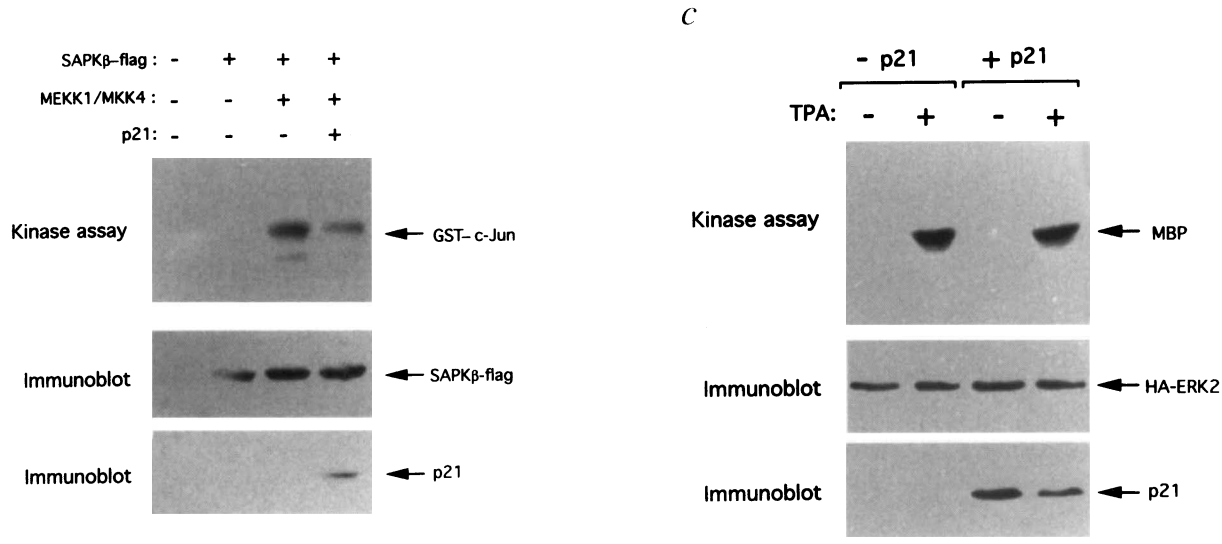

FIG. 4 p21 inhibits SAPK activity in intact cells. *a*, Coexpression of p21 results in a decrease in the UV-activated SAPKβ-flag activity. 293 cells were transfected with SAPKβ-flag or SAPKβ-flag and p21 as indicated. After exposing transfected cells to UV, cell lysates were assayed for SAPKβ-flag activity and immunoblotted for either SAPKβ-flag or p21. *b*, Effect of coexpressed p21 on the MEKK1/Mkk4-stimulated SAPKβ-flag activity. 293 cells were transfected with plasmids expressing indicated proteins. *c*, TPA-activated HA–Erk2 activity was not changed by coexpression of p21. METHODS. 293 cells were transiently transfected with pcDNAI-neo-SAPKβ-flag, pcDNA3-p21, pSRα-MEKK1, pcDNA3-MKK4, or pCEP4-HA-ERK2, as indicated. For UV activation of SAPKβ-flag, transfected cells were exposed to UV (40 J m^{-2}) and the cells lysed after 1 h. For TPA activation of HA-ERK2, transfected cells were exposed to 200 nM TPA for 30 min. Immunocomplex kinase assays for SAPKβ-flag activity were performed as for Fig. 1. HA–Erk2 activity was isolated from cell lysates by immunoprecipitation with anti-HA monoclonal antibody (Boehringer Mannheim) and the immunocomplex kinase assayed using MBP as a substrate. Cell lysates were also immunoblotted with anti-flag, anti-HA or anti-p21 (Oncogene Science) monoclonal antibody using an enhanced chemiluminescence system (Amersham).

signal transduction for different cellular processes, p21 will be valuable for understanding the role of each MAP kinase subfamily in a network of complicated intracellular signalling cascades. Furthermore, our findings indicate that p21 may be a converging point for the regulation of cellular stress, cell cycle[8], tumour suppression[7], and senescence[9]. □

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Reduction of transcription by homologue asynapsis in *Drosophila* imaginal discs

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THE interactions between enhancers and promoter elements that control gene expression are generally considered to act in *cis* only, but genetic studies suggest that they can also function in *trans* between non-contiguous DNA molecules. Termed transvection[1], such *trans* interactions have been proposed to be responsible for several examples of intragenic complementation in *Drosophila*[1–9]. Transvection is thought to depend on the physical proximity of sister chromosomes[10,11], because it is inhibited when chromosome rearrangements reduce the pairing of homologues[1,3,5]. This led to the suggestion that transvection occurs when enhancer elements on one chromosome regulate expression on the other[7,12], with the pairing dependence resulting from a need for proximity between the two copies of the gene. Here we have analysed the levels of transcription from both alleles of the *Drosophila Ultrabithorax (Ubx)* gene, and report that the predictions of this simple model are not supported. Our findings indicate a more complex level of *trans* regulation that may have implications for the aetiology of genetic disorders that are influenced by chromosome rearrangements.

