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Consistent association between *sigma* elements and tRNA genes in yeast

(repetitive DNA/transposable elements/genome organization)

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ABSTRACT *sigma* is a recently described family of transposable elements in yeast (*Saccharomyces cerevisiae*). The most striking feature of the seven *sigma* elements that have been previously identified is that all are located 16–18 base pairs upstream from tRNA-encoding regions. Because these cases were all encountered in the process of studying specific tRNA genes, the full extent of the association between *sigma* elements and tRNA genes could not be assessed. In this paper, we report a more global characterization of the *sigma* family in a typical laboratory yeast strain: of the 30 copies of *sigma* that we estimate to be present in the haploid genome, we have cloned and analyzed 25 loci. Although in two cases a pair of *sigma* elements were found within several kilobases of each other, the majority occur as individual elements at widely dispersed sites. Moreover, in all 25 cases analyzed, the *sigma* elements are closely associated with tRNA genes. Thus, the *sigma* transposable element has been shown to have an absolute association with another gene family.

A transposable element in yeast called *sigma* has been independently described in two recent reports (1, 2). Evidence for the transposition of *sigma* is based on DNA sequence analysis of naturally occurring variants in closely related yeast strains. This 341-base-pair (bp) element is of special interest because, in all seven occurrences of *sigma* described, the element is 16–18 bp upstream from the 5′ end of a tRNA gene (1, 2). *sigma* elements have been found adjacent to six different types of tRNA genes, encoding $tRNA^{Glu}$, $tRNA^{His}$, $tRNA^{Leu}$, $tRNA^{Lys}$, $tRNA^{Ser}$, and $tRNA^{Tyr}$, and in six of the seven analyzed cases the elements are in the same orientation relative to the tRNA gene. *sigma* has 8-bp inverted repeats at its ends and is flanked by 5-bp direct repeats of the target sequence. Moreover, the absence of sequence homology between known and inferred *sigma* "target" sequences suggests that the specificity of *sigma* insertion depends on recognition of the adjacent tRNA-encoding region.

Because all these cases were encountered during the analysis of clones selected to contain specific tRNA genes, the full extent of the association between *sigma* and tRNA genes could not be assessed. Therefore, we have undertaken a more global characterization of the *sigma* family of repetitive elements in a haploid strain of *Saccharomyces cerevisiae*. From our analysis, we estimate that there are 30 copies of *sigma* in the yeast nuclear genome. Furthermore, all the elements we have cloned, representing 25 different loci, are closely associated with tRNA genes.

MATERIALS AND METHODS

Screening of Yeast Recombinant Library. A λ–yeast recombinant library was screened by plaque hybridization (3) with two pBR322-derived recombinant plasmids known to contain a complete *sigma* sequence (pPM15 and pPM55, see below). The construction and detailed characterization of the library will be described elsewhere. Briefly, the yeast DNA was prepared from strain AB972, a derivative of S288C that lacks mitochondrial DNA. The library consisted of 12- to 20-kilobase (kb) *Sau* IIIA partial-digest fragments of this yeast DNA cloned in the *Bam*HI site of a Charon 30/λ1059 hybrid λ vector.

Ninety-eight *sigma*-hybridizing clones obtained from primary screening were plaque-purified for subsequent analysis. DNA was prepared from these clones (4) and analyzed by digestion with the restriction enzymes *Eco*RI, *Hin*dIII, and *Xho* I and combinations thereof. Because these enzymes do not cleave the arms of the vector, all the digests produce two large fragments, consisting of the λ arms fused to yeast sequences of different lengths, as well as several smaller (<10-kb) fragments, consisting entirely of yeast sequences. These smaller fragments should correspond exactly to naturally occurring yeast restriction fragments. The fragments were separated by electrophoresis on agarose gels of various compositions (0.7–2.0%) and transferred to nitrocellulose filters by a modification of the method of Southern (5). Hybridizations were carried out at 65°C as described (6–8). After hybridization, filters were washed and then exposed for 1–48 hr to Kodak XAR-5 film at −70°C with an intensifying screen.

