



Generation of a Widespread *Drosophila* Inversion by a Transposable Element

Mario Cáceres *et al.*

Science **285**, 415 (1999);

DOI: 10.1126/science.285.5426.415

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of July 24, 2012):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/285/5426/415.full.html>

This article **cites 22 articles**, 5 of which can be accessed free:

<http://www.sciencemag.org/content/285/5426/415.full.html#ref-list-1>

This article has been **cited by** 86 article(s) on the ISI Web of Science

This article has been **cited by** 41 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/285/5426/415.full.html#related-urls>

This article appears in the following **subject collections**:

Genetics

<http://www.sciencemag.org/cgi/collection/genetics>

would have to have developed only in the $\beta_2M^{-/-} \rightarrow B6$ and $C3H.SW \rightarrow B6$ chimeras, and not in the $B6 \rightarrow B6$ controls. In any event, if suppressor cells were involved, host APCs would be required for their development.

These results contrast with those of Sprent and colleagues (4). They found that in a $CD8^+$ T cell-dependent MHC-compatible but miHA-incompatible GVHD model donor \rightarrow host bone marrow chimeras that were reirradiated and injected with donor bone marrow and lymph node cells developed GVHD. They suggested that either the chimeras were not devoid of host APCs because of insufficient radiation, or that host antigens were processed by donor marrow cells. Our data support the former explanation. Sprent and colleagues also found that heavily irradiated allogeneic parent $\rightarrow F_1$ bone marrow chimeras developed GVHD in response to very high doses of parental T cells (up to 8×10^7 T cells per recipient) and concluded that nonhematopoietic cells functioned as APCs. Given the increased precursor frequency of T cells recognizing allogeneic MHC molecules in comparison to miHA on self MHC and the large dose of T cells used, they may have unmasked the presence of small numbers of residual host APCs. Alternatively, nonhematopoietic cells when challenged with very large numbers of allogeneic T cells may cause T cell activation sufficient to induce GVHD.

Our results suggest that depleting host APCs before the conditioning regimen should abrogate GVHD without the need for prolonged T cell-targeted immunosuppression. Such an approach, perhaps using toxin-conjugated or radiolabeled antibodies, could expand the range of diseases treated with alloBMT. To test the feasibility of in vivo antibody-mediated depletion of host dendritic cells, we injected mice with N418, a hamster monoclonal antibody to the β integrin CD11c expressed on murine dendritic cells (17). The injected anti-CD11c bound to all CD11c-expressing dendritic cells in both lymph node and spleen (15), supporting the feasibility of antibody-mediated APC depletion.

A subset of alloBMT recipients have self-limited GVHD, which was presumed to reflect acquired T cell tolerance. Our data suggest another explanation: replacement of host with donor APCs abrogates T cell activation. Infusions of T cells from original bone marrow donors given to relapsed leukemia patients months to years after the initial alloBMT (18) cause less GVHD than has been observed when T cells are given at the time of transplantation (19). Although there may be other explanations (20), we suggest that the replacement of host with donor APCs reduces the chance of a donor $CD8^+$ T cell interacting with a GVHD-inducing host APC. In addition to suggesting explanations for these clinical observations, our data provide the

foundation for a different strategy for reducing GVHD-host APC depletion. This approach may avoid the problems associated with T cell depletion of marrow allografts: failure of engraftment, poor immune reconstitution, and lack of immunoreactivity against the tumor.

References and Notes

1. M. M. Horowitz *et al.*, *Blood* **75**, 555 (1990); D. Gaziev *et al.*, *Transplantation* **63**, 854 (1997).
2. J. M. M. den Haan *et al.*, *Science* **268**, 1476 (1995); W. Wang *et al.*, *ibid.* **269**, 1588 (1995).
3. N. J. Chao and H. J. Deeg, in *Graft-Vs.-Host Disease*, J. L. Ferrara, H. J. Deeg, S. J. Burakoff, Eds. (Dekker, New York, 1996), pp. 639–666.
4. R. Korngold and J. Sprent, *J. Exp. Med.* **155**, 872 (1982); H. Kosaka, C. D. Surh, J. Sprent, *ibid.* **176**, 1291 (1992).
5. J. W. Streilein and R. E. Billingham, *ibid.* **132**, 163 (1970); *ibid.*, p. 181.
6. P. L. Weiden *et al.*, *J. Immunol.* **116**, 1212 (1976).
7. R. L. Truitt and A. A. Atasoylu, *Blood* **77**, 2515 (1991).
8. R. N. Germain, *Cell* **76**, 287 (1994).
9. P. Matzinger and M. J. Bevan, *Cell. Immunol.* **33**, 92 (1977); M. J. Bevan, *J. Exp. Med.* **182**, 639 (1995); P. K. Srivastava, H. Udono, N. E. Blachere, Z. Li, *Immunogenetics* **39**, 93 (1994); C. Kurts, J. F. Miller, R. M. Subramaniam, F. R. Carbone, W. R. Heath, *J. Exp. Med.* **188**, 409 (1998).

