the 3′ junction and extends 215(±1) nucleotides into the 3′ exon. The likelihood that it codes for a protein is reinforced by the fact that the other frames of the intron contain 28 and 30 stop codons. Moreover, two putative Shine–Dalgarno sequences (5′ AGGA 3′ and 5′ GGGGT 3′) precede the ORF; these lie 3 and 7 nucleotides, respectively, from the start codon and both can interact with the sequence 5′ ACCTCCT 3′ at the 3′ end of the 16S RNA (data not shown). Furthermore, a 30-nucleotide region preceding these putative Shine–Dalgarno sequences is very A+T-rich (80%); this feature is common to ORFs of introns in the rRNA genes of *Neurospora crassa*[21]. The extension of the ORF by 215(±1) nucleotides into the 3′ exon suggests either that it is translated prior to splicing or that the RNA circularizes after splicing, as was found for intron II of *T. pigmentosa*[22], such that translation is terminated by stop codons upstream from the initiation codon.

In conclusion, this is the first intron to be found in a rRNA gene of any prokaryotic system. The result complements those introns found in archaebacterial tRNAs, that is, the small putative introns in $tRNA^{Leu}$ and $tRNA^{Ser}$ of *Sulfolobus solfataricus*[23] and a 105-bp intron in the $tRNA^{Trp}$ gene of *Halobacterium volcanii*[24]; all exhibit important differences compared with their eukaryotic counterparts. Collectively, though, these results reinforce the view that the archaebacteria, in general, share more characteristics with the eukaryotes than do the eubacteria, where an intron has been reported only for the thymidylate synthase gene of the phage T4[25].

We thank Professor Wolfram Zillig for providing *D. mobilis* cells and Dr Wilhelm Ansorge for advice on gel systems for DNA sequencing. We thank Henrik Leffers and Niels Larsen for advice and help with the sequence alignments and Lisbeth Heilesen for her assistance with the manuscript. The research was supported by grants from the Danish Natural Science Research Council.

Received 19 August; accepted 22 October 1985.

1. Fox, G. E. *et al. Science* **209**, 457–463 (1980).
2. Woese, C. R. *Scient. Am.* **7**, 94–106 (1981).
3. Zillig, W., Schabel, R. & Stetter, K. O. *Curr. Topics Microbiol.* (in the press).
4. Tu, J. *et al. J. molec. Evol.* **18**, 109–114 (1982).
5. Zillig, W. *et al. Zentbl. Bakt. Hyg. I. Abt. Orig.* C**3**, 304–317 (1982).
6. Nomiyama, H., Kuhara, S., Kukita, T., Otsuka, T. & Sakaki, Y. *Nucleic Acids Res.* **9**, 5507–5519 (1981).
7. Cech, T. R. *Cell* **34**, 713–716 (1983).
8. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
9. Neumann, H. *et al. Molec. gen. Genet.* **192**, 66–72 (1983).
10. Andersen, A., Larsen, N., Leffers, H., Kjems, J. & Garrett, R. in *3-D Structure and Dynamics of RNA* (eds Van Knippenberg, P. & Hilbers, C.) (Plenum, London, in the press).
11. Noller, H. F. *A. Rev. Biochem.* **53**, 119–162 (1984).
12. Wild, M. A. & Sommer, R. *Nature* **283**, 693–694 (1980).
13. Branlant, C. *et al. Nucleic Acids Res.* **9**, 4303–4324 (1981).
14. Michel, F. & Dujon, B. *EMBO J.* **2**, 33–38 (1983).
15. Waring, R. B. & Davies, R. W. *Gene* **28**, 277–291 (1984).
16. De La Salle, H., Jacq, C. & Slonimski, P. P. *Cell* **28**, 721–732 (1982).
17. Inoue, T. & Cech, T. R. *Proc. natn. Acad. Sci. U.S.A.* **82**, 648–652 (1984).
18. Johnson, J. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 2546–2568 (1980).
19. Colby, D., Leboy, P. S. & Guthrie, C. *Proc. natn. Acad. Sci. U.S.A.* **78**, 415–419 (1981).
20. Koch, W., Edwards, K. & Kössel, H. *Cell* **25**, 203–213 (1981).
21. Burke, J. M. & RajBhandary, U. L. *Cell* **31**, 509–520 (1982).
22. Zaug, A., Grabowski, P. J. & Cech, T. R. *Nature* **301**, 578–583 (1983).
23. Kaine, B. P., Gupta, R. & Woese, C. R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3309–3312 (1983).
24. Daniels, C., Gupta, R. & Doolittle, W. F. *J. biol. Chem.* **260**, 3132–3134 (1985).
25. Belfort, M. *et al. Cell* **41**, 375–382 (1985).
26. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
27. Ansorge, W. & Labeit, S. *J. biochem. biophys. Meth.* **9**, 33–47 (1984).
28. Staden, R. *Nucleic Acids Res.* **12**, 521–538 (1984).

