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1. Freese, E. *et al. Teratology* **20**, 413-440 (1979).
2. Waddell, W. & Butler, T. *J. clin. Invest.* **38**, 720-729 (1959).
3. Robert, E. & Rosa, F. *Lancet* **ii**, 1142 (1983).
4. Koch, S., Jäger-Roman, E., Rating, D. & Helge, H. *J. Pediatr.* **103**, 1007 (1983).
5. Nau, H., Zierer, R., Spielmann, H., Neubert, D. & Gansau, Ch. *Life Sci.* **29**, 2803-2814 (1981).
6. Kao, J., Brown, N. A., Schmid, B., Goulding, E. H. & Fabro, S. *Teratogen, Carcinogen, Mutagen.* **1**, 367-382 (1981).
7. Ong, L. L. *et al. Fundamental appl. Tox.* **3**, 121-126 (1983).
8. Nau, H. *et al. J. Chromat.* **226**, 69-78 (1981).
9. Brown, N., Holt, D. & Webb, M. *Tox. Lett.* **22**, 93-100 (1984).
10. Ritter, E. J., Scott, W. J., Randall, J. L. & Ritter, J. M. *Teratology* **32**, 25-31 (1985).
11. Keberle, H., Loustalot, P., Maller, R. K., Faigle, J. W. & Schmid, K. *Ann. N.Y. Acad. Sci.* **123**, 253-265 (1965).
12. Danielsson, B. R. G., Ghantous, H. & Dencker, L. *Acta pharmac. tox.* **55**, 410-417 (1984).
13. Tagashira, E. *et al. Japan J. Pharmac.* **31**, 563-571 (1981).
14. Willhite, C. & Shealy, F. *J. natn. Cancer Inst.* **72**, 689-694 (1984).
15. Löscher, W. & Nau, H. *Neuropharmacology* **24**, 427-435 (1985).
16. Sullivan, F. M. & McElhatton, P. *Tox. appl. Pharmacol.* **40**, 365-378 (1977).
17. Fabro, S., Shull, G. & Brown, N. A. *Teratogen, Carcinogen, Mutagen.* **2**, 61-76 (1982).
18. Nau, H., Löscher, W. & Schäfer, H. *Neurology* **34**, 400-401 (1984).
19. Helm, F.-Ch., Frankus, E., Fridereichs, E., Graudums, I. & Flohe, L. *Arzneimittel-Forsch.* **31**, 941-949 (1981).
20. Nau, H. & Löscher, W. *Fundamental appl. Tox.* **6**, 669-676 (1986).
21. Mirkin, B. L. & Singh, S. in *Perinatal Pharmacology and Therapeutics* (ed. Mirkin, B. L.) 1-69 (Academic, New York, 1976).
22. Mast, T. J., Cukierski, M. A., Nau, H. & Hendrickx, A. G. *Toxicology* **39**, 111-119 (1986).

Maternal control of *Drosophila* segmentation gene expression

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Several genes have been identified that are involved in establishing the segmented body pattern during development of the fruit-fly *Drosophila melanogaster*. These fall into several classes on the basis of the kind of alteration to the wild-type segmentation pattern observed in mutant embryos. For example, mutations of the pair-rule¹ class, such as *fushi tarazu* (*ftz*)¹⁻³, cause the deletion of pattern elements with a two-segment periodicity; those of the gap class¹, such as *knirps*^{1,4}, cause the deletion of contiguous groups of segments. The availability of antibodies against the *ftz* protein has allowed its spatial pattern of expression to be studied during the development of wild-type⁵ and mutant⁶ embryos. The aim of the latter kind of experiment is to investigate possible interactions between these important genes. We have recently reported that *knirps* mutations cause a striking alteration to the pattern of transverse stripes of *ftz* expression usually seen during embryogenesis⁶. *Knirps* is a zygotically-expressed gene, but recently a class of maternally-active genes has been identified that causes similar defects in pattern formation⁷⁻⁹. We have now investigated the pattern of *ftz* expression in mutants of this class and have found that while they do have features seen in *knirps* mutants, they also exhibit significant differences between the different mutations reflecting the distinct but overlapping domains of gene activity. These observations demonstrate that maternally-active segmentation genes regulate zygotic gene expression, and that some of their effects on *ftz* may be directed through the *knirps* gene.