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Ubx specifies haltere and abdominal identities in the *Drosophila* epidermis. Mutations that reduce its activity (for example null alleles such as Ubx^1, deletions of the locus, and some regulatory mutations; see Fig. 1*a*) transform these cells to wing[13]. In contrast, mutations that increase its activity (such as Cbx^1, which causes ectopic expression of *Ubx* in wing imaginal discs) transform wings to halteres. Unexpected interactions between Ubx^1 and Cbx^1 led to the initial observation of transvection. Cbx^1 Ubx^1/+ + heterozygotes, in which the *Ubx* gene linked to the Cbx^1 mutation is nonfunctional, nevertheless exhibit a transformation of wing to haltere. In such flies, the wild-type *Ubx* gene is presumably activated in *trans* by Cbx^1. Such transvection is suppressed by alleles of the *zeste* gene, for example z^a, and by chromosome rearrangements[1].

We developed a way to measure the distinct pools of transcripts produced by heterozygous alleles[14], and used a variation of this

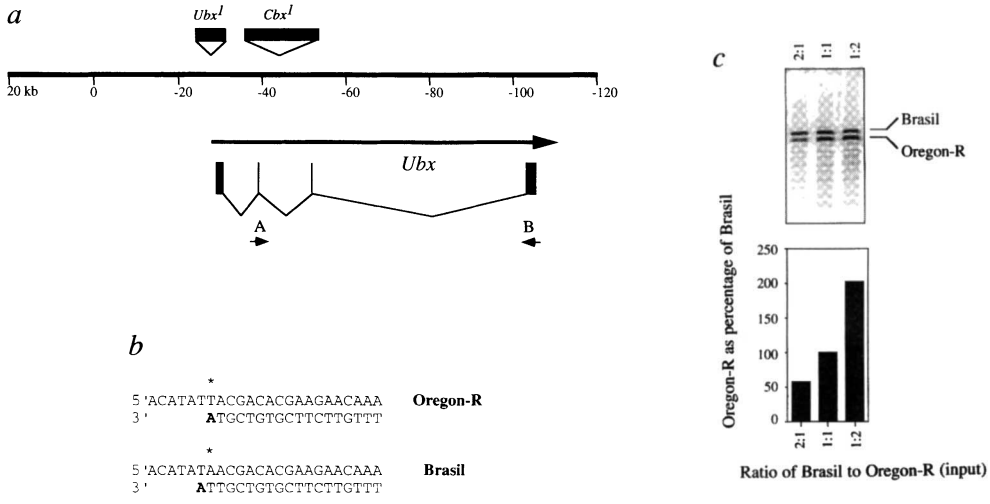

FIG. 1 *a*, Map of *Ubx* mutations. Ubx^1 and Cbx^1 are indicated above the solid line, which represents genomic DNA, and the large horizontal arrow indicates the primary transcript; numbers refer to kilobases. Arrows A and B represent the two PCR primers used to amplify *Ubx* cDNAs; A is in microexon 1 (nucleotides 1,762–1,782), B is in the 3′ UTR (nucleotides 2,898–2,917). *b*, Portion of the *Ubx* 3′ UTR sequence including the T → A polymorphism (asterisk at nucleotide 2,779) that distinguishes transcripts derived from the Brasil, R(B)3, R(B)4, R(B)8 and R(B)13 chromosomes from OR, Cbx^1, TM6b and 31316,607. Only two possible sequencing products, both terminated by ddA and differing from each other by a single nucleotide, can be made. The antisense sequencing primer shown below each template (2,797–2,819) was designed so that the first dT encountered by polymerase is at position 2,778 for Brasil and 2,779 for Cbx^1/OR cDNAs, providing unambiguous length distinction between sequencing products derived from Brasil (19 bp) or Cbx^1 and OR (18 bp) templates. *c*, Allele-specific sequencing of *Ubx* cDNAs showing products derived from different ratios of OR and Brasil cDNA templates. cDNA prepared from either OR or Brasil haltere discs was prepared and amplified as described below, purified, quantified spectrophotometrically and mixed in the indicated ratios. For each sample, 1 ng was reamplified by PCR and processed as described below. The expected ratios (input) of OR to Brasil templates are plotted against experimental results (OR as per percentage of Brasil). Sequencing products of 18 bp (OR) and 19 bp (Brasil) are the only bands on the sequencing gel.

