t(X;21) translocation patient, sequence analysis by Bodrug in our laboratory has revealed a small deletion of ~70 bp at the site of exchange[22] but no exons are deleted. The dystrophic phenotype can be entirely accounted for by the fact that the translocation breaks through an intron of the gene separating a few exons at the 5′ end from the remainder of the gene. Non-random X-inactivation of the normal X chromosome[3] results in a manifesting heterozygote. With the exception of the oncogene rearrangements in cancer cells, this is the first documented case of a translocation that splits a gene causing a genetic disease.

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1. Moser, H. *Hum. Genet.* **66,** 17-40 (1984).
2. Worton, R. G. & Burghes, A. H. M. *Int. Rev. Neurobiol.* **29** (in the press).
3. Verellen-Dumoulin, C. *et al. Hum. Genet.* **67,** 115-119 (1984).
4. Boyd, Y., Buckle, V., Holt, S., Munro, E., Hunter, D. & Craig, I. *J. med. Genet.* **23,** 484-490 (1986).
5. Davies, K. E. *et al. Nucleic Acids Res.* **11,** 2303-2312 (1983).
6. Goodfellow, P. N., Davies, K. E. & Ropers, H. H. *Cutogenet. Cell Genet.* **40,** 296-352 (1985).
7. Francke, U. *et al. Am. J. hum. Genet.* **37,** 250-267 (1985).
8. Monaco, A. P. *et al. Nature* **316,** 842-845 (1985).
9. Ray, P. N. *et al. Nature* **318,** 671-675 (1985).
10. Kunkel, L. M. *et al. Nature* **322,** 73-77 (1986).
11. Thomas, N. S. T., Ray, P. N., Worton, R. G. & Harper, P. S. *J. med. Genet.* **23,** 509-515 (1986).
12. Hart, K. *et al. J. med. Genet.* **23,** 516-530 (1986).
13. Monaco, A. P. *et al. Nature* **323,** 646-650 (1986).
14. Ray, P. N. *et al. Am. J. Hum. Genet.* (submitted).
15. Lloyld, J. C., Isenberg, H., Hopkinson, D. A. & Edwards, Y. H. *Ann. hum. Genet.* **49,** 241-251 (1985).
16. Burmeister, M. & Lehrach, H. *Nature* **324,** 581-585 (1986).
17. VanOmmen, G. J. B. *et al. Cell* **47,** 499-504 (1986).
18. Kenwrick, S., Patterson, M., Speer, A., Fishbeck, K. & Davies, K. *Cell* **48,** 351-357 (1986).
19. Boyd, Y. *et al. Clin. Genet.* **19,** 108-115 (1987).
20. Monaco, A. P., Bertelson, C. J., Colletti-Feener, C. & Kunkel, L. M. *Hum. Genet.* **75,** 221-227 (1987).
21. Thompson, M.W. *J. med. Genet.* **23,** 548-555 (1986).
22. Bodrug, S. E. *et al. Science* (in the press).
23. Worton, R. G., Duff, C., Sylvester, J. E., Schmickel, R. D. & Willard, H. F. *Science* **224,** 1447-1449 (1984).
24. Southern, E. M. *J. molec. Biol.* **98,** 503-517 (1975).
25. Feinberg, A. & Vogelstein, B. *Analyt. Biochem.* **132,** 6-13 (1982).
26. Korneluk, R. G. *et al. J. biol. Chem.* **261,** 8407-8413 (1986).
27. Chirgwin, J. M., Przybyla, R. J., Macdonald, T. & Rutter, W. J. *Biochemistry* **18,** 5294-5299 (1979).
28. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* 1408-1412 (1972).
29. Maniatis, T., Fritisch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* 202-203 (Cold Spring Harbor, New York, 1982).

