staining comprising a fusion of stripes one and two, a normal stripe three, a weaker fourth stripe, very faint fifth and sixth stripes and a relatively normal stripe posterior (Fig. 4E). In the second abnormal pattern, the two
Fig. 4. **fitz** and **eve** protein expression in embryos carrying duplications and/or deficiencies for entire arms of chromosome two. Embryos deficient for the right arm of chromosome two, with either four copies of the left arm (A), or two copies of the left arm (B) show a **Kruppel** mutant phenotype when stained with **fitz** (see Fig. 2C in accompanying paper for comparison). A complete deficiency of the left arm of chromosome two generates novel **fitz** and **eve** patterns in embryos with four copies (C, **fitz**; E, **eve**) as well as with two copies (D, **fitz**; F, **eve**) of the right arm.

The anteriormost stripes are again almost fused and are the most intense, stripes three and four are weak but appear normal in width and spacing, the fifth stripe appears to be completely absent, and the sixth and seventh stripes seem shifted anteriorly, nearer to the fourth (Fig. 4F). The abnormal **eve** protein patterns in embryos lacking this arm indicates the presence of at least one more zygotically required regulator of pair-rule gene expression on the left arm of chromosome two.

To positively identify the embryos with the 2R, 0L genotype, another compound chromosome, C(2)EN (entire compound) was used. The C(2)EN chromosome consists of two homologous autosomes joined together with a single centromere in the order right arm, left arm, centromere, left arm, right arm (Novitski et al.
wild-type embryos. The second novel ftz pattern is stronger labelled. Embryos from the second compound cross (C(2)EN males and females receive a normal chromosome two content, one fourth receive four copies of each arm and are genotypically 4R,4L, and one fourth are completely deficient for all of the second chromosome (Fig. 3B).

A cross between compound virgin females (C(2)RM,C(2)RM) and entire compound males (C(2)EN) results in embryos of four different genotypes: 4R,2L; 4L,2R; 2R,0L and 2L,0R, all of which were generated by the first compound cross (Fig. 3B). All but the 2L,0R embryos stain with eve, and those with genotypes of 4R,2L or 4L,2R (approximately 2/3 of the embryos) displayed the seven-stripe nearly wild-type eve pattern. The remaining stained embryos exhibited the second novel eve mutant pattern described in first cross above and must be genotypically 2R,0L (Fig. 4F). Distinguishing the 4R,0L from the 2R,0L embryos was necessary, as we described earlier, variations in chromosome arm dosage also cause deviations in these protein patterns. This cross proves that the novel eve pattern is generated because of a missing activity of a gene (or genes) on the left arm, and not because there are two extra copies of the right arm.

This same conclusion was reached by studying the ftz protein patterns in embryos from both compound second chromosome crosses. We could identify embryos from the first compound cross (Fig. 3A) as follows: one fourth of the embryos obtained are phenotypically wild-type and presumably 2L,2R; one fourth exhibit the nearly wild-type pattern, with slight deviations in the width or spacing of some stripes, these are the 4R,2L and 4L,2R embryos; approximately one-half of the remaining embryos have the very distinct ftz pattern of a Krüppel mutant (Fig. 4A,B; Carroll and Scott, 1986). These embryos must be of the genotype 4L,0R or 2L,0R since Krüppel is on the right arm of the second chromosome. No differences were detected that would distinguish the 4L,0R or 2L,0R embryos. The remaining embryos are either 4R,0L or 2R,0L and exhibit two novel six stripe ftz patterns (Fig. 4C,D). The stripes vary in width, intensity and spacing in both patterns. In one pattern (Fig. 4C), the two anteriormost stripes are slightly broader, more intense and are separated by a wider than normal interstripe than the third, fourth and fifth stripes, and the sixth stripe is shifted even more posteriorly than the seventh stripe of wild-type embryos. The second novel ftz pattern (Fig. 4D) exhibits slightly more uniform spacing of the stripes with the two anteriormost stripes being more strongly labelled. Embryos from the second compound cross (C(2)RM,C(2)RM females and C(2)EN males) were stained with ftz antibody to positively identify which of these patterns were the 2R,0L embryos (Fig. 3B). The 2R,0L embryos exhibited six ftz stripes with the first two being the most intense, and the remaining four being distributed evenly to the posterior end of the embryo as in Fig. 4D. This ftz pattern appears to be complementary to the 2R,0L eve pattern and confirms that at least one more zygotically active pair-rule regulatory (segmentation?) gene is located on the left arm of chromosome two.

