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A transposable element can drive the concerted evolution of tandemly repetitious DNA

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ABSTRACT **Recombination and conversion have been proposed to drive the concerted evolution of tandemly repeated DNA sequences. However, specific correction events within the repeated genes of multicellular organisms have not been observed directly, so their nature has remained speculative. We investigated whether the excision of transposable *P* elements from tandemly repeated sequences would induce unequal gene conversion. Genetically marked elements located in a subtelomeric repeat were mobilized, and the structure of the region was analyzed in progeny. We observed that the number of repeats was frequently altered. Decreases were more common than increases, and this bias probably resulted from intrinsic mechanisms governing *P* element-induced double-strand break repair. Our results suggest that transposable elements play an important role in the evolution of repetitious DNA.**

A significant fraction of eukaryotic genomes consists of tandemly repeated DNA sequences such as ribosomal RNA genes, histone genes, satellite DNAs, and a wide variety of other heterochromatic sequences. Comparison of ribosomal genes between closely related species revealed that both gene and spacer DNA sequences undergo concerted evolution (1, 2). A wide variety of multicopy DNAs show similar properties, whether or not they are tandemly repeated (3). Potentially, unequal sister chromatid exchange (4) and gene conversion (5) could correct some repetitive sequence copies against others to maintain their similarity. Meiotic sister chromatid exchanges occur frequently within yeast ribosomal DNA (6, 7). However, the tandemly repetitious genes of multicellular organisms have not been directly observed to undergo correction events, so the mechanisms driving their concerted evolution remain uncertain.

Transposable elements are potential agents of gene conversion. Upon excision from unique sequences, *Drosophila P* elements induce double-strand breaks that are subsequently repaired by using homologous sequences located on the sister strand, on the homologue, or at an ectopic site as template (8, 9). *P*-element transposons containing tandem arrays of 5S ribosomal genes frequently undergo internal changes following activation that are consistent with this mechanism (10). Sequences duplicated internally within a *P* element are often lost during the repair process (11), most likely because single strands extended from the two invading ends can "anneal" at sites of local homology (12). The effects of *P*-element excision on tandemly repetitious genomic sequences have not previously been examined.

To investigate the nature of the events catalyzed within heterochromatic, repetitive DNA by transposon-induced double-strand breaks, we mobilized *P* elements located within a characterized subtelomeric tandem repeat on the *Dp1187* minichromosome (13, 14). Arrays with an altered number of repeats were recovered frequently, but intrinsic properties of *P* element-induced conversion appeared to favor certain classes of products. These studies imply that transposable element activity may be an important factor controlling the concerted evolution of DNA sequences.

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MATERIALS AND METHODS

***Drosophila* Stocks.** Strains 9901 and 0801, containing *P*-element insertions in the TAS-C repeat, were generated as described (13). Strains derived from 9901 and 0801 are denoted by the prefixes 9- and 8-, respectively. Further information about genetic markers is available (15).

Mobilization of the *PZ* Transposon in Lines 9901 and 0801 and Recovery of Progeny Minichromosomes. The genetic crosses used to mobilize the *P*[*lacZ*, ry^+] element (referred to as "*PZ*") in the 0801 and 9901 strains have been described (14). Briefly, a cross was carried out to generate males of genotype *X/Y;SbΔ2-3/ry;Dp* containing both the integrated *PZ* element and the transposase-producing *Δ2-3* transposon. Individual progeny males that lacked the *Δ2-3* element were crossed to *y;ry* females in vials. All lines that still showed evidence of rosy expression were scored for segregation, and 400 of 521 lines containing a $rosy^+$ gene linked to the minichromosome were subsequently analyzed by Southern blotting to determine the structure of the tandemly repeated region. A limited number of lines that appeared to have lost the transposon ($rosy^-$) were similarly studied.