METHODS. Allele-specific amplification was performed as follows. cDNA was prepared from 1–3 mg imaginal discs as described[14] using 50 ng *Ubx* reverse transcriptase primer (GCAAACGGTTTGTCGCACTCC; nucleotides 3,051–3,072). A first strand cDNA sample (1 μl) was added to a PCR reaction (30 μl) with final concentrations of 0.4 mM of primers CGGCATATCAACAGACATGGG and TAGTCGTTTTGGGCGAGAAC (nucleotides 1,762–1,782 and 2,898–2,917, respectively). Parameters for PCR were: 60 s at 94 °C, 60 s at 60 °C, 120 s at 72 °C for 36 cycles. The PCR product was purified by Geneclean following agarose gel electrophoresis and sequenced with primer AATATTCGATACTCCCGAAAACG (nucleotides 2,797–2,819) as described[14] with the following modifications. Both template DNA and primer (~200 ng) were mixed in a total volume of 4.5 μl 10% DMSO. DNA was denatured at 94 °C for 1 min and frozen on dry ice. The combined labelling and termination reaction was performed in the presence of 1.5 mM dTTP, dCTP, ^{35}S dGTP (6,000 Ci mmol^{-1}) and ddATP. After electrophoretic separation, the peak height for each band was measured with a Phosphorimager and background (typically less than 2% of signal) was subtracted.

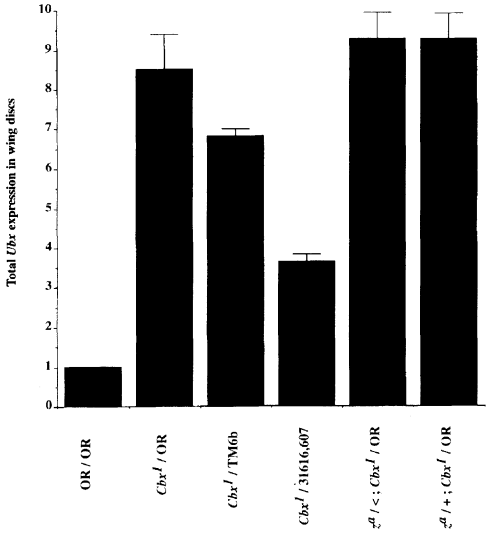

FIG. 2 *Ubx* transcription in Cbx^1 wing imaginal discs is enhanced by chromosome synapsis. cDNA (2 μl) prepared from 4 or 8 similarly sized late third instar wing discs from the indicated genotypes was used for allele-specific amplification in the presence of 1 or 0.5 arbitrary unit of Brasil cDNA template prepared from haltere imaginal discs. PCR amplification produced different amounts of product from either the Brasil and non-Brasil templates, as indicated. At least three independent experiments were performed for each genotype.

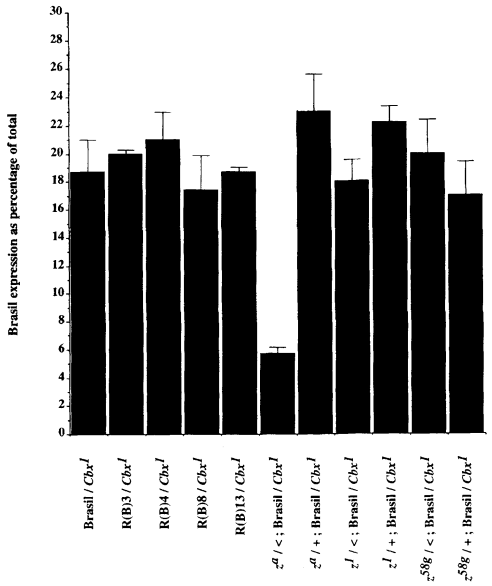

FIG. 3 *Trans*-activation requires *z* function but not chromosome synapsis. RNA isolated from mature third instar wing imaginal discs of the indicated genotypes was subjected to allele-specific amplification (as Fig. 1). The sequence reaction products are expressed as a percentage of the total contributed by the Brasil template. R(B)3, R(B)4, R(B)8 and R(B)13 are Brasil strains with third chromosomes that have translocation and/or inversion breakpoints. These rearrangements suppress transvection.
METHODS. Flies with rearrangement chromosomes were identified among the progeny of 150 Brasil males that had been irradiated with X-rays (4,000 rads) and then mated with Cbx^1 Ubx^1/TM1 females. Flies with the indicated rearrangement chromosomes had weakened transformations of wing to haltere, and had rearrangement breaks proximal to 89E, the cytological location of *Ubx*. Transcript analysis was as Fig. 1.