Hybridization Probes. The five different plasmids used during this analysis are derivatives of pBR322. pPM15 has a 1.4-kb *Bam*HI/*Hin*dIII yeast fragment containing *sigma* and the tRNA gene *SUQ5* (6). pPM15s contains a 204-bp internal *Xho* I/*Alu* I *sigma* fragment from pPM15 inserted into the *Bam*HI site of pBR322 with synthetic *Bam*HI linkers. This fragment starts at position 16 relative to the end of *sigma* that is adjacent to the *Xho* I site and extends up to position 220 (2). pPM16 has a 1.1-kb *Bam*HI/*Hin*dIII yeast fragment homologous to the 1.4-kb fragment in pPM15 but cloned from a yeast strain that lacks *sigma* at the *SUQ5* locus. pPM55 has a 1.7-kb *Eco*RI/*Hin*dIII yeast fragment containing *sigma* and the associated $tRNA^{Tyr}$ gene *SUP2* (8), and pPM57 contains the homologous 1.4-kb *Eco*RI/*Hin*dIII fragment cloned from a strain that lacks *sigma* at the *SUP2* locus. The plasmids were labeled for use as probes by nick-translation (9) with [α-^{32}P]dATP to a specific activity greater than 10^8 dpm/μg.

pPM55 was used for the initial screening to obtain the first 26 of the 98 clones. Only one of these clones also hybridized to pPM57, which contains *SUP2* without *sigma*; the AB972 *SUP2* locus was already known to be adjacent to a *sigma* element (2). The 72 additional clones were obtained by using pPM15; clones that also hybridized with pPM16 were excluded because *SUQ5* was known not to be adjacent to a *sigma* sequence in this strain.

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Abbreviations: bp, base pair(s); kb, kilobase(s); R+H, *Eco*RI plus *Hin*dIII.

The *sigma* sequences of pPM15 and pPM55 differ by only 5 of 341 bp (1.5%).

For all subsequent analyses, *sigma* hybridizations were done with the *sigma*-specific probe pPM15s, which contains the internal *Xho* I/*Alu* I *sigma* fragment from pPM15. For tRNA gene hybridization, purified total yeast tRNA (Boehringer Mannheim) was 3'-end-labeled by using RNA ligase and cytidine 3'-phosphate 5'-[^{32}P]phosphate by a modification of the technique of Bruce and Uhlenbeck (10, 11).

RESULTS

Grouping of *sigma* Clones. The 98 *sigma*-hybridizing clones were grouped into 23 families based on restriction digest patterns. The members of each family were related by overlapping subsets of restriction fragments that produced an internally consistent map of the genomic DNA surrounding each particular *sigma* locus. Most families are represented by 2–4 clones each, but five are represented by 1 clone each and two other families had 21 and 14 clones each. The latter two families, designated A and B, were overrepresented for unknown reasons among the *sigma* clones obtained by using either pPM15 or pPM55 as a probe.

To correlate these families of *sigma*-containing λ clones with particular genomic *sigma* sequences, we relied primarily on *Eco*RI plus *Hin*dIII (R+H) double digestions both of the clones and of total yeast DNA. This combination of enzymes does not cleave any of the characterized *sigma* sequences, and it is particularly effective at distributing the *sigma*-bearing fragments over a wide range of sizes. For 18 of our 23 clone families, R+H digestion separated the *sigma* sequence from the λ arms and resulted in an R+H fragment of diagnostic size. In five cases, R+H digestions left the *sigma* sequences of all available family members fused to the vector arms, thereby preventing the direct association of those *sigma* elements with a particular R+H fragment size.

Association of *sigma* Sequences and tRNA Genes. To determine the extent to which *sigma* elements and tRNA genes are associated, we carried out parallel hybridizations of restriction digests of the *sigma* clones to the *sigma*-specific probe pPM15s and to labeled total yeast tRNA. Fig. 1 shows the results of hybridizations of *sigma*- and tRNA-specific probes to digests of plasmids that contain two well-analyzed tRNA loci with or without *sigma* elements on different restriction fragments. This control experiment demonstrates that the two probes have the expected specificities.