10. M. L. Albert, B. Sauter, N. Bhardwaj, *Nature* **392**, 86 (1998).
11. B6 mice received 200 μ g of antibody to NK1.1 (anti-NK1.1) on consecutive days (21) followed by two 500-cGy fractions and injection with 10^7 T⁴⁹⁶BM cells from B6 $\beta_2M^{-/-}$ or B6 mice. Four months later, chimeras were reirradiated with two 375-cGy fractions, then injected with 7×10^6 C3H.SW T⁴⁹⁶BM cells with or without 10^6 or 2×10^6 C3H.SW $CD8^+$ T cells.
12. B. H. Koller, P. Marrack, J. W. Kappler, O. Smithies, *Science* **248**, 1227 (1990).
13. T cells were depleted with anti-Thy1.2-labeled magnetic microbeads (Miltenyi Biotec) according to the manufacturer's protocol.
14. $CD8^+$ T cells were isolated by staining lymph node cells with biotin-labeled antibodies to CD4, B220, and CD11b, followed by depletion with streptavidin-conjugated magnetic beads (Miltenyi Biotec). Ninety-five percent of cells were $CD8^+$; less than 0.25% of cells were $CD4^+$.
15. W. Shlomchik, data not shown.
16. T. Maier and H. N. Claman, in (3), pp. 75–94; H. J. Deeg and M. Cottler-Fox, in (3), pp. 311–335.
17. J. P. Metlay *et al.*, *J. Exp. Med.* **171**, 1753 (1990).
18. H. J. Kolb *et al.*, *Blood* **86**, 2041 (1995).
19. K. M. Sullivan *et al.*, *N. Engl. J. Med.* **320**, 828 (1989).
20. J. L. Ferrara, *Bone Marrow Transplant.* **14**, 183 (1994).
21. M. Bix *et al.*, *Nature* **349**, 329 (1991).
22. Supported by P50HL-54516 from the National Heart, Lung and Blood Institute and the Leukemia Society of America.

3 March 1998; accepted 16 June 1999

Generation of a Widespread *Drosophila* Inversion by a Transposable Element

Mario Cáceres,^{1,2*} José María Ranz,¹ Antonio Barbadilla,¹ Manyuan Long,² Alfredo Ruiz¹

Although polymorphic inversions in *Drosophila* are very common, the origin of these chromosomal rearrangements is unclear. The breakpoints of the cosmopolitan inversion *2j* of *D. buzzatii* were cloned and sequenced. Both breakpoints contain large insertions corresponding to a transposable element. It appears that the two pairs of target site duplications generated upon insertion were exchanged during the inversion event, and that the inversion arose by ectopic recombination between two copies of the transposon that were in opposite orientations. This is apparently the mechanism by which transposable elements generate natural inversions in *Drosophila*.

Most *Drosophila* species are naturally polymorphic for inversions in one or more chromosomes (1, 2), but little is known about the molecular mechanisms underlying the generation of these inversions. Indirect evidence suggests that transposable elements (TEs) cause *Drosophila* inversions: TEs mediated chromosomal rearrangements in laboratory populations (3), and in situ hybridization studies detected the transposon *hobo* around the breakpoints of four endemic inversions of *D. melanogaster*

(4). However, in *D. subobscura* and *D. pseudoobscura* there was no cytological association between middle repetitive sequences and inversion breakpoints (1, 2), and the two direct studies that sequenced the breakpoints of naturally occurring inversions did not detect any TE (5).

The species *D. buzzatii* belongs to the *D. repleta* group of the *Drosophila* subgenus (6). Two chromosomal arrangements are commonly observed in chromosome 2 of *D. buzzatii*: the ancestral one or *2 standard* (*2st*), and the *2j*, which derived from the *2st* by inversion *2j* and is distributed throughout the species range at high frequencies (7). Here, we cloned and sequenced the breakpoints of inversion *2j* of *D. buzzatii*. First, the region of the proximal breakpoint in the *2st* chromosome (designated as

¹Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain. ²Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, USA.

