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# A Yeast Sigma Composite Element, TY3, Has Properties of a Retrotransposon*

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**Sigma is a 340- or 341-base pair repetitive element which is located almost exclusively within 19 base pairs of the 5′ ends of various tRNA genes in the *Saccharomyces cerevisiae* genome. Although most sigma elements characterized to date are isolated insertions, a few of the elements occur relatively closely spaced. One of these pairs is a direct repeat of the sigma element separated by an internal domain 4.7 kilobase pairs in length. Not only does this structure resemble a composite transposable element, but regions within the sigma elements and intervening domain are homologous to conserved regions in retroviruses and retrotransposons of yeast and other organisms. Two features suggest that the sigma elements and intervening DNA transposed in a concerted event: only one of the two sigma elements is associated with a tRNA gene, and only the outside ends of the two elements are flanked by the 5-base pair direct repeats that usually flank individual sigma insertions. Examination of genomic DNA from five laboratory strains indicates that the 4.7 kilobase pair internal domain is present in one to four copies per haploid genome and that the genomic location of this domain differs from strain to strain. In addition, Northern blot analysis showed the presence of a 5.2 kilobase poly(A) transcript which hybridizes to both sigma and internal domain-specific probes. The existence of this composite element may suggest new ways to consider the mechanisms by which retrotransposons select their targets.**

Transposable elements are DNA sequences which are capable of movement from one position to another in the genome. Depending upon their sites of insertion, they can modify both the expression and arrangement of host genes (for reviews, see Kleckner, 1981; Finnegan, 1985; Roeder and Fink, 1983; Williamson, 1983; and Varmus, 1983). One class of transposable elements, which includes the retroviruses of birds and mammals and the retrovirus-like (retrotransposon) elements of yeast and fruit flies, transposes through an RNA-mediated process. This common mode of transposition is reflected in the similar structures of the integrated elements. Retrotransposons are flanked by 4–6-bp[1] direct repeats of target sequence. Each element is a composite consisting of an internal region of several kbp, flanked by LTRs of several hundred bp each. The ends of these LTRs are inverted repeats, up to 10 bp in length, which terminate with the conserved dinucleotides TG-CA. The LTRs contain initiation, termination, and polyadenylation signals which direct transcription of the internal coding sequences. Hormone-response sequences and/or enhancer sequences (Laimins *et al.*, 1983; Errede *et al.*, 1985; Roeder *et al.*, 1985) which regulate this transcription have also been identified, both in the LTRs and in the promoter-proximal portion of the internal domain. The retrotransposon gene products that have been identified include proteins with homology to the retroviral *gag*- and *pol*-encoded proteins (Saigo *et al.*, 1984; Mount and Rubin, 1985; Clare and Farabaugh, 1985; Hauber *et al.*, 1985; and Warmington *et al.*, 1985).

Two closely related sets of yeast retrotransposons have been described, Ty1, or Class I (Cameron *et al.*, 1979; Farabaugh and Fink, 1980; Gafner and Philippsen, 1980), and Ty2, or Class II (Kingsman *et al.*, 1981). They are present in a total of 30–35 copies per haploid genome in standard laboratory strains. Ty1 and Ty2 elements consist of an internal domain, called epsilon, which is 5.3 kbp in length, and two flanking direct repeats, called delta elements, which are 334–338 bp in length. Ty insertion in the 5′-flanking sequence of a gene can inactivate that gene (Chaleff and Fink, 1980; Roeder and Fink, 1980; Eibel and Philippsen, 1984) or increase its expression, an increase which is correlated with the expression of Ty itself (Errede *et al.*, 1980; Williamson *et al.*, 1981). Ty elements contain blocks of sequence (Roeder *et al.*, 1985; Errede *et al.*, 1985) with homology to the SV40 enhancer core sequence (Laimins *et al.*, 1983) that overlap blocks of sequence with homology to mating-type control regions (Strathern *et al.*, 1981; Miller *et al.*, 1985; Siliciano and Tatchell, 1986). Regions containing these elements mediate at least some of the observed regulation of Ty transcription. The expression of Ty in yeast has also been correlated with levels of reverse transcriptase activity (Garfinkel *et al.*, 1985; Mellor *et al.*, 1985). Boeke *et al.* (1985) recently showed that increased transcription of a particular Ty element resulted in increased levels of Ty transposition. In addition, when a yeast intron was introduced into a marked Ty, it was spliced out during transposition, thereby demonstrating an RNA intermediate.

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*The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J03500.*

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[1] The abbreviations used are: bp, base pairs; kbp, kilobase pairs; kb, kilobase(s); LTR, long terminal repeat; SNV, spleen necrosis virus; SDS, sodium dodecyl sulfate.

Resolution of sequence differences between the two delta termini of a single Ty in the course of transposition was observed by Boeke *et al.* (1985) and is consistent with a retrovirus-like mechanism of transposition.

In addition to delta, two other short, repetitive elements, sigma (del Rey *et al.*, 1982; Sandmeyer and Olson, 1982) and tau (Genbauffe *et al.*, 1984), are found in the yeast genome. Isolated delta, sigma, and tau elements are present in 15–30 copies each in the yeast haploid genome. Sigma and tau elements are related to delta elements by sequence similarities at their termini and by 6–11 short (5–10 bp) similar sequences spaced between the termini (Genbauffe *et al.*, 1984). Sigma, tau, and delta elements are all flanked by direct 5-bp repeats of their insertion sites; however, no *de novo* transposition of any of these isolated elements has ever been demonstrated. Unlike delta elements, sigma and tau elements have not been found associated with large repetitive coding domains, such as epsilon. This has encouraged speculation that sigma and tau elements are not active transposable elements. On the other hand, the possibility remains that an unidentified subset of these elements might exist as part of a larger, mobile genetic element.

Sigma elements are distinguished from delta and tau elements by their extremely close association with tRNA genes (Brodeur *et al.*, 1983). A survey of the position and orientation of sigma elements in one strain of yeast has shown that 15 of 17 sigma elements are found starting at −16 to −19 bp upstream of tRNA genes.[2] While most sigma elements are separated in the genome, a few are found in proximity to one another (Brodeur *et al.*, 1983). We have investigated the properties of one of these pairs of sigma elements, and we report here that, together with a 4.7-kbp intervening DNA sequence, it possesses the structural features of a retrotransposon described above. In addition to these properties, almost full-length transcripts from this element are present in the poly(A) RNA. These observations suggest that this sequence constitutes a retrotransposon with sigma termini. If this element is a member of an uncharacterized subset of sigma elements which are transpositionally active, then this subset has retained the unusual tRNA-gene association which characterizes the isolated elements previously described. The present studies should aid in the construction of an appropriate selection system in which the position-specific transposition specified by the sigma element could be observed.

## EXPERIMENTAL PROCEDURES

*Bacterial and Yeast Strains*—Lambda 112 (V family, Brodeur *et al.*, 1983) was grown in *Escherichia coli* C600 [$F^-$ *thi-1 thr-1 leu B6 lacY1 tonA21 supE44* $\lambda^-$]. Plasmids were cultured in the *E. coli* strain JM109 [*recA1 endA1 gyrA96 thi, hsdR17 supE44 relA1* $\lambda^-$ Δ(*lac-proAB*)] [F′ *traD36 proAB lacI*$^q$*Z* Δ *M15*] (Yanisch-Perron *et al.*, 1985). Bacterial culture conditions were as described by Miller (1972).

AB972, a rho° derivative of the *S. cerevisiae* strain BJ 1983 (Woolford *et al.*, 1986), which is a *trp1* derivative of X2180-1B (*MAT α suc2 mal mel gal2 CUP1*) was the source of the yeast genomic insert in lambda 112 (Brodeur *et al.*, 1983). X2180-1A (*MATa SUC2 mal mel gal2 CUP1*), a sister segregant of X2180-1B (Mortimer and Johnston, 1986); X2180-1B; and ySBS500, a diploid constructed by crossing X2180-1B and X2180-1A, were the sources of poly(A) RNA. X2180-1B, B596 [*MATa lys2 his1 trp2 cyc1-9* (Sherman *et al.*, 1974)], Xp8-4A [*MATa his6 leu1 met1 trp5-1 gal2 can1* (Kushner *et al.*, 1979)], Y4A (prototroph of unknown ploidy and mating type) and YNN281 [*MATa trp1Δ-901 ura3-52 his3Δ200 ade2-101 lys2-801* (Hieter *et al.*, 1985)] were the sources of yeast genomic DNA. Yeast were cultured as described by Sherman *et al.* (1982).