Near-reciprocal phenotypes caused by inactivation or indiscriminate expression of the *Drosophila* segmentation gene *ftz*

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Early in development, *Drosophila* embryos express the segmentation gene *fushi tarazu* (*ftz*)[1,2] in a 'zebra' pattern of active and inactive stripes, each about the width of a segment primordium[3]. If the *ftz* gene is prevented from functioning, alternating portions of the body normally derived from the active stripes fail to develop, resulting in larvae which lack the denticle bands normally formed by the mesothorax and odd-numbered abdominal segments (that is, thoracic segment T2 and abdominal segments A1, A3, A5 and A7). Here, using the *Drosophila* heat shock protein 70 (*hsp70*) gene promoter[4–7] to drive widespread expression of *ftz* transcripts on heat shock, I find that unrestricted *ftz* activity can cause a reciprocal 'pair-rule' phenotype—that is, the absence of the denticle bands which are normally derived from segments T1, T3, A2, A4, A6 and A8. These results show that both the 'on' and 'off' states of *ftz* gene expression have instructive roles in the development of alternating regions of the body, and hence suggest that the *ftz* gene acts combinatorially with other pair-rule genes (for example, *even-skipped*, *odd-skipped*, *paired*)[8–10] to establish the metameric pattern of the body.

To examine the consequences of indiscriminate *ftz* expression, a sequence coding for most of the mature *ftz* messenger RNA (including the entire open reading frame of the *ftz* protein)[11] was fused to the promoter of the *hsp70* gene[5–7], and this hybrid gene was incorporated into the germ line by P-element-mediated transformation[12,13] (see Fig. 1). Four independent transformant lines were obtained: two, called HSF2 and HSF3, have been examined in detail. The HSF2 and HSF3 transformants result from insertions of a single copy of the *P(hsp70-ftz, Adh⁺)* element into chromosomes 2 and 3, respectively (segregation and Southern blotting data not shown): in both cases, animals homozygous for the transduced element are viable and fertile.

The *hsp70* promoter can be induced by heat shock throughout the life cycle except for the first few hours of embryogenesis (up to the blastoderm stage, ~2.5–3.5 h after egg laying, 25 °C) and the late stages of oogenesis[5–7,14]. Both HSF transformants expressed the *hsp70–ftz* hybrid transcript in response to heat shock during the blastoderm stage as well as in older embryos (see, for example, Fig. 2). When HSF embryos were heat-shocked during the blastoderm stage, approximately half of the resulting larvae displayed pair-rule phenotypes (described below and in Fig. 3). Similar segmentation phenotypes were not observed when HSF embryos were exposed to heat shock after the blastoderm stage, nor were they observed when control embryos lacking the *hsp70–ftz* hybrid gene were heat-shocked at any time during embryogenesis.

Previous studies have shown that almost all cells of the body induce the *hsp70* promoter on heat shock[5–7]. Because wild-type embryos normally display a tightly restricted zebra pattern of *ftz* transcripts during the blastoderm stage, the altered segment patterns caused by heat-shocking HSF embryos during this period probably result from ectopic expression of the *hsp70–ftz* transcripts.

Before describing the HSF phenotypes, it is helpful to consider the pair-rule phenotype caused by apparent null mutations in the *ftz* gene. *ftz*⁻ embryos develop into larvae which appear to delete every other segment[1,2] (that is, segments T2, A1, A3, A5 and A7; see Fig. 3*b*, *c*). This assessment is based primarily on the presence or absence of conspicuous belts of ventral hairs (denticle bands) which are formed by anterior portions of each segment. However, examination of sensory organs such as Keilin's organs, which are positioned at or near the anteroposterior compartment boundary[15], as well as the patterns of dorsal hairs, which display segment-specific differences in both anterior and posterior compartments of several segments[15,16], indicates that the deleted regions are composed of segment-length domains which begin and end near the anteroposterior compartment boundaries within adjacent segments (Fig. 3; see also refs 9, 10). Note that the regions deleted