method (Fig. 1*b*, *c*) to analyse transvecting interactions at *Ubx*. To quantify *Ubx* expression in wing and haltere imaginal discs, we took advantage of a single-base polymorphism in the *Ubx* 3′ untranslated regions (UTRs) of Oregon R wild-type (OR) and Brasil wild-type flies. Complementary DNA was prepared from either a fixed number of OR or Cbx^1/OR imaginal discs, and these cDNAs were mixed separately with an arbitrary but fixed amount of cDNA standard prepared from Brasil discs. We observed that wing discs contain low levels of *Ubx* RNA, which is consistent with previous studies on Ubx protein[15]. Given that wing discs contain five times as many cells as halteres, we estimate that, on average, haltere cells express 43-fold more *Ubx* RNA per cell than do wing disc cells.

We then compared *Ubx* RNA in Cbx^1/OR and OR/OR wing discs, and examined the effects of chromosome rearrangements and z^a. Using Brasil cDNA for standardization, we determined that Cbx^1/OR wing discs had approximately 8.5-fold more *Ubx* RNA than did OR/OR wing discs (Fig. 2). This elevated level of *Ubx* RNA in Cbx^1/OR discs is consistent with the transformation of wing to haltere in these discs, and not unexpectedly the level fell in discs with transvection-suppressing chromosome rearrangements. Chromosome rearrangements that affected a wild-type (non-Cbx^1) chromosome, such as TM6b (which has multiple rearrangements that break sites distant from *Ubx*, and suppresses transvection moderately) and T(2;3)31616,607 (a strong suppresser of transvection), reduced total levels of *Ubx* RNA in a Cbx^1 background by 20% and 58%, respectively. Thus chromosome rearrangements can reduce expression in a manner that directly correlates with the degree of chromosome asynapsis. In contrast, the level of *Ubx* RNA in wing discs did not change detectably in z^a mutants. We note that z^a;Cbx^1/+ flies do not differ significantly in phenotype from Cbx^1/+, whereas the wings of z^a;Cbx^1 Ubx^1/++ flies are considerably less like halteres than those of Cbx^1 Ubx^1/++ flies. We presume that the z^a mutation also reduces transactivation in Cbx^1/+ flies, but that it does not lower the total level of *Ubx* expression sufficiently for it to fall below a phenotypic threshold.

To assess the contributions of the two *Ubx* genes to the elevated levels of *Ubx* RNA in Cbx^1/+ wing discs, we assayed RNA extracted from Cbx^1/Brasil wing discs, and used the polymorphic sequence difference (Fig. 1*b*) in their *Ubx* 3′ UTRs to distinguish their *Ubx* transcripts. Brasil transcripts contributed almost 20% of the total (Fig. 3), indicating that their abundance had been enhanced more than threefold compared to normal, and thus that Cbx^1 had caused *trans*-activation. We then examined the identity of *Ubx* RNA in Cbx^1/Brasil heterozygotes that carried various *z* alleles. z^a, but not z^1 or z^{58g}, suppresses transvection[16] and z^a, but not z^1 or z^{58g}, reduced the transcripts from the Brasil *Ubx* gene to normal levels (Fig. 3). The amount of *Ubx* transcript transcribed from the Cbx^1 gene was not altered in any of these mutants (Fig. 2). These data indicate that z^a suppresses transactivation of the wild-type *Ubx* gene by Cbx^1.

We next examined flies with chromosome rearrangements that alter homologue pairing at *Ubx*. We isolated flies with rearranged Brasil chromosomes in a screen for mutations that suppressed transvection in Cbx^1 Ubx^1/++ flies. Among those selected in the screen, all had chromosome breaks in 3R, the chromosome arm containing *Ubx*. None of the chromosome breaks in these flies was in the cytological location of *Ubx*, 89E. These rearranged chromosomes (R(+)) suppressed transvection strongly (for example,