*Enzymes and Chemicals*—Restriction endonucleases (with the exception of *Nde*I), DNA polymerase I (large fragment), and calf alkaline phosphatase were purchased from Boehringer Mannheim. *Nde*I and T4 DNA ligase were obtained from New England Biolabs. Polynucleotide kinase, S1 nuclease, and T4 RNA ligase were obtained from P-L Biochemicals. Sequenase was obtained from U. S. Biochemical Corporation. Radionuclides, including [$\gamma$-$^{32}$P]ATP (5000 Ci/mmol), [$\alpha$-$^{32}$P]dATP (3000 Ci/mmol), [$\alpha$-$^{32}$P]GTP (3000 Ci/mmol) and cytidine [5′-$^{32}$P] 3′,5′-bisphosphate (3000 Ci/mmol), were obtained from Amersham Corp. The hexanucleotide primer mixture for randomly primed synthesis of radioactive probes was obtained from P-L Biochemicals. Total bakers' yeast tRNA (type X-S), used as a probe for tRNA genes, and yeast $\alpha$-factor were obtained from Sigma. Oligo(dT)-cellulose was obtained from Collaborative Research. Plasmid pIBI20 was purchased from International Biotechnologies, Inc. The synthetic oligonucleotide, 5′ GTAACCTTTAACGCTACCAAG 3′, was synthesized by Louise Schmidt, University of California, San Diego, PON Facility. It is complementary to the Ty3 transcript in the left-most region of the internal domain. The set of RNA markers was obtained from Bethesda Research Laboratories.

*Manipulations of Recombinant Clones*—Rapid isolation of analytical amounts of plasmid DNA was by the alkaline lysis procedure of Birnboim and Doly (1979). Large scale preparation of DNA was carried out using a modification of the boiling method (Holmes and Quigley, 1981). Supercoiled plasmid DNA was further purified by centrifugation to equilibrium in a cesium chloride density gradient.

The 6.5-kbp *Hin*dIII/*Eco*RI fragment from lambda clone 112, which contained the two sigma elements, was subcloned by standard procedures into the *Hin*dIII and *Eco*RI sites of the pIBI20 polylinker. The recombinant plasmid is referred to as pSBS12. Single-stranded pSBS12, of the same sense as the Ty3 transcript, was produced by infecting NM522 cells transformed with pSBS12 with the single-stranded helper phage M13K07 (IBI). The 112 lambda clone was identified originally as a clone containing two sigma elements associated with tRNA genes (Brodeur *et al.*, 1983); the present studies indicate unambiguously that only one tRNA is present on this *Hin*dIII/*Eco*RI fragment (see "Results"). Restriction sites in the yeast insert were mapped by digestion with various combinations of enzymes including *Hin*dIII, *Eco*RI, *Acc*I, *Nde*I, *Pst*I, *Sal*I, and *Xho*I. Digestions were carried out under standard conditions specified by suppliers' instructions. The cloning of pPM15s, a 204-bp internal fragment from the sigma element associated with *SUQ5*, into the *Eco*RI site of pBR322, was described previously (Brodeur *et al.*, 1983). The *Xho*I/*Taq*I 339-bp fragment, containing almost the complete *SUP2* sigma element (Sandmeyer and Olson, 1982), was cloned in this study into the *Eco*RI and *Hin*dIII sites of pSP64 yielding the plasmid, pSBS6. The Ty probes were derived from Ty1 (S13) (Cameron *et al.*, 1979) from R. Davis, Stanford University.

*Sequence Analysis*—Sequence analysis of the two sigma elements and flanking DNA in pSBS12 was by the chemical modification method of Maxam and Gilbert (1980). The sequencing strategy is shown in Fig. 1. Sequence was compiled, edited, and translated using the DNA sequence analysis programs of A. Goldin (California Institute of Technology) and G. Gutman (University of California, Irvine). Comparisons with the GenBank Data Base were made through the core library programs of BIONET (Intelligenetics, Inc.). The homologous sequence ladder used for size determinations in the 5′-mapping experiment was primed with the synthetic oligonucleotide described above and synthesized by the dideoxy-chain termination method (Sanger *et al.*, 1977) on a double-stranded pSBS12 DNA template with reverse transcriptase. The sequence ladder used for 3′ mapping was produced by chemical modification and degradation of the homologous end-labeled DNA.

*DNA and RNA Hybridization Analysis*—DNA and RNA hybridization probes were prepared from restriction fragments by randomly primed synthesis in the presence of [$\alpha$-$^{32}$P]dATP, as described by Feinberg and Vogelstein (1983; 1984). The tRNA probe was prepared by reacting the 3′ ends of yeast tRNA with [5′-$^{32}$P]cytidine 3′,5′-bisphosphate in the presence of T4 RNA ligase under conditions specified in a modification of the procedure of Bruce and Uhlenbeck (1978; Pirtle *et al.*, 1980). Unincorporated label was separated from tRNA by chromatography over Sephadex G-50. RNA size markers were made by radiolabeling the Bethesda Research Laboratory RNA ladder in the same manner. The synthetic oligonucleotide was end-labeled by reacting it with [$\gamma$-$^{32}$P]ATP in the presence of polynucleotide kinase.

Yeast genomic DNA was prepared from cells grown to early stationary phase in 1% yeast extract, 2% peptone, and 2% glucose medium, following the protocol described by Boeke *et al.* (1985). DNA samples for hybridization analyses were digested with restriction

[2] S. B. Sandmeyer, V. W. Bilanchone, D. J. Clark, P. Morcos, G. F. Carle, and G. M. Brodeur, unpublished data.

endonucleases and fractionated by electrophoresis in 1% agarose gels, buffered in 2.5 mM EDTA, 45 mM borate, and 133 mM Tris-HCl, pH 8.3. Electrophoresis was at 2 V/cm for 14–16 h. The DNA was transferred to nitrocellulose by the method of Southern (1979). Radioactive probes were denatured by boiling for 3 min and chilling immediately on ice. Blots were hybridized in 1% SDS, 0.5 M NaCl, 2.5 mM EDTA, and 0.1 M $Na_2HPO_4$, pH 7.0, for 14–16 h at 65 °C (DNA probe) or 55 °C (tRNA probe). Size markers were lambda DNA cut with *Hin*dIII, and 5′ end-labeled with $\gamma$-$^{32}$P or hybridized to a radioactive lambda probe. After hybridization, filters were washed four times, for 5 min each, in 10 mM Tris, pH 8.0. The first wash included 0.1% SDS. Filters were exposed to Kodak XAR-5 film at room temperature or at −70 °C with a Cronex Quanta III intensifying screen, manufactured by Du Pont.

Yeast RNA was isolated from cultures grown to $OD_{660}$ = 0.7 to 0.8 (approximately 3 × $10^7$ cells/ml of broth) by vortexing the cells with glass beads in the presence of buffer, chloroform, and phenol as described by Elder *et al.* (1983). Cells were treated, where noted, with 0.4 μM α-factor for 30 min before RNA isolation, as described by Stetler and Thorner (1984). Poly(A) RNA was further purified by chromatography over oligo(dT)-cellulose as described by Aviv and Leder (1972). Poly(A) RNA samples for hybridization analyses were denatured by reaction with glyoxal as described (McMaster and Carmichael, 1977) and subjected to electrophoresis in a 1.1% agarose gel in 10 mM NaP, pH 7, at 6 V/cm for 3 h. The RNA was transferred directly to nitrocellulose, hybridized, and washed as described by Thomas (1980). Nitrocellulose filters with bound RNA were preincubated in hybridization buffer for 1–2 h. Radiolabeled probes were denatured as before and added to the hybridization buffer. Hybridization buffer was 4 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 10% dextran sulfate, 0.8% Denhardt's solution, 40 mM $Na_2PO_4$, pH 8.6, and 40% formamide. Hybridizations were for 14–16 h at 38 °C. Following hybridization, the filters were washed four times in 2 × SSC and 0.1% SDS, at room temperature, two times in 0.1 × SSC and 0.1% SDS, at 38 °C, and once in 2 × SSC at room temperature. The washes were 5 min each, except the washes at 38 °C, which were 15 min each. Hybridization with the 5′-labeled oligonucleotide probe was as described by Berent *et al.* (1985).

*Determination of the 5′ Ends of the Ty3 Transcript by Reverse Transcription Extension of a Synthetic Oligonucleotide Primer*—The 5′ ends of the Ty3 RNA were determined by reverse transcription from an end-labeled synthetic oligonucleotide primer (5′ GTAACCTTTAACGCTACCAAG 3′) hybridized to poly(A) RNA (Ghosh *et al.*, 1978). This oligonucleotide is complementary to the Ty3 transcript for the first 21 bases within the internal domain and downstream of the left sigma element. 15 μg of poly(A) RNA was annealed to 2 × $10^6$ cpm of oligonucleotide primer for 2 h at 37 °C in 5 × reverse transcription buffer (1 × is 50 mM Tris, pH 8.3, 10 mM $MgCl_2$, 0.4 mM dithiothreitol, and 80 mM KCl). The synthetic reaction was carried out for 1 h at 37 °C with 6.5 units of reverse transcriptase per reaction volume of 21 μl in 1 × buffer. Actinomycin D was present at 100 μg/ml and dNTPs were present at 500 μM in the final reaction. One-third of the precipitated reaction, redissolved in electrophoresis sample buffer, was loaded per lane on a denaturing gel. Electrophoresis conditions were as described by Sanger *et al.*, 1977.