*To whom correspondence should be addressed (in Spain). E-mail: mariocs@cc.uab.es

REPORTS

CD) was cloned from a line homozygous for the *2st* arrangement (*st*-1). Then the proximal and distal breakpoints in the *2j* chromosome (designated as *BD* and *AC*, respectively) were isolated from a line homozygous for the *2j* arrangement (*j*-1). Finally, the region of the distal breakpoint in the *2st* chromosome (named *AB*) was obtained from the *st*-1 line.

The proximal breakpoint of inversion *2j* in the *2st* arrangement lies between the *nAcRβ-96A* and *Pp1α-96A* genes (Fig. 1A), which are 4 kb apart in *D. melanogaster* (8). A cDNA clone of the *D. melanogaster nAcRβ-96A* gene (9) was used to screen a *st*-1 lambda genomic library (10). Three positive clones were identified, and one of these, λ st9, contained the *Pp1α-96A* gene (Fig. 1A) and spanned the breakpoint (Fig. 1B). By sequential subcloning of λ st9 and in situ hybridization of the different subclones to *2st* and *2j* chromosomes, the *CD* breakpoint was further located in a 2-kb Hind III fragment, pGPE102.2, and a 0.9-kb Dra I fragment, pGPE102.2.2 (Fig. 1A). The *AC* and *BD* breakpoints in the *2j* arrangement were recovered by screening a *j*-1 lambda genomic library with pGPE102.2 used as a probe. Five positive phages were identified, and the two with the most divergent restriction patterns, λ j3.1 and λ j10, were selected. In situ hybridization of both phages to *st*-1 and *j*-1 chromosomes yielded multiple signals (Fig. 1B), showing that they include repetitive sequences. However, the stronger signals on *2j* chromosomes at the proximal breakpoint with λ j3.1 and at the distal breakpoint with λ j10 indicated that the inserts of these phages came from the *BD* and *AC* regions, respectively. Fragments of λ j3.1 containing *D* sequences (pGPE105.4.1) and of λ j10 containing *C* sequences (pGPE107.2.1.1) were identified by hybridization of digested DNA from each phage with a probe of the *CD* breakpoint (Fig. 1A). Next, a series of contiguous fragments of λ j3.1 adjacent to pGPE105.4.1 and of λ j10 adjacent to pGPE107.2.1.1 were subcloned. Most showed a repetitive in situ hybridization pattern, similar to that of the entire phages. A 1.2-kb Pst I–Bam HI fragment of λ j3.1 (pGPE109.2), which contained *B* sequences and gave a strong signal at the *AB* region of *st*-1 chromosomes, was found 3.3 kb away from pGPE105.4.1 (Fig. 1A). Likewise, a 2.4-kb Pst I–Xba I fragment of λ j10 (pGPE107.3), containing *A* sequences and giving a unique signal in the *AB* region of *st*-1 chromosomes, was found 1.9 kb away from pGPE107.2.1.1 (Fig. 1A). All these fragments were sequenced. A 95% identity at the amino acid level was found between the proximal end of pGPE107.3 and the 44 COOH-terminal residues of the *D. melanogaster* Rox8 protein (11), thus revealing the location of the *D. buzzatii* *rox8* gene (Fig. 1A). The *AB* region in the *2st* arrangement