In large-scale genetic screens for maternal genes required for normal embryonic pattern formation⁷⁻⁹, at least five (*vasa*, *valois*,

staufen, *tudor* and *oskar*) have been identified which are required for normal abdominal segmentation. Embryos produced by females homozygous for mutations in any of the five genes exhibit deletions of abdominal segments that resemble, to various degrees, defects observed in embryos homozygous for mutations of the zygotically-active gap locus *knirps*^{1,4} (Fig. 1). The abdominal deletions caused by the maternal-effect mutations are somewhat variable; the largest deletions are caused by mutations in *vasa* and *oskar*. In *vasa* and *oskar* embryos, all the abdominal segments are deleted and the thorax appears juxtaposed to very posterior structures including the telson and posterior spiracles⁷ (Fig. 1*b,f*). Mutations in the other three genes usually leave the abdominal denticle belts corresponding to the first and eighth abdominal segments intact but delete and fuse the intervening abdominal segments to different extents. One particular deletion pattern, which can result from any of the five mutations, is a large field of abdominal denticles followed by a region of naked cuticle. The broad band of denticles, representing one enlarged abdominal segment, closely resembles the cuticle pattern formed by the zygotic segmentation mutation *knirps* (Fig. 1*g*), and was most frequently found in *valois* (Fig. 1*c*) and *staufen* (Fig. 1*e*). In addition to the abdominal deletions, *staufen* also causes deletions of head structures. Mutations in all five of the genes result in the absence of the germline precursor cells, the pole cells⁷⁻⁹.

To investigate how maternal genes may exert an influence on the zygotic segmentation program, antibodies have been used to localize the protein encoded by the *ftz*^{2,3,10,11} pair-rule segmentation gene in whole-mount mutant embryos. The *ftz* protein is normally expressed in seven transverse stripes of nuclei during cellularization of the embryo. These stripes correspond to parts of alternate double-segment units^{5,12}. A segment (or parasegment) primordium is about four cells across, in the anterior-posterior axis, at the blastoderm stage. At the beginning of gastrulation the *ftz* stripes are about three nuclei in width and are separated by an 'interstripe' of five relatively unstained nuclei (Fig. 2*a*). The posterior stripe is widest, remaining about five nuclei in width throughout germ-band elongation.

Each of the mutants has a distinctive pattern of *ftz* expression, but there are some common features exhibited by mutations in all of the five loci. The first (most anterior) two stripes of *ftz* expression are relatively normal in all five mutants (Fig. 2), and are found at the position of the primordia for the normal labial-maxillary and T1-T2 segments. These segments appear normal in larvae from mutant mothers (Fig. 1). The seventh stripe is also normal (Fig. 2) and corresponds to the posterior segmental region of A8-A10 which gives rise to the telson, a structure which forms normally in the embryos. The major aberrations occur in the region between the second and seventh *ftz* stripes. Either the second 'interstripe' (*vasa*), or the third stripe (*valois*, *tudor*), or both (*staufen*, *oskar*) appear(s) wider than in a normal embryo, and the fourth through sixth stripes become narrower or disappear altogether (Fig. 2*b-f*).

There are a number of significant differences between the effects of the different mutations. In embryos from homozygous *vasa*^{PD} mothers, *ftz* protein is present only as a weak, partial band in the central abdominal region (Fig. 2*b*). In embryos from homozygous *valois*^{RB} (Fig. 2*c*) and *tudor*¹ (Fig. 2*d*) mothers, *ftz* expression in the fourth, fifth and sixth stripes is restricted to fewer cells and the spaces between the stripes are narrowed. In the major pattern of *staufen* defects (90% of the embryos), there is a wide patch of *ftz* protein in the abdominal primordium and abnormal spacing of the two anterior stripes (Fig. 2*e*). However, in about 10% of the progeny of *staufen*^{HI} mothers, a *valois*-type pattern of *ftz* stripes is exhibited (Fig. 2*f*). In embryos from females homozygous for *oskar*¹⁶⁶, *ftz* expression is spread over a wide band of cells (Fig. 2*f*).

The cuticular phenotypes of these maternal effect mutants (Fig. 1*b-f*) resemble, to varying degrees, that of strong mutants of the gap locus *knirps*^{1,4} (Fig. 1*g*). Homozygous mutant *knirps*