*Determination of the 3′ Ends of the Ty3 Transcript by S1 Nuclease Protection Mapping*—The 3′ ends of the Ty3 transcripts present in α-factor-treated cells were determined by S1 nuclease protection experiments performed essentially as described (Berk and Sharp, 1978). The end-labeled DNA used for determination of the 3′ end of the Ty3 transcript was a 1-kbp *Acc*I restriction fragment which spans the downstream sigma element (see Fig. 1). This fragment was specifically labeled on the 3′ end that occurs within the Ty3 internal domain, by extension with Klenow polymerase in the presence of [α-$^{32}$P]dCTP and cold dGTP, dTTP, and dATP. The degeneracy of the *Acc*I recognition site made it possible to label the internal end of this fragment specifically. 5 × $10^5$ cpm of denatured, end-labeled DNA was hybridized to 15 μg of poly(A) RNA and digested with 1000 units or 1500 units of S1 nuclease per reaction. The sizes of the end-labeled, protected fragments were determined by electrophoretic migration on denaturing acrylamide gels compared to a sequence ladder of the same strand. The sequence ladder was produced by chemical modification, followed by pyridine degradation (Maxam and Gilbert, 1980) of the end-labeled *Acc*I fragment.

## RESULTS

*A Repeated Sequence Flanked by Sigma Elements*—In a survey of sigma elements from yeast strain AB972, one family of lambda clones, the V family (including clone 112), was identified which contains two sigma elements separated by 4.7 kbp of DNA (Brodeur *et al.*, 1983). In order to facilitate discussion, we will refer to the region between these two sigma elements as the internal domain and the entire structure, including the sigma elements, as the sigma composite element (Ty3). This composite structure is reminiscent of the Ty1 and Ty2 elements, which are composed of an internal domain flanked by direct repeats of delta elements. Because delta elements have some similarities with sigma elements, a structural study of the composite sequence in lambda clone 112 was undertaken to determine whether it constitutes a sigma-flanked, transposed sequence. The 6.5-kbp *Hin*dIII/*Eco*RI fragment from the lambda V family, which contains the two sigma elements, 4.7 kbp of intervening DNA and 1.2 kbp of flanking DNA, was subcloned into the *Hin*dIII/*Eco*RI site of pIBI20. This clone was designated pSBS12.

Several approaches were pursued to determine if the two sigma elements in pSBS12 were functionally associated or were only coincidentally closely positioned in the genome. First, the yeast DNA insert was digested with various combinations of *Acc*I, *Nde*I, *Xho*I, *Pst*I, and *Sal*I, and the fragments were analyzed to deduce a restriction map (Fig. 1). The internal domain between the two sigma elements was then analyzed for possible sequence homology to the epsilon region of Ty. The fragment containing the yeast insert in pSBS12 was isolated by digestion with *Eco*RI and *Hin*dIII followed by electrophoresis in a 1% agarose gel and was then transferred to nitrocellulose by the method of Southern (1979). The DNA was hybridized with a $^{32}$P-labeled, 1.6-kbp *Eco*RI/*Sal*I frag-

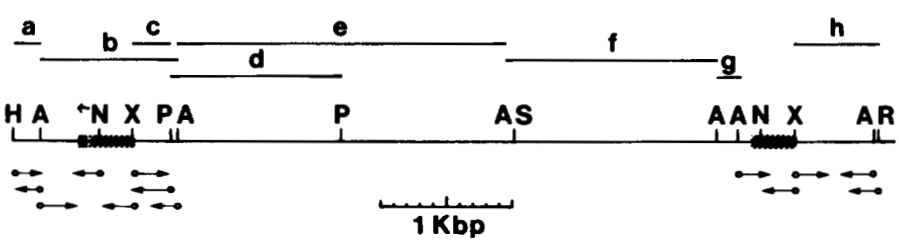


FIG. 1. **Restriction site map of the 6.5-kbp *Hin*dIII/*Eco*RI yeast insert in plasmid pSBS12.** The pSBS12 yeast insert was digested according to manufacturers' specifications with various combinations of restriction enzymes and fractionated by electrophoresis in a 1% agarose gel in Tris/borate/EDTA buffer. The fragment sizes derived from these digests were analyzed and a restriction site map was deduced. The *boxed* regions indicate sigma (*lined*) and $tRNA^{Cys}$ gene (*stippled*) sequences. These regions were identified by Southern blot and by sequence analysis. The *arrow* indicates the direction of transcription of the tRNA gene, 5′–3′. Sigma elements are distinguished in the text as left and right, as shown in this diagram. The fragments used as probes (*a–h*) are indicated by a *horizontal line* extending the length of the fragment. The DNA sequencing strategy is shown at bottom with $^{32}$P-labeled 5′ ends of strands indicated as *circles*, and the 3′ ends indicated as *arrows*. Abbreviations are: *H*, *Hin*dIII; *A*, *Acc*I; *N*, *Nde*I; *P*, *Pst*I; *X*, *Xho*I; *S*, *Sal*I; and *R*, *Eco*RI.

ment from the internal region of Ty1 element S13 (Cameron *et al.*, 1979) and with a $^{32}$P-labeled, 2.5-kb *Sal*I fragment, which includes a delta element from the S13 Ty. The second probe includes sequences which are homologous to Ty1 and Ty2. Neither the epsilon nor the epsilon plus delta probes hybridized to the internal domain of the sigma composite. The internal domain also failed to hybridize to a plasmid which contained a copy of the tau element.[2]

Because the internal domain did not show homology to Ty1 or Ty2 by the criterion of hybridization under these conditions, the alternative possibility, that the two sigma elements in pSBS12 are part of a repetitive element which is not closely related to Ty1 and Ty2, was investigated. Genomic DNA from X2180–1A was digested with *Eco*RI, fractionated by electrophoresis, and transferred to nitrocellulose by the method of Southern. Restriction fragments that together spanned the entire yeast insert in pSBS12 were labeled and used as hybridization probes (Fig. 1). A sigma-specific probe, the pPM15s insert, was used to visualize the genomic fragments with sigma similarity. The autoradiograph of these hybridizations is shown in Fig. 2. Probes derived from fragments located outside the sigma elements hybridized only to a fragment 9.7 kbp in length (*probes a* and *h, lanes 2* and *8*). Probe b, derived from the region containing the entire left-hand sigma and some flanking DNA, produced multiple bands on the autoradiograph (*lane 3*). This pattern differed from that generated by the internal sigma probe (*lane 1*) only by the presence of an additional band, indicated by the *arrow* in lane 3. The origin of the additional band in the probe b pattern is discussed below. Probes *c–f* (*lanes 4–7*) are derived from the DNA between the two sigma elements and do not have homology to the sigma element. Interestingly, all of these internal probes hybridized to both the DNA fragment 9.7 kbp in length, which hybridized to probe a, and to another fragment 6.5 kbp in length, which is not represented in the probe a pattern. The patterns generated by the sigma-specific probe and probe b are more complex but also have a band at both 9.7 and 6.5 kbp, suggesting that the internal domain is associated with sigma elements on the 6.5-kbp fragment as well. These results indicate that the region between the two sigma elements in the pSBS12 insert is repeated in the genome, whereas the regions outside the sigma repeats are represented only once. This hybridization pattern is consistent with a repeated domain, approximately 5 kbp in length, which terminates near or within flanking sigma elements.

*Number and Location of Composite Elements in Different Strains of Yeast*—Transposable elements, by definition, have polymorphic genomic loci. The genomic constancy of the internal domain of pSBS12 was investigated by comparing hybridization patterns among five laboratory strains of *S. cerevisiae*. The genomic DNA from these strains was prepared as described under "Experimental Procedures" and digested with *Eco*RI. The restriction fragments were separated by electrophoresis and transferred to nitrocellulose. The various DNAs were each hybridized with probe e (as defined in Fig. 1) from the internal domain. The rather striking result is shown in Fig. 3: the number of fragments which hybridize to the internal domain sequence ranges from one to four in the five different genomes tested, and the sizes of the hybridizing fragments from different strains are dissimilar. Because neither probe hybridized to any fragments smaller than 6.5 kbp, the pattern is consistent with elements of 5.4 kbp which do not have internal *Eco*RI sites. If *Eco*RI sites are absent in the genomic copies of the pSBS12 composite sequence, then these different numbers of bands correspond to different numbers of copies of sequences closely related, or identical to, the

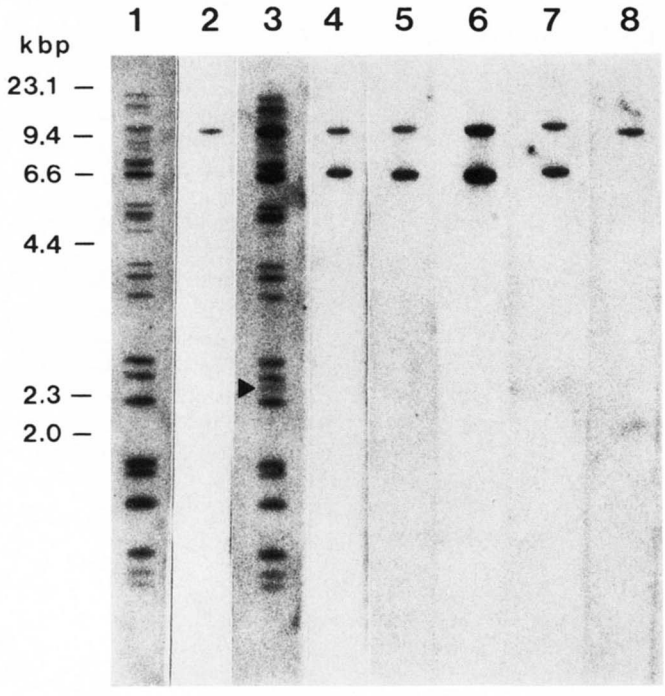


FIG. 2. **Blots of genomic DNA probed with repeated and unique regions of the pSBS12 yeast insert.** Genomic DNA from yeast strain X2180–1A was digested with *Eco*RI. Replicate samples (10 μg) were electrophoresed in a 1% agarose gel and transferred to nitrocellulose. The DNA in individual lanes was hybridized with $^{32}$P-labeled probes: *lane 1,* DNA was probed with the radiolabeled sigma fragment insert from pPM15s; *lanes 2–8,* DNA samples were probed with radiolabeled fragments a–h, respectively. Derivation of these fragments is shown in Fig. 1. *Bars* in the left margin indicate the position of lambda DNA digested with *Hin*dIII and used as size markers.