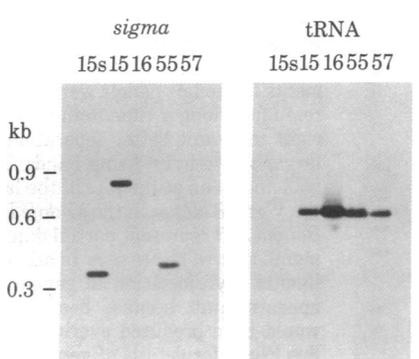

FIG. 1. Demonstration of the specificities of the *sigma* and tRNA hybridization probes. pPM15s (internal *sigma* fragment), pPM15 (*SUQ5*, *sigma*$^+$), and pPM16 (*SUQ5*, *sigma*$^-$) were digested with *Hae* III; pPM55 (*SUP2*, *sigma*$^+$) and pPM57 (*SUP2*, *sigma*$^-$) were digested with *Mnl* I. These enzymes were chosen because they were known to separate the *sigma* elements from the tRNA-coding regions in the two plasmids (pPM15 and pPM55) that contained both types of sequences. (*Left*) Hybridization pattern obtained by using the 5'-end-labeled 204-bp internal *sigma* fragment from pPM15s as a probe. Hybridization was seen to 0.32-, 0.85-, and 0.34-kb fragments from pPM15s, pPM15, and pPM55, respectively. (*Right*) Pattern obtained by using labeled total yeast tRNA as a probe. Hybridization was seen to a 0.60-kb fragment in pPM15 and pPM16, and to a 0.58-kb fragment in pPM55 and pPM57. The tRNA probe appeared to hybridize equally well to both the tRNA^{Ser}- (*SUQ5*) and tRNA^{Tyr}- (*SUP2*) encoding regions. There was no cross-hybridization between the *sigma* probe and the tRNA-encoding regions or *vice versa*.

A representative clone was chosen from each of the 18 families that gave rise to a *sigma*-hybridizing R+H fragment of unique size. These representative clones were arranged on gels in order of decreasing size of the *sigma*-hybridizing fragment. Because two separate R+H fragments hybridized with the *sigma* probe in the F family, clones containing only one or the other of the two fragments (F_1 or F_2) were chosen for inclusion in the series at the appropriate places. Shown in Fig. 2 *Top* are ethidium bromide-stained R+H digests of the 19 representative clones electrophoresed on 1% agarose gels. The center lane of both gels contains R+H digests of yeast genomic DNA. Duplicates of these gels were transferred to nitrocellulose filters and hybridized with either the *sigma* or the tRNA probe.

Fig. 2 *Middle* contains autoradiograms that show the distribution of fragments containing sequences homologous to the *sigma* probe. These fragments ranged in size between 6.8 and 0.85 kb. Fig. 2 *Bottom* shows autoradiograms produced by hybridizing duplicate filters with labeled total yeast tRNA. Comparison of the *Middle* and *Bottom* autoradiograms shows that, in all but one case, the *sigma*-positive fragments also hybridize with the tRNA probe. The only exception was the clone representing the G family. This exception appears to reflect the fortuitous occurrence of an *Eco*RI restriction site between a tRNA gene and an adjacent *sigma* element, or in the tRNA-encoding region: the 3.4-kb R+H fragment that hybridizes to *sigma* has been shown by restriction mapping to be adjacent to the 3.7-kb *Eco*RI fragment that hybridizes to the tRNA probe.

There were five clones in which both *sigma* and tRNA probes hybridized to yeast–λ fusion fragments after R+H digestion. Therefore, these clones were analyzed with the same two probes as above, but after *Bam*HI/*Xho* I digestion. In all five cases this placed the *sigma* sequences on fragments composed of yeast DNA. In four of them, both probes hybridized to the same double-digested fragment, whereas in the fifth case the probes hybridized to adjacent fragments separated by an *Xho* I site.

