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Biochemistry

Insertion of a repetitive element at the same position in the 5'-flanking regions of two dissimilar yeast tRNA genes

(DNA polymorphism/transposition/target sequence/nonsense suppressor/*sigma* sequence)

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ABSTRACT The regions 5' proximal to many yeast tRNA genes exhibit a high frequency of DNA sequence polymorphisms. DNA sequence analysis of polymorphic variants of *SUQ5*, a $tRNA^{Ser}_{UCA}$ gene, and *SUP2*, a $tRNA^{Tyr}$ gene, shows that in each case one sequence variant of the tRNA gene is 346 base pairs longer than the other. The longer variants appear to have arisen from the shorter ones by the insertion of nearly identical copies of a 341-base pair *sigma* element into a site 16 base pairs upstream from the 5' ends of the tRNA-coding regions. The sequences of the two copies of the *sigma* element differ at only five positions. The element has a number of properties that are typical of many transposable elements: (*i*) there is a perfect eight-base-pair inverted repeat at its ends, (*ii*) these ends are flanked by a five-base-pair direct repeat of a sequence that occurs only once in the target DNA, (*iii*) there are approximately 20 copies of the element in the yeast genome, and (*iv*) there is considerable strain-to-strain variation in the sizes of the restriction fragments on which these copies lie. The presence of the *sigma* element has no gross effect on the phenotype of a *SUP2 ochre* suppressor. Analysis of the *SUQ5* and *SUP2* sequences favors the hypothesis that *sigma* is a transposable element with a novel type of insertion specificity, which is primarily based on the presence of a tRNA-coding region a fixed distance from the insertion site, rather than on the immediate target sequences.

Transposable elements, which were first extensively studied in bacteria, are now known to be widespread in eukaryotic organisms also (1). Considerable progress has been made, both in the structural analysis of these elements and in establishing their participation in a wide variety of genetic events, ranging from somatic mutation in maize embryos (2), hybrid dysgenesis in *Drosophila* (3), and the alteration of yeast gene regulation (4, 5) to—in the case of retroviruses—the transformation of animal cells (6). Nonetheless, the study of these elements is still at an early stage, and little is as yet understood about their roles in either the normal functioning or the evolution of eukaryotic cells. Under these circumstances, further examples of eukaryotic transposable elements are of continuing interest, particularly if they have novel properties and offer opportunities to relate transposon biology to other better-understood aspects of eukaryotic molecular genetics.

In a recent paper, del Rey *et al.* (7) reported a repeated element, *sigma*, that has many properties in common with transposable elements. The *sigma* elements were found 16-18 base pairs (bp) from the 5' end of several tRNA genes. In this report, we provide further evidence that *sigma* is a transposable element. The *sigma* element was encountered in the course of characterizing the yeast tRNA genes that correspond to tyrosine- and serine-inserting nonsense suppressors. As previously reported, the sizes of the restriction fragments that contain many of these tRNA genes vary among different laboratory yeast strains (8-11). In this paper, we show that two of these polymorphisms, involving a $tRNA^{Ser}_{UCA}$ gene, *SUQ5*, and a $tRNA^{Tyr}$ gene, *SUP2*, can be explained by hypothesizing that a *sigma* sequence has inserted into both genes at a site 16 bp upstream from the 5' end of the region that codes for the mature tRNA. Retrospective analysis of yeast tRNA gene sequences in the literature reveals two other cases in which the same sequence occurs at the same position in still other tRNA genes.

MATERIALS AND METHODS

Yeast Strains and Clones. Most of the λ and plasmid clones used in this work have been described. The *SUQ5* allele that contains the *sigma* element was present in pPM15, a pBR322 clone that contains a 1.4-kilobase (kb) *Bam*HI/*Hin*dIII fragment from the *suq5*$^+$ yeast strain Y4A (11). The *SUQ5* allele that lacks the *sigma* element was present in pPM16, a pBR322 clone that contains a 1.1-kb *Bam*HI/*Hin*dIII fragment from the *SUQ5-o* yeast strain 4093-4A (11). The *SUP2* allele that contains the *sigma* element was present in pPM55, a pBR322 clone that contains a 1.7-kb *Eco*RI/*Hin*dIII fragment from the *sup2*$^+$ yeast strain S288C; this plasmid had been previously subcloned from λgt1-Sc463 (ref. 9; unpublished work). The *SUP2* allele that lacks the *sigma* element was present in pPM57, a pBR322 clone that contains a 1.4-kb *Eco*RI fragment from the *sup2*$^+$ yeast strain B596; this plasmid was subcloned for the present work from λPM57, a *SUP2* λ clone isolated from the λgt5/B596 pool previously described (ref. 9; unpublished work).