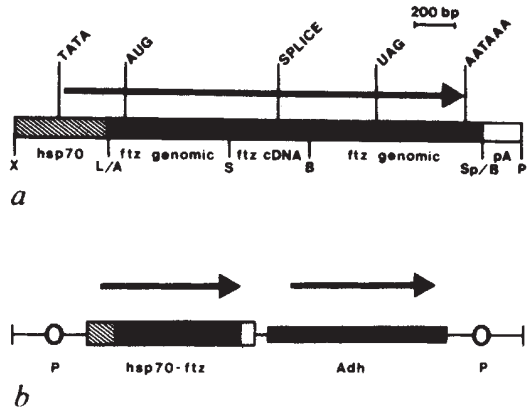

Fig. 1 Composition of the *hsp70-ftz* fusion gene (*a*) and generation of germline transformants (*b*). *a*, The fusion gene is composed of three pieces: (1) a 450-base pair (bp) sequence containing the *hsp70* promoter (hatched bar), (2) a composite *ftz* gene which contains the uninterrupted open reading frame present in the mature *ftz* transcript (solid bar), and (3) a 150-bp fragment of the 3′ end of a *Xenopus* β-globin complementary DNA including a 23-bp poly(A) tail (open bar). The *hsp70* (*132E3*) and globin fragments are described in refs 33 and 34; the composite *ftz* gene was generated by substituting a 580-bp region of the genomic clone *Dm437* (ref. 35) containing the single *ftz* intervening sequence, with the corresponding 400-bp region of the cDNA clone *G20*$^{+}$ (ref. 11) as shown (X, A, S, B, Sp and P represent, respectively, the *Xba*I, *Ava*II, *Sal*I, *Bgl*II, *Sph*I and *Pst*I sites;/indicates that the fragments are joined by blunt-end ligation) (see ref. 11 for the *ftz* cDNA and genomic sequences). The 5′ portion of the fusion gene transcript (arrow) consists of 200 bp of the 5′-untranslated *hsp70* transcript joined by a 10-bp linker sequence (L= AAGCTTGGGC) to the *ftz* gene about 80 bp in front of the start of the major open reading frame (AUG). The 3′ end of the fusion gene, beginning at the end of the open reading frame (UAG), consists of ~400 bp of the *ftz* 3′-untranslated region, the putative polyadenylation signal (AATAAA), and the next 100 bp of the *ftz* genomic sequence. *b*, The *hsp70-ftz* fusion gene was inserted into the P-element transformation vector pPA-1 (J. Posakony, unpublished) which carries the *Adh* (alcohol dehydrogenase) gene as a selectable marker. The directions of transcription of the *hsp70-ftz* and *Adh* genes are indicated by arrows; P, sites for P-factor-mediated integration. Germline transformants were then generated in *Adh*^{fn6} *cn*;*ry* hosts by standard means[12,13]. The same hosts were used as untransformed controls for the heat-shock experiments.

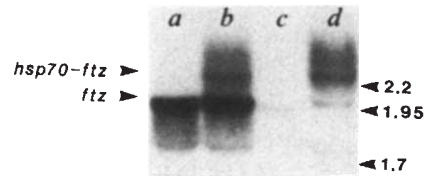

Fig. 2 Heat-shock control of the *hsp70-ftz* fusion transcript. The presence of normal *ftz* and hybrid *hsp70-ftz* transcripts was assayed by Northern blot analysis using a probe complementary to the composite *ftz* gene (solid bar in Fig. 1*a*). *a*, HSF2 embryos aged $2\frac{3}{4}$-$3\frac{1}{4}$ h after egg laying express the normal *ftz* transcript (~2.0 kilobases (kb)[11,35,36]) and, at a low concentration, the 2.2-kb hybrid *hsp70-ftz* transcript (not visible in this exposure). *b*, HSF2 embryos of a similar age to those in *a*, but heat-shocked for 20 min, express the 2.0-kb *ftz* transcript as well as a series of larger transcripts, including a discrete 2.2-kb transcript: the 2.2-kb transcript almost certainly corresponds to the *hsp70-ftz* transcript (which should be ~200 bp longer than the normal *ftz* transcript if terminated near the putative polyadenylation signal), and the larger transcripts may correspond to hybrid transcripts which terminate farther downstream. Note that the *hsp70-ftz* transcripts are less abundant than the native *ftz* transcripts; this difference may be due to poor induction of the fusion gene in some embryos (consistent with the finding that only about half of the HSF embryos heat-shocked during this period give rise to larvae showing reciprocal *ftz* phenotypes). It may also indicate that the levels of ectopic *hsp70-ftz* transcripts necessary to alter the segment pattern may be lower than the levels of *ftz* transcripts normally expressed in the zebra pattern. *c*, Control embryos (6-10 h after egg laying) heat-shocked for 20 min express low levels of the normal *ftz* transcript. *d*, HSF2 embryos of a similar age to those in *c*, but heat-shocked, express low levels of the normal *ftz* transcript as well as high levels of the fusion transcripts.
Methods. Embryos were grown at 25 °C and heat-shocked at 35 °C for 20 min. Northern blot analysis was performed as described previously[37] (~8-10 μg of total nucleic acid was loaded per lane). The *ftz* probe was generated by gel extracting and then nick-translating of the composite *ftz* gene. Neighbouring lanes loaded with RNA from $2\frac{3}{4}$-$3\frac{1}{4}$-h-old HSF2 embryos were probed with sequences complementary to the major actin transcripts (1.7, 1.95 and 2.2 kb)[38], providing size markers for the *ftz* and *hsp70-ftz* transcripts. Similar results were obtained using HSF3 embryos. Low levels of an additional 1.75-kb transcript were detected in all four lanes; whether this represents a *bona fide ftz* transcript is unknown.