Orientation of *sigma* Elements Relative to tRNA Genes. To assess the orientation of *sigma* elements relative to the associated tRNA genes, DNAs from representative clones for the 23 distinct *sigma* families were digested with *Xho* I. Duplicate gels were transferred to nitrocellulose and hybridized with both *sigma* and tRNA probes. *Xho* I digestion was chosen because all five of the *sigma* elements whose sequences have been determined have a single *Xho* I site 16 bp from one end of the element, and in all cases *sigma* is upstream from the tRNA-encoding region. In four of these cases, the *Xho* I site is at the distal end of *sigma* relative to the adjacent tRNA-encoding region, whereas in one case the *sigma* is inserted in the same position but in the opposite orientation (1, 2). Given the high degree of homology among the *sigma* elements of known sequence (98%), it is likely that a high proportion of *sigma* elements contain this site. Furthermore, *Xho* I sites are sufficiently infrequent in yeast DNA that this enzyme is expected to cleave very rarely in the short interval that characteristically separates a *sigma* sequence and the adjacent tRNA-encoding region. On the basis of these assumptions, *Xho* I digestion provides a simple means of determining the orientation of *sigma* relative to its adjacent tRNA gene. Clones that contain *sigma* in the orientation that has been most commonly observed should contain an *Xho* I fragment that hybridizes with both *sigma* and tRNA