Zygotic genes on the third chromosome

The third chromosome contains four zygotically active genes necessary for normal segmentation and proper expression of the wild-type ftz and eve protein patterns (Carroll and Scott, 1986; Frasch and Levine, 1987; Mahoney and Lengyel, 1987). The gap gene knirps and the pair-rule gene hairy are both located on the left arm of chromosome three, while the gap and terminal genes, hunchback (hb) and tailless (tl/l), respectively, lie on the right arm, as does the ftz gene (Fig. 1).

To screen the third chromosome for additional genes, compound third chromosomes similar to the types used above for the second chromosome were used to generate embryos deficient for each entire third chromosome arm (Fig. 3A,B). Progeny from the C(3)XC(3) cross were identified as follows: the ftz gene is on the right arm of chromosome three, therefore only those embryos with at least one copy of the right arm will stain with ftz. Roughly one-third of the stained embryos show the seven-stripe wild-type ftz pattern and are genotypically 2R,2L, while another one-third show a nearly wild-type seven-stripe ftz pattern. These embryos are genotypically 4R,2L or 4L,2R, with ftz pattern deviations due to two extra copies of either the left arm or the right arm. The remaining stained embryos that are the genotypes 4R,0L or 2R,0L each exhibited two different spatial ftz patterns according to their stage of development (Fig. 5E,F). Early on, the embryos display a large nearly continuous domain of ftz expression between 13 % and 68 % egg length, as seen in a hairy mutant (Carroll and Scott, 1986). This spread of ftz expression begins to develop gaps, and eventually deteriorates into a pattern similar to that found in knirps embryos (Carroll and Scott, 1986). The ftz patterns in these embryos (4R,0L and 2R,0L) correspond to the absence of knirps and hairy, with no evidence of other genes on 3L. However, the mutant phenotype of an additional gene or genes could be masked by the strong knirps- and/or hairy- effects.

To positively identify embryos of genotype 2R,0L, we crossed C(3) females (C(3)RM,C(3)RM) and entire compound males (C(3)EN) to generate embryos with the genotypes 4R,2L; 4L,2R; 2R,0L and 2L,0R (Fig. 3B). Embryos of genotype 2L,0R are deficient for the ftz gene and therefore show no staining leaving only the 2R,0L class as the most deviant pattern. The pattern in these embryos is similar to the two-phase pattern in the first cross. The only difference noted was that more embryos exhibited the knirps- type pattern than the hairy- type or intermediate pattern, suggesting a more rapid transition to the knirps- type pattern in the 2R,0L versus the 4R,0L embryos.

Embryos from each cross were also stained with the
Fig. 5. *ftz* and *eve* protein expression in embryos carrying duplications and/or deficiencies for entire arms of chromosome three. *eve* stained embryos deficient for the right arm of chromosome three carrying either four copies of the left arm (A), or two copies of the left arm (B) show pattern defects in regions of the embryo affected in *hunchback* and *tailless* mutants. Embryos deficient for the left arm of chromosome three have *eve* protein patterns that undergo a rapid transition. Embryos with four copies of the right arm (C,E) as well as two copies of the right arm (D,F) exhibit the same transition patterns. Panels C and E are the early *eve* and *ftz* patterns; panels D and F are the later patterns.

