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17. In all experiments, BDNF antisera as well as control sera were used at a concentration of 10 μl per 1 ml of medium.
18. Antibodies to NT3 were generated in turkeys with human recombinant NT3 as antigen. By the same criteria described for BDNF antibodies, NT3 antibodies specifically block NT3 function without recognizing or interfering with signaling by NGF, BDNF, or NT4/5. Antibodies to BDNF are not generally cytotoxic to cells: anti-BDNF has no apparent morphological effects on NGF-differentiated PC-12 cells or on cortical neurons cultured from neonatal animals.
19. Preliminary experiments suggest that recombinant BDNF can promote the survival of embryonic cortical neurons (*10*).
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Long-Range Cis Preference in DNA Homology Search over the Length of a *Drosophila* Chromosome

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P element–induced chromosome breakage on the X chromosome of *Drosophila melanogaster* was repaired six times more frequently when a homologous template was located anywhere on the X chromosome rather than on an autosome. Cis-trans comparisons confirmed that recombinational repair was more frequent when the interacting sequences were physically connected. These results suggest that the search for homology between the broken ends and a matching template sequence occurs preferentially in the cis configuration. This cis advantage operates over more than 15 megabases of DNA.

Most genetic recombination events are preceded by a homology search to align the interacting DNA sequences. This alignment can take place on the level of whole chromosomes, such as when two homologs synapse for meiosis, but it can also occur on a much smaller scale involving short sequence matches. Only a few hundred base pairs of homology are needed for efficient homology searching in *Drosophila* and mammalian cells (*1–4*), and fewer than 100 base pairs (bp) are required in *Escherichia coli* and yeast (*5–7*). One way to study this process is provided by P transposons in *D. melanogaster*. Excision of these elements initiates recombinational repair, which requires a genome-wide homology search for a template (*1*, *8–10*). This template is normally the corresponding gene on the sister chromatid or homolog, but it can also be an ectopic sequence carried on a transposable element anywhere in the genome (*9*). In the experiments reported here, we use ectopic templates to examine the process of genome-wide homology searching.

A comparison of gene conversion frequencies for 10 template sites on the X chromosome and 28 on the two major autosomes is shown in Fig. 1. For each site, we measured the rate at which a P element insertion mutation at the *white* locus was restored to the wild type in the presence of a *white* sequence template at the indicated genomic position (*11*). Such reversions occur when the P element in *white* excises and sequences from the template are copied into the vacated site, replacing some of the flanking sequence (*9*). These events, which occur in the premeiotic germ cells when P transposase is present, are interpreted as repair of double-strand DNA

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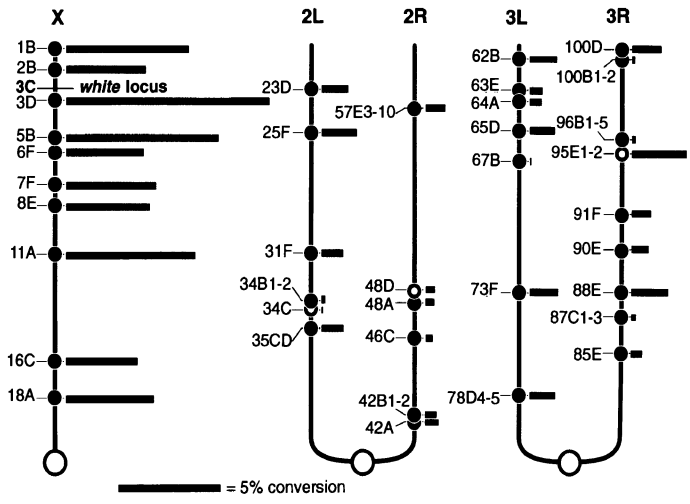