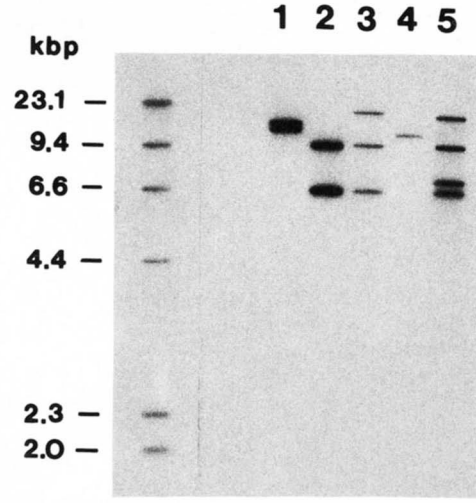


FIG. 3. **Southern blot of genomic DNA from different yeast strains digested with *Eco*RI and hybridized to an internal fragment from the sigma composite.** *Lanes 1–5,* approximately 5 μg of miniprep genomic DNA was digested with *Eco*RI, subjected to electrophoresis, blotted, and hybridized to probe e (as defined in Fig. 1). Yeast strains used as sources of genomic DNA are: *lane 1,* B596; *lane 2,* X2180–1A; *lane 3,* Xp8–4A; *lane 4,* Y4A; and lane 5, YNN281. Lambda DNA restricted with *Hin*dIII and $^{32}$P-labeled was fractionated for size standards in the left lane. Genomic fragments hybridizing to the internal probes are lane 1, 15.5 and 13.5 kbp; *lane 2,* 9.7 and 6.5 kbp; *lane 3,* 20.0, 9.7, and 6.5 kbp; *lane 4,* 12.7 kbp; and *lane 5,* 18.0, 9.7, 7.4, and 6.5 kbp. The pattern of hybridization of genomic DNA from X2180–1B is the same as shown for X2180–1A,[2] a cosegregant from diploidized S288C (Mortimer and Johnston, 1986).

pSBS12 internal domain. The size variation observed for hybridizing fragments suggests that most of these copies are in different genomic locations in different strains. Although restriction site polymorphisms could also explain these observations, hybridization of the composite probe to separated chromosomal DNAs has shown that, at least in some strains with two and four hybridizing fragments, the hybridizing sequences are located on different chromosomes.[2]

*Coordinate Insertion of Sigma Elements and the Internal Domain*—The genomic Southern blot shown in Fig. 2 places the ends of the repeated region of pSBS12 within or near the sigma elements. We hypothesized that this composite structure could be explained by the coordinate transposition of the sigma elements and internal domain into this genomic locus. We therefore examined the postulated end points of the composite element for structural evidence of a transposition event. Of the 17 sigma elements we have sequenced from AB972, all but two are within 19 bp of the 5′ end of a tRNA gene[2]; thus, a tRNA gene is one of the most conserved features of a sigma insertion site. We examined the regions surrounding the two sigma elements in pSBS12 for the expected tRNA gene target(s) by hybridization with a $^{32}$P-labeled total yeast tRNA probe. In order to assess each sigma-containing region separately, the pSBS12 plasmid was digested with *Hin*dIII and *Acc*I, and a combination of *Hin*dIII, *Acc*I, and *Xho*I, and subjected to electrophoresis on a 1% agarose gel. Fig. 4*A* shows the pattern produced by the ethidium-stained DNA. This DNA was transferred to nitrocellulose and hybridized to the tRNA probe. The autoradiograph of the filter is shown in Fig. 4*B*. The tRNA probe hybridized only to the *Acc*I fragment (*lane 2*) and to the smaller 700-bp *Xho*I/*Acc*I fragment containing the *Hin*dIII site proximal-flanking sequence and sigma (*lane 3*). This result is consistent with a single transposition which inserted two sigma elements and intervening DNA near one tRNA gene target.

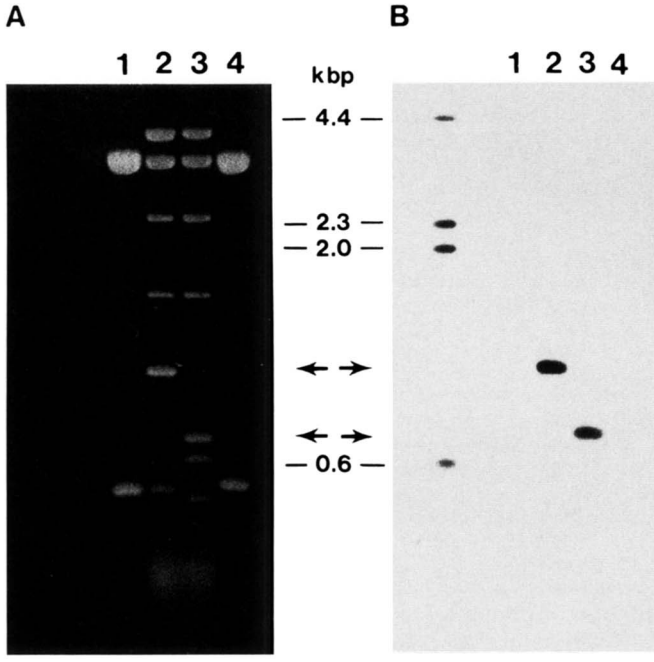


FIG. 4. **Hybridization of radioactive yeast total tRNA to fragments containing left and right sigma elements.** *A*, ethidium bromide-stained restriction digest of pSBS12. 1.5 μg of pSBS12 DNA and of vector (pIBI20) DNA were digested with *Hin*dIII and *Acc*I plus or minus *Xho*I, electrophoresed on a 1% agarose gel, and stained with ethidium bromide. *Lanes 1* and *4*, pIBI20 digested with *Hin*dIII and *Acc*I; *lane 2*, pSBS12 digested with *Hin*dIII and *Acc*I; and *lane 3*, pSBS12 digested with *Hin*dIII, *Acc*I, and *Xho*I. *B*, autoradiograph of total yeast tRNA hybridized to the insert in pSBS12. The DNA shown in *A* was transferred to nitrocellulose by the method of Southern and hybridized to total yeast tRNA labeled at the 3′ end. *Lanes 1–4* are the same as above. *Arrows* indicate the fragments which were hybridized. The *left lane* shows lambda DNA restricted with *Hin*dIII and $^{32}$P-labeled.

Sigma insertions are flanked by a 5-bp direct repeat of the target sequence (Sandmeyer and Olson, 1982). The regions of DNA surrounding the two sigma elements in pSBS12 were sequenced by the method of Maxam and Gilbert (1980). The strategy is indicated in the restriction map of Fig. 1. The sequence of the two sigma elements in pSBS12 and their immediate flanking regions is shown in Fig. 5*A*. These sigma elements are shown in the opposite orientation compared to sigma elements previously described (del Rey *et al.*, 1982; Sandmeyer and Olson, 1982). This sequence and the restriction map in Fig. 1 are oriented to correspond to the direction of transcription of the major sigma-related transcripts (left to right, 5′–3′) as determined by Northern blot hybridization with single-stranded probes[3] (Van Arsdell *et al.*, 1987). In contrast to other cases of sequenced sigma elements (del Rey *et al.*, 1982; Sandmeyer and Olson, 1982), neither of these elements is immediately flanked by 5-bp direct repeats; instead, the 5-bp sequence on the outside end of the right sigma reiterates the 5-bp sequence found on the outside end of the left sigma. These data are consistent with a single staggered cleavage of the insertion site from which direct repeats of the insertion site sequence on the outside ends of the sigma composite element resulted.