Southern Blots. DNA was prepared from $yellow^+$ (y^+) adult males as described (14). After digestion with *EcoRI*, DNA from 48 lines was separated per gel and transferred to filters. Hybridization was carried out sequentially by using probes specific for the 5′ (pBS5′PendHR0.55) and 3′ (pBS5′ryHR3.2) ends of *PZ* insertions and also for the 1.8-kb TAS repeats (13, 14). Control DNAs from strains lacking a minichromosome and from the starting 9901 and 0801 strains were present on each gel. To map the number of 1.8-kb TAS repeats using Southern blots, DNA was digested with *Xba* I or *Xba* I and *Spe* I, and Southern transfers were hybridized with the 5′ or 3′ *PZ* probes. The sizes of the bands were compared to control DNAs from strains bearing *PZ* insertions in TAS-B–TAS-D (13). To map the number of 173-bp repeats, restriction fragments flanking the 3′ side of each insertion were sized on Southern blots and compared to the corresponding fragments from the starting strains and from strains containing *PZ* insertions at known locations in different 173-bp repeats.

DNA Sequencing. Plasmid rescue, restriction mapping, and DNA sequencing was carried out as described (13, 14). A clerical error was discovered in the reporting of the 0801 insertion site (13). The correct position is 4938/9 (rather than 4936/7), which corresponds to nucleotide 98 in 173-bp repeat C3.

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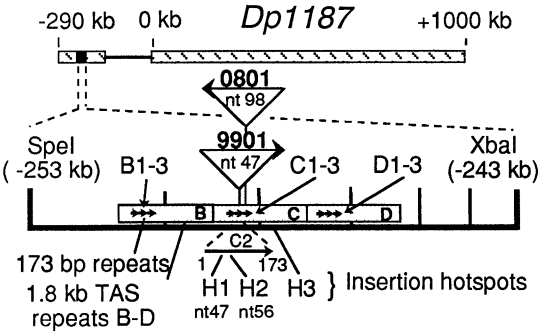

FIG. 1. The *Dp1187* minichromosome and its subtelomeric repetitive region. The 10-kb *Spe* I–*Xba* I region from the *Dp1187* minichromosome (16) is expanded below the diagram to illustrate 1.8-kb TAS repeats B–D (boxes) and their three 173-bp subrepeats 1–3 (arrows). The locations of the *PZ* insertion (inverted triangles) in strains 9901 and 0801 are shown [nucleotide (nt) coordinates are relative to the start of the relevant 173-bp repeat]. The C2 173-bp repeat is expanded to show the *P*-element insertion hotspots: H1 (nucleotide 47) and H2 (nucleotide 56); H3 is located outside the subrepeats. Vertical lines represent *Eco*RI sites.

RESULTS

Repetitive TAS Repeats on *Dp1187* Provide a System for Studying Concerted Evolution. To investigate the role transposable elements play in concerted evolution, we utilized a 1300-kb minichromosome derived from the X chromosome, *Dp1187*, that contains a well-characterized subtelomeric region (Fig. 1) (13, 16). The 10-kb *Spe* I–*Xba* I region contains a 1.8-kb repetitive element called "TAS" (telomere-associated sequence) in three complete tandem copies ("TAS-B–TAS-D") that are more than 95% identical in sequence. Within each 1.8-kb TAS repeat are 173-bp subrepeats, which can be distinguished by a small number of sequence polymorphisms. The three 173-bp subrepeats within TAS-B are called B1, B2, and B3; those in TAS-C are called C1–C3, etc. (Fig. 1). The TAS repeats on *Dp1187* are frequent targets of *P* element insertion, and each contain three particular hotspots denoted H1, H2, and H3 (Fig. 1). The corresponding region on the X chromosome probably contains *P* insertion(s) in certain wild populations (13, 17). Consequently, the TAS repeat cluster on *Dp1187* represents a model tandem array for which *P*-element insertions are available, and which is likely to be a frequent target of *P*-element insertion in wild strains.