Exchange of terminal portions of X- and Y-chromosomal short arms in human XX males

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In most human 'XX males'[1], DNA sequences normally found on Yp, the short arm of the Y chromosome, are present on Xp, the short arm of the X chromosome[2–6]. To establish whether this transfer involves a terminal portion of Yp, and whether a terminal portion of Xp is lost in the process, we followed the inheritance of pseudoautosomal restriction fragment length polymorphisms in two XX-male families. One XX male apparently inherited the entire pseudoautosomal region of his father's Y chromosome and no part of the pseudoautosomal region of his father's X chromosome. The second XX male also inherited the entire pseudoautosomal region of his father's Y, but in addition inherited a proximal portion of the pseudoautosomal region of his father's X. These findings argue that XX males result from the transfer of a terminal portion of Yp onto Xp in exchange for a terminal portion of Xp (ref. 7). This implies that the testis-determining factor gene (*TDF*) maps distally in the strictly sex-linked portion of Yp, near the pseudoautosomal domain. The XX males described here appear to result from single (and, at least in the second case, unequal) crossovers proximal to the pseudoautosomal region on Yp and proximal to or within the pseudoautosomal region on Xp.

In man, the most distal portions of Xp and Yp are 'pseudoautosomal'. They undergo frequent X-Y recombination during normal male meiosis. As a result, these portions of Xp and Yp are homologous, and their inheritance is not strictly sex-linked[8–12].

The *MIC2* gene maps proximally in the pseudoautosomal region, recombining with sex phenotype at a frequency of only 2% in male meiosis[12]. Plasmid pDP1001, which detects a restriction fragment length polymorphism (RFLP) at *MIC2*, was hybridized to DNAs from the family of XX male LGL163 (Fig. 1*a*). The father is heterozygous; his 1.4- and 2.0-kilobase (kb) fragments must be on his Y chromosome, because that allele is present in the grandfather but not the grandmother. The XX male inherited the father's Y allele. Densitometry reveals that the XX male has one copy of the 3.3-kb allele. This copy must be from the mother, because she is homozygous for that allele. Thus, at *MIC2*, XX male LGL163 inherited his father's Y allele but not his father's X allele.

The second family was typed for a *MIC2* RFLP detected by pDP1002 (Fig. 1*b*). XX male LGL1358 has two copies of the 2.6-kb allele, for which the father is homozygous, and one copy of the 3.3-kb allele, for which the mother is homozygous. (Compare, in Fig. 1*b*, the relative intensities of the 2.6- and 3.3-kb fragments in XX male LGL1358 with those in LGL1364 and LGL1362, both presumably normal heterozygotes.) A similar result was obtained with the pDP1001 RFLP: the XX male has two copies of the allele for which the father is homozygous and one copy of the allele for which the mother is homozygous (not shown).

These studies did not reveal the chromosomal origin of the two copies of *MIC2* that XX male LGL1358 received from his father. This information was obtained using an RFLP detected by pSG1, a *MIC2* complementary DNA clone[12]. The father is heterozygous (Fig. 1*c*). His 2.7-kb fragment must be on his Y chromosome, because that allele is present in the grandfather but not the grandmother. Conversely, the father's 2.4-kb allele must be on his X chromosome. XX male LGL1358 appears to have two copies of the 2.4-kb allele and one copy of the 2.7-kb allele. (Compare, in Fig. 1*c*, the relative intensities of the 2.4- and 2.7-kb fragments in LGL1358 with those in LGL1359, a normal heterozygote.) The XX male inherited the 2.7-kb fragment from his father's Y chromosome. The XX male presumably inherited one copy of the 2.4-kb fragment from his mother, who is homozygous for that allele. Given the results with pDP1001 and pDP1002, he must have inherited the second 2.4-kb fragment from his father's X chromosome. That is, XX male LGL1358 inherited both his father's X and Y alleles at *MIC2*.