The sequence analysis corroborated and extended the conclusions from the tRNA hybridization shown in Fig. 4*B*. A 72-bp sequence with perfect homology to tRNA$^{Cys}$ (Holness and Atfield, 1976) is found extending from position −16 to position −87 (*overlined* in Fig. 5*A*). The alignment of this sequence with the tRNA sequence is shown in Fig. 5*B*. This coding sequence does not have an intervening sequence and is perfectly homologous to the coding region sequenced by Genbauffe *et al.* (1984). Absence of homology in the flanking sequence indicates that this tRNA$^{Cys}$ gene is not allelic with the one they described. Because probe b (Fig. 1) actually contains this gene in addition to the sigma element, a plausible explanation of the additional band in the probe b genomic hybridization pattern (Fig. 2, *lane 3, arrow*) is the presence of at least one additional genomic copy of an homologous tRNA$^{Cys}$ gene. The sigma-flanking sequences shown in Fig. 5*A* were also inspected for conserved patterns in tRNA genes (Galli *et al.*, 1981), compared to National Institutes of Health GenBank yeast tRNA sequences for homology, and searched for potential intrastrand pairing by BIONET core programs (Intelligenetics). Although there were regions of short homology to tRNA-coding sequences in and around the sigma elements, no evidence was discovered for any additional tRNA genes in these regions.

The properties described above characterize a repeated element which terminates with direct repeats of the sigma element and which is organizationally homologous to the previously described yeast transposable elements, Ty1 and Ty2. Because of this organizational homology and because of properties this composite element shares with eukaryotic transposable elements, we refer to this element as Ty3. A *de novo* transposition of this element in standard laboratory strains has not yet been demonstrated.

*Ty3 Structural Homology with Retroviruses and Retrotransposons*—The organization of Ty3 as an internal domain

[3] D. J. Clark, V. W. Bilanchone, and S. B. Sandmeyer, unpublished data.

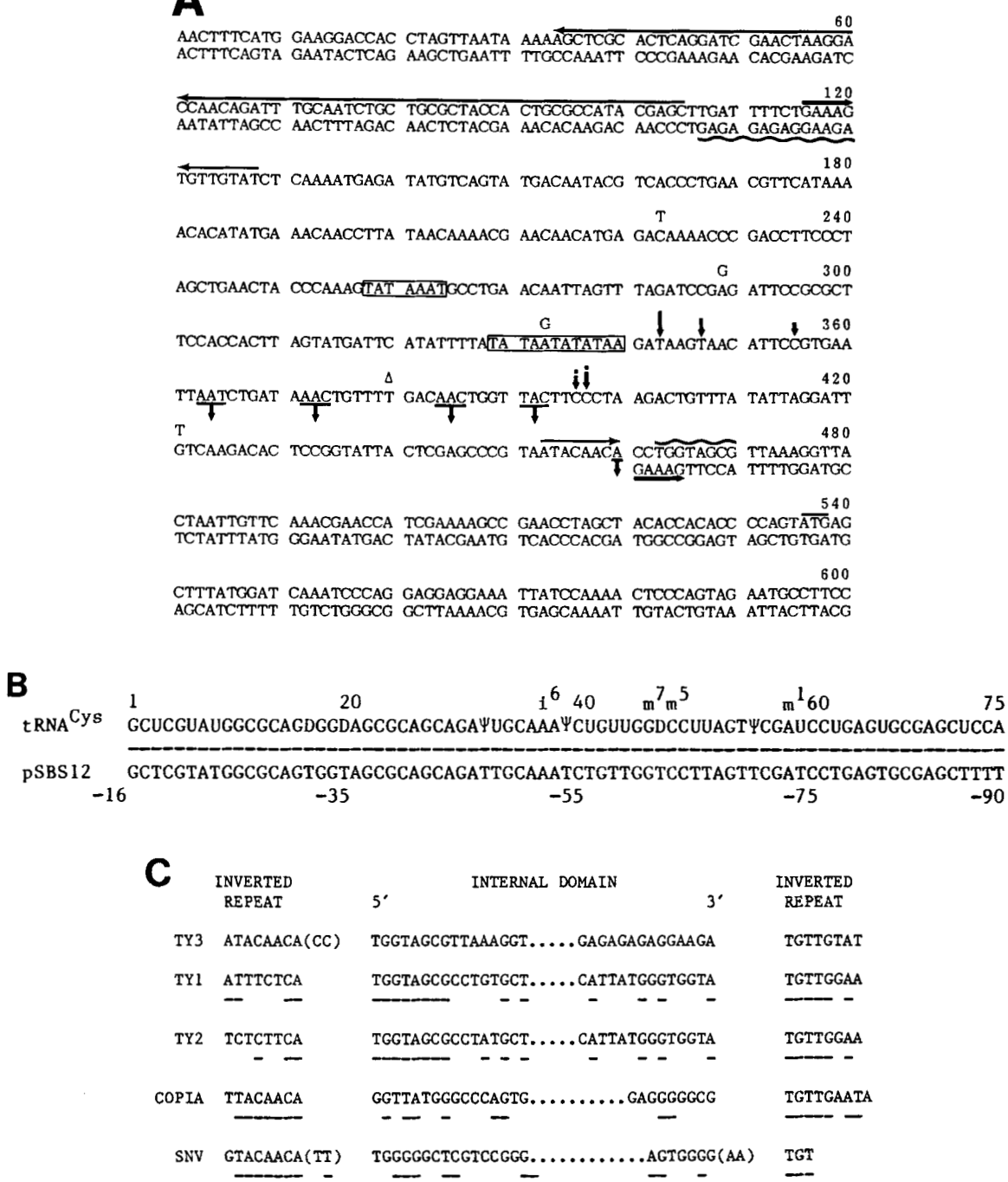


FIG. 5. **DNA sequence of sigma elements and flanking DNA from pSBS12.** *A*, DNA sequence of pSBS12 sigma elements and flanking regions. *Lines 1* and *2* are the sequences flanking the upstream and downstream sigma elements (left and right, respectively, in Fig. 1). The identical sigma elements are shown as a single sequence (121–460). 8-bp terminal inverted repeats are indicated by light arrows; 5-bp direct repeats flanking the outside ends of the sigma elements are indicated by *heavy arrows*. The $tRNA^{Cys}$-coding sequence in line 1 (−90 to −16 relative to the upstream sigma) is *overlined* with an *arrow* indicating the direction of transcription. The first ATG (76 bp downstream from the end of the first sigma) in the open reading frame that extends into the internal domain is *overlined* once. Differences between the sequence of the Ty3 sigma elements and the sequence of the sigma element described by Van Arsdell *et al.* (1987) are indicated above the sigma sequence. Sites of 5′ ends of transcripts are indicated by *arrows* above the sequence; 5′ ends of transcripts found predominantly in RNA from diploids are indicated by *dotted arrows*; sites of termination are indicated by *bars* and *arrows* below the sequence. Sequences similar to the TATA consensus are *boxed*. The plus strand primer site and minus strand primer-binding site are *underlined*, and *overlined*, respectively, with *wavy lines*. *B*, alignment of $tDNA^{Cys}$ in pSBS12 with $tRNA^{Cys}$ (Holness and Atfield, 1976). *Numbers* above the sequence are relative to mature tRNA sequence. Numbers below the sequence are relative to the start of the sigma sequence. Abbreviations for modified bases in the tRNA sequence are as follows: *D*, dihydrouridine; Ψ, pseudouridine; $A^{i^6}$, *n*-6-isopentenyladenosine; $G^{m^7}$, 7-methylguanosine; $C^{m^5}$, 5-methylcytidine; *T*, 5-methyluridine; and $A^{m^1}$, 1-methyladenosine. *C*, selected conserved domains of several retrotransposons and one retrovirus. Sequences shown are the coding strands. Left to right, LTR-inverted repeat terminus at the 5′ internal domain junction; 5′ end of internal sequence (minus strand primer-binding site); 3′

flanked by sigma elements is similar to the organization of the retrotransposon class of transposable elements. The sequence similarity between the termini of sigma elements and retrotransposons has already been noted. In Fig. 5*C* we compare the terminal repeat-internal domain junction sequences of Ty3 to analogous junction regions which are conserved among retrovirus-like elements and retroviruses. The Ty1 (Farabaugh and Fink, 1980; Elder *et al.*, 1983), Ty2 (Warmington *et al.*, 1985), copia (Levis *et al.*, 1980), and SNV (Shimotohno *et al.*, 1980) sequences are aligned to allow maximum correspondence of bases. Starting at the right-hand terminus of the left sigma, the 8 base pairs which form the terminal inverted repeats are homologous to copia and SNV termini, with the exception of the first base. The left ends of the internal domains of all five elements have some sequence homology. This homology is greatest among Ty1, Ty2, and Ty3 elements. The 8 base pairs which are identical among the three yeast elements perfectly complement a region at the 3′ end of $tRNA_i^{Met}$ (Venegas *et al.*, 1982; previously noted by Eibel *et al.*, 1980, for Ty1). In retrovirus RNA this region is the minus strand primer-binding site and is typically complementary to a tRNA. The left-most 2 bases of the internal domain are not part of the region with tRNA homology. Thus if Ty3 is replicated from a tRNA primer, it is predicted, as has been shown for retroviruses such as SNV, to lose its terminal dinucleotide sequences upon integration. At the 3′ end of the internal domain of retroviruses is an oligopurine sequence postulated to play a role in plus-strand priming (see Varmus, 1983 for review). Similarly, at the right end of the transcript strand of the internal domain flanked by sigma elements is a sequence of 14 purine residues. This sequence of purines is followed by the sigma terminal repeat in the inverted orientation. Unlike the other elements shown here, the sigma termini are perfect inverted repeats of each other. This right-hand end is also identical to the Ty1- and Ty2-terminal regions at 6 out of 8 positions.