The yeast strain 4093-1B (from D. C. Hawthorne) is a segregant from the same diploid as 4093-4A (11); by both genetic and physical criteria, it contains the same allele of *SUQ5* as did 4093-1A, which is no longer viable. The strain J13-8B (from J. Kurjan) is a *SUP2-o* segregant from the diploid 952RW87-27C (12). AB972 was derived from S288C by a complex series of steps that involved no outcrossing; it contains no known restriction fragment size variations when compared with other S288C isolates. Note that in yeast suppressor notation *suq5*$^+$ and *sup2*$^+$ are wild-type alleles while *SUQ5-o* and *SUP2-o* are ochre-suppressing alleles.

Recombinant DNA Methods. DNA sequences were determined by the method of Maxam and Gilbert (13). The methods used for such standard procedures as gel electrophoresis, λ and plasmid cloning, nick-translation, DNA·DNA hybridization, yeast DNA preparation, and Southern blots have all been described (9-11, 14).

RESULTS

Definition of the *SUQ5*- and *SUP2*-Linked Polymorphisms by Restriction Enzyme Analysis. Previous studies have shown

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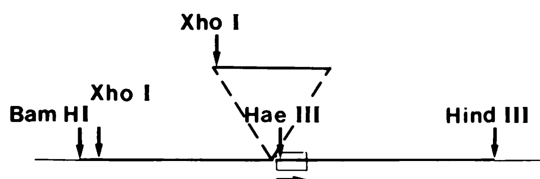
Abbreviations: bp, base pair(s); kb, kilobase(s).

that restriction fragment sizes in the *SUQ5* and *SUP2* regions are poorly conserved in different laboratory yeast strains (10, 11). Fig. 1 is based on detailed restriction mapping of clones bearing long and short variants of the two genes. It reveals similar situations at the two loci: the long variants differ from the short ones by the presence of an extra 0.34 kb of DNA in the immediate vicinity of the 5′ ends of the tRNA-coding regions.

Both long variant clones have previously been shown to contain uncharacterized yeast repetitive sequences (11, 15). To determine whether or not these sequences were related to the observed polymorphisms, we studied the hybridization among all four clones and genomic yeast DNA. The results show that the repetitive sequence present on the long variant clone of *SUQ5* is altogether lacking in the short variant clone (Fig. 2*A*). The strain to which the long variant clone was hybridized in lane 1 contains the long variant itself. This circumstance accounts for the strong hybridization to a 1.4-kb *Hin*dIII/*Bam*HI fragment that correspnds exactly in size to the *Hin*dIII/*Bam*HI insert in the long variant cloned probe. However, there was also strong hybridization to many other *Hin*dIII/*Bam*HI fragments. When the same digest was hybridized to the short variant probe (lane 2), the only strong band was the 1.4-kb *Hin*dIII/*Bam*HI long variant *SUQ5* fragment. The faint bands in lane 2 have not been thoroughly analyzed, but they probably correspond to hybridization between the tRNA-coding region of *SUQ5* and fragments that contain other closely related $tRNA^{Ser}$ genes (11, 16). Finally, the short variant *SUQ5* probe was hybridized to *Bam*HI/*Hin*dIII-cleaved DNA from a yeast strain that contains the short variant of the *SUQ5* gene (lane 3). As expected, the only strong band corresponded to a 1.1-kb fragment that was identical to the yeast insert in the short variant cloned probe.