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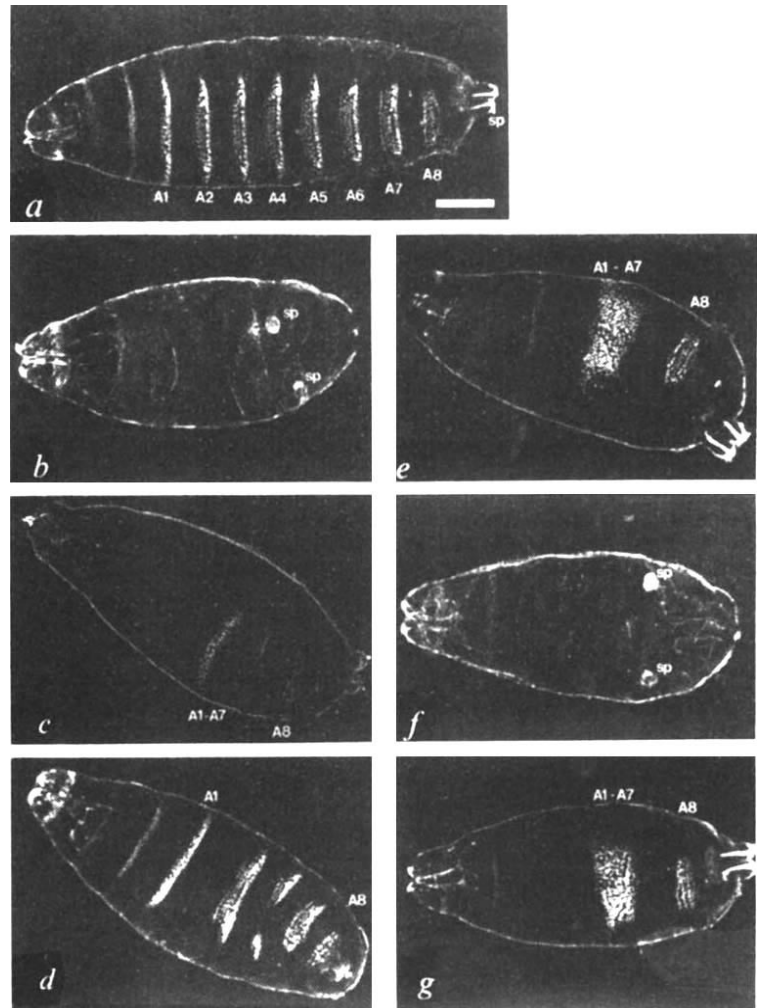


Fig. 1 The phenotypes of progeny of females homozygous for mutations at five loci controlling abdominal segmentation, and of embryos homozygous for *knirps*. *a-g*, Whole mounts of larval cuticles obtained from wild-type or homozygous mutant mothers, fixed and mounted as described by Van der Meer¹³. The identity and position of some abdominal denticle belts are indicated (A1-A8). *a*, Wild-type larva. Scale bar represents 100 μ m. *b*, *vasa*^{PD}. Most of the anterior abdominal segments are deleted; sp, spiracles. *c*, *valois*^{RB}. Segments A1-A7 are fused. *d*, *tudor*¹. Segments A2-A6 defective. *e*, *staufen*^{HL}. Segments A1-A7 fused. *f*, *oskar*¹⁶⁶. Most denticle bands are deleted; sp, spiracles. *g*, Homozygous *knirps*^{11E72} larva, obtained from a balanced heterozygous mutant stock. Segments A1-A7 fused.

embryos exhibit a band of *ftz* protein about 27 nuclei in width in the region normally comprising the third through sixth stripes (Fig. 2*h*). Since the absence of *knirps* gene activity leads to a uniform 'on' state of *ftz* across most of the abdominal region⁶, a primary defect of mutations at the *staufen* and *oskar* loci may be the failure to properly activate the *knirps* gene in certain parts of the embryo. The differences between the *ftz* patterns resulting from mutations in *knirps*, *staufen* or *oskar* could be due to differences in allele strengths or in the exact spatial limits of influence of the genes.

In *vasa* embryos, *ftz* expression is affected in a manner opposite to that of *staufen*, *oskar*, and *knirps* in that *ftz* protein is not produced in most of the anterior abdominal cells. Since *vasa* and *oskar*, for example, produce very similar larval phenotypes (Fig. 1*b* and *f*), quite different alterations in the *ftz* pattern can lead to similar terminal cuticular phenotypes. The *ftz* pattern suggests that *vasa* influences a different pathway of abdominal segmentation gene regulation or that *vasa* interacts with *knirps* differently than do the other genes.

Observed at the level of *ftz* expression, the maternal segmentation mutations result in a shift of cellular fates at the blastoderm stage: it appears that anterior regions become expanded at the expense of posterior regions. The common effect of all five mutations is the enlargement of certain anterior stripes and interstripes coupled with the increasingly substantial compression and eventual elimination of more posterior stripes. The particular stripes or interstripes which are enlarged vary between individual loci, indicating that the exact domains of influence of their products may differ.