The findings in these XX males have implications for the localization of the testis determining factor gene, *TDF*, on the Y chromosome. An eight-interval deletion map of the Y has

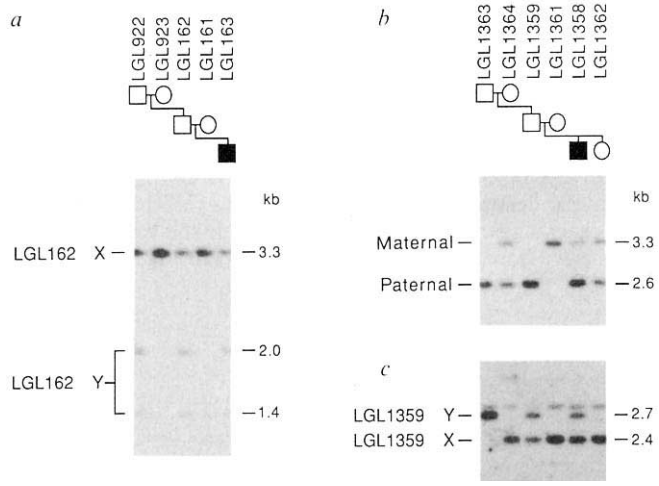

Fig. 1 Inheritance in two XX male families of RFLPs at *MIC2*. Squares, males; circles, females; filled squares, XX males. Both XX males carry DNA sequences derived from the strictly sex-linked portion of Yp (ref. 3 and unpublished results). Study of the paternal grandparents allows the phase of the pseudoautosomal alleles in the father to be determined. Each autoradiogram lane corresponds to the individual above that lane in the pedigree. *a*, Family 1: probe pDP1001 hybridized to *Taq*I-digested genomic DNAs. Allelic restriction fragments marking the X or Y chromosomes of LGL162, the father, are indicated. *b*, Family 2: pDP1002 hybridized to *Taq*I-digested DNAs. Allelic fragments present in father, LGL1359, or in mother, LGL1361, are indicated. *c*, Family 2: an 0.7-kb *Eco*RI–*Stu*I fragment purified from plasmid pSG1 was hybridized to *Msp*I-digested DNAs. Fragments that distinguish the X and Y chromosomes of LGL1359, the father, are indicated.
Methods. Plasmids pDP1001 and pDP1002 were subcloned from recombinant phages identified by screening a human genomic library with plasmid pSG1, a *MIC2* cDNA clone[21]. Plasmid pDP1001 contains a 1.5-kb genomic *Eco*RI-*Pst*I fragment and detects a *Taq*I RFLP with fragments of, in one allele, 3.3 kb and, in a second allele, 1.4 and 2.0 kb. Plasmid pDP1002 contains a 0.8-kb genomic *Eco*RI fragment. It detects an insertion/deletion RFLP (not shown) and a *Taq*I RFLP with allelic fragments of 2.6 and 3.3 kb. These clones were mapped to the region Xp22.32-pter and to the Y by hybridization to DNAs from hybrid somatic cell lines, consistent with their being derived from *MIC2*. Human genomic DNAs were prepared from leukocytes or cultured fibroblasts[22], digested with restriction endonuclease, electrophoresed on 0.7% agarose gels, and transferred[23] to nylon membrane. Human inserts purified from the plasmids were labelled with ^{32}P by random-primer synthesis[24], prehybridized with an excess of sonicated human genomic DNA[25], and hybridized overnight to genomic DNA transfers at 47 °C in 50% formamide, 5 × SSC (1 × SSC = 0.15 M NaCl, 15 mM Sodium citrate *p*H 7.4), Denhardt's (0.02% Ficoll 400, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin), 1% SDS, 20 mM $NaPO_4$ *p*H 6.6, 50 μg ml^{-1} denatured salmon sperm DNA and 10% dextran sulphate. Membranes were washed three times for 15 min each at 65–70 °C in 0.1 × SSC, 0.1% SDS and exposed at −80 °C for 1–3 days with X-ray film backed by an intensifying screen.