Parallel results are shown for the *SUP2* locus in Fig. 2*B*. In this, case *Eco*RI/*Pvu* II double digests of the yeast DNAs were used so that the results of the hybridizations to genomic DNA

A SUQ5



B SUP2

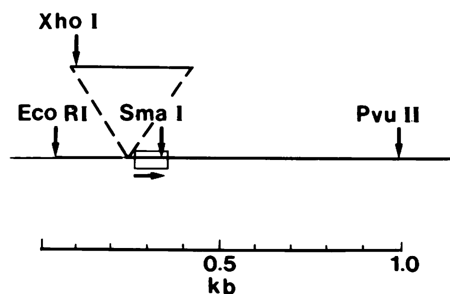


FIG. 1. Restriction maps of the *SUQ5*- and *SUP2*-containing plasmids. The region of each tRNA gene that codes for mature tRNA is indicated by an open box; the underlying arrow indicates the direction of transcription of the tRNA.

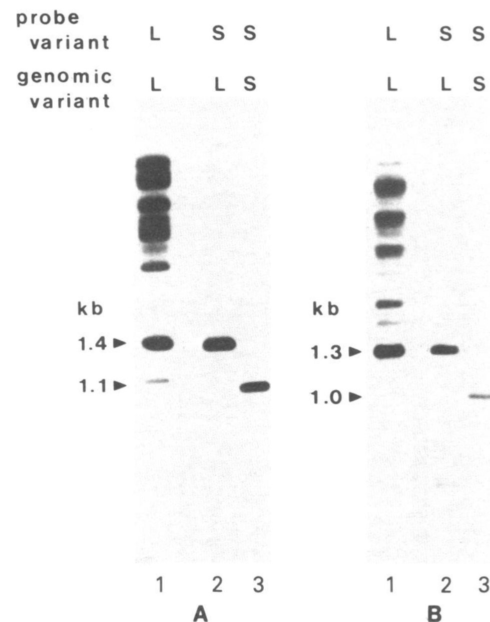


FIG. 2. Southern blot analysis of yeast genomic DNA hybridized to nick-translated plasmid DNA. Total DNA from strains containing the long (L) and short (S) variants of the *SUQ5* and *SUP2* loci were digested with restriction enzymes and electrophoresed on a 0.7% agarose gel. DNA was transferred to nitrocellulose by the method of Southern (14) and hybridized to nick-translated DNA from clones containing the long (L) and short (S) variants of *SUQ5* or *SUP2*. The nitrocellulose sheet was exposed to x-ray film, and the autoradiogram is shown here. (*A*) Polymorphism at the *SUQ5* locus. Total yeast DNA was cleaved with *Hin*dIII/*Bam*HI. DNA in lanes 1 and 2 is from strain Y4A; DNA in lane 3 is from strain 4093-1B. (*B*) Polymorphism at the *SUP2* locus. Total yeast DNA was cleaved with *Eco*RI/*Pvu* II. DNA in lanes 1 and 2 is from strain J13-8B; DNA in lane 3 is from strain B596.

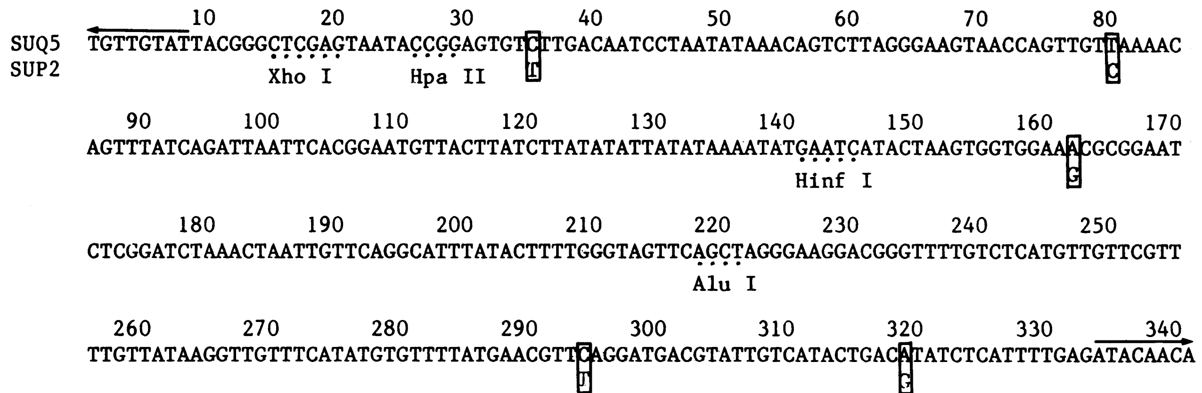
could be correlated with the plasmid restriction maps (Fig. 1*B*). Taken together, the results in Fig. 2 show that the long variant clones of both the *SUQ5* and *SUP2* genes contain repetitive DNA sequences while the short variant clones contain predominantly single-copy DNA.