in *ftz*$^{-}$ embryos cannot be precisely demarcated, partly because of the lack of sufficient cuticular landmarks, but also because they are somewhat variable in extent (see Fig. 3 legend). Also, a small proportion of *ftz*$^{-}$ embryos (usually <20%) show more extreme segmental fusions in which the denticle bands associated with one or more additional segments appear to be partially or completely deleted.

In contrast to *ftz*$^{-}$ embryos, HSF2 and HSF3 embryos heat-shocked during the blastoderm stage can give rise to abnormal larvae which partially or completely lack the denticle bands normally formed by segments T1, T3, A2, A4, A6 and A8—exactly those denticle bands which are retained in *ftz*$^{-}$ embryos (see, for example, Fig. 3). Thus, conditional, and presumably unrestricted, expression of the *hsp70-ftz* hybrid gene can cause a pair-rule phenotype which is superficially reciprocal to that resulting from absence of the *ftz* gene.

Both the frequency and extents of these reciprocal phenotypes are variable, perhaps because an extreme phenotype results only when the embryos are heat-shocked during a brief, optimal period during the blastoderm stage (for example, late enough for the *hsp70* promoter to be fully inducible, but early enough to allow sufficient *ftz* gene product to accumulate before it has to act). This variability has the fortunate consequence that a broad range of phenotypes can be examined. As in the case of *ftz*$^{-}$ embryos, there seems to be no absolute boundary between deleted and retained regions; however, the deletion pattern shown in Fig. 3*e* illustrates the boundaries which are usually respected. Note that the deletion patterns of *ftz*$^{-}$ and heat-shocked HSF larvae are not perfectly reciprocal, but appear to overlap to some extent (Fig. 4).

The *ftz* gene is one of about 10 'pair-rule' genes that, when mutant, cause the apparent deletion or fusion of homologous portions of every other segment[8-10]. Moreover, each of these genes is associated with a particular deletion pattern, suggesting that they are responsible for the development of overlapping, but different, repeating intervals of the segment pattern[8-10]. The spatial distributions of transcripts of two pair-rule genes, *ftz* and *hairy* (*h*), have been determined; both are expressed in zebra patterns in which the active stripes correspond approximately to the regions of the body that are deleted in mutant embryos[3,17]. The emerging picture therefore seems to be that the deletion patterns associated with the different pair-rule genes define alternating regions of the body where these genes are both expressed and required for normal development. Why then are the *ftz* and *h* genes (and possibly the remaining pair-rule genes) silent in the reciprocal portions of the body where their activities seem to be dispensable?