been constructed from studies of Y chromosome DNA from XX males and other individuals with sex chromosome anomalies; *TDF* maps to interval 1, on Yp (refs 3 and 13). Uncertainty regarding the order of intervals 1, 2, and 3 on Yp stems from uncertainty as to whether XX males have received terminal or internal portions of Yp (ref. 3). Given that *MIC2* recombines with sex phenotype in only 2% of normal male meioses[12], the 'terminal' and 'internal' models make opposite predictions as to the inheritance of *MIC2* in XX males. According to the terminal model, an XX male would receive the end of the strictly sex-linked portion of Yp from his father; he would then be very likely to inherit his father's Y-chromosomal allele at the closely-linked *MIC2* locus (probability 98%). According to the internal model, an XX male would not receive the end of the strictly sex-linked portion of Yp; he would inherit his father's Y-chromosomal *MIC2* allele only in the unlikely event of recombination with *MIC2* (probability 2%). That both XX males inherited their father's Y allele at *MIC2* strongly suggests that they received terminal portions of Yp. In turn, this argues that deletion interval 1, containing *TDF*, is just proximal to the pseudoautosomal region. Similarly, that XX male LGL163 did not inherit his father's X allele at *MIC2* suggests that the end of the strictly sex-linked portion of Xp has been lost.

We also followed the inheritance in these families of five other pseudoautosomal RFLPs (Table 1). Three of these polymorphic loci, *DXYS17*, *DXYS15* and *DXYS28*, show partial sex linkage, recombining with sex phenotype in male meiosis at frequencies of about 14%, 32% and 38%, respectively (ref. 11 and D.P. *et al.*, in preparation). The other two loci, *DXYS20* and *DXYS14*, show no detectable sex linkage and map to the most distal portion of the pseudoautosomal region, near the telomeres of Xp and Yp (refs 9 and 11; D.P. *et al.*, in preparation).

All five RFLPs are informative in the first family, and at each locus the XX male inherited from father the Y but not the X allele (Table 1). For example, the father is heterozygous for the *DXYS14* RFLP (Fig. 2*a*). The father's X and Y alleles are identified by their presence in, respectively, the grandmother and grandfather. XX male LGL163 inherited from his father the Y but not the X allele at *DXYS14*.

Thus, at six loci, together spanning nearly the entire pseudoautosomal portion of the sex chromosomes, XX male LGL163 inherited from father the Y but not the X alleles. These results argue that LGL163 inherited the pseudoautosomal region of his father's Y chromosome intact and unrecombined. A double crossover between two of the pseudoautosomal markers for which we tested is unlikely, given that double recombinants

Table 1 X- or Y-chromosomal origin of pseudoautosomal RFLP alleles transmitted from fathers to XX-male sons

Locus	Probe	Recombination with sex phenotype (%)	Reference	Allele from father: LGL163 (XX male 1)	Allele from father: LGL1358 (XX male 2)
DXYS14	29C1	50	9,11	Y	Y
DXYS20	pDP230	50	Page *et al.* (in preparation)	Y	NI
DXYS28	pDP411a	38	Page *et al.* (in preparation)	Y	NI
DXYS15	113D	32	10, 11	Y	NI
DXYS17	601	14	11	Y	NI
MIC2	pSG1 pDP1001 pDP1002	2	12	Y	X and Y

DNA probes detecting pseudoautosomal RFLPs were hybridized to *Taq*I, *Msp*I, or *Eco*RI-digested DNAs from two XX males, their parents, and paternal grandparents. NI, not informative.