Fig. 1. Conversion rates for template sites. The five major chromosome arms (X, 2L, 2R, 3L, and 3R) of *Drosophila* are shown with template sites indicated according to their cytological position (*21*). Bars represent estimated conversion rates associated with each template site, defined as the proportion of X-bearing gametes in which the w^{hd} allele had reverted to the wild type and had acquired sequences from the template gene (*11*). For sites marked with a solid dot (●), the template was P[*w*alter], a modified *white* gene carried on a P element (*9*). The *white* gene in P[*w*alter] has 12 single-base modifications that alter restriction sites but do not prevent expression of the gene (*9*). We confirmed conversion events by verifying that at least one of these marker sites had been copied into the reverted w^{hd} gene (*11*). Three templates (○) carried a slightly modified form of P[*w*alter] that had lost its mobility or in which some of the markers had been substituted (*9*). (The immobile element at 34C is missing part of its 5′ P end.) All reversion events occurred in the germ line of w^{hd} males with a single copy of one of the template elements and the Δ2-3 (99B) element to provide transposase (*14*). A total of 3298 single-male crosses were performed to yield 209,057 potential revertant progeny. The results were pooled with an earlier data set obtained by identical methods and reported previously (*9*), accounting for 43% of the data shown here. All data for sites 1B, 2B, 3D, 5B, 7F, 8E, 11A, 16C, 18A, 23D, 48A, and 57E3-10 are new, and data from sites 25F, 31F, 35CD, 64A, 65D, 73F, 78D4-5, 85E, 91F, 96B1-5, 100B1-2, and 100D include both experiments. The remaining sites were tested only in the first experiment. The new P[*w*alter] insertion sites were obtained by mobilizing previous insertions with transposase and selecting for jumps (*14*). Cytological positions were determined by in situ hybridization to polytene chromosomes with a labeled *white* DNA probe (*23*).

breaks at the *white* locus. Each such event requires a homology search in which the sequence flanking the P insertion is paired with homologous sequences in the template. The ectopic template, designated P[*w*alter], carries only 2456 bp of contiguous *white* locus sequence on one side of the site of P element excision in w^{hd} and 999 bp on the other (*1*, *9*, *10*). These lengths define the maximum sequences that can be used for the homology search. If there is gap enlargement before the search, the flanking homology would be correspondingly less than 2456 or 999 bp.

An advantage of using X-linked templates for repair was clear (Fig. 1). Each of the X chromosomal template sites induced higher conversion frequencies than any of the autosomal sites. The average conversion rate for the 10 X-linked sites was 5.5%, as compared with 0.9% for the 28 autosomal sites. The *white* locus is also on the X chromosome, so that this sixfold difference can be interpreted as a preference for cis interactions. However, because the repair events occurred in males, it is also possible to explain the sixfold difference in terms of an X-specific effect. For example, male *Drosophila* exhibit dosage compensation in which the X chromosome is more transcriptionally active than the autosomes (*12*). This enhanced transcription could increase recombinational activity (*13*). Another possibility is that the lack of a homolog makes the X chromosome in males more active in recombinational repair.

To distinguish among these possibilities, we selected four of the X chromosomal template sites for further study. For each site, we repeated the conversion test as above except that females instead of males were used, and the test was performed with the target and template sites in both the cis and trans configurations. The two mating schemes (Fig. 2) produced females in generation G_1 carrying (i) the P insert in *white*, designated w^{hd}; (ii) one of the X-linked templates in either cis or trans configuration relative to w^{hd}; and (iii) an immobile source of P transposase (*14*) designated Δ2-3. Molecular tests (Fig. 2) were required to confirm the presence of w^{hd} in the trans G_1 females.

The results (Fig. 3) showed that repair occurred more frequently when the template was present on the same chromosome (or on the sister chromatid) as the target site. This finding implies a long-range cis effect linking the target and template sequences. This effect can explain at least some of the sixfold X chromosome–autosome differences. In addition, the cis preference will be advantageous in gene replacement procedures (*9*). Thus, one can select a template located in cis relative to the target site in order to maximize the frequency of the desired gene replacement event.

The average conversion rate in the cis configuration was 2.5 times that in the trans configuration (Fig. 3). This difference is less than the sixfold difference observed between the X chromosome and the autosomes in males (Fig. 1). This discrepancy suggests that a homolog preference or the X-specific factors mentioned above could be important in addition to the cis effect.

The conversion rates of the four sites relative to each other were also consistent within each test, falling in the order 3D-18A-1B-11A for both the cis and trans configurations (Fig. 3). This consistency