The results also suggest that expansion of a given *ftz* stripe does not necessarily result in gross abnormalities in the differentiated cuticle, while compression does. In wild-type embryos, the third stripe of *ftz* expression marks the posterior part of the third thoracic and the anterior portion of the first abdominal segment. In spite of the fact that the third stripe is enlarged in blastoderm embryos derived from *valois*, *tudor*, *staufen*, *oskar* and *knirps*, the third thoracic segments of the larvae have normal cuticular morphology. The first abdominal denticle band also usually appears to be normal in many of the embryos with large cuticular deletions and is present, though abnormally large, in embryos with only one denticle field. These observations argue for the existence of some regulatory mechanism which can compensate to produce relatively normal segmental pattern when primordial cell pools are too large. By contrast, the compression of the *ftz* pattern leads to deletion of segments from the final cuticular pattern. It appears that within the region of pattern compression, the cells cannot differentiate their corresponding pattern elements and may therefore assume different fates or be eliminated. The fourth abdominal segment is most often deleted, followed by the fifth, and then the third and sixth abdominal segments⁷⁻⁹. In wild-type embryos at the blastoderm stage, the posterior part of segment A4 and the anterior part of segment A5 are represented by the fifth stripe of *ftz* expression. This stripe is in the middle of the compressed stripe region in the mutants. In the stronger mutants, the pattern compression is so extreme that, even at the blastoderm stage, no trace of the compressed stripes is visible and correspondingly all traces of abdominal segments 2-7 have disappeared from