DNA Sequences. The sequences of the four clones containing the long and short variants of the *SUQ5* and *SUP2* genes were determined throughout the regions in which the allelic pairs differ. The results show that the two polymorphisms are manifestations of strikingly similar DNA sequence alterations (Fig. 3): Both of the two long variant genes are 346 bp longer than the corresponding short variants and the extra sequences in the two long variants are nearly identical. Furthermore, both of these extra sequences are in the same orientation and in the same position relative to the adjacent tRNA-coding regions. The extra sequences are homologous to the *sigma* sequences that del Ray *et al.* (7) have found next to still other yeast tRNA genes.

The data in Fig. 3 favor a simple insertion model for the origins of the two polymorphisms. We have arranged the sequences in a way that reflects the most natural choices for the exact ends of the *sigma* element. These choices define the two *sigma* sequences as 341-bp elements that differ by only five single-bp substitutions and end in identical eight-bp inverted repeats with the sequence 5′-T-G-T-T-G-T-A-T...A-T-A-C-A-A-C-A-3′. These ends are flanked by direct repeats of five-bp sequences that occur only once in the target DNA.

This model for the *sigma* insertions gains further support from limited data in the literature on two other occurrences of the *sigma* element. In one case, the element is present next to

A Sigma sequence



B Sigma insertion into the 5'-flanking sequence of two tRNA genes

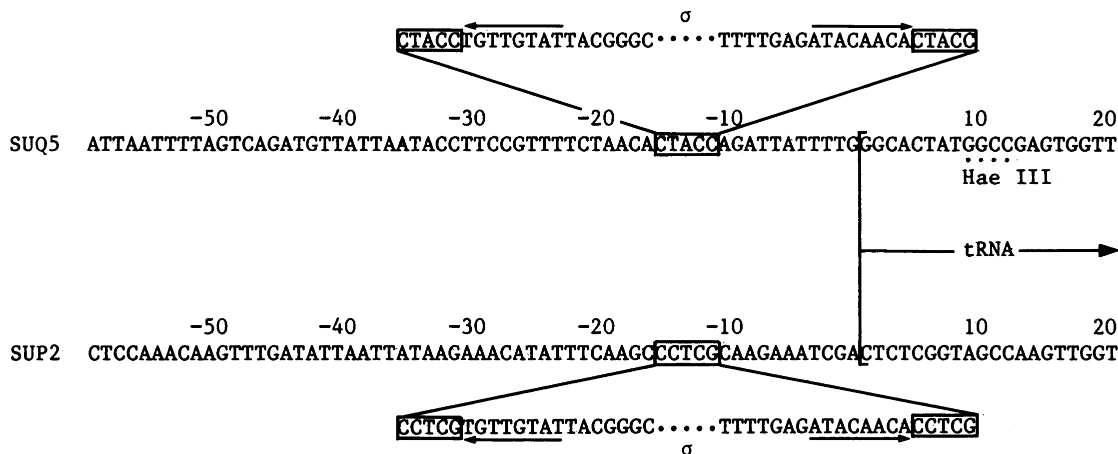


FIG. 3. Relationship between the DNA sequences of the σ^+ and σ^- alleles of *SUQ5* and *SUP2*. (*A*) The perfect inverted repeats at the ends of the *sigma* element are indicated by arrows. The five differences between the *SUQ5*- and *SUP2*-associated *sigma* sequences are boxed. (*B*) The five bp that are present once in the σ^- alleles ("target DNA") and twice in the σ^+ alleles are boxed. The sequences of the σ^+ alleles are related to those of the σ^- alleles by replacing the boxed regions with the indicated *sigma*-associated sequences. In the case of the *SUQ5* σ^+ allele, there was a single additional difference between the two sequences: The cytidine at −33 was replaced by thymidine. The origins used for the sequence analyses, which are labeled, were as follows: *SUQ5* (σ^+), *Xho* I, *Hpa* II, and *Hinf*I; *SUQ5* (σ^-), *Hae* III; *SUP2* (σ^+), *Xho* I, *Alu* I; *SUP2* (σ^-), *Sma* I (position 81 in the tRNA-coding region). The continuity of all the sequences shown was established by overlapping with sequences obtained from adjacent origins. Three of the sequences overlap with sequences previously determined for the immediate 5'-flanking regions of the tRNA genes: *SUQ5* (σ^+), ref. 11; *SUQ5* (σ^-), refs. 11, 16; *SUP2* (σ^+), S. Goh and M. Smith (personal communication).