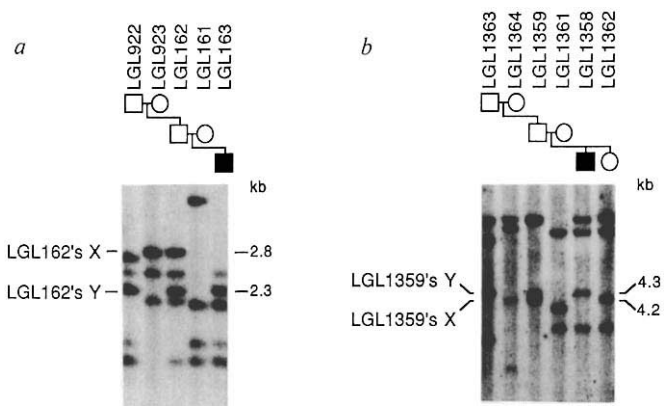

Fig. 2 Inheritance in two XX-male families of RFLPs at *DXYS14*. *a*, Family 1: probe 29C1 was hybridized at 42 °C to *Taq*I-digested genomic DNAs. After washing at 60 °C, the transfer membrane was exposed with X-ray film for 3 days. Probe 29C1 detects a polymorphic family of related sequences, resulting in complex hybridization but simple, single-locus inheritance; a typical allele comprises a collection of restriction fragments[9,11]. Some of the allelic restriction fragments that distinguish the X and Y chromosomes of LGL162, the father, are indicated. *b*, Family 2: 29C1 hybridized to *Eco*RI-digested DNAs. Some of the allelic restriction fragments marking the X and Y chromosomes of LGL1359, the father, are indicated.

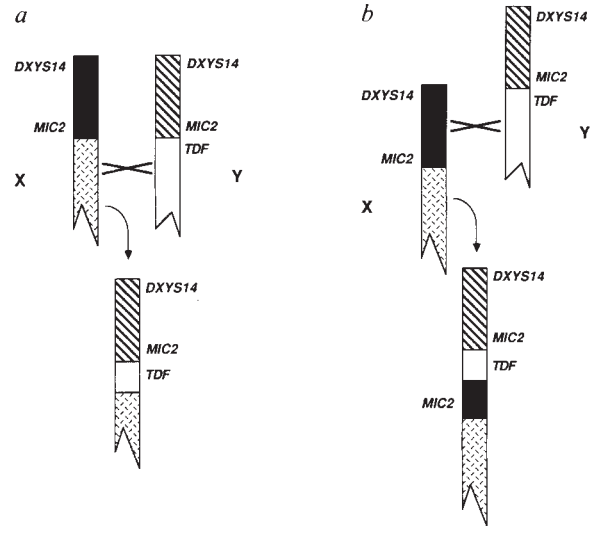

Fig. 3 Genesis of *TDF*-bearing X chromosomes in XX males by single crossovers between Xp and Yp during or prior to paternal meiosis. Differentially shaded regions depict the pseudoautosomal (black and striped) and strictly sex-linked (stippled and white) portions of Xp and Yp. On Yp, the point of crossing over is proximal to *TDF*, which in turn is proximal to the pseudoautosomal region. On Xp, crossing over can occur either *a*, proximal to *MIC2*, perhaps in the X-specific region, or *b*, distal to *MIC2*, in the pseudoautosomal region. Unequal crossing-over depicted in *b* produces an X-Y interchange product carrying two copies of *MIC2*.

occur rarely if ever within the pseudoautosomal region in man (ref. 11; D.P. *et al.*, in preparation). The results also suggest that XX male LGL163 inherited no part of the pseudoautosomal region of his father's X chromosome.

In the second family, apart from *MIC2*, one pseudoautosomal locus was informative (Table 1). At the distal locus *DXYS14*, XX male LGL1358 inherited from his father the Y allele but not the X allele (Fig. 2*b*).

We propose that sex reversal in these XX males is the result of aberrant Xp-Yp exchanges (Fig. 3). XX male LGL163 appears to have inherited the entire pseudoautosomal region of his father's Y chromosome but no part of the pseudoautosomal region of his father's X chromosome. This is probably the result of a single crossover proximal to *MIC2* on Xp and proximal to *TDF* on Yp (Fig. 3*a*). The result is a transfer of a terminal, male-determining portion of Yp onto distal Xp, in exchange for a terminal portion of Xp, as predicted by the 'X-Y interchange' model[7,14].

XX male LGL1358 also seems to have inherited the entire pseudoautosomal region of his father's Y chromosome. In addition, however, he inherited the proximal portion (*MIC2*) but not the distal portion (*DXYS14*) of the pseudoautosomal region of his father's X chromosome. This is probably the result of a single crossover in the pseudoautosomal region (between *MIC2* and *DXYS14*) on Xp and proximal to *TDF* on Yp (Fig. 3*b*), as has been hypothesized to occur in some XX males[14].