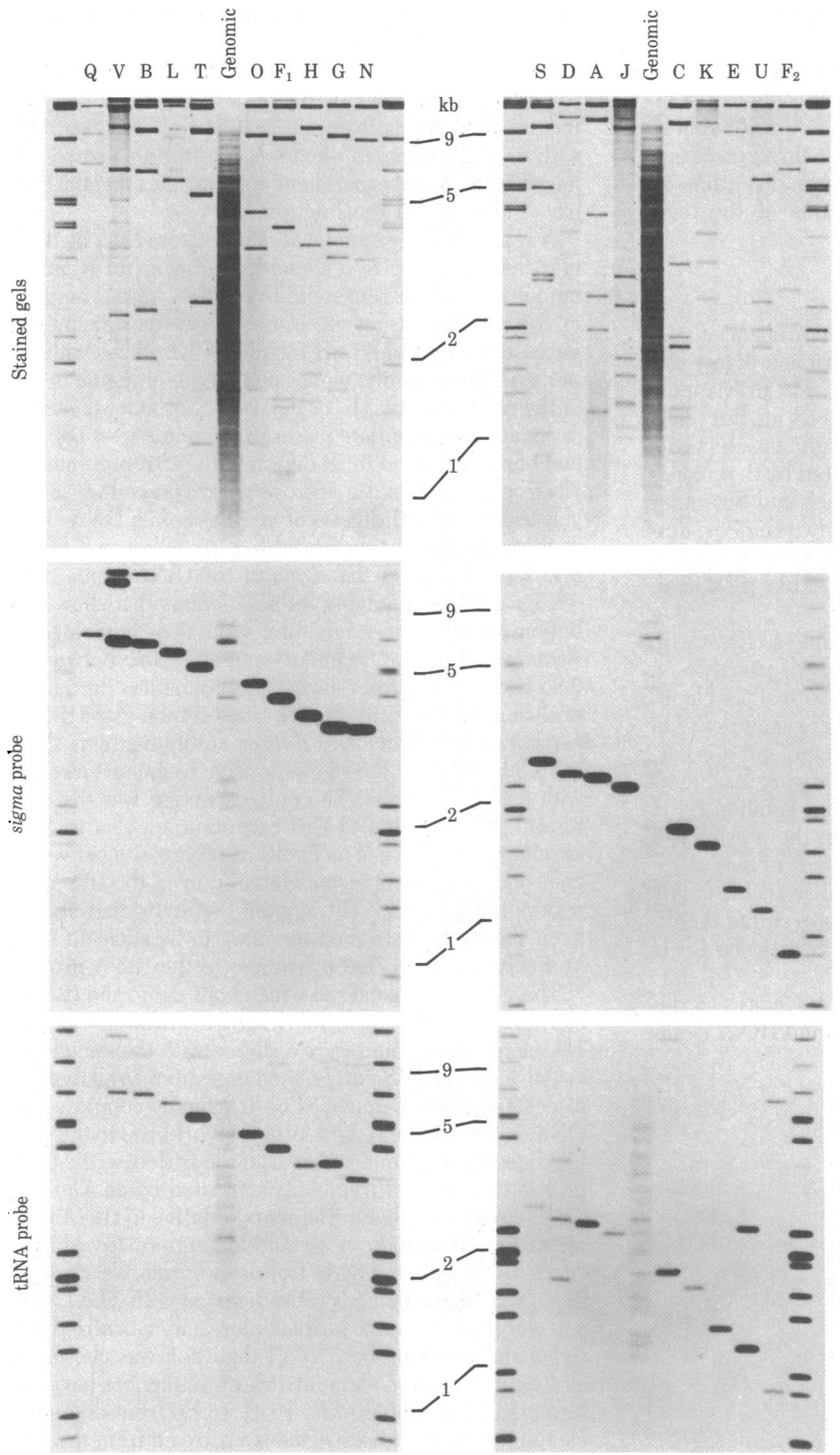

FIG. 2. Hybridization of DNA from 19 *sigma*-containing clones with *sigma*- and tRNA-specific probes. DNA from the 19 yeast–λ clones was arranged in order of decreasing size of the *sigma*-hybridizing R+H fragment on two 1% agarose gels. (*Top*) Ethidium bromide-stained R+H restriction digest patterns of these 19 clones. Also shown in the middle lane of each gel are R+H digests of total yeast genomic DNA. The outside lanes are size markers (an equal mixture of cI857 λ DNA digested with *Hin*dIII and the same DNA digested with R+H) (5). (*Middle*) Pattern of hybridization of the *sigma* probe to blots of these gels. (*Bottom*) Pattern of hybridization of duplicate filters with the tRNA probe. With the exception of the G clone, the single band with *sigma* hybridization also hybridizes with the tRNA probe. Additional bands also hybridize with the tRNA probe in clones representing families D, U, and F_2. Faint hybridization of the tRNA probe to the smaller λ vector arm was also consistently seen for unknown reasons. The gels used for all the experiments in the left panels were electrophoresed for a longer time than those on the right to permit better separation of the larger *sigma*-hybridizing bands. The extra bands seen at the tops of the lanes for the V and B clones in the *Middle Left* and *Bottom Left* represent partial digest fragments that were not seen in other experiments. Hybridization to genomic lanes appears faint, because heavier loading would have produced overload artifacts. See Fig. 3 for details of genomic hybridization with the *sigma* probe.

probes, whereas the two probes will hybridize to separate fragments when the opposite orientation occurs.

Two *sigma* loci (U and F_2) did not appear to be cleaved by *Xho* I. This was determined by comparing the sizes of *sigma*-hybridizing fragments produced by R+H digests and by R+H plus *Xho* I. An additional case gave ambiguous results due to multiple tRNA-hybridizing bands, so the relative orientation of the element in these three cases could not be determined. However, the results for the remaining cases, which are summarized in Table 1, show that 16/22 or 73% of the loci gave results consistent with the "+" orientation (*Xho* I site distal to tRNA), and the remainder appear to be in the "−" orientation (*Xho* I site proximal to tRNA). Thus, although the orientational bias of the five *sigma* elements of known sequence appears to be a general property of the family, both orientations are well represented.