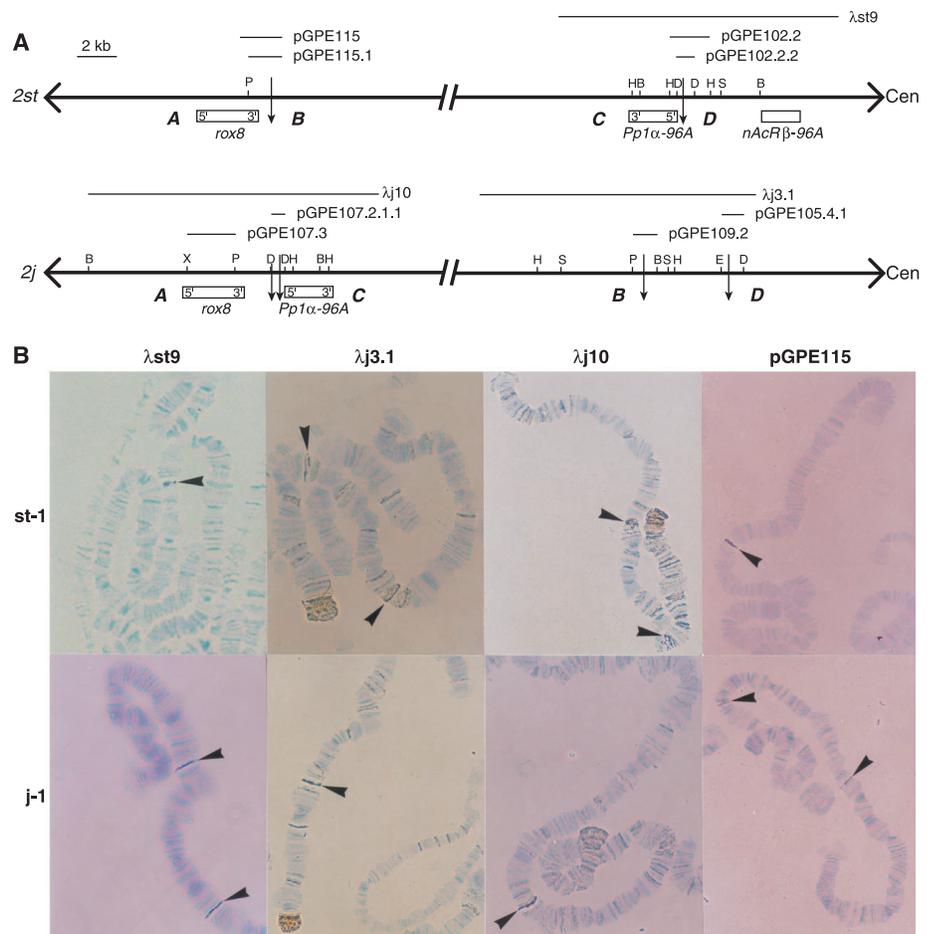


Fig. 1. Cloning strategy and in situ hybridization of the regions spanning the breakpoints of inversion *2j*. (A) Physical map of *2st* and *2j* chromosomes showing the breakpoint regions (*AB* and *CD* in *2st* and *AC* and *BD* in *2j*) and different clones obtained (thin lines shown above map). Vertical arrows mark the limits of the A, B, C, and D regions (in *2j* chromosomes there are insertions between A and C and between B and D). Empty boxes below map represent the genes flanking the breakpoints according to the sizes of the respective cDNAs in *D. melanogaster*. The direction of transcription, when known, is indicated as 5' and 3'. Cen, centromere; B, Bam HI; D, Dra I; E, Eco RI; H, Hind III; P, Pst I; S, Sal I; X, Xba I. (B) In situ hybridization to the salivary gland chromosomes of the *st*-1 and *j*-1 *D. buzzatii* lines of the four clones containing the breakpoint regions of inversion *2j* (arrowheads). Clones spanning the breakpoints in the *2st* arrangement (λ st9 and pGPE115) give one signal in *st*-1 and hybridize to both breakpoints in *j*-1. Clones spanning the breakpoints in the *2j* arrangement (λ j3.1 and λ j10) give two signals in *st*-1 and one signal in *j*-1.

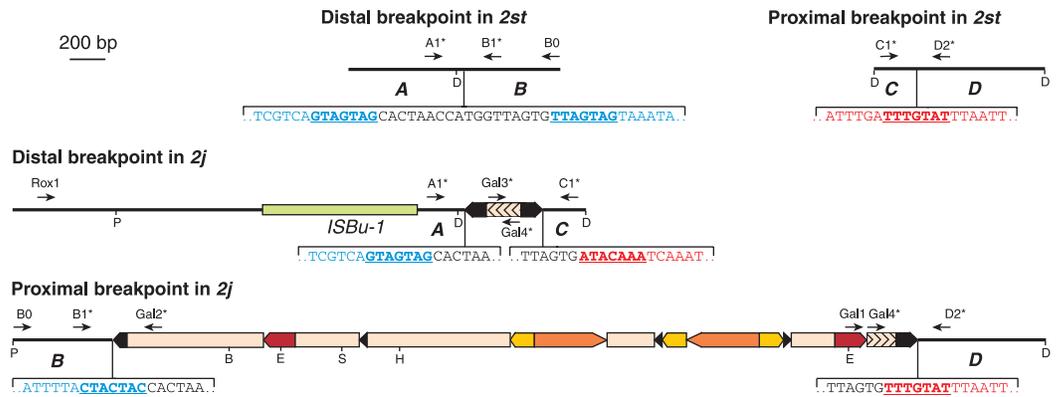
was finally obtained by polymerase chain reaction (PCR) amplification from *st*-1 genomic DNA using primers Rox1 and B0, located at the 3' end of the *rox8* coding region and the distal end of pGPE109.2, respectively. In situ hybridization to *st*-1 and *j*-1 chromosomes confirmed that the resulting 2.1-kb PCR product (pGPE115) spanned the *2j* distal breakpoint (Fig. 1B).