Structure of Derivatives with Altered TAS Repeats. Evidence that *P*-element excision can be accompanied by unequal genetic exchange arose during previous studies in which we mobilized *P* elements inserted within TAS-C (14). Because the presence of inserted single-copy sequences greatly simplifies the structural analysis of the *Spe* I–*Xba* I region, these studies focused on 521 lines in which the minichromosome still retained a rosy (*ry*) gene following mobilization (Table 1). Southern blotting revealed that the configuration of TAS and 173-bp repeats in most lines was unchanged, including 36 lines that had undergone short-range ("local") *P*-element transposition events (14), while others had suffered terminal deletions. However, in 34 lines the number of TAS and/or 173-bp repeats appeared to be altered in a manner inconsistent with a simple terminal deletion (Fig. 2).

To further investigate the structures inferred from the initial Southern blots, DNA between the 5′ end of the resident *PZ* element and the *Xba* I or *Spe* I site was cloned by plasmid rescue (18) and analyzed by restriction mapping. The junction between the *P* element and chromosomal DNA was sequenced. Additional Southern blots were carried out to estimate the number of TAS and 173-bp repeats 3′ to the insertion. Changes in the size of 3′ restriction fragments that corresponded to integral multiples of 173 bp were assumed to be due to a homologous change in the number of 173-bp repeats. The structures so determined for most of these derivatives are shown in Fig. 3.

Results obtained with lines 9-205 and 8-086 illustrate the mapping experiments. Line 9-205 differed from the progenitor 9901 strain on the initial Southern blots by a reduction in the size of the 5′ *Eco*RI band from 1.0 kb (Fig. 2 *Left*, fragment "c") to about 0.85 kb (Fig. 2 *Left*, fragment "d"). No changes were seen in the 3′ flanking band or the bands labeled by the TAS probe. The loss of one 173-bp repeat 5′ to the insertion was confirmed by restriction mapping of the rescued plasmid and by sequencing. Thus, line 9-205 differed from 9901 only by the precise loss of a single 173-bp repeat flanking the insertion (Fig. 2).

The changes giving rise to line 8-086 were more extensive. The *Eco*RI fragment 5′ to the 8-086 insertion had increased from about 2.1 kb (Fig. 2 *Left*, fragment "a") to >15 kb (Fig. 2 *Left*, fragment "b"), while the 3′ flanking fragment increased by <100 bp (Fig. 2 *Center*, compare fragments "e" and "f"). Hybridization with the TAS probe showed that a 1.8-kb TAS repeat was still present (Fig. 2 *Right*, fragment "i"), but the 2.1-kb 5′ fragment flanking the 0801 insertion had been lost (Fig. 2 *Right*, fragment "a"). These changes suggested that the *PZ* element was now located in the first TAS repeat and at a different position within the 173-bp repeats. The restriction map of 5′ DNA in the rescued plasmid verified that the insertion was in the TAS repeat closest to the *Spe* I site. Sequence analysis confirmed that the *P* element was located in 173-bp repeat C3 and mapped the junction at nucleotide 54 rather than at nucleotide 98, explaining the slight increase in the 3′ flanking fragment (see Fig. 2).

A total of 12 lines with altered TAS and/or 173-bp repeats were observed (Fig. 3 *Upper*). Most of the lines had lost a single TAS repeat, but line 9-569 had gained two additional repeats. Line 9-205 had lost a single 173-bp repeat, while line 9-357 had gained a fourth copy. The *PZ* element in many of these lines had relocated as well. In 16 other lines, changes in the location of the *PZ* element were accompanied by terminal deletion of distal sequences (Fig. 3 *Lower*).