Genomic Organization of *sigma* Elements. To determine if *sigma* elements occur in pairs or clusters, representative clones for the 23 families were digested either with *Eco*RI, *Hin*dIII, and *Xho* I or with *Bam*HI and *Xho* I; as before, the latter combination was used for the five clones that had *sigma* hybridization to λ–yeast fusion fragments after R+H digestion. Neither combination of enzymes cuts within the λ arms, and the multiple digestion should result in many small fragments and thus have a high likelihood of separating multiple copies of *sigma* occurring together in the same clone. In addition, the inclusion

Table 1. Properties of *sigma*-hybridizing fragments

Genomic R+H bands, kb	*sigma* family	No. of isolates	tRNA hybridization*	Orientation†	Comments‡
10.3					
8.3					
8.0	I	2	+	+	Arm
7.8	X	1	+	+	Arm
6.8	Q	2	+	+	
6.6	V	1	+	++	2-*sigma* loci
6.5	M	2	+	?	Arm
6.4	B	14	+	+	
6.0	L	4	+	+	
5.8					
4.7	O	2	+	−	
4.2	F_1	7§	+	+	
3.7	H	3	+	+	
3.40	G	4	−	+	
3.35	N	2	+	−	
2.80	R	2	+	−	Arm
2.65	S	2	+	+	
2.60					
2.45	D	5	+	−	
2.40	A	21	+	+	Largest family
2.25	J	4	+	+	
1.75	C	8	+	−	
1.70					
1.65	K	4	+	+	*SUP2*
1.55					
1.25	E	4	+	+	
1.15					
1.10	U	1	+	?	No *Xho* I site
0.85	F_2	7§	+	?	No *Xho* I site
—	T	1	+	−	See text
—	W	1	+	+	Arm

The 29 *sigma*-hybridizing R+H fragments visualized in the total yeast DNA digests are listed in the left column. In the seven cases in which the corresponding band has not yet been cloned, no entries appear to the right. The two clones that could not be correlated with genomic loci are listed at the bottom.

*A + indicates that *sigma* and tRNA probes hybridized to the same fragment in the R+H digestion; a − indicates that the tRNA probe did not hybridize to the same fragment.

†A + indicates that the same fragment hybridized to both *sigma* and tRNA probes after *Xho* I digestion, suggesting a relative orientation in which the end of *sigma* near the *Xho* I site is distal relative to the tRNA gene. A − indicates that the *Xho* I site is proximal to the tRNA gene. A ? indicates that the orientation of *sigma* relative to the tRNA gene could not be determined.

‡"Arm" designates the five *sigma*-hybridizing clone families in which *sigma* hybridization was to λ–yeast fusion fragments after R+H digestion. The method of tentatively assigning these cloned *sigma* elements to genomic loci is described in the text.

§There are seven clones in the F family: one clone contains only F_1, three contain only F_2, and three contain both F_1 and F_2.

of *Xho* I in both of these combinations should maximize our ability to detect clustering, because this well-conserved site is particularly likely to separate neighboring *sigma* elements from one another: only inverted repeats of *sigma* elements with the *Xho* I sites distal to each other would not be separated by this enyzme.

Nitrocellulose filters, prepared from gels containing the multiple digests described above, were hybridized with the *sigma* probe and revealed *sigma*-hybridizing fragments that ranged in size between 0.45 and 8.0 kb (median 1.3 kb). Only in the case of the V family did this procedure reveal more than one *sigma* element on one of the *sigma*-hybridizing R+H fragments characterized in Fig. 2. The 6.6-kb R+H fragment of the V family clone was resolved into two *sigma*-hybridizing fragments in the triple digest. Assuming that an *Xho* I site is present in these *sigma* elements, our analysis would place the two *sigma* elements about 5 kb apart.

As previously mentioned, R+H digestion had demonstrated that the F family had two separate *sigma*-hybridizing fragments (F_1 and F_2). More detailed restriction mapping revealed that the F_1 and F_2 *sigma*-hybridizing fragments are separated by a 1.7-kb fragment that does not hybridize with either the *sigma*-specific or the tRNA-specific probe. Moreover, because 2 of the 23 families had 2 *sigmas* each, the total number of cloned loci from this yeast strain is 25.