Alignment of *AB* and *CD* sequences in *st*-1 (2086 nucleotides) and *AC* and *BD* sequences in *j*-1 (8668 nucleotides) showed that there are large insertions at both *2j* inversion breakpoints that are not present in the *2st* arrangement (Fig. 2). The insertion between A and C is 392 base pairs (bp) long and has inverted terminal repeats (ITRs) of 106 bp. The insertion between B and D is 4319 bp long, with a complex internal structure. It has

ITRs like those of the *AC* insertion, of 106 and 47 bp, the latter corresponding to the beginning of the 106-bp repeat. The central 180 bp of the *AC* insertion and the *BD* sequence that follows the proximal ITR are homologous (95% identity) but are oppositely oriented (Fig. 2). Flanking each insertion are 7-bp sequences that resemble target site duplications that were produced during the insertion event. However, the duplicated sequences are separated and inverted (Fig. 2), which strongly suggests that inversion *2j* appeared by intrachromosomal pairing and recombination between the two homologous sequences inserted in distant sites in opposite orientations (Fig. 3). Although the precise structure of the original insertions is not known, they were likely homologous at least over a span of 274 bp, and this relatively

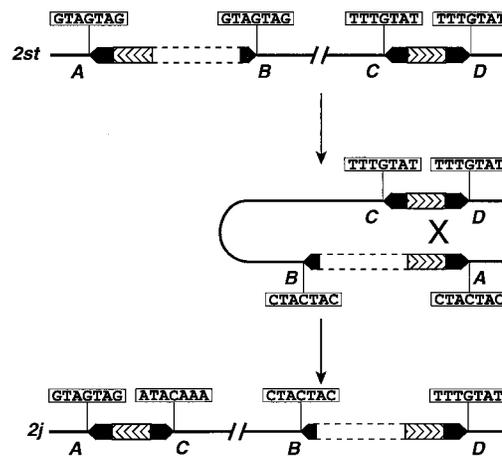
REPORTS

Fig. 2. Features of the sequenced regions of the distal and proximal breakpoints of inversion *2j* in the *2st* and *2j* arrangements. Horizontal lines correspond to the A, B, C, and D sequences, and insertions within them are represented as colored boxes. The insertions at the breakpoints are delimited by black blocks corresponding to the ITRs. Orientation of the homologous sequences of the two insertions is indicated by angle bracket patterns (<<<< >>>>). Internal repeats within the BD insertion are represented as sharp-pointed blocks with different colors. *ISBu-1* is a nondescribed, highly dispersed repeated sequence inserted in the *2j* arrangement in the A region, 256 bp away from the insertion at the breakpoint. Details of the breakpoint sequences in st-1 and j-1 are shown below each diagram (A and B sequences in blue, C and D sequences in red, and insertions in black). The target site duplications generated during insertion are underlined and in boldface. The distal breakpoint in *2st* shows a structure with 7-bp imperfect (one nucleotide difference) direct repeats flanking two adjacent 9-bp inverted repeats, which resembles



the footprints left by class II TEs after excision and incomplete repair of the double strand break (30). The A sequence in the *2j* arrangement is that of the j-14 line, because this sequence is absent in the j-1 line as the result of a 17-bp deletion. Horizontal arrows represent different PCR primers; those used for sequencing are marked with an asterisk. (Sequences of all primers can be found at Science Online, www.sciencemag.org/feature/data/1039043.shl.) Codes for restriction sites are as in Fig. 2.

Fig. 3. Model for the generation of inversion *2j*. First, the two homologous sequences became inserted between the A and B sequences and between the C and D sequences of a *2st* chromosome, generating direct duplications of seven nucleotides (boxed sequences). Then, ectopic recombination between the homologous sequences in opposite orientation generated the inversion. As a result of this process, target site duplications were exchanged between the insertions and acquired their present orientation in *2j* chromosomes, with each insertion flanked by its original target site and an inverted target site of the other element. Dashed lines refer to sequences not drawn to scale.



short sequence appears to be long enough to sustain ectopic recombination in *Drosophila* (12).

The duplication of a 7-bp sequence upon insertion, the presence of ITRs, and the moderately repetitive in situ hybridization pattern identify the breakpoint insertions as copies of a class II TE (13) that we designated as *Galileo* (14). Furthermore, we have observed variation among *D. buzzatii* lines both in the number of in situ hybridization signals and in their chromosomal location, which suggests recent transpositional activity of this TE (15). The AC insertion is probably a defective copy that derived from a complete element by an internal deletion but retained the terminal sequences needed for transposition (16). The BD copy may have degenerated partially, but it contains open reading frames (ORFs) that could code for a protein involved in transposition. The largest ORF has a predicted sequence of 172 amino acids, displaying 32% identity with eight gaps (53% similarity) to the putative transposase of the transposon *Gandalf* of *D. koepferae*, a

sibling species of *D. buzzatii* (17). Ectopic recombination between repeated DNA sequences has been implicated in inversion generation in diverse organisms, such as yeast (18) and humans (19), and TE activity could stimulate this recombination (20). However, precedents of TE involvement in the generation of naturally occurring inversions were restricted to bacteria (21). A TE-like sequence was found at one breakpoint of a polymorphic inversion of *Anopheles arabiensis*, but its implication in the origin of the inversion was not clear (22). Evidence from inversion *2j* confirms the role of TEs in *Drosophila* chromosomal evolution.