The identity of the two sigma sequences in Ty3 is consistent with the proposed retrotransposon analogy. The RNA intermediate, in retrovirus transposition, contains only a portion of the proviral LTR at each end, the R and U5 regions at the 5′ end, and the R and U3 regions at the 3′ end. The complete terminal repeats present in the proviral DNA are regenerated by a mechanism of redundant copying of the U5 and U3 regions (for discussion see Varmus, 1983). One consequence of this mechanism of replication is that the LTRs of a single provirus are highly conserved. In the case of yeast Ty elements, for example, the delta elements flanking a particular Ty are often identical or differ by only a few nucleotides (see Williamson, 1983, for a comparison of delta sequences). Although sigma elements are generally more conserved than delta elements, the two sigma sequences present at the ends of this Ty3 are not only identical, but they have in common differences with previously published sigma element sequences. The features described above, suggest that Ty3 is, or was, a member of the retrovirus-like class of transposable elements.

*Composite Element Transcription*—Poly(A) RNA was prepared from X2180–1A (*MATa*), X2180–1B (*MATα*), and ySBS500 (*MATa/α*) cells. Poly(A) RNA was also prepared from all three cell types treated with α-factor as described under "Experimental Procedures." Poly(A) RNA was denatured by glyoxylation, fractionated by electrophoresis, and transferred to nitrocellulose as described by Thomas (1980). The filter-bound RNA was hybridized to a sigma-specific probe, an internal Ty3 probe, and a synthetic DNA oligonucleotide with homology to the internal region of Ty3 (Fig. 6). Hybridization to the sigma-specific probe is shown in Fig. 6*A*, *lanes 1–4*. The major sigma-hybridizing transcripts in *MATa*, and *MATα* cells (*lanes 1* and *3*, respectively) are 6.0, 5.2, 3.1, 0.96, 0.88, and 0.46 kb in length. The effect on transcript

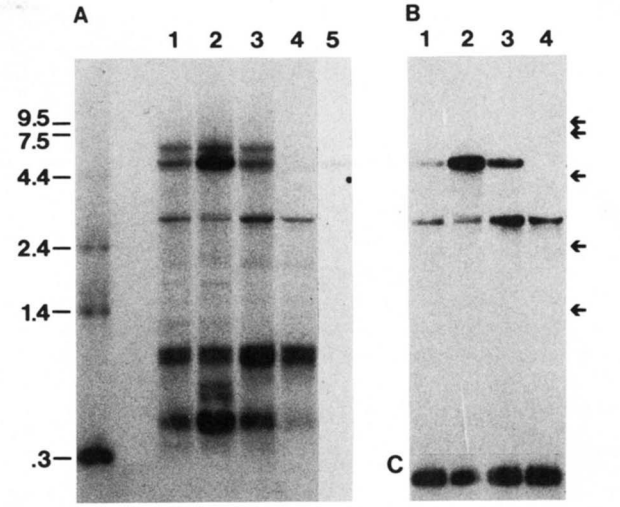


FIG. 6. **Transcription of sigma and Ty3.** Poly(A) RNA was prepared from cells grown to mid-log phase. RNA was denatured by glyoxylation, fractionated by electrophoresis, and transferred to nitrocellulose as described by Thomas (1980). 10 μg of RNA was loaded per lane. The nitrocellulose-bound RNA was hybridized to sigma or Ty3 probes. Sizes were determined based on migration relative to end-labeled size marker RNAs. The sizes of these RNA markers are 9.5, 7.5, 4.4, 2.4, 1.4, and 0.3 kb. Size markers and hybridized transcripts were visualized by autoradiography. *A*, hybridization of poly(A) RNA to a radiolabeled sigma fragment and to a radiolabeled synthetic oligonucleotide with homology to the Ty3 minus strand primer-binding site. The *left lane* shows migration of marker RNAs. The RNA in *lanes 1–5* was prepared from strains differing at *MAT*: *lanes 1, 2,* and *5*, X2180–1A (*MATa*); *lane 3*, X2180–1B (*MATα*), and *lane 4*, ySBS500 (*MATa/α*). X2180–1A cells were treated with α-factor as described under "Experimental Procedures" before preparation of RNA in lanes 2 and 5. Samples in *lanes 1–4* were hybridized to the pSBS6 sigma insert, radiolabeled by random primer synthesis. The sample in lane 5 was hybridized to the $^{32}$P-end-labeled synthetic oligonucleotide with homology to the Ty3 internal domain (see "Experimental Procedures"). *B*, hybridization of poly(A) RNA to a radiolabeled Ty3 internal domain probe. The position of migration of size marker RNAs described in A is indicated in the right margin. Poly(A) RNA samples in *lanes 1–4* are the same as in *lanes 1–4* of A, above. Nitrocellulose-bound RNA was hybridized to the 1600-bp *Acc*I fragment from the Ty3 internal domain (Fig. 1, *probe f*) labeled by random primer synthesis. *C*, control hybridization of poly(A) RNA to a *URA3* sequence probe. *Lanes 1–4* are the same as in *B*. The nitrocellulose blot which was exposed to give the autoradiograph shown in *B* was subsequently hybridized to a 1.1-kb *Hin*dIII fragment containing the *URA3* gene (Bach *et al.*, 1979) labeled by random primer synthesis. Only the region of exposure showing the *URA3*-hybridizing transcript is shown here.

end of internal sequence; and 5′-inverted terminal repeat from LTR. *Line 1* shows the Ty3 internal domain junctions with the left sigma and right sigma. *Lines 2–5* show conserved junction regions of Ty912, a Ty1 (class I) element, Ty1–17, a Ty2 (class II) element, copia, and SNV, respectively. Except for Ty3, these alignments have been considered previously (Varmus, 1983; Warmington *et al.*, 1985).The bases shown in parentheses are present at the ends of the full-length linear form of SNV but are deleted during proviral integration; the analogous nucleotides occurring in Ty3, *i.e.* the nucleotides between the minus strand primer-binding site and the LTR, are also shown in *parentheses.*

patterns of treating *MATa* cells with α-factor is shown in *lane 2*. The 5.2- and 0.46-kb transcript bands are increased at least in intensity and two new classes of transcripts, 0.67 and 0.61 kb in length appear. Van Arsdell *et al.* (1987) observed induction of three sigma-hybridizing transcripts, 5.2, 0.65, and 0.45 kb in length in *MATa* cells treated with α-factor. These may correspond to the 5.2-kb, the 0.67-and 0.61-kb and the 0.46-kb transcripts we observed. Alternatively, differences in the transcription patterns among strains may account for the differences in the observed sizes of transcripts. *Lane 4* shows that the 6.0-, 5.2-, and 0.46-kb transcripts are present at reduced levels, if at all, in the *MATa/α* diploid. The position of the high molecular weight band in *lane 4* is slightly but distinctly ahead of the position of the 5.2 kb transcript in *lanes 1–3*. *MATα* and *MATa/α* cells were also treated with α-factor, with no effect.[3]

A synthetic DNA oligonucleotide homologous to 21 bp of the left-most region of the Ty3 internal domain was used to probe poly(A) RNA from *MATa*, *MATα*, and *MATa/α* cells and α-factor-treated cells. Hybridization was visible only in the RNA prepared from *MATa* cells treated with α-factor (*lane 5*). The hybridizing transcript in *lane 5* co-migrates with the α-factor-sensitive 5.2-kb transcript. Background hybridization to rRNA was observed after long exposures of the filter hybridized to the synthetic DNA oligonucleotide.[3]

An *AccI* 1600-bp restriction fragment from Ty3 (Fig. 1, *fragment f*) was used to monitor poly(A) transcripts with similarity to the Ty3 internal domain (Fig. 6*B*). Hybridization of the same poly(A) RNA samples as used in Fig. 6*A* with this probe shows a different result. This internal domain probe hybridizes only to transcripts 5.2 and 3.1 kb in length. Although the apparent increase in the level of the 5.2-kb transcript after α-factor treatment is greater, when measured with the internal probe than when measured with the sigma probe, the pattern revealed by the two probes is qualitatively similar and is consistent with the common identity of the 5.2-kb transcript which hybridizes to sigma and the transcript seen here. As was observed with the sigma probe, this transcript is absent in the diploid cells. The levels of the 3.1-kb transcript visualized with these probes is affected neither by α-factor treatment nor by the presence of *MATa/α*. Incubation of poly(A) RNA from treated and untreated cells with radiolabeled fragments from the regions flanking Ty3 (Fig. 1, *probes a* and *h*) did not show any hybridization,[2] suggesting that the 5.2-kb transcript begins and ends in, or near, the terminal sigma repeats. A radiolabeled *URA3* probe was hybridized to the blot shown in Fig. 6*B* in order to corroborate the loading of equivalent levels of poly(A) RNA in the different lanes. Hybridization, shown in Fig. 6*C* for the region of the gel containing the *URA3* transcripts, was similar in all four lanes.