The results presented here argue strongly that the *ftz* gene is

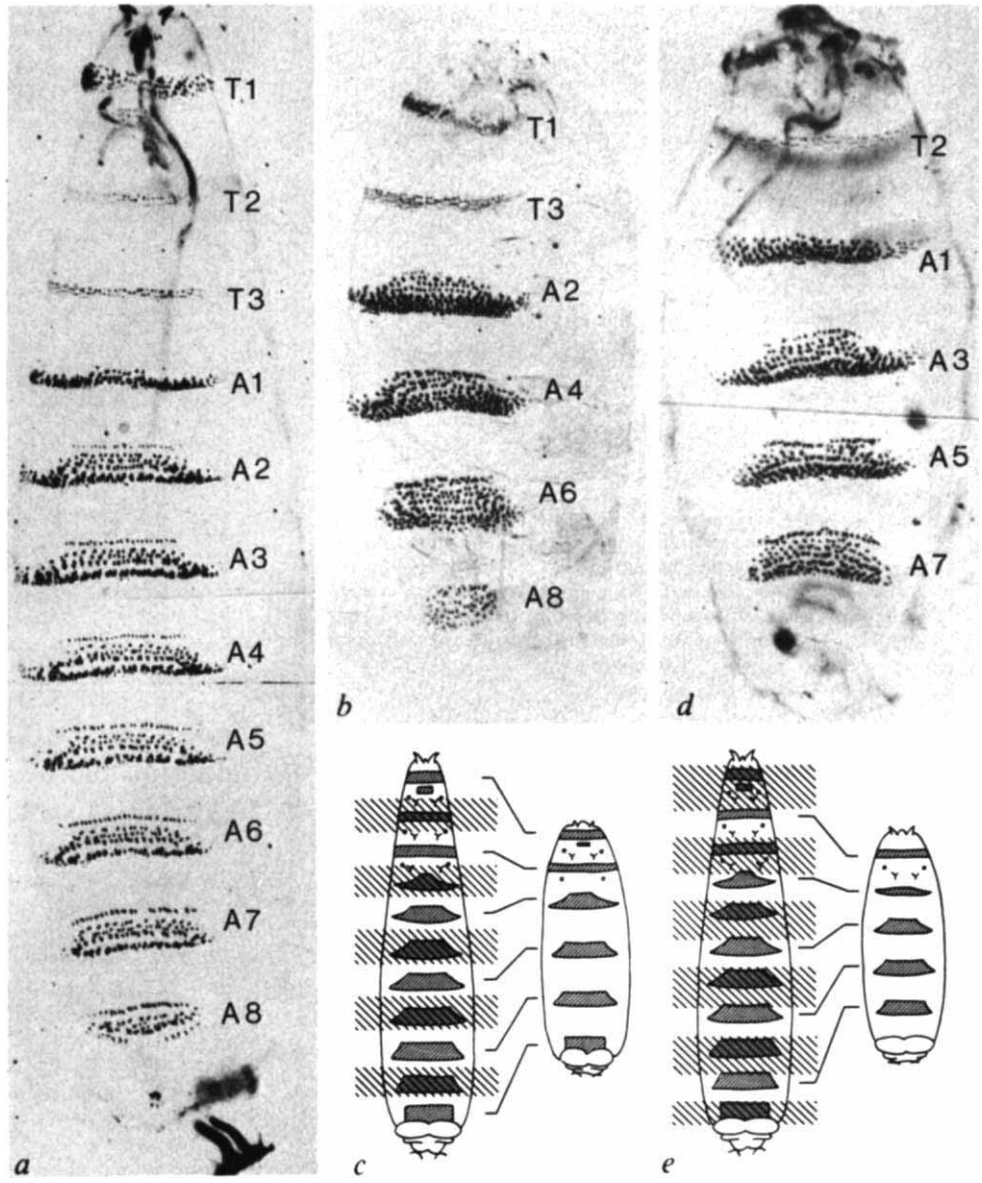

Fig. 3 Near-reciprocal pair-rule phenotypes of *ftz*$^-$ and heat-shocked HSF embryos. *a*, Wild-type first-instar larva. The three thoracic and first eight abdominal segments (T1–T3, A1–A8) each bear a characteristic band of ventral hairs (denticles) formed by the anterior compartment, *b*, *c*, *ftz*$^-$ larva (homozygous for an apparent null allele, *ftz*^{9093}; ref. 39). As described in the text, mutant larvae form only half the number of denticle bands (those belonging to segments T1, T3, A2, A4, A6 and A8)[1,2] and hence appear to lack every other segment. In fact, the deleted regions are not segments, but rather segment-length units which begin close to the anteroposterior compartment boundary in one segment and end close to the boundary within the next segment. This is particularly clear in the thoracic segments because: (1) the patterns of dorsal and ventral hairs in each 'double segment' are composites of the anterior (a) and posterior (p) portions of adjacent segments (that is, T1a+T2p, T3a+A1p, A2a+A3p, etc.), (2) two sets of lateral sensory hairs[40] are often formed in each 'double segment' (normally each segment has a single set positioned just anterior to the compartment boundary within the segment), suggesting that a region slightly less than a segment's length lying between the lateral hairs in adjacent segments has been deleted, and (3) partial or complete Keilin's organs (which normally lie on or near the anteroposterior compartment boundary in each thoracic segment[15]) are usually present in the T1aT2p double segment, but are present only rarely in the T3aA1p double segment (deletion of a segment-length unit beginning and ending around the compartment boundaries within adjacent segments might be expected to leave behind partial or complete Keilin's organs more frequently when both segments are thoracic than when one is thoracic and the other abdominal). The exact boundaries between deleted and retained portions of the body seem to vary somewhat. For example, Keilin's organs are composed of three sensory hairs, two derived from the anterior compartment and a third derived from the posterior compartment[15]. In the T1aT2p double segment of *ftz*$^-$ embryos, the Keilin's organs are sometimes rudimentary di-hairs or mono-hairs, or are absent. A further aspect of the *ftz* phentoypes is that some pattern elements, such as specific sensory hairs or rows of denticles, appear to be duplicated in each metameric unit[2]. One consequence of this phenomenon, which has also been observed with mutations in other pair-rule genes[41], is that the denticle bands appear broader than in wild-type embryos. *d*, *e*, Heat-shocked HSF3 larva. In contrast to *ftz*$^-$ larvae, HSF larvae derived from embryos heat-shocked during the blastoderm stage often partially or completely lack the denticle bands associated with segments T1, T3, A2, A4, A6 and A8. Partial deletions often appear as fusions