The previous results were corroborated by PCR amplification of the breakpoint regions and sequencing of the PCR products in *2st* lines and *2j* lines of different origin (23). Primer pairs A1-B1 and C1-D2 (Fig. 2) were used with genomic DNA of four *2st* lines, whereas primer pairs A1-C1, B1-Gal2, and Gal1-D2 (Fig. 2) were used with DNA from 21 *2j* lines. In each case PCR products of the appropriate sizes

were obtained, indicating that the breakpoint insertions are present in all *2j* lines and absent in all *2st* lines (in the amplification of the AC segment only, j-9 and j-10 yielded no PCR product). We sequenced 521 bp of the A, B, C, and D regions in three *2st* lines and five *2j* lines and 800 bp of the *Galileo* insertions in the *2j* lines (24). All the *2j* chromosomes in this geographically diverse set had the same insertions and identical breakpoints with regard to *2st* chromosomes. Nucleotide diversity (25) over the 489 aligned sites in the eight sequences (excluding gaps) was 0 and 0.0279 for the *2j* and *2st* arrangements, respectively, and *2j* chromosomes shared 22 fixed differences when compared to *2st* chromosomes (including six small insertions of 1 to 4 bp). *Galileo* sequences showed more variation among the *2j* lines, with a nucleotide diversity over the 794 aligned sites of 0.0053. Thus, it appears that despite the transposon-mediated origin, all *2j* arrangements descend from the product of a single ectopic recombination event: They are monophyletic.

Finally, we tested the effect of the inversion on the nearby genes *rox8* and *Pp1 α -96A* (Fig. 1A). The *Pp1 α -96A* transcriptional startpoint (26) is just 156 bp away from the proximal breakpoint in the *2st* arrangement, and a semi-quantitative RT-PCR was carried out to compare *Pp1 α -96A* expression between the st-1 and j-1 lines at four developmental stages (27). No apparent differences in expression between st-1 and j-1 were seen. As in *D. melanogaster* (26), the *Pp1 α -96A* transcript was present in embryos, was detected with a lower expression in adults, and was barely visible in larvae and pupae. We also performed Northern (RNA) analyses (28) using probes of the AB (pGPE115.1) and CD regions (pGPE102.2).

Both probes hybridized to the transcripts of *Pp1 α -96A* [2.3 kb (26)] and *rox8* [3 to 3.3 kb (11)] only, and no detectable differences in the expression of these genes between st-1 and j-1 were observed. It seems that inversion 2*j* neither disrupted any transcriptional unit nor affected the expression of the closest genes, ruling out a relation between the mutational effect of the inversion and its adaptive value (29).