*Termini of the 5.2-kb Ty3 Transcript*—The 5′ end of the Ty3 5.2-kb transcript was mapped by reverse transcription extension of a primer with homology to the left end of the internal domain. The DNA oligonucleotide used as the primer in the reverse transcription analysis hybridized to the 5.2-kb transcript, as shown in Fig. 6*A*, *lane 5*. Southern blot analysis showed that this oligonucleotide also hybridizes to both composite element-containing fragments in the X2180 background but not to any other fragments.[3] The results of the reverse transcriptase-mediated extension using this oligonucleotide primer are shown in Fig. 7. The sizes of the extended molecules were determined by comparison to an homologous sequence ladder. Extensions of the oligonucleotide primer hybridized to the poly(A) RNA prepared from *MATα* and *MATa* cells, *lanes 1* and *3*, and *MATa* cells treated with α-factor (*lane 4*) showed the presence of a strong band corresponding to the 5′ end of a transcript at a position 118 bp (*T*)

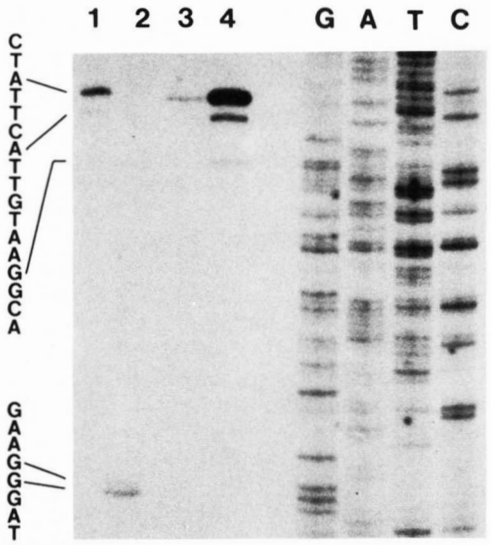


FIG. 7. **Determination of the 5′ ends of the Ty3 transcripts by reverse transcription from a synthetic oligonucleotide primer.** The preparations of poly(A) RNA described in the legend of Fig. 6 were also the sources of RNA in these experiments. The end-labeled synthetic oligonucleotide with homology to the left end of the Ty3 internal domain was used to prime reverse transcription. The cells used for poly(A) RNA preparations which provided the templates for the extensions were: *lane 1*, X2180–1B (*MATα*); *lane 2*, ySBS500 (*MATa/α*); *lane 3*, X2180–1A (*MATa*); and *lane 4*, X2180–1A cells treated with α-factor. The same synthetic oligonucleotide was used to prime synthesis from single-stranded Ty3 DNA (plasmid, pSBS12) with the same sense as the Ty3 transcript in the presence of dideoxy chain terminators; *lanes G, A, T,* and *C*. This sequence is indicated to the left of *lane 1*. Electrophoresis conditions for experiments shown in *A* and *B* were as described under "Experimental Procedures."

upstream from the left end of the internal domain, as shown in Fig. 5*A*. Fainter bands appeared at positions corresponding to a 5′ end 114 bp (*T*) from the left end of the internal domain, and in the α-factor-treated cell RNA analysis, corresponding to 106 bp (*C*) from the left end of the internal domain. The intensity of the band corresponding to a putative transcription initiation site at position 118 was much more intense in the α-factor-treated cell preparation than in the untreated haploid cell preparations. The positions of these sites in the upstream sigma are indicated in Fig. 5*B*. In the poly(A) RNA sample prepared from diploid cells, bands appeared which correspond to 5′ ends of transcripts at positions 64 and 65 bp (*C*) upstream from the left end of the internal domain; of these, the band corresponding to position 64 was more intense. In the several reverse transcription experiments performed, a higher molecular weight band was sometimes observed. This band was intense and migrated at a position just below the position of the full length DNA, as indicated by the sequence ladder. Although this band seemed to be dependent on poly(A) RNA, it was not consistently present in any sample. The results of the reverse transcription experiment presented here indicate that a major fraction of the transcripts of one or both Ty3 elements have 5′ ends in the upstream sigma element.

S1 nuclease protection experiments were performed with heteroduplexes of poly(A) RNA from *MATa* cells treated with α-factor and DNA synthesized *in vitro* starting with the 5′-end-labeled primer described above.[3] These experiments gave results which were consistent with those obtained by primer extension mapping. Protected fragments were observed which correspond to the 5′ ends of transcripts both within the lefthand sigma element at the position (plus or minus 2 bp) indicated by the most intense band in the reverse transcrip-

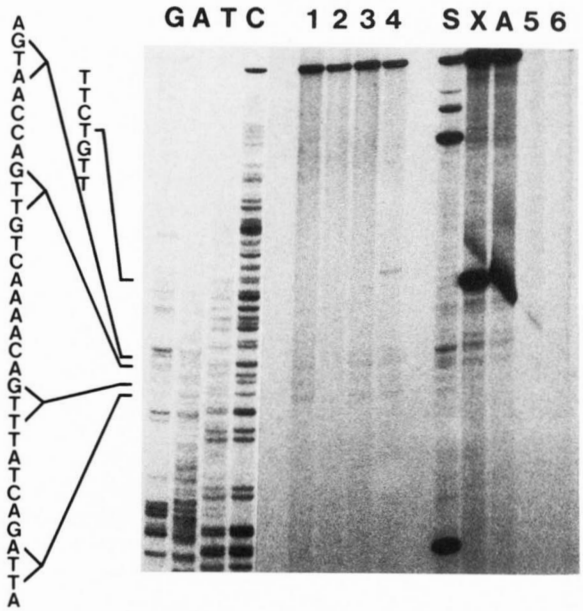


FIG. 8. **Identification of the 3′ ends of Ty3 transcripts.** The preparations of poly(A) RNA described in the legend of Fig. 6 were the sources of RNA in these experiments. The 3′ ends of Ty3 transcripts were determined by S1 nuclease protection. A 1.0-kb *Acc*I fragment was labeled in the 3′ end, inside the Ty3 internal domain, as described under "Experimental Procedures." This end-labeled DNA was hybridized to poly(A) RNA from different cells, *lanes 1–4,* and no RNA, *lane 5,* as described for Fig. 7, and the duplexes treated with S1 nuclease. *Lane 6* contains the control with no S1 added. Samples were electrophoresed in a denaturing acrylamide gel and exposed to x-ray film as described. The end-labeled *Acc*I fragment was modified and degraded by the chemical method and electrophoresed on the same gel in order to obtain the sequence shown in the left four lanes. Lanes labeled *G* and *C* are specific for nucleotides at those positions. Lanes labeled *A* and *T* are purine and pyrimidine cleavages, respectively. The sequence shown on the left was deduced by electrophoresing samples for different lengths of time. The end-labeled *Acc*I fragment (*lane A*) was also partially digested with *Sau*3A (*lane S*) and completely with *Xho*I (*lane X*) in order to provide markers corresponding to the *Sau*3A site inside the sigma element and the *Xho*I site 14 bp within the downstream end of the element. These samples were separated on the same denaturing gel as those described above.

tion experiment and at a position at least 150 bp upstream. The position of the upstream end point was not mapped. The three start sites indicated by primed reverse transcription against the *MATa* α-factor-induced cell RNA template are the same as the three sites observed by Van Arsdell *et al.* (1987) for the 5′ ends of α-factor-induced transcripts from an isolated sigma element. The coincidence of at least a set of 5′ end points determined by S1 nuclease and reverse transcription experiments suggests that the 5.2-kb transcript is not spliced between the position of the oligonucleotide primer and the 5′ end of the transcript mapped by primer extension.

The 3′ end of the Ty3 transcript was also mapped by protection from S1 nuclease degradation (Fig. 8). In this case, the labeled DNA was the *Acc*I fragment which spans the downstream sigma element. It was labeled at its 3′ end, within the internal domain sequence. One class of protected fragments extended from the *Acc*I site of labeling to the end of the sigma element plus or minus one or two nucleotides. Four smaller classes of protected fragments migrated close together at positions corresponding to 75–100 bp within the end of the sigma element (see Fig. 5*A*). Although there is slight contamination of the uncut *Acc*I fragment with DNA migrating at this position, all four "protected" fragments were absent in samples digested in the absence of RNA and were intensified after digestion with nuclease in the presence of RNA, arguing that these termini represent actual heterogeneity in the 3′ ends of the transcripts. Thus, at least some Ty3 transcripts begin in the leftward sigma element and end in or beyond the rightward sigma element.