a $tRNA_3^{Leu}$ gene, while the other involves a $tRNA_3^{Glu}$ gene.* Unless there is terminal heterogeneity in the *sigma* family, the ends of the *sigma* element are completely defined by the *SUQ5* and *SUP2* data together with these two additional cases, and these uniquely defined ends agree with the assignments in Fig. 3. It is intriguing that the immediate ends of the *sigma* element, specifically the inverted repeat sequences 5'-T-G-T-T-G...C-A-A-C-A-3', are identical to those of the *Drosophila copia* element (19) and show more limited similarities to the ends of certain other eukaryotic transposable elements, including the δ sequences that flank yeast Tyl elements (20).

All four of the *sigma* sequences discussed above—those present at *SUQ5* and *SUP2* and those associated with the $tRNA_3^{Leu}$ and $tRNA_3^{Glu}$ genes—have the same orientation relative to the adjacent tRNA-coding regions. Furthermore, the end of the *sigma* element that is proximal to the tRNA-coding region is always at position −17 relative to the nucleotide that codes for the 5' end of the mature tRNA. This positional conservation is particularly interesting because there is no obvious similarity between the immediate target sequences at *SUQ5* and *SUP2* or the "half-target" sequences that can be inferred for the $tRNA_3^{Leu}$ and $tRNA_3^{Glu}$ cases. It is possible to find a few very short homologies among these four sequences, as well as between the various target sequences and the ends of the *sigma* element, but even these homologies are poorly conserved in position.

Given the extraordinary specificity of the *sigma* insertions and the general dissimilarity of the immediate target sequences, we hypothesize that the specificity of the *sigma* insertions derives primarily from recognition of the tRNA-coding region *per se* rather than a specific sequence upstream from it.

Because *sigma* sequences are very similar in length to yeast δ sequences and there is extensive sequence heterogeneity within the δ family (20), we carried out computer-based com-

* In the $tRNA_3^{Leu}$ case (17), the published sequence ends only 24 bp into the *sigma* sequence. The $tRNA_3^{Glu}$ sequence extends 1.5 kb upstream from the tRNA-coding region (18), but the *sigma* sequence is interrupted 68 bp from the end that is proximal to the tRNA-coding region. The interruption is due to insertion into the *sigma* sequence of a δ sequence and, although extensive sequence distal to the δ sequence is reported in ref. 18, the expected remaining portion of the *sigma* sequence is not present.