X-Y exchanges like that in Fig. 3*b* produce XX males with two copies of all strictly X-linked loci. In contrast, exchanges of the sort seen in LGL163 (Fig. 3*a*) might produce XX males with a single copy of strictly sex-linked loci on distal Xp. This may explain why some XX males express their fathers' alleles for *Xg*, a dominant, X-linked marker on distal Xp, whereas most, including LGL163, do not[1,15]. Exchanges like that in Fig. 3*b* would yield *TDF*-bearing X chromosomes whose length is greater than that of normal X chromosomes. Depending on the positions of Xp and Yp breakpoints, exchanges like that in Fig. 3*a* might yield abnormally long or short *TDF*-bearing X chromosomes. Such alterations may explain the observation that, in many XX males, one of the two X chromosomes seems to be abnormally long[16] or has an altered high-resolution banding pattern[17].

Distal portions of Xp and Yp pair during male meiosis[18,19]. Does this pairing contribute to the rather high frequency (1 in 20,000 males[1]) at which XX males occur? Various mechanisms can be envisaged. First, homologous X-Y recombination initiated in the pseudoautosomal region might, on occasion, give rise to branch migration into the strictly sex-linked portions of the sex chromosomes, with resolution proximal to *TDF*. This seems unlikely, however, because branch migration seems to require that recombining chromosomes have very similar DNA sequences; the Yp-specific DNA sequences that XX males inherit are not homologous to Xp.

A second possibility is more likely. The Xp-Yp synaptonemal complex extends far beyond the pseudoautosomal region, involving the distal quarter of Xp and virtually all of Yp (ref. 20). This synapsis might occasionally produce exchanges between the strictly sex-linked portions of the X and Y chromosomes. These exchanges may or may not be legitimate recombination events occurring at sites of limited X-Y homology. To account for XX males in whom the point of recombination on X is pseudoautosomal, one would have to suppose that Xp-Yp synapsis is compatible with the pseudoautosomal portions of X and Y chromosomes being grossly out of register. Perhaps the pseudoautosomal regions of the X and Y chromosomes, so highly recombinogenic in male meiosis, retain this property when misaligned.

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1. de la Chapelle, A. *Hum. Genet.* **58**, 105-116 (1981).
2. Guellaen, G. *et al. Nature* **307**, 172-173 (1984).
3. Vergnaud, G. *et al. Am. J. Hum. Genet.* **38**, 330-340 (1986).
4. Affara, N. A. *et al. Nucleic Acids. Res.* **14**, 5375-5387 (1986).
5. Muller, U. *et al. Nucleic Acids. Res.* **14**, 6489-6505 (1986).
6. Andersson, M., Page, D. C. & de la Chapelle, A. *Science* **233**, 786-788 (1986).
7. Ferguson-Smith, M. A. *Lancet* **ii**, 475-476 (1966).

8. Burgoyne, P. S. *Hum. Genet.* **61,** 85-90 (1982).
9. Cooke, H. J., Brown, W. R. A. & Rappold, G. A. *Nature* **317,** 687-692 (1985).
10. Simmler, M.-C. *et al. Nature* **317,** 692-697 (1985).
11. Rouyer, F. *et al. Nature* **319,** 291-295 (1986).
12. Goodfellow, P. J., Darling, S. M., Thomas, N. S. & Goodfellow, P. N. *Science* **234,** 740-743 (1986).
13. Page, D. C. *Cold Spring Harb. Symp. quant. Biol.* **51,** 229-235 (1986).
14. Polani, P. E. *Hum. Genet.* **60,** 207-211 (1982).
15. de la Chapelle, A., Tippett, P. A., Wetterstrand, G. & Page, D. *Nature* **307,** 170-171 (1984).
16. Evans, H. J., Buckton, K. E., Spowart, G. & Carothers, A. D. *Hum. Genet.* **49,** 11-31 (1979).
17. Magenis, R. E. *et al. Hum. Genet.* **62,** 271-276 (1982).
18. Pearson, P. L. & Bobrow, M. *Nature* **226,** 959-961 (1970).
19. Chen, A. & Falek, A. *Nature* **232,** 555-556 (1971).
20. Moses, M. J., Counce, S. J. & Paulson, D. F. *Science* **187,** 363-365 (1975).
21. Darling, S. M., Banting, G. S., Pym, B., Wolfe, J. & Goodfellow, P. N. *Proc. natn. Acad. Sci. U.S.A.* **83,** 135-139 (1986).
22. Kunkel, L. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 1245-1249 (1977).
23. Southern, E. M. *J. molec. Biol.* **98,** 503-517 (1975).
24. Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **137,** 266-267 (1984).
25. Litt, M. & White, R. L. *Proc. natn. Acad. Sci. U.S.A.* **82,** 6206-6210 (1985).