which join the densicle bands of particular pairs of segments (T1+T2, T3+A1, A2+A3, etc.). In progressively more extreme deletions, the denticle bands belonging to the more anterior segments (T1, T3, A2, etc.) appear progressively narrower or are eliminated, leaving the denticle bands derived from the more posterior segments (T2, A1, A3 etc.) largely intact (as in *d*). Thus, the deleted regions seem to be slightly larger than segment-length units which begin and end just outside the boundaries of segments T1, T3, A2, A4, A6 and A8. As in the case of *ftz*$^-$ embryos, the limits of the deletion pattern illustrated in *e* seem to define the usual stopping line, but some embryos show more extreme deletion phenotypes. Because of the variation in phenotype, these limits should be considered provisional. *a*, *b*, *d*, Dark-field photomicrographs (ventral aspects, positive images; ×120). *c*, *e*, The regions deleted from the normal pattern (after ref. 8). The denticle bands are shaded and the missing regions indicated by hatched bars; Keilin's organs and ventral pits are shown as Y and o symbols, respectively. Keilin's organs are found only rarely in the T3aA1p double segment of *ftz*$^-$ larvae and are therefore not indicated in this position in *c*. No attempt has been made to map the deleted regions anterior to T1 or posterior to the A8 denticle band, though some portions of these terminal regions fail to develop in each case.

Methods. *d*, *e*, Embryos were collected and allowed to develop at 25 °C and were usually heat-shocked for 20 min at 35 °C, although shocks of 5 or 10 min were also effective at generating reciprocal *ftz* phenotypes. Typically, 500–1,000 fertilized eggs were collected during a 30-min period and shocked between $2\frac{3}{4}$ and $3\frac{1}{4}$ h after egg laying. In these conditions, ~50% of the resulting larvae showed partial or complete deletions of the denticle bands of at least three alternating segments; fewer than 1% of the embryos heat-shocked between $3\frac{1}{4}$ and $3\frac{3}{4}$ h after egg laying (or at any time thereafter) developed into larvae showing reciprocal *ftz* phenotypes. HSF2 and HSF3 embryos responded similarly to heat shock.

normally silent in the 'off' stripes because it must be off to allow these regions to develop. Thus, the alternating on and off stripes of *ftz* activity seem to be equally critical, in their respective domains, for organizing the metameric pattern of the body. Similar evidence is not yet available for the *h* gene. However, dosage studies of another pair-rule gene, *runt*[8,18], have shown that extra wild-type copies can cause pair-rule deletions which are nearly reciprocal to the deletions resulting from loss of gene function (ref. 10 and J. P. Gergen and E. Wieschaus, personal communication). Though the interpretation is less clear in this case, it is possible that these 'anti-runt' phenotypes are caused by elevated levels of *runt* expression in regions where the gene must normally be either silent or expressed at a low level.