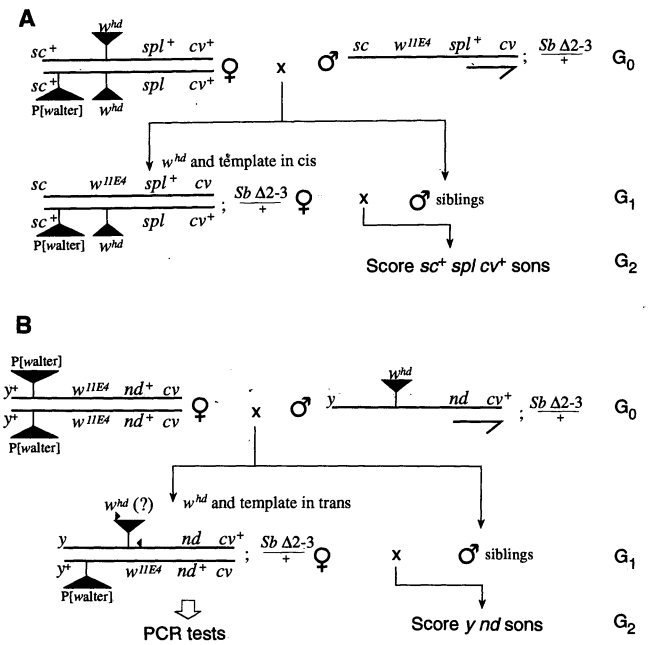

Fig. 2. Mating schemes for cis-trans comparison. Crosses are shown for the template at position 1B, and analogous crosses were used for templates 3D, 11A, and 18A. Conversion events occur in the premeiotic germ line of the females of generation G_1. The Δ2-3 element served as the transposase source (*14*). These females were allowed to mate with their brothers and were placed singly in vials to produce the G_2 offspring. The mutations *y*, *sc*, *nd*, *spl*, *cv*, and *Sb* were used as markers to follow the chromosomes and are as described (*21*). Note that linkage between *sc* and *spl* and between *y* and *nd* is sufficiently close that double recombination is not expected (*10*, *21*). The w^{11E4} allele carries a deletion for the entire *white* gene (*21*) and thus cannot serve as a template for repair of w^{hd}. Revertants were identified as sons in generation G_2 with wild-type eye color among males with the indicated marker combinations. Note that the *white* gene in P[*w*alter] is only partially expressed and therefore does not interfere with this scoring (*9*). For the cis configuration (**A**), the revertants were crossed to *w spl*+ females, and the daughters were examined for eye color. This step eliminated false positives that can result from phenotypic interactions between *w* and *spl*. For the trans tests (**B**), the transposase source and w^{hd} are both present in the same G_0 males, resulting in an opportunity for the P insertion in w^{hd} to excise in females before the G_1 generation. To avoid using females in G_1 that were not carrying w^{hd}, it was necessary for us to test each individually to ensure that w^{hd} was intact. Each G_1 female was allowed to lay eggs for at least 5 days, after which her DNA was extracted as described (*24*). The resulting DNA samples were then analyzed by PCR with primer 5′-GCACATCGTCGAACACCACG from the *white* locus (◀) and primer 5′-GAGTGTCGTATTGAGTCTGAG from the P sequence (▶). This amplification yields a 641-bp fragment only if the w^{hd} allele is intact. Each of 1810 G_1 females was tested in this way, and 930 of them were found to have the 641-bp fragment. The G_2 progeny was scored only when the 641-bp fragment was observed after PCR with the mother's DNA. All flies were reared on standard cornmeal-molasses-agar medium at room temperature (approximately 21°C).

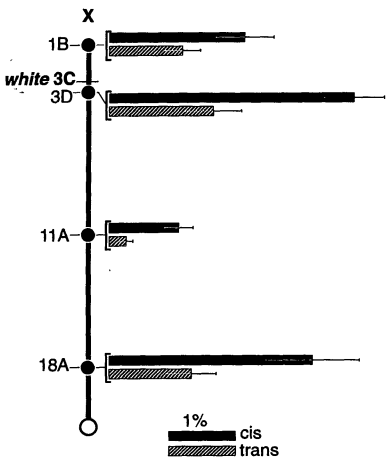

Fig. 3. Reversion rates for cis and trans configurations. Each of the four X-linked templates was tested by the two schemes in Fig. 2. Reversion rates are defined as the proportion of G_2 sons of the indicated marker combination that had wild-type eye color. Standard errors were computed as described (*25*) in order to be unbiased by clustering caused by premeiotic reversion events. The reversion rates were consistently lower than the comparable ones in Fig. 1. This difference is expected from previous work in which there was generally a lower conversion rate in the female germ line relative to that in males (*25*). The numbers of crosses, each with a single G_1 female, were 198, 237, 215, and 198 starting with site 1B for the cis configuration, and 210, 211, 229, and 280 for the trans configuration. In the cis tests, we scored totals of 3302, 5149, 4920, and 3329 G_2 sons with the *sc*+ *spl cv*+ combination, and for the trans tests we scored 4414, 4199, 3960, and 4500 of the *y nd* sons.

indicates that local site-specific effects are the same in both configurations. However, these local effects can be sex-specific, because the order changes to 3D-11A-1B-18A in males (Fig. 1). Both observations are consistent with the possibility that the local effects are related to the transcriptional activity of nearby genes.