Borders of parasegments in *Drosophila* embryos are delimited by the *fushi tarazu* and *even-skipped* genes

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One of the earliest molecular signs of segmentation in *Drosophila* embryos is the striped expression of some pair-rule genes[1] during the blastoderm stage. Two of these genes, *fushi-tarazu* (*ftz*) and *even-skipped* (*eve*) are expressed during this stage in complementary patterns of seven stripes[2–8] which develop and disappear in concert[8]. Here, we map the cells expressing each of these two pair-rule genes with respect to the 14 stripes of cells expressing the *engrailed* gene. We find that both *ftz* and *eve* generate stripes which have sharp boundaries at the anterior margin, but fade away posteriorly. The anterior boundaries correspond cell by cell with the anterior boundaries of expression of the *engrailed* gene. We therefore suggest that a key function of early *ftz* and *eve* gene activity is the formation of a sharp stable boundary at the anterior margin of each stripe. These boundary lines, rather than the narrowing zonal stripes, would delimit the anterior boundaries of *engrailed* and other homoeotic genes and thereby subdivide the embryo into parasegments[9].

The *engrailed* gene product was mapped directly by staining embryos for binding of an antibody[10] against the engrailed product ('anti-*engrailed*' antibody). The embryos we used contained constructs consisting of the promoter of either the *ftz* gene[4] or the *eve* gene joined to the β-galactosidase gene from *Escherichia coli*. The patterns of expression of the *ftz* and *eve* genes were detected using monoclonal antibodies against β-galactosidase. The *engrailed* gene product is seen in the nuclei and the β-galactosidase in the cytoplasm; this allows us to score single cells independently for both proteins. Compared with the native *ftz* and *eve* products β-galactosidase seems to be relatively stable[4,11]; hence, the earlier activity of both the *ftz* and *eve* promoters in particular cells is signalled by the presence of β-galactosidase which continues to accumulate in those cells or in their descendents.

During the entire period when the germ band is extended, and also later when the germ band has shortened[12], the *engrailed* gene is expressed in nuclei which are arranged in sharply demarcated stripes that constitute the posterior compartments of the ectoderm[10,13–15]. For *ftz*, the β-galactosidase staining is found only in even-numbered parasegments and is graded in intensity, starting abruptly at the anterior margin of the stripe and fading away gradually in the more posterior cells. The pattern of *eve*-β-galactosidase expression is similar, except that the stripes are restricted to the odd-numbered parasegments. At the anterior margins the expression of *engrailed* and β-galactosidase are precisely coextensive (Figs 1–3). Later stages show the same pattern but the *ftz* and *eve* stripes have narrowed further and the stain is concentrated in the anterior parts of the parasegment (Fig. 1*d*). The anterior borders of *engrailed* and *ftz* expression also coincide (S. Carroll and S. DiNardo, personal communication).