These results can be explained by positing that the pair-rule genes act combinatorially to initiate the development of repeating portions of the segment pattern (see also ref. 10). For example, each cell along the anteroposterior axis of the blastoderm may express a specific combination of pair-rule genes (perhaps in response to a periodic spatial cue). This combination of genes then initiates the development of a specific interval of

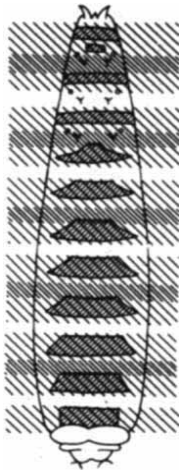

Fig. 4 Overlap between the *ftz*$^-$ and heat-shocked HSF pair-rule phenotypes. The regions of the body deleted in heat-shocked HSF larvae (Fig. 3*e*) are largely anterior to the regions deleted in *ftz*$^-$ larvae (Fig. 3*c*). Note that the deletion patterns are not perfectly reciprocal: regions of common overlap (heavy shading) and common exclusion (unshaded) are found in alternating intervals along the body.

the repeat pattern, perhaps by activating the correct patterns of expression of other genes such as *engrailed*[8,19–22] and the segment polarity genes[8,9] which function subsequently to control growth and cell pattern within segments. Accordingly, both the on and off states of pair-rule gene function would have equal instructive roles because the developmental behaviour of any given cell or region would depend on the initial code-word of on and off pair-rule genes. In this regard, pair-rule genes may be acting in a manner functionally analogous to that of homoeotic genes of the bithorax and *Antennapedia* complexes[23–31].

Though this interpretation is prompted in part by the reciprocal nature of the *ftz*$^-$ and heat-shocked HSF phenotypes, it should be noted, as shown in Fig. 4, that these phenotypes are not perfectly reciprocal: some regions of the body are deleted in both *ftz*$^-$ and heat-shocked HSF larvae while other regions appear unaffected in either case. Regions of overlap between the two deletion patterns suggest that *ftz* and perhaps other pair-rule genes do not function simply as binary switches, but rather that differences in relative levels of their gene products, or perhaps temporal sequences of alternating expression, may also be important in defining specific portions of the segment pattern. Note that the stripes of cells expressing *ftz* transcripts narrow towards the end of the blastoderm stage[3,32], indicating that the gene may be on to different extents, or first on and then off, in some cells of each stripe.

I thank Matthew Scott, Hugh Pelham, Paul Krieg, Christiane Nusslein-Volhard and Jim Posakony for making their cloned genes, cloning vectors and mutations freely available; Paul Macdonald for help with constructing the *hsp70-ftz* hybrid gene; Janice Fischer for generating the germline transformants by microinjection; and Doug Melton for catalysing the initial phases of this work. I also thank Peter Lawrence and Peter Gergen for their critical comments on the manuscript, Tom Maniatis for allowing me to work in his laboratory and Harvard University and the USNIH (program project grant NIH 5 PO1 GM29301) for financial support.

Received 9 September; accepted 23 October 1985.