Mechanism of Unequal Conversion Events. Several possible conversion mechanisms are suggested by these results that differ in the source of the recovered *P* element. Derivatives such as line 9-205 may have resulted from double-strand break repair that extended through the *P* element on the sister strand (Fig. 4 *Left*). More commonly, as illustrated by line

Table 1. Summary of mutagenesis screen

Progenitor strain	Total chrom.*	ry+ sublines*	Total analyzed†	Candidates‡	Conversions		Terminal deletions
					Gain	Loss	
0801	1900	263	142	8	1	2	4
9901	1700	258	258	26	1	8	12

*Data from ref. 14. chrom. Chromosomes.
†Lines subjected to Southern blot analysis as described in text.
‡Lines selected based on initial Southern blots; those not listed as conversions or terminal deletions were other events or were lost prior to completion.

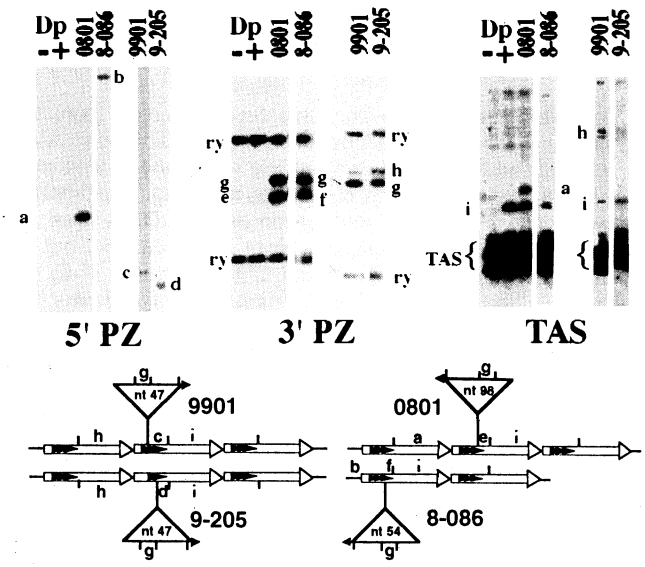

FIG. 2. Analysis of TAS arrays by Southern blotting. Adult male DNA was digested with *Eco*RI and hybridized sequentially with probes specific for the 5′ *PZ* element end (5′ *PZ*), the 3′ *PZ* element (3′ *PZ*), and the TAS repeat (TAS). Results are shown for lines 8-086 and 9-205 and include control males that lacked (−Dp) or contained (+Dp) *Dp1187* or males from the 0801 or 9901 starting strains. *Eco*RI fragments are indicated by lowercase letters at the sides of the blots; their positions within the tandemly repeated region are shown on the diagrams below. Vertical lines represent relevant *Eco*RI sites. nt, Nucleotide.

8-086, short-range ("local") transposition to a nearby site on the gap-containing strand appeared to be the source of the new element (Fig. 4 *Right*). Several similar pathways could lead to the observed structures, depending on (*i*) which repeat copy was invaded by the free ends, (*ii*) whether the ends were trimmed by exonuclease prior to invasion, and (*iii*) whether the single-stranded molecules extended from the left and right ends annealed together at regions of local homology to short circuit the repair process (11, 12). These pathways differ in the predicted location of conversion tracts (Fig. 4). Failure of a right strand bearing a locally transposed *PZ* element to rejoin to the left strand after repair on the sister strand could explain most of the terminal-deletion derivatives. The fact that the *PZ* element was inserted within the distal-most TAS repeat in all of the lines of this class suggested that the presence of the transposon in some way contributed to the failure of double-strand break repair.

DNA Sequences of Flanking 173-bp Repeats. We determined the DNA sequences flanking the 5′ end of 12 of the derivatives (data not shown), including most of those in Fig. 3 *Upper*, to look for direct evidence of sequence conversion. Despite sequencing 15 173-bp repeats, including those flanking line 9-205, no hybrid repeats were found. These data supported the view that the conversion tracts were not usually located close to the *PZ* element, most likely because it had transposed away from the site where double-strand break repair initiated.