*eve* antibody, both to confirm the *ftz* results and to observe patterns in embryos missing the entire right arm of chromosome three that could not be stained for *ftz* protein expression. Embryos deficient for the right arm of chromosome three lack the *hunchback* and *tailless* genes. These embryos show an altered *eve* pattern in regions affected by *hb* and *ill* (Fig. 5A,B) (Frasch and Levine, 1987). A broad band of *eve* expression is seen in the anteriormost region of pair-rule expression, covering roughly the domain of the first three *eve* stripes. The fourth and fifth stripes are almost normal, but stripes six and seven are fused and
shifted to the extreme posterior end of the embryo. As the \textit{ill} gene is necessary for the formation of the eighth and ninth terminal abdominal segments, it seems likely that the deviant posterior expression is due to the absence of both the \textit{ill} and \textit{hb} genes (Frasch and Levine, 1987). We were able to screen the right arm genes distal to the \textit{ftz} gene for their influence on \textit{ftz} expression by crossing a Y-chromosome translocation covering the \textit{ftz} locus to the C(3L) RM chromosome. The pattern of \textit{ftz} expression in those embryos is consistent with \textit{hb} and \textit{ill} being the principal pair-rule regulatory genes on the right arm of chromosome three (data not shown).

The \textit{eve} pattern of the 4R,0L and 2R,0L embryos exhibits pattern alterations seen in both \textit{knirps} and \textit{hairy} mutants (Fig. 5C, D; Frasch and Levine, 1987). The first stripe appears normal, while the second is very faint, almost absent, and is followed by a solid block of expression covering the region of stripes 3–7. This block is generally very faint and fades rapidly, leaving only one strong anterior stripe and a weak second stripe.

Embryos from C(3)×C(3)EN cross stained with \textit{eve} antibody exhibited patterns identical to those seen in the C(3)×C(3) cross for the corresponding genotypes. The deviant \textit{eve} and \textit{ftz} patterns can be largely explained by the absence of known genes. However, it is possible that the strong pattern alterations caused by the gap genes or \textit{hairy} could conceal the existence of any other third chromosome genes. Therefore, while most third chromosome pair-rule regulatory genes are accounted for, we do not exclude the possibility that additional zygotic regulators of pair-rule genes exist on the third chromosome.

\textbf{Chromosome IV}

Analysis of embryos lacking the fourth chromosome showed no evidence of any zygotically active genes that affect the \textit{ftz} or \textit{eve} patterns. All classes of progeny produced in the C(4) cross appeared wild-type with respect to the \textit{ftz} and \textit{eve} patterns.

\textbf{Discussion}

From our analysis of pair-rule gene expression in embryos lacking large portions of the zygotic genome we have drawn three principal conclusions about the genetic circuitry that establishes the periodic patterns of pair-rule gene expression. First, the general activation of the pair-rule genes is not dependent on any strictly zygotic gene. Second, some maternal factors directly regulate the spatial domains of pair-rule genes. And finally, most but not all zygotically required regulators of pair-rule gene expression have been identified. In addition, we point out that our approach of directly analyzing gene expression in aneuploid embryos may be of general use in screening for genes that regulate other early zygotic functions, such as those involved in establishing dorsalventral polarity.

\textbf{The general activation of pair-rule gene expression}

Since each pair-rule gene was expressed in some parts of the embryo regardless of which portion of the zygotic genome was removed, there does not appear to be a general zygotically required activator of the pair-rule genes. Their initial expression, which is evident by the tenth nuclear cycle (Weir and Kornberg, 1985), appears to involve a general activation that could be stimulated by specific maternally provided factors or perhaps by the general activation of the zygotic genome. Since injection of protein synthesis inhibitors into the late syncytial blastoderm embryo only inhibits the spatial repression of the pair-rule pattern and not gene transcription (Edgar et al. 1986), it is likely that the more general pair-rule transcription factors are relatively long-lived compared with the short-lived spatial regulatory proteins.

\textbf{Maternal control of pair-rule gene expression}

One activity that we expected to uncover but did not in the course of the aneuploid screen was the gene(s) responsible for repression of \textit{eve} and \textit{ftz} expression in the anterior third of the embryo. Previous studies of \textit{ftz} (Carroll and Scott, 1986) and \textit{eve} (Frasch and Levine, 1987) protein expression in all known zygotic segmentation mutants failed to reveal how these genes are kept off in the anterior region of the embryo. It is clear that there is active repression of both genes in this region because injection of protein synthesis inhibitors during blastoderm formation leads to \textit{eve} and \textit{ftz} mRNA accumulation there (Edgar et al. 1986, 1989).