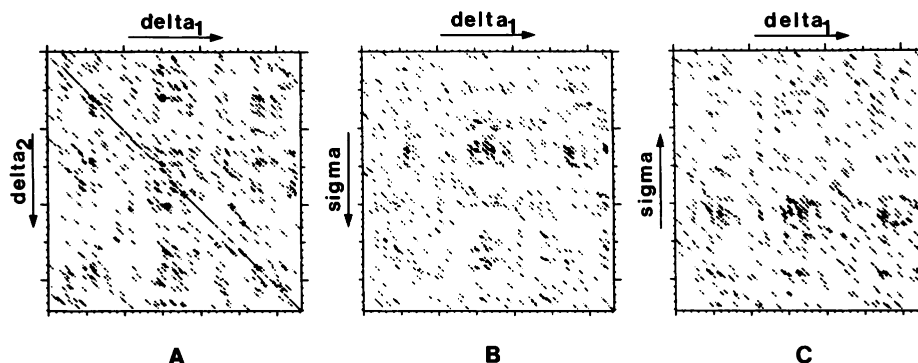


FIG. 4. Dot-matrix display of a computerized comparison of *sigma* and delta sequences. Homologies of greater than five out of six bp are indicated by a dot at every position of identity. The axes are marked in units of 10 bp. (*A*) Dissimilar delta sequences. $Delta_1$ is from Ty1-917 and $delta_2$ is from Ty1-B10/D15 (20). (*B* and *C*) The $delta_1$ sequence versus the *SUQ5*-associated *sigma* sequence; both orientations are shown.

parisons of *sigma* and δ sequences to establish that *sigma* sequences are not simply a special subclass of δ sequences. The results of a "dot-matrix" (21) search for homology between representative *sigma* and δ sequences is shown in Fig. 4. In this presentation, homologies between two sequences result in diagonal lines with a negative slope. In Fig. 4*A*, two dissimilar published δ sequences, which are only 81% homologous, are shown to give a strong diagonal component when matches of five out of six adjacent nucleotides are plotted. At the same comparison "stringency," the *SUQ5 sigma* sequence shows no significant homology to a particular δ sequence in either orientation (Fig. 4 *B* and *C*). Similar results were obtained regardless of which *sigma* and δ sequences were used for this analysis.

Analogous comparisons were made between the *sigma* sequence and the following sequences: yeast ARS2 (22), and 5S RNA (23); mouse NTS (24); rat U-1 nuclear RNA (25); adenovirus VAI RNA (26); primate *Alu* (27); and *Drosophila copia* (28). In no case was any striking similarity detected.

As a final point of sequence analysis, neither strand of the *SUQ5*- or the *SUP2*-linked *sigma* sequences has an open reading frame starting with an ATG that is longer than 19 codons.

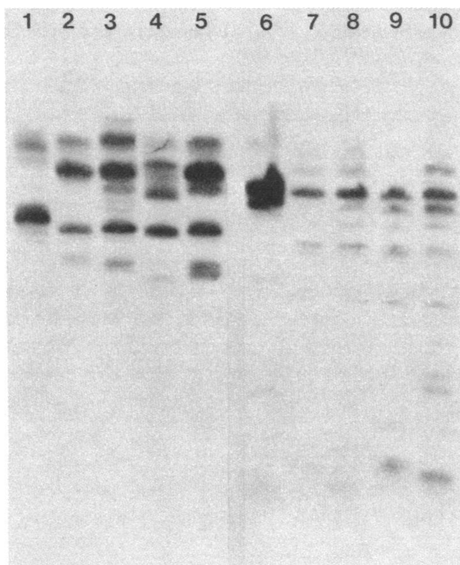


FIG. 5. Southern blot analysis of nick-translated *sigma* DNA hybridized to total DNA from five yeast strains. Total yeast DNA was digested with *Hin*dIII (lanes 1–5) or *Xho* I (lanes 6–10). All lanes were hybridized to nick-translated DNA from the *sigma*-containing *SUQ5* plasmid. Lanes: 1 and 6, Y4A DNA; 2 and 7, 4093-1B DNA; 3 and 8, J13-8B DNA; 4 and 9, B596 DNA; 5 and 10 AB972 DNA.

It is unlikely, therefore, that either of these sequences encodes a protein product.

Size of the *sigma* Family. To obtain an estimate of the number of *sigma* sequences in the yeast genome, the *sigma*-containing *SUQ5* clone was used as a hybridization probe to analyze *Hin*dIII and *Xho* I digests of DNA from several yeast strains. The results suggest that approximately 20 copies is a reasonable estimate of the number of *sigma* sequences present in these strains. Although numerous strain-to-strain differences are evident in Fig. 5, there are also many cases of bands that are conserved among all five strains, a result suggesting that the frequency at which most *sigma* sequences move is not extraordinarily high.