1. Wakimoto, B. T. & Kaufman, T. C. *Devl Biol.* **81**, 51-64 (1981).
2. Wakimoto, B. T., Turner, F. R. & Kaufman, T. C. *Devl Biol.* **102**, 147-172 (1984).
3. Hafen, E., Kuroiwa, A. & Gehring, W. *Cell* **37**, 833-841 (1984).
4. Pelham, H. R. B. *Cell* **30**, 517-528 (1982).
5. Lis, J. T., Simon, J. A. & Sutton, C. A. *Cell* **35**, 403-410 (1983).
6. Dudler, R. & Travers, A. A. *Cell* **38**, 391-398 (1984).
7. Bonner, J. J. *et al. Cell* **37**, 979-991 (1984).
8. Nusslein-Volhard, C. & Wieschaus, E. *Nature* **287**, 795-801 (1980).
9. Nusslein-Volhard, C., Wieschaus, E. & Jurgens, G. *Verh. dt. zool. Ges.*, 91-104 (1982).
10. Gergen, J. P., Coulter, D. & Wieschaus, E. *Symp. Soc. dev. Biol.* (in the press).
11. Laughon, A. & Scott, M. P. *Nature* **310**, 25-31 (1984).
12. Rubin, G. M. & Spradling, A. C. *Science* **218**, 348-353 (1982).
13. Spradling, A. C. & Rubin, G. M. *Science* **218**, 341-347 (1982).
14. Zimmerman, J. L., Petri, W. & Meselson, M. *Cell* **32**, 1161-1170 (1983).
15. Struhl, G. *Nature* **308**, 454-457 (1984).
16. Hayes, P. M., Sato, T. & Denell, R. E. *Proc. natn. Acad. Sci. U.S.A.* **87**, 545-549 (1984).
17. Ingham, P. W., Howard, K. & Ish-Horowicz, D. *Nature* (in the press).
18. Gergen, J. P. & Wieschaus, E. F. *Devl Biol.* **109**, 321-335 (1985).
19. Lawrence, P. A. & Morata, G. *Devl Biol.* **50**, 321-337 (1976).
20. Kornberg, T. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1095-1099 (1981).
21. Lawrence, P. A. & Struhl, G. *EMBO J.* **1**, 827-833 (1982).
22. Kornberg, T. *et al. Cell* **40**, 45-53 (1985).
23. Lewis, E. B. *Am. Zool.* **3**, 33-56 (1963).
24. Lewis, E. B. *Nature* **276**, 565-570 (1978).
25. Lewis, E. B. in *Embryonic Development: Genes and Cells* (ed. Burger, M.) 269-288 (Liss, New York, 1982).
26. Duncan, I. & Lewis, E. B. in *Developmental Order: Its Origin and Regulation* (ed. Subtelny, S.) 533-554 (Liss, New York, 1982).
27. Kaufman, T. C., Lewis, R. & Wakimoto, B. T. *Genetics* **94**, 115-133 (1980).
28. Struhl, G. *Nature* **293**, 36-41 (1981).
29. Struhl, G. *Nature* **292**, 635-638 (1981).
30. Struhl, G. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7380-7384 (1982).
31. Struhl, G. *J. Embryol. exp. Morph.* **76**, 297-331 (1983).
32. Martinez-Arias, A. & Lawrence, P. A. *Nature* **313**, 639-642 (1985).
33. Karech, F., Torok, I. & Tissieres, A. *J. molec. Biol.* **148**, 219-230 (1981).
34. Krieg, P. A. & Melton, D. A. *Nucleic Acids Res.* **12**, 7057-7070 (1984).
35. Wiener, A. J., Scott, M. P. & Kaufman, T. C. *Cell* **37**, 843-851 (1984).
36. Kuroiwa, A., Hafen., E. & Gehring, W. J. *Cell* **37**, 825-831 (1984).
37. Struhl, G. & Akam, M. *EMBO J.* (in the press).
38. Fyrberg, E. A. *et al. Cell* **33**, 115-123 (1983).
39. Jurgens, G. *et al. Wilhelm Roux Arch. dev. Biol.* **193**, 283-295 (1984).
40. Lohs-Schardin, M., Cremer, C. & Nusslein-Volhard, C. *Devl Biol.* **73**, 239-255 (1979).
41. Sander, K., Lohs-Schardin, M. & Baumann, M. *Nature* **287**, 841-843 (1980).

Simian virus 40-mediated *cis* induction of the *Xenopus* β-globin DNase I hypersensitive site

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Regions in chromatin which are hypersensitive to the action of DNase I appear to be associated with sites of genetic activity; the association between DNase I hypersensitivity and transcriptional activation is well known[1]. In the case of the chicken β-globin gene the establishment of a DNase I hypersensitive site is dependent on tissue-specific *trans*-acting factors[2,3]. Such factors have also been implicated in the action of viral and cellular enhancers[4–10], which are themselves hypersensitive to DNase I[11–14]. Enhancers have been defined operationally as DNA sequences which act in *cis* to potentiate transcription from their own, heterologous or cryptic promoters. This activity is essentially unaffected by changes in the orientation, position (5′ or 3′) or distance of the enhancer element with respect to its cognate promoter (ref. 15 for review). We demonstrate here that the transcriptional rescue of the *Xenopus laevis* β-globin gene by simian virus 40 (SV40) sequences including the enhancer coincides with the conferment of DNase I hypersensitivity upon that gene, and that this occurs in the absence of any change in the complement of *trans*-acting factors. These results suggest that a propensity to form sites hypersensitive to the action of DNase I is encoded in the primary sequence of DNA[16], and that this predilection is aggravated by SV40 sequences, perhaps through a mechanism dependent on supercoiling.

Neither human nor rabbit β-globin genes are expressed when they are introduced by transfection into HeLa cells on plasmid vectors in a transient assay, but transcription of these genes is restored by linkage in *cis* to the SV40 enhancer[17,18]. We have examined the behaviour of the *X. laevis* β_1-globin gene in similar conditions. HeLa cells were transfected with recombinant plasmids and left for 48 h, after which both nuclei and total cytoplasmic RNA were prepared. The RNA was analysed for the presence of correctly initiated globin transcripts by quantitative S_1

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