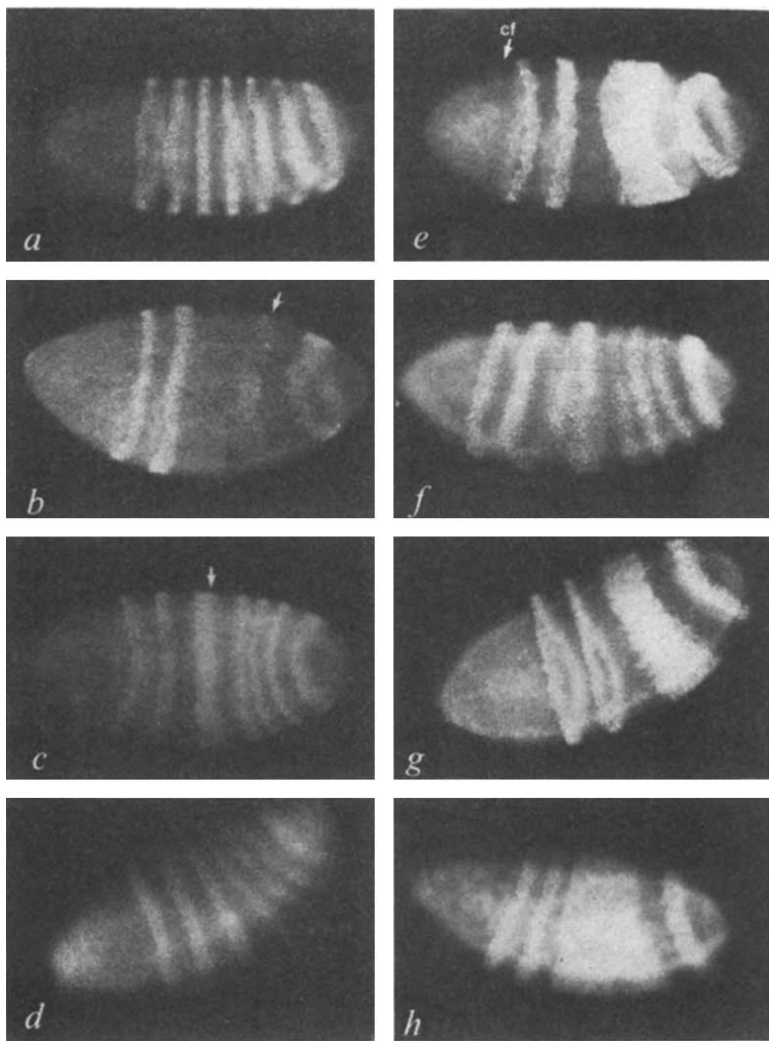


Fig. 2 Expression of the *ftz* protein in embryos from females homozygous for mutations in abdominal segmentation genes. In each photograph, the anterior end of the embryo is at the left, and the ventral side, when visible, is at the bottom. The alleles used were the strongest available, but may not be null alleles. *a-h*, Whole-mount embryos were collected at 25 °C and fixed, incubated with anti-*ftz* antibodies and stained with fluorescein conjugated goat anti-rabbit antibody⁵. *a*, A wild-type embryo just beginning gastrulation. The *ftz* protein stripes are roughly three nuclei wide except for the posterior stripe which is about five nuclei wide. *b*, *vasa*^{PD}: The enlarged second gap between the *ftz* stripes is followed by a region of weak *ftz* expression where the boundary between expressing and non-expressing cells is difficult to see (arrow). Often the band of weak *ftz* expression extends more anteriorly on the dorsal than on the ventral surface. *c*, *valois*^{RB}: The third stripe extends slightly farther to the posterior than in normal embryos, and the fourth, fifth, and sixth stripes are compressed. *d*, *tudor*: The second stripe is slightly wider (by about one cell) than in a wild-type embryo, whereas the first stripe is normal. The fourth, fifth and sixth stripes of *ftz* protein are narrow and more compressed than three normal stripes. *e, f*, *staufen*^{HL}: The two anterior stripes are spaced farther apart from each other than in wild-type embryos. Embryos from *staufen* mothers lack anterior head structures⁷, an alteration which is probably related to the anterior shift of the cephalic furrow (*e*, arrow) and the anterior shift of the first stripe of *ftz* protein. Posterior to the second stripe, the most common pattern observed (90% of the embryos) consists of an unstained region of about 7-9 nuclei followed by a third stripe that is 14-20 nuclei in width (*e*). In the second pattern (10% of the embryos), the wide band is split into four stripes, the overall pattern resembling that of *valois* embryos (*c*), but with a larger number of nuclei staining in each stripe (*f*). *g*, *oskar*¹⁶⁶: The first two stripes are farther apart than in wild-type embryos, but are not shifted anteriorly as in *staufen*. The third stripe in *oskar* consists of a broad band of *ftz* expression about 12-16 nuclei in width. The space between this band and the apparently normal posterior stripe is 3-5 cells wider than the space between the wild-type sixth and seventh stripes. *h*, Homozygous *knirps*^{1E72} embryo, exhibiting a wide band of *ftz* protein in the A1-A7 segment primordia. The first, second, and seventh *ftz* protein stripes are normal.

the cuticle.

We have previously shown that four zygotic gap genes (including *knirps*) and three of the seven pair-rule¹ genes influence *ftz* expression, whereas four other pair-rule genes and three segment polarity¹ genes have no effect⁶. These results place *ftz* in the middle of the zygotic segmentation gene hierarchy, with *ftz* acting after or in parallel to the seven zygotically-active genes that influence it, but before or independently of those that do not affect its expression. The maternal segmentation genes studied in this work appear to be at the top of the hierarchy, since they are expressed during oogenesis and alter *ftz* expression. It is likely that the different larval and molecular phenotypes of the maternally-active genes reflect the different effects of the maternal genes on other segmentation genes as well as on *ftz*. The identification of any direct regulatory interac-

tions between the maternally-active genes and *ftz* is complicated by the genetic hierarchy problem.

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1. Nüsslein-Volhard, C. & Wieschaus, E. *Nature* **287**, 795-801 (1980).
2. Wakimoto, B. T. & Kaufman, T. C. *Dev Biol.* **81**, 51-64 (1981).
3. Wakimoto, B. T., Turner, F. R. & Kaufman, T. C. *Dev Biol.* **102**, 147-172 (1984).
4. Jürgens, G., Wieschaus, E., Nüsslein-Volhard, C. & Kluding, H. *Wilhelm Roux Arch.* **193**, 283-295 (1984).
5. Carroll, S. B. & Scott, M. P. *Cell* **43**, 47-57 (1985).

6. Carroll, S. B. & Scott, M. P. *Cell* **45**, 113-126 (1986).
7. Schüpbach, T. & E. Wieschaus, *Wilhelm Roux Arch. dev. Biol.* (in the press).
8. Lehmann, R. Thesis, Univ. Tübingen (1985).
9. Boswell, R. E. & Mahowald, A. P. *Cell* **43**, 97-104 (1985).
10. Kuroiwa, A., Hafen, E. & Gehring, W. J. *Cell* **37**, 825-831 (1984).
11. Weiner, A. J., Scott, M. P. & Kaufman, T. C. *Cell* **37**, 843-885 (1984).
12. Hafen, E., Levine, M. & Gehring, W. J. *Cell* **37**, 833-841 (1984).
13. Van der Meer, J. M. *Drosophila Info. Serv.* **52**, 160 (1977).