We have not been able to map *ftz*-β-galactosidase or *eve*-β-

Fig. 1 *a*, Lateral view of embryo with germ band extended (stage 11, ref. 12). Numbers indicate the *engrailed* (grey nuclei) stripes of particular parasegments[9]. Cytoplasmic staining for *ftz*-β-galactosidase is brown, and marks the anterior regions of even-numbered parasegments. *b*, Section of same embryo shown in Fig. 1. The section gives a higher resolution. *c*, Glancing section of dorsal posterior region of an extended germ band embryo to show precise coextension of *engrailed* (greyish nuclei) and *ftz*-β-galactosidase (brown cytoplasm) expression. This is particularly clear at the anterior border of parasegment 14 (arrow). Note that the 15th *engrailed* stripe[37] is exceptional and falls within the broader posterior *ftz* stripe. Bright field. *d*, Lateral glancing section of embryo with germ band shortened (stage 13, ref. 12). The anterior boundaries of the parasegments are visible on the surface, behind them cells dip out of the plane of section into the segmental grooves. Note, at much of the anterior boundaries of the even-numbered parasegments, the *engrailed* expression (greyish nuclei) and the *ftz*-β-galactosidase (brown cytoplasm) are clearly coextensive (arrows). In each even-numbered stripe there is a small ventral patch of β-galactosidase staining that extends anteriorly. We do not know what this signifies. *e*, Another picture of the section in Fig. 3 (Nomarski interference contrast microscopy).
Methods. For immunohistochemistry, embryos were fixed in 4% paraformaldehyde in heptane/methanol[38], treated with anti-*engrailed* antibody overnight (dilution 1:100), then with biotinylated secondary antibody and stained wtih Vectorlabs Vectastain ABC kit using diaminobenzidine; makers instructions were followed except that 30 μl of 1% nickel sulphate and 1% cobalt chloride in water were added to 1 ml staining mix. This gave a slate grey colour[39]. Embryos were then treated with anti-β-galactosidase antibody (C. Doe and C. Goodman) (dilution 1:100), biotinylated secondary antibody and stained as above except the metal ions were omitted. This gave an orange-brown colour. Individual embryos were mounted on slides and photographed in various orientations, removed, embedded in agar, oriented and embedded in araldite. Thick sections (20–40 μm) were cut, mounted and photographed. These photographs are the best we were able to prepare to show what is easily seen down the microscope simply by focusing up and down. In sections and whole mounts there is a trade-off between colour intensity and resolution. For immunofluorescence, embryos fixed as above were incubated with the anti-*engrailed* and anti-β-galactosidase primary antibodies, washed and incubated in both fluoresceinisothiocyanate (FITC)-linked-anti-rabbit and rhodamine isothiocyanate (RITC)-linked-anti-mouse secondary antibodies. The *eve*-β-galactosidase hybrid gene consists of 6.3 kilobases (kb) of the *eve* 5′ flanking sequence extending into the structural gene to the *Ava*II site in codon 22 of the *eve* coding sequence, fused with the β-galactosidase structural gene. To ensure consistent polyadenylation of the fusion gene transcript, 800 base pairs (bp) of the 3′ flanking region of the tubulin α1 gene[40] (beginning at the *Sca*I site at position 1,960 and including the poly(A) addition site at position 2,185) was fused 3′ to the stop codon of the β-galactosidase coding sequence. The *eve*-β-galactosidase fusion gene was inserted into the Carnegie 20 vector and incorporated into the genome by P-element mediated transformation. Flies used in this study were homo- or hemizygous for a single insertion on the X chromosome. We cannot be certain that either the *ftz* or *eve*-β-galactosidase gene fusions are expressed exactly like the native *ftz* and *eve* genes. However, within the limits of resolution of previous studies, the major features of expression, notably the seven-striped zebra patterns, appear very similar, if not identical. Both the *ftz* and *eve* genes are actually expressed at a low level throughout much of the syncytial blastoderm and the *eve* gene also generates seven additional minor stripes after the onset of gastrulation[8]. We could not detect these additional aspects of *ftz* and *eve* expression possibly because they are too ephemeral or insubstantial. Hence our analysis is limited to the prominent seven stripes of both genes.