References and Notes

1. C. B. Krimbas and J. R. Powell, Eds., *Drosophila Inversion Polymorphism* (CRC Press, Boca Raton, FL, 1992).
2. J. R. Powell, *Progress and Prospects in Evolutionary Biology: The Drosophila Model* (Oxford Univ. Press, New York, 1997).
3. W. R. Engels and C. R. Preston, *Genetics* **107**, 657 (1984); S. Schneuwly, A. Kuroiwa, W. J. Gehring, *EMBO J.* **6**, 201 (1987); E. A. Montgomery, S.-M. Huang, C. H. Langley, B. H. Judd, *Genetics* **129**, 1085 (1991); J. K. Lim and M. J. Simmons, *Bioessays* **16**, 269 (1994); V. Ladeveze, S. Aulard, N. Chaminade, G. Periquet, F. Lemeunier, *Proc. R. Soc. London Ser. B* **265**, 1157 (1998).
4. T. W. Lyttle and D. S. Haymer, *Genetica* **86**, 113 (1992).
5. C. S. Wesley and W. F. Eanes, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3132 (1994); S. Cirera, J. M. Martín-Campos, C. Segarra, M. Aguadé, *Genetics* **139**, 321 (1995).
6. M. Wasserman, in (7), pp. 455–541; A. Ruiz and M. Wasserman, *Heredity* **70**, 582 (1993).
7. A. Fontdevila, A. Ruiz, G. Alonso, J. Ocaña, *Evolution* **35**, 148 (1981); A. Ruiz, H. Naveira, A. Fontdevila, *Genet. Iber.* **36**, 13 (1984); E. Hasson et al., *J. Evol. Biol.* **8**, 369 (1995).
8. J. M. Ranz, C. Segarra, A. Ruiz, *Genetics* **145**, 281 (1997); J. M. Ranz, M. Cáceres, A. Ruiz, *Chromosoma* **108**, 32 (1999).
9. E. Sawruk, C. Udri, H. Betz, B. Schmitt, *FEBS Lett.* **273**, 177 (1990).
10. For the construction of genomic libraries, ~30 μ g of high molecular weight genomic DNA were partially digested with Sau 3AI and electrophoresed on a 0.4% agarose gel. Fragments in the size range 15 to 23 kb were eluted from the gel, ligated with LambdaGEM-11 Bam HI arms, and packaged in vitro using Packagene extracts (Promega).
11. S. Brand and H.-M. Bourbon, *Nucleic Acids Res.* **21**, 3699 (1993).
12. I. Busseau, A. Pelisson, A. Bucheton, *Mol. Gen. Genet.* **218**, 222 (1989); N. Nassif and W. R. Engels, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1262 (1993).
13. D. J. Finnegan, *Trends Genet.* **5**, 103 (1989); P. Capy, C. Bazin, D. Higuert, T. Langin, *Dynamics and Evolution of Transposable Elements* (Springer-Verlag, Heidelberg, 1998).
14. The insertions at the 2*j* inversion breakpoints do not show homology at the nucleotide level to any known sequence in the databases and thus appear to correspond to a previously undescribed TE. We have named this TE after the Italian astronomer, mathematician, and physicist Galileo Galilei (1564–1642).
15. A 5-kb probe of the λ j3.1 proximal end that contains most of the BD insertion was in situ hybridized to the polytene chromosomes of seven *D. buzzatii* lines. The total number of euchromatic signals found per genome was 55 in st-1, 44 in st-3, 47 in st-4, 34 in j-1, 34 in j-8, 37 in j-11, and 28 in j-13 (23). On average, 11.8% of each line's signals were exclusive.
16. K. O'Hare and G. M. Rubin, *Cell* **34**, 25 (1983); R. D. Streck, J. E. MacGaffey, S. K. Beckendorf, *EMBO J.* **5**, 3615 (1986).
17. I. Marín and A. Fontdevila, *Mol. Gen. Genet.* **248**, 423 (1995).
18. G. S. Roeder and G. R. Fink, in *Mobile Genetic Elements*, J. Shapiro, Ed. (Academic Press, Orlando, FL, 1983), pp. 299–328; T. D. Petes and C. W. Hill, *Annu. Rev. Genet.* **22**, 147 (1988).
19. D. Lakich, H. H. Kazazian, S. E. Antonarakis, J. Gitschier, *Nature Genet.* **5**, 236 (1993); J. A. Naylor et al., *Hum. Mol. Genet.* **4**, 1217 (1995); K. Small, J. Iber, S. T. Warren, *Nature Genet.* **16**, 96 (1997).

20. W. B. Eggleston, N. R. Rim, J. K. Lim, *Genetics* **144**, 647 (1996).
21. Y. Komoda, M. Enomoto, A. Tominaga, *ibid.* **129**, 639 (1991); M.-L. Daveran-Mingot, N. Campo, P. Ritzen-thaler, P. le Bourgeois, *J. Bacteriol.* **180**, 4834 (1998).
22. K. D. Mathiopoulos, A. della Torre, V. Predazzi, V. Petrarca, M. Coluzzi, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12444 (1998).
23. The *D. buzzatii* lines used are homokaryotypic for four different chromosome arrangements: 2st, 2*j*, 2*jz*³, and 2*jq*⁷ (2*jz*³ and 2*jq*⁷ derive from the 2*j* arrangement and bear inversions 2*z*³ and 2*q*⁷, respectively). Their geographic origins cover a major part of the species distribution; lines that were sequenced are indicated in boldface. Spain: st-1, st-2, j-1, j-2, j-3, j-4, j-5, j-6, jz³-1, jz³-2, and jq⁷-1 (Carboneras), j-7 and jq⁷-3 (Caldetas), and jq⁷-2 (Mogan, Canary Islands). Tunisia: jz³-3 (Kariouan). Argentina: st-3 (Vipos), j-8 (San Luis), j-9 (Quilmes), j-10 (Palo Labrado), and jq⁷-4 (Otamendi). Bolivia: j-11 (Los Negros). Brazil: st-4, j-12, and j-13 (Guaritas, RS). Australia: j-14. All lines (except jq⁷-3 and jq⁷-4) were made isogenic by repeated sib-mating or by using the second chromosome balancer stock *Antp/Δ5* [J. S. F. Barker, *Genetica* **92**, 165 (1994)].
24. The PCR products were purified from 1% agarose gels, reamplified with one of the sequencing primers shown in Fig. 2, and sequenced in an ALFexpress DNA automated sequencer (Pharmacia Biotech). Both strands of each fragment were sequenced completely using primers separated by 323 to 466 nucleotides.
25. M. Nei, *Molecular Evolutionary Genetics* (Columbia Univ. Press, New York, 1987).
26. V. Dombrádi et al., *Eur. J. Biochem.* **194**, 739 (1990).
27. Two sets of primers were used to amplify simultaneously a 271-bp 5' fragment of the *Pp1 α -96A* cDNA and 438 bp of the *Gapdh* cDNA (internal control)