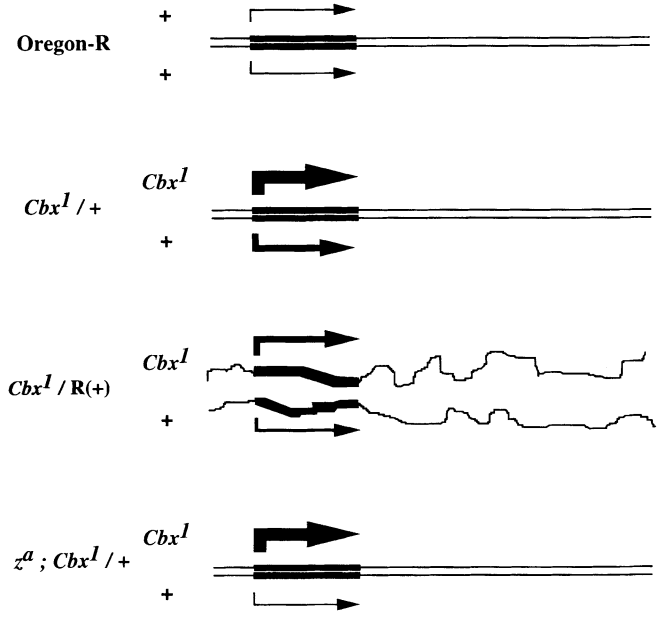

FIG. 4 Transcription of Ubx in wing imaginal discs. The quantitative relation between the amount of transcript contributed by each of the Ubx templates in the indicated heterozygotes is shown. Transcripts are symbolized by arrows, with their amount being indicated by the relative arrow size.

R(B)3; comparable to T(2;3)31616,607), moderately (for example, R(B)13), or weakly (for example, R(B)4, R(B)8), and they were used to construct Cbx^1/R(+) strains for analysis. In all of the R(+)/OR heterozygotes we studied, each of the *Ubx* genes produced half of the *Ubx* RNA in both wing and haltere discs (data not shown). As described above, the +*Ubx* gene produces approximately 20% of the *Ubx* RNA in Cbx^1/+ wing discs, and transvection-suppressing rearrangements reduce the total level of *Ubx* RNA in wing discs (Fig. 2). Surprisingly, the proportion of *Ubx* RNA derived from the + chromosome did not change significantly in any of the R(+)/Cbx^1 heterozygous wing discs (Fig. 3). This indicates that the rearrangements reduce *Ubx* expression from both homologues but do not eliminate transactivation specifically.

Our results show that the Cbx^1 mutation activates *Ubx* expression in the wing disc on both homologues, confirming the prediction that regulatory elements can function in *trans* (summarized in Fig. 4). Wing disc expression induced by Cbx^1 was sensitive to *z* function and to chromosome asynapsis, observations that are consistent with previous proposals based on either phenotypic or molecular assays[2,17,18]. However, we found an unexpected difference between the mechanisms through which the z^a mutation and chromosome rearrangements affect expression. Although chromosome rearrangements and z^a have essentially indistinguishable phenotypic consequences, namely, suppression of the *Cbx* phenotype, only z^a specifically disrupts *trans*-activation. Unexpectedly, chromosome rearrangements reduced expression from both homologues. These results suggest that, in normal wild-type flies, chromosome synapsis enhances expression of the *Ubx* genes on both homologues, implying that association between homologous chromosomes has a general enhancing effect on transcription. We presume that the systems in *Drosophila* in which transvection has been observed[1–9] are those in which gene expression levels can be measured with particular sensitivity. Transvection may therefore provide a useful means of studying the interactions between homologous chromosomes that can subtly influence gene expression. Our finding that interactions between homologous chromosomes seem to enhance expression levels from both chromosomes has many possible implications. In particular, we note that gross chromosome rearrangements, such as translocations, could give rise to global reductions in gene expression on the affected chromosomes. They could therefore contribute to disease states associated with haplo-insufficiency and cancer, and could modulate fitness during speciation. □

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ERRATA

Non-chondritic platinum-group element ratios in the Earth's mantle

L. Pattou, J. P. Lorand & M. Gros

Nature **379,** 712–715 (1996)

THE date of submission of this Letter for publication was given incorrectly as 16 March 1994; this date should have been 25 September 1995. □

A neural basis for lexical retrieval

Hanna Damasio, Thomas J. Grabowski, Daniel Tranel, Richard D. Hichwa & Antonio R. Damasio

Nature **380,** 499–505 (1996)

AN ambiguity was introduced during editing into the opening sentence of the heading of this Article. This should read "Two parallel studies, one conducted in neurological patients with brain lesions, the other using positron emission tomography in normal individuals, indicate that the normal process of retrieving words that denote concrete entities depends in part on multiple regions of the left cerebral hemisphere, located outside the classic language areas." □

A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125^{FAK}

Alan Richardson & Thomas Parsons

Nature **380,** 538–540 (1996)

FIGURE 1, panel *i*, which was accidentally omitted from this Letter during the page-make-up process, is presented below. This figure shows the number of cells spread on fibronectin after 20 min. □