DISCUSSION

***P*-Element Activation Is Associated with Unequal Gene Conversion Events Within a Flanking Tandem Repeat.** The experiments reported here demonstrated that mobilization of a resident *P* element frequently changes the number of tandem TAS repeats and 173-bp subrepeats located near the excision site. At lest 12 derivatives among the 400 analyzed differed from their progenitor in repeat copy number, and

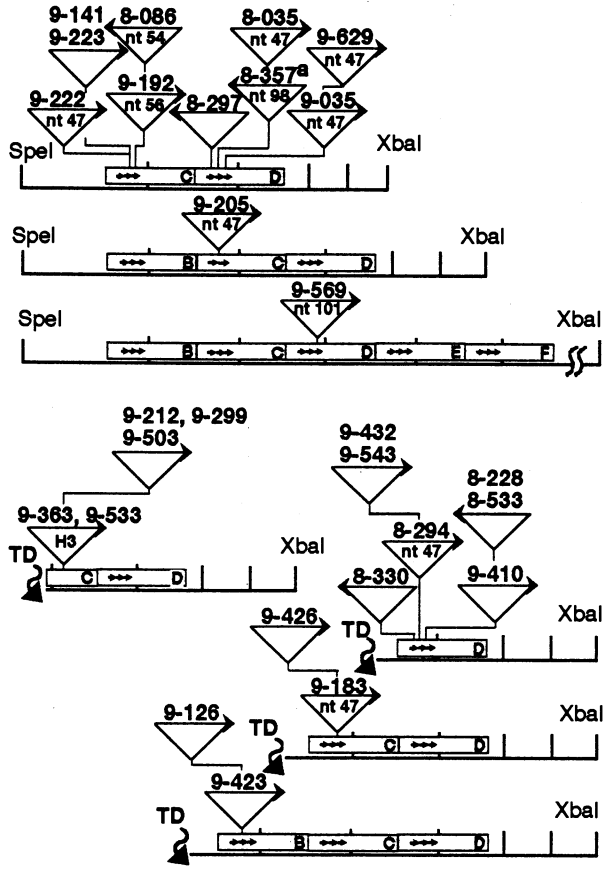

FIG. 3. Structure of the *Spe* I–*Xba* I regions in lines that underwent (*Upper*) or may have undergone (*Lower*) unequal conversion events. Each line contains only a single insertion within the region shown, but to save space the insertions (inverted triangles) in derivatives with the same number of TAS repeats are plotted on a single map (arrowhead = 5′ *P* end). Lines 8-357 and 9-205 contain an altered number of 173-bp subrepeats. When the number of TAS repeats was changed, letter designations were given to individual repeats for purposes of notation only; these names do not reflect the hybrid nature of some repeats relative to the starting strains. Note: Lines starting with 9 were derived from 9901; those starting with 8 were derived from 0801. Large stippled boxes are 1.8-kb TAS repeats; small arrows are 173-bp subrepeats. TD, terminal deletion (not shown are variations in the exact position of the deletion breakpoint distal to the insertion); superscript a in *Upper*, line 8-357 inserted in a TAS repeat with four 173-bp subrepeats. nt, Nucleotide.

these changes could be explained by double-strand break repair using the sister strand as template. This mechanism would alter the DNA sequences of individual repeats; however, we were unable to document this directly.

Unequal conversion events associated with *P*-element mobilization occurred at relatively high frequency. The 12 chromosomes with altered repeats represent 0.33% of the 3600 chromosomes whose *P* elements were mobilized (14); 120 transpositions to other chromosomes, 128 terminal deletions, and 36 local transpositions were recovered in the same sample. However, this probably underestimates the true frequency of unequal conversion events, since only 400 of the chromosomes that could potentially have undergone conversion events were studied because of the technical requirement that derivatives retain a *PZ* insertion. If unequal exchanges occurred at a similar frequency in all chromosomes containing a double-strand break regardless of the fate of the excised *P* element, then its true frequency would be substantially higher than 0.33%.