Cleavage with *Xho* I, which cuts within the terminal region of each *sigma* sequence analyzed thus far, produced multiple bands of similar intensity. The one exceptional band actually contains the *SUQ5* locus (unpublished results) and thus has more extensive homology with the probe. These results suggest that *sigma* sequences—in contrast to δ sequences—probably do not occur frequently as a flanking repeat in a large conserved element because the band(s) derived from that DNA would be intensified relative to other bands in the Southern blot.

DISCUSSION

The most reasonable interpretation of our sequence analyses of *sigma*-related polymorphisms at the *SUQ5* and *SUP2* loci is that *sigma* is a transposable element. In analyzing naturally occurring variants of this kind, there is no way to establish which sequence is most similar to the ancestral one but, by analogy with better-understood tranposition systems (1), there is a high likelihood that the long variants of these two genes arose from the short ones by transposition of a *sigma* element into single-copy DNA.

Two previously studied genetic properties of the *SUP2* locus are of renewed interest in light of our data. First, it has been shown by both meiotic and mitotic segregation experiments, using Southern hybridization to score the segregants, that the long and short variants of *SUP2* are allelic (10), thus ruling out the possibility that the association of *SUP2* with a *sigma* element involved a transposition of the tRNA gene itself. Second, at least one widely used ochre-suppressing allele of *SUP2* was clearly isolated in a genetic background that contains *sigma* at this locus;† this suppressor has a normal phenotype when compared

† See ref. 10 for evidence that the diploid W87, in which R. Rothstein isolated a complete set of tyrosine-inserting ochre suppressors, is homozygous for the *Eco*RI restriction variant that is characteristic of the *sigma*-containing *SUP2* allele. The strain J13-8B, which we analyze in Fig. 2*B*, contains a *SUP2*-o allele derived from W87.

with other tyrosine-inserting ochre suppressors, suggesting that the presence of *sigma* has no gross effect on expression of the tRNA gene.

The specific association between *sigma* sequences and tRNA genes is a unique feature of this family of repetitive elements. All seven of the *sigma* sequences for which data are available are immediately adjacent to tRNA-coding regions (refs. 7, 17, and 18 and this work). Furthermore, there are preliminary indications that the same relationship holds for most or all *sigma* elements (unpublished data). The association between *sigma* sequences and tRNA genes raises two intriguing issues about which we can only speculate. The first concerns the molecular basis of the extraordinary insertional specificity of the *sigma* element, and the second relates to the teleology of the tight linkage between the *sigma* sequences that have been analyzed so far and tRNA-coding regions.

The fixed positional relationship between tRNA-coding regions and sites of *sigma* insertions is reminiscent of the specificity of RNA polymerase III, which is known to select its transcription initiation sites primarily by measuring "backward" from recognition sites in the tRNA-coding region that appear to be quite highly conserved in all eukaryotic nuclear tRNA genes (29–31). A reasonable hypothesis is that the *sigma* site-specific recombination machinery somehow borrows from or mimics the specificity of the RNA polymerase III transcription apparatus. For example, the *sigma* system might recognize a complex between the tRNA-coding region and a transcription factor that has properties analogous to those of the 5S gene transcription factor (32). Alternately, the 5′ end of the primary transcript of the tRNA gene, which has not been identified for any of the genes discussed here, could provide the basis of the specificity either by defining one end of an R-loop intermediate in the transcription process or by means of an RNA·RNA ligation event. Whatever the mechanism of *sigma* insertions, the lack of observable coding capacity in any of the *sigma* elements for which sequences have been determined argues that this family of insertion sequences achieves its novel insertional specificity by some means that relies heavily on enzymes encoded by other genes.

With respect to the teleology of the *sigma*–tRNA gene association, two opposing hypotheses suggest themselves. On the one hand, *sigma* sequences may be directly involved in the regulation, transcription, processing, or transport of certain yeast tRNAs. In this view, the proximity between the elements and certain tRNA genes could be rationalized in the same way as are many other examples of tight linkage between two DNA sequences that are functionally related. Alternatively, a "selfish DNA" hypothesis (33, 34) might hold that *sigma* exploits its close linkage to a class of dispersed repetitive genes that has an indispensable function to reduce the risk of loss that is posed by its own selective neutrality.

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