from the total cDNA. In both cases the primer sites were selected to span introns (of 61 bp and 69 bp, respectively). *Gapdh* primers were designed on the basis of the sequence of *D. hydei* [K. M. Wojtas, L. von Kalm, J. R. Weaver, D. T. Sullivan, *Genetics* **132**, 789 (1992)]. PCRs were performed with varying amounts of the reverse transcription reaction product as template and different concentrations of the two sets of primers.

28. The Northern blot hybridization was done with ~30 μ g of total RNA from embryos, larvae, pupae, and adults of the st-1 and j-1 lines as described [A.-K. Rost, in *Quantitation of mRNA by Polymerase Chain Reaction: Nonradioactive PCR Methods*, Th. Köhler et al., Eds. (Springer-Verlag, Berlin, 1995), pp. 93–114].
29. A. Ruiz et al., *Genetics* **128**, 739 (1991); E. Betrán, M. Santos, A. Ruiz, *Evolution* **52**, 144 (1998).
30. W. R. Engels, D. M. Johnson-Schlitz, W. B. Eggleston, J. Sved, *Cell* **62**, 515 (1990); R. H. A. Plasterk, *EMBO J.* **10**, 1919 (1991); E. Takasu-Ishikawa, M. Yoshihara, Y. Hotta, *Mol. Gen. Genet.* **232**, 17 (1992).
31. Supported by a doctoral fellowship and a travel grant from the Comissionat per a Universitats i Recerca (Generalitat de Catalunya, Spain) (M.C.) and grant PB95-0607 from the Dirección General de Investigación Científica y Técnica (Ministerio de Educación y Cultura, Spain) (A.R.). We thank J. Zhang for his help in the RNA work; J. Barbé, O. Cabré, and A. Tapias for material and technical help; B. Schmitt for the *nAcR β -96A* clone; J. S. F. Barker, E. Betrán, A. Fontdevila, and F. M. Sene for *D. buzzatii* stocks; and M. Aguadé, M. Ashburner, A. Berry, F. Brunet, B. Charlesworth, and R. de Frutos for valuable comments and helpful discussions.

4 February 1999; accepted 9 June 1999

Identification of a Vertebrate Sister-Chromatid Separation Inhibitor Involved in Transformation and Tumorigenesis

Hui Zou, Thomas J. McGarry, Teresita Bernal, Marc W. Kirschner*

A vertebrate securin (vSecurin) was identified on the basis of its biochemical analogy to the Pds1p protein of budding yeast and the Cut2p protein of fission yeast. The vSecurin protein bound to a vertebrate homolog of yeast separins Esp1p and Cut1p and was degraded by proteolysis mediated by an anaphase-promoting complex in a manner dependent on a destruction motif. Furthermore, expression of a stable *Xenopus* securin mutant protein blocked sister-chromatid separation but did not block the embryonic cell cycle. The vSecurin proteins share extensive sequence similarity with each other but show no sequence similarity to either of their yeast counterparts. Human securin is identical to the product of the gene called pituitary tumor-transforming gene (*PTTG*), which is overexpressed in some tumors and exhibits transforming activity in NIH 3T3 cells. The oncogenic nature of increased expression of vSecurin may result from chromosome gain or loss, produced by errors in chromatid separation.

The metaphase to anaphase transition is the final discrete event in duplication and separation of the genetic material of a cell. Its

timing is regulated by the activation of the anaphase-promoting complex (APC), which mediates selective proteolysis of various mitotic regulators (1–3). Experiments with *Xenopus* egg extracts indicated that a putative protein factor might exist whose degradation was required for the onset of sister-chromatid separation (4). Proteins with such an activity

Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: marc@hms.harvard.edu