The three TAS repeats in the *Spe* I–*Xba* I fragment have remained unchanged for several years in numerous stocks not

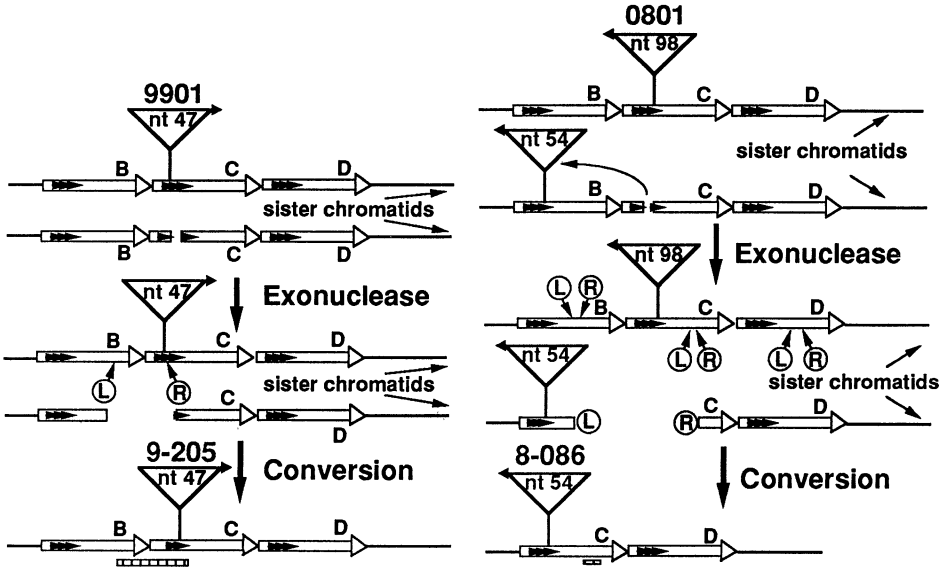

FIG. 4. Models of unequal TAS conversion. (*Left*) Repair of a double-strand break on the sister strand containing the starting insertion. Two sister strands from the starting 9901 line are shown after excision of one of the *P* elements. Exonuclease trimming of the right end proceeds only into the adjacent 173-bp subrepeat and is followed by strand invasion at the indicated sites and repair to yield the structure observed in line 9-205. The expected conversion tract (striped box) would span the *P* element and both flanks. (*Right*) Repair of a broken strand bearing a new *P*-element insertion. Two sister strands from the starting 0801 line are shown after excision and local transposition of one of the *P* elements from TAS-C to TAS-B. After exonuclease trimming, the left (L) and right (R) ends invade the sister at any of the indicated sites of homology. Simple repair (if the strands invade the same TAS repeat) or repair according to the single-strand annealing model (11, 12) would yield the structure observed in line 8-086. The conversion tract (small striped box) predicted by this model would usually lie some distance from the *P* element. nt, Nucleotide.

exposed to *P*-element transposase (13). However, it would be worthwhile to screen large numbers of individuals from these stocks for changes in TAS copy number to determine if spontaneous conversion occurs at measurable levels. *P* elements can serve as unique genetic and molecular markers within tandem arrays, greatly facilitating such studies. The insertion of such markers was a key step in analyzing recombination events within tandemly repeated yeast genes (6, 7).

Are the Conversions Biased? The number of TAS repeats decreased in 10 derivatives but increased in only 1 case. It is unlikely that the experimental design favored reductions in TAS copy number, since rosy$^+$ expression did not correlate with the number of TAS copies (data not shown). Rather the preference for reductions probably resulted from an inherent bias of the conversion mechanism. Extensive exonuclease trimming of the ends created by excision prior to invasion of the sister strand or single-strand annealing of the invading strands prior to the synthesis of multiple repeats would give rise to such a bias.