Since zygotic removal of chromosome segments covering the entire genome did not lead to \textit{eve} and \textit{ftz} expression in the anterior, we conclude that maternally expressed gene products must be responsible for \textit{eve} and \textit{ftz} repression. The best candidate for an anteriorly acting repressor of \textit{eve} and \textit{ftz} is the \textit{bcd}+ gene product, which has been shown to affect the anterior limit of both \textit{ftz} (Frohnhofer and Nüsslein-Volhard) and \textit{eve} (Driever and Nüsslein-Volhard, 1988) expression as well as being a regulator of the \textit{Kr} (Gaul et al. 1987) and \textit{hb} (Driever and Nüsslein-Volhard, 1989) gap genes. Previously, we might have believed that \textit{bcd}+ acted indirectly upon \textit{ftz} and \textit{eve} through some anterior zygotic gap gene, but in the absence of evidence for such a gene, we hypothesize that \textit{bcd}+ works directly.

\textbf{Have all of the zygotically required segmentation genes been identified?}

Previous genetic screens for embryonic visible mutations (Nüsslein-Volhard and Wieschaus, 1980; Nüsslein-Volhard et al. 1984; Jürgens et al. 1984; Wieschaus et al. 1984) are believed to have approached near saturation for mutable loci that give rise to homozygous visible phenotypes. It is possible, though, that certain segmentation genes may have escaped detection in these screens for one of several possible reasons. First, there was a slight statistical chance that not all loci were mutated. Second, redundancy of function, involving duplicated genes as exhibited by loci such as \textit{gooseberry} (Baumgartner et al. 1987) and perhaps \textit{invected} (Coleman et al. 1987) may render these loci insensitive to chemical mutagens. Third, some genes may be ex-
pressed both maternally and zygotically, and the maternal contribution could mask a zygotic function (Per
termon and Mahowald, 1986). Finally, some mutants causing visible defects may not be interpretable as segmen
tation mutants because other morphological abnormalities could conceal an underlying segmenta
tion defect.

The aneuploid screen may circumvent some of these limitations because it does not rely upon mutagenesis to remove functions of all parts of the genome and because the phenotypic assay involves direct inspection of pair-
rule gene expression at the blastoderm stage. If a segmen
t of the genome carries a zygotically active gene re
duced for the resolution of the eve or ftz (or hairy) patterns, we would detect these through their specific effects on each gene pattern. In the experiments pre
ented here, we have shown that all previously identi
fied genes manifest themselves in the aneuploid em
bryos and that a new pair-rule regulatory gene could exist on chromosome arm 2L. It should be borne in
mind, however, that we have only screened for genes at the top of the pair-rule hierarchy, namely those affecting eve, ftz, or hairy. Segmentation genes that are downstream from these genes would not be detected by our screen. We could not eliminate other possibilities such as the effect of the absence of one gene on a chromosome segment masking the requirement of another gene, or that some functions could be redund
antly encoded on different chromosome arms. For these reasons, we may conclude that most, but not necessarily all, zygotic segmentation genes have prob
ably been found and that most spatial regulators of hairy, eve, and ftz have been identified.

We would like to localize and identify the locus (loci) responsible for the abnormal expression patterns of embryos lacking chromosome arm 2L. Since the initial eve expression pattern is relatively refractory to most pair-rule mutations (Frasch and Levine, 1987; Ingham and Gergen, 1988; Carroll and Vavra, 1989), and is significantly affected all along the anteroposterior axis of 2L- embryos, we suspect that we are searching for a gap-type gene or for more than one gene. Since the initial screen to us; Jim Kennison for lots of advice and patience on the genetic manipulations; Barry Ganetzky for pointers on compound chromosomes and their behavior and several key fly stocks; Manfred Frasch and Mike Levine for their eve antibody; and two anonymous reviewers for their constructive help on the presentation of the information in both papers. We also thank Bruce Thalley and Allen Laughon for their critical review of the manuscript and Pat Hanson and Carmen Huston for typing it. S.H.V. is a predoctoral trainee supported by a NIH training grant to the Department of Genetics. This research was supported by NSF grant DCB-

References


(Accepted 17 August 1989)