Our method of analysis provides information about the presence or absence of multiple *sigma* elements within intervals of 10–30 kb of one another, with the precise amount of DNA analyzed depending on the family in question. At this level of analysis, the F and V families appear to be the only examples in our collection of *sigma* clustering, and in these two cases the DNA stretches separating the neighboring elements are dissimilar in length and restriction map. Thus, the general rule appears to be that *sigma* occurs as an individual element.

Correlation of Cloned *sigma* Sequences with Genomic Copies of the Element. The data in Fig. 2 suggest that most of the cloned families contain a *sigma*-hybridizing R+H fragment that corresponds reasonably well in size with a *sigma*-hybridizing R+H fragment in total yeast DNA. We used two additional approaches to make more precise correlations between cloned and genomic *sigma* sequences. First, we analyzed R+H digests of yeast nuclear DNA on agarose gels of different compositions in order to optimize separation of *sigma*-hybridizing bands throughout the relevant size range; at least 29 bands could be distinguished by this method. The two band clusters at about 8 and 6.6 kb (Fig. 2) are multiplets that could be readily resolved into at least three and four bands, respectively, on low-percentage agarose gels. Second, R+H digests of all 19 clones analyzed in Fig. 2 were mixed to allow the direct juxtaposition of two gel lanes containing the entire sets of genomic and cloned *sigma* elements (Fig. 3). In 18/19 cases the *sigma*-hybridizing R+H bands from the composite clone lane comigrate with bands in the genomic lane. The only obvious exception to this rule is a 5.4-kb band in the composite clone lane, which is derived from the single-member T family (see below).

The five families that gave *sigma* hybridization to a λ–yeast fusion fragment in R+H digests had to be analyzed by a less direct method. The entire λ clones were nick-translated and hybridized to R+H-digested genomic DNA. These hybridizations gave a light background of *sigma*- and tRNA-related bands and an intense series of bands corresponding to genomic R+H fragments that have single-copy homology with the probe. For each clone, this intense series of bands contained all the bands seen on R+H digestion of the clone, and in most cases two additional bands as well. These extra bands correspond in size to the yeast R+H fragments in the genome that are partly represented in the λ–yeast fusion fragments of the clones. In four cases, one of the two new bands appeared to migrate with a *sigma* band in the genomic lane that had not been accounted for by any of the clones analyzed above; no specific assignment could be made in the fifth case (W family). Including the four tentative assignments described above, we were able to correlate 22 of the cloned *sigma*-hybridizing fragments with 22 of the 29 bands visible in the genomic hybridization experiments. Because two *sigma* elements were detected on the R+H fragment from the V family, this represents 23/30 *sigma* loci identified in the genome. No assignment could be made in the case

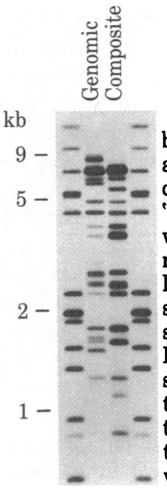

FIG. 3. Comparison of the sizes of the *sigma*-hybridizing R+H fragments present in yeast DNA and a mixture of DNAs derived from *sigma*-containing λ clones. The outside lanes contain size markers. The "genomic" lane contains yeast nuclear DNA digested with R+H, run on a 1% agarose gel, transferred to nitrocellulose, and hybridized to the *sigma* probe. At least 24 of the 29 *sigma*-positive bands can be resolved on this autoradiogram. The "composite" lane shows the pattern obtained after hybridization of R+H-digested DNA from the combined 19 clones shown in Fig. 2. Some variability in the intensity of the genomic bands was consistently seen: in addition to the multiplets at about 8.0 and 6.6 kb, the most intense band was 4.2 kb, and the least intense bands were 1.10, 1.15, 2.8, and 10.3 kb.

of one cloned *sigma* locus (W family), and the T family does not have a corresponding genomic fragment with *sigma* homology.