The single-strand annealing model (12, 20) most simply explains why TAS copy number was frequently reduced as well as several other aspects of our results. If truncation by single-strand annealing made it unlikely that the starting element would be completely copied, the only way a *PZ* insertion could be retained in an array undergoing conversion would be through local transposition onto the starting strand, explaining the high frequency of such events in our collection. In the absence of a local jump, single-strand annealing would be expected to produce arrays containing one or two TAS repeats but no *PZ* insertion. These would not have been analyzed in our experiments, further suggesting that the frequency of unequal conversions may be significantly higher than was observed here.

These considerations also explain why the local transposition events recovered previously on invariant sets of TAS repeats usually contained both the original and a new *PZ* element (14). They likely resulted from transpositions onto the sister chromatid lacking a double-strand break, precluding the opportunity for the repeat number to change by an unequal repair process. Surprisingly, the transposing element usually inverted its orientation only when jumping to the sister strand (compare Fig. 3 and ref. 14), providing further evidence that excision and local reintegration occur within a transposition complex that holds the *P* element in a defined orientation relative to the donor and recipient chromosomes (14, 19).

Transposon-Induced Conversion May Drive the Evolution of Tandemly Repeated Sequences. Conversion was frequently observed following *P*-element excision from tandemly repeated TAS sequences and is likely to occur at other *P* insertion sites rich in repetitive sequences (21). However, the ability to induce unequal conversion events is unlikely to be limited to this one family of transposable elements. *P* elements are members of a large and phylogenetically widespread class of inverted-repeat transposons, including *Ac*, *hobo*, and *mariner* that probably transpose by related mechanisms (22). It would be worthwhile to determine directly whether any of these elements can induce similar events to those described here.

Transposons that transpose and excise via RNA intermediates may also catalyze gene conversions within repetitive DNA at some level. Non-long-terminal-repeat retrotransposons constitute a large class of elements that are abundant in heterochromatic regions (22). For example, a variable fraction of the tandemly repeated rRNA genes of many insect species contain *R1* or *R2* element insertions at specific sites within the 28S rRNA gene (23, 24). The *R2* retrotransposon encodes an integrase-like protein that specifically cleaves its target site *in vitro* (25). Site-specific breaks within uninserted rDNA repeats may occasionally be generated by these elements *in vivo* and lead to unequal repair.

If the flux of integrating and excising transposons makes a major contribution to concerted evolution, several interesting properties would hold. The rate at which repetitive arrays homogenize would vary and would depend on the properties

and activity of specific transposon families resident within individual genomes. A transposon's insertion site specificity as well as the cellular and element-encoded mechanisms controlling its activity would be influential. The introduction of even a single new transposon family by horizontal transmission might drastically alter the evolution of particular arrays into which it could efficiently insert or cut. Properties of DNA-repair pathways and the efficiency of single-strand annealing might influence whether reductions were favored over increases, as in the studies reported here. Under such conditions, which our results suggest may be common, high transposon activity might limit the size of repetitive sequence arrays susceptible to insertion. Thus, repetitive DNAs are likely to evolve nonuniformly in response to internal and external biological influences rather than by uniform stochastic processes. Transposon-catalyzed conversion may not be limited to tandemly repeated genes (9) and might similarly influence the evolution of other multigene families susceptible to transposon activity (26, 27).

If unequal conversion produces useful variation, transposable elements that induce such changes might be of selective value. Their activity would not have to be limited to the germ line. Since the transcription and transposition of many transposable elements is developmentally regulated, addition and loss of repeated sequences might be catalyzed in somatic cells during embryogenesis under the control of element-specific functions (see refs. 28 and 29 for further discussion). HeT and TART transposons have recently been suggested to function in place of telomerase to maintain *Drosophila* telomeres (30, 31). Our results raise the possibility that certain transposable elements provide another valuable service by mediating the concerted evolution of repetitive gene families.

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