We interpret the results shown in Figs. 1 and 3 to indicate that the search for homology in *Drosophila* is more effective when the matching sequences are physically connected, even if the distance between them is more than 15 million base pairs. We favor this view because the homology search is the only step in the recombinational repair process with an apparent genome-wide interaction. If repair happens after the S phase of the cell cycle, it is possible that the preferred template is actually on the sister chromatid and is not physically connected to the break. However, the process would still require some long-range mechanism to distinguish that chromatid from either of the two sister chromatids of the homologous chromosome.

Much of the recent work on homologous recombination has used systems in which one or both of the interacting sequences reside on a multicopy extrachromosomal plasmid (*2*, *4*, *5*, *7*, *15*) or in which both sequences are closely linked (*2*, *3*, *6*, *7*). These strategies eliminate the need for a genome-wide homology search and thus would not reveal the long-range cis effect seen here. Other experiments on recombination in yeast have indicated that there is a cis effect over distances of 20 to 70 kb, but it diminishes rapidly over longer ranges (*16*–*18*). It is possible that the very long range effects we observe occur only in species with more complex genomes than *Saccharomyces cerevisiae*. In the present data, even the nearest site, position 3D, is still several hundred kilobases away from the *white* locus. It may be significant that the 3D site also had the highest reversion rates (Figs. 1 and 3).

It is unclear what biological advantage might exist for a homology search mechanism that scans the genome preferentially in cis. One possibility is that the homology search occurs as a succession of increasingly specific steps. Rao and Radding (*19*) have shown that the RecA-catalyzed homology search recognizes both complementary and identical sequences and even sequences in which the DNA backbone is reversed. They suggest that this recognition represents a preliminary scanning to be followed by a more specific comparison. It is possible that the long-range cis effect we observe reflects an even cruder step that occurs early in the refinement process.

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11. We determined the conversion rate for each insertion site by crossing males bearing the w^{hd} allele [$w^{hd80k17}$ in previous work (*20*)], one copy of P[*walter*], and the Δ2-3 transposase source (*14*) with C(1)DX,*y f* females (*21*) and scoring the progeny as described (*9*). The w^{hd} allele has a 629-bp P element inserted into exon 6 of the gene (*20*). Its phenotype is bleach-white. Precise excision of the P element results in full reversion of w^{hd} (*8*). There is no measurable reversion of w^{hd} in the absence of P transposase (*8*). The phenotype of P[*walter*] in a *white* mutant background is intermediate between bleach-white and that of the wild type and is easily distinguished from either. For each site, a sample of the revertants was tested by PCR [polymerase chain reaction (*22*)] and restriction mapping as described (*9*) to change reversion rates to conversion rates. Previous results indicate that background reversion of w^{hd} in the absence of a template is essentially absent in females and very rare (0.1%) in males (*8*).
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Mutation of a *mutL* Homolog in Hereditary Colon Cancer

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Some cases of hereditary nonpolyposis colorectal cancer (HNPCC) are due to alterations in a *mutS*-related mismatch repair gene. A search of a large database of expressed sequence tags derived from random complementary DNA clones revealed three additional human mismatch repair genes, all related to the bacterial *mutL* gene. One of these genes (*hMLH1*) resides on chromosome 3p21, within 1 centimorgan of markers previously linked to cancer susceptibility in HNPCC kindreds. Mutations of *hMLH1* that would disrupt the gene product were identified in such kindreds, demonstrating that this gene is responsible for the disease. These results suggest that defects in any of several mismatch repair genes can cause HNPCC.

Hereditary nonpolyposis colorectal cancer is one of the most common genetic diseases of man, affecting as many as one in 200 individuals (*1*). Definitive evidence that the disease is inherited in a Mendelian fashion was provided when markers on chromosome 2p were found to segregate with disease in two large HNPCC kindreds (*2*). A further clue to pathogenesis emerged from the observation that a subset of sporadic colorectal cancers and most colorectal cancers from HNPCC patients exhibit widespread alterations of short, repeated sequences (microsatellites) distributed