The single clone in the T family is of particular interest in light of the evidence suggesting that *sigma* is a transposable element. This clone may contain *sigma* at a new locus as a result of a transposition event; a minor population of the yeast cells whose DNA was packaged during preparation of the library might have contained this insertion. If this hypothesis is true, *sigma* has retained specificity for insertion adjacent to a tRNA gene, because this fragment also hybridizes with the tRNA probe (Fig. 2). However, other possibilities such as other genomic DNA rearrangements or an outright cloning artifact have not yet been ruled out.

None of our families has the restriction pattern that would be expected if *sigma* had inserted next to the gene encoding 5S RNA. The λ–yeast library that we employed, however, is underrepresented in clones derived from the ribosomal repeat (unpublished data), so our results have little bearing on whether or not *sigma* is ever associated with this other class of RNA polymerase III-transcribed genes.

DISCUSSION

We report here substantial progress towards a global analysis of the *sigma* family of transposable elements in a typical laboratory yeast strain. Analysis of *sigma* hybridization to restriction digests of yeast nuclear DNA indicates that there are about 30 copies of *sigma* in the haploid genome. Our 98 *sigma*-hybridizing yeast–λ clones can be grouped into 23 families, representing 25 distinct loci cloned from this strain. Further analysis of these clones by hybridization experiments with *sigma* and tRNA probes provided answers to the following questions: (*i*) Is there a consistent, close association between *sigma* and tRNA genes? (*ii*) If a tRNA-coding region is found in close proximity to *sigma*, is there a preferred relative orientation between these two sequences? (*iii*) Do different copies of *sigma* have any tendency to occur in pairs or clusters?

On the first point, the evidence for a consistent pattern of close association between *sigma* and tRNA genes is overwhelming. It is estimated that there are about 300 tRNA genes dispersed in the yeast genome (7, 12). Assuming about 1.5×10^4 kb per haploid genome, there would be an average of one tRNA gene for every 50 kb. Because the λ clones in our yeast library have inserts of 12–20 kb, only one out of every three or four randomly selected clones would be expected to have a tRNA gene. Our observations on *sigma*-containing clones are in sharp contrast to this expectation. Not only did every *sigma*-containing clone have a tRNA gene, but also in all but one case the same R+H double-digest fragment hybridized to both *sigma* and tRNA probes. Furthermore, the *sigma*-hybridizing band was frequently the only yeast restriction fragment with tRNA homology in the clone, although, not unexpectedly, occasional clones had two or three tRNA-hybridizing fragments. Because there are almost 10 times as many tRNA genes as *sigma* elements, it is clear that not all tRNA genes have an adjacent *sigma*. Conversely, however, it appears probable that every *sigma* element is adjacent to or closely associated with a tRNA gene.

With respect to orientation, in all five cases in which the sequences of *sigma* are known the elements are 16–18 bp upstream from the 5′ end of the tRNA-encoding region, and in four of them the conserved *Xho* I site is at the end of *sigma* distal to the tRNA gene (1, 2). Of the 22 loci that we could analyze, 16 (73%) of the *sigma* loci appear to be in this orientation relative to the adjacent tRNA-encoding region.

The conserved *Xho* I restriction site also helped to determine if pairing or clustering of *sigma* elements occurs: in only two cases (F and V) were a pair of *sigma* elements found on the same clone, and in these instances the *sigma* elements appear to be separated by about 2 and 5 kb, respectively. Thus, our analysis indicates that *sigma* rarely if ever occurs as the flanking ends of a larger element, in contrast to the *delta* sequences that flank Ty elements in yeast (13–16); *sigma* usually occurs as an individual element.

We have previously discussed the structural and evolutionary evidence that *sigma* is a transposable element (2). Although this evidence is compelling, a *sigma* transposition *de novo* has not yet been demonstrated. Nevertheless, our data emphasize that the association of *sigma* with tRNA-coding regions is a central feature of this element that must be explained. Because we have cloned nearly the entire set of *sigma* sequences, the groundwork is in place for a detailed examination of the basis of *sigma* insertion specificity and the possible effects of *sigma* on tRNA gene transcription and function.

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