## DISCUSSION

*Ty3 Homology to Retroviruses and Retrotransposons*—We show here that the sigma element occurs as terminal repeats flanking a larger element; we have named this composite element Ty3. Ty3 has the properties of a transposable element and shares additional features with retroviruses and retrotransposons. These features include: conserved terminal short inverted repeats, a tRNA-primer-binding region, a composite structural organization, and regulatory signals which direct synthesis of an almost full-length, polyadenylated transcript. Ty3 is further distinguished by an absolute insertional specificity for the 5′ ends of tRNA genes.

*Analysis of the Major Ty3 Transcript*—Ty3 is transcribed into an almost full-length transcript, approximately 5.2 kb in length. One class of the Ty3 transcripts have been shown to begin in the upstream sigma element and end in, or just beyond, the downstream sigma element. Primer extension and S1 nuclease experiments indicated, in addition to the 5′ ends mapped in the downstream half of the left sigma element, 5′ ends further upstream which were not mapped. The 5.2-kb transcripts then contain at least the minimum information required in a retrotransposition intermediate.

The sigma element was examined for sequence motifs associated with transcription initiation and transcription termination and polyadenylation. In yeast, DNA sequences homologous to the canonical TATAA/TAA/T sequence (Breathnach and Chambon, 1981) are observed 60–100 nucleotides upstream of transcription initiation sites (Dobson *et al.*, 1982). The 5′ ends of the major Ty3 transcripts mapped by primer extension coincide with DNA sequence beginning 118 and 64 bp from the right end of the sigma element in haploid and diploid cells, respectively. Promoter sequences, TATAAA and TATAATATATAA, occur 85 bp upstream of the haploid transcription initiation site and 68 bp upstream of the diploid transcription initiation site, respectively. Therefore, both of the Ty3 putative transcription initiation sites appear to be preceded by an appropriately positioned DNA promoter consensus sequence. The sequence PyAAG also has been found close to the site of transcription initiation of a number of yeast genes (Burke *et al.*, 1983; Dobson *et al.*, 1982; Russell *et al.*, 1983, 1986). The sequence TAAG occurs twice near the Ty3 transcription initiation site observed in haploid cells: once starting at position −5 and once starting at position +1.

It is interesting to compare the positions of these consensus promoter sequences with the positions of the consensus promoter sequences and transcription initiation sites of Ty1. The transcription initiation sites of Ty1 occur 95 and 107 bp from the right end of the delta element (Elder *et al.*, 1983). If delta and sigma elements are aligned by the short regions of homology noted by Genbauffe *et al.* (1984), then the transcription initiation sites of Ty1 transcripts coincide almost exactly with the Ty3 transcript initiation site observed in diploid cells, and the TATAAACATATAA sequences 73 bp upstream of the Ty1 transcription initiation site in delta coincides with the TATAATATATAA sequence 68 bp upstream of the Ty3 initiation site in sigma.

Two sequences have been proposed to specify transcription termination in yeast: TAG···TAGT(TATG)···TTT (Zaret and Sherman, 1982) and TTTTTATA (Henikoff and Cohen, 1984). These sequences are found in the downstream end of

the sigma element. The sequence TAG···TAGTATG ·· TTT occurs between 85 and 35 bp upstream of the first Ty3 polyadenylation site, and the sequence TTTTATA occurs approximately 35 bp upstream of that polyadenylation site.

The polyadenylation sites of the Ty3 transcripts are closely spaced and this kind of heterogeneity has been reported for several yeast genes (Zaret and Sherman, 1982; Bennetzen and Hall, 1982). S1 nuclease protection analysis of a restriction fragment labeled in the pSBS12 Ty3 internal domain revealed five positions of heterology between the transcripts and end-labeled fragment. These positions, which are indicated in Fig. 5A, occur in the second sigma element between 20 and 50 nucleotides downstream from the position of the major transcription initiation site in the upstream sigma element and at the end of the second sigma element. Interpretation of this pattern is complicated by the presence of two Ty3 elements in the strain used in these experiments (Figs. 2 and 3). Determination of the sequence of the downstream sigma element in the second Ty3 showed that the sequence from this Ty3 and the pSBS12 Ty3 are identical in this region, but that they are flanked by different 5-bp direct repeat sequences. We conclude, therefore, that at least four of the fragments generated by protection from S1 nuclease degradation correspond to sites of transcript polyadenylation, while the fifth fragment, which has its unlabeled terminus close to the end of the second sigma element, may correspond to the position of polyadenylation of transcripts from either or both Ty3 elements in this strain, or to the point of heterology between the pSBS12 Ty3 end-labeled fragment and transcript from the nonallelic Ty3.

The extent of the linkage of transcription termination and polyadenylation in yeast is unclear (reviewed by Birnstiel *et al.*, 1985). The sequence AATAAA that signals polyadenylation 11–20 nucleotides downstream, frequently within the dinucleotide PyA, which occurs in the genes of metazoans, is not consistently present near the ends of yeast genes. Nonetheless, the sequence, TAAATAAA/G, has been found 28–34 nucleotides upstream of some message polyadenylation sites in yeast and has been proposed to be related to polyadenylation (Bennetzen and Hall, 1982). The sequence TATATAAG, which differs by only one nucleotide from the consensus, occurs approximately 29 bp upstream of the first Ty3 polyadenylation site. Despite the considerable ambiguity regarding signals for transcription termination and processing of the 3′ end of the Ty3 transcript, it is clear that these transcripts do fulfill a basic requirement for a retrotransposition intermediate: Ty3 transcripts begin in the left long terminal repeat and terminate within a redundant sequence, downstream of the homologous position in the right long terminal repeat.

*Expression of Sigma Elements*—Differential expression of the multiple sigma elements is implicit in the pattern of hybridization observed by Northern blot analysis with various probes and with RNA from different cells. There are, depending on the strain, from five to ten transcription products that are easily visualized with sigma-specific DNA probes. The 5.2-, 0.67-, 0.61-, and 0.46-kb transcripts show dramatic increases in levels in *MATa* cells treated with $\alpha$-factor. The levels of the 6.0-, 5.2-, and 0.46-kb transcripts are decreased in *MATa*/$\alpha$ cells. Of the transcripts visualized with the sigma probe, only the 5.2-kb transcript and the 3.1-kb transcript, which is present at relatively constant levels, hybridize to DNA probes specific for the Ty3 internal domain. The number of transcript classes which hybridize to sigma-specific DNA probes contrasts with the larger number of sigma elements. The different flanking sequences of the 34 or so isolated sigma elements should result in distinct transcripts, at least in cases where the transcript is larger than the element itself. Therefore, we tend to support the notion that relatively few independent sigma elements may be actually expressed. If only a subset of this highly conserved family of elements is active, it is possible that the sequences responsible for this differential expression reside outside the sigma element itself. This situation would be at least partially analogous to the regulation of Ty1 and Ty2, which is effected, in part, by enhancer and mating-type control sequences within the internal domain (Errede *et al.*, 1985; Roeder *et al.*, 1985).

The reduced levels of some sigma transcripts which we observed in RNA from diploid cells, compared to haploid cells, is consistent with *a*1-$\alpha$2 regulation (Strathern *et al.*, 1981; Miller *et al.*, 1985; and reviewed by Herskowitz, 1987), which is observed for Ty1 and Ty2 (Elder *et al.*, 1983). Van Arsdell *et al.* (1987) have attributed the low level of sigma-hybridizing transcripts in untreated *MATa* strains that they have studied to low levels of $\alpha$-factor, synthesized by small populations of switched (*MAT*$\alpha$) cells. These levels of sigma-hybridizing transcripts are apparently higher in the haploid strains which we studied, which leads us to propose that transcription of sigma elements may be under mating-type control. Preliminary experiments show that the sigma transcripts that are present in low levels in *MATa*/$\alpha$ cells, compared to haploid cells, are also reduced in levels in *MATa*/$\alpha$ cells compared to *MATa*/*a* and *MAT*$\alpha$/$\alpha$ cells.[3] This result is consistent with *a*1-$\alpha$2 regulation. Although blocks of sequence with strict homology to the *a*1-$\alpha$2 regulation consensus sequence are not found within the sigma element, this is perhaps not surprising, because the comparable sequences in Ty1 and Ty2 are found within the internal domain (Errede *et al.*, 1985; Roeder *et al.*, 1985). Thus, the inferred differences in expression among different sigma elements imply a contribution to the regulation of sigma element transcription from sequences outside the element itself.

*Ty3 Protein-coding Potential*—The RNA genomes of retroviruses code for protein products having functions in viral replication, capsid formation, and integration (reviewed by Varmus, 1983). Ty1 and Ty2 elements have two long open reading frames, TYA and TYB, that code for proteins which bind the RNA and have reverse transcriptase activity (Clare and Farabaugh, 1985; Hauber *et al.*, 1985; Warmington *et al.*, 1985; Garfinkel *et al.*, 1985; Mellor *et al.*, 1985; and Adams, *et al.*, 1987). In Ty3 there is an open reading frame which begins 41 bp downstream of the transcription initiation site observed in haploid cells and continues for the 200 bp for which the DNA sequence has been determined. The first ATG translation start codon occurs 76 bp inside the internal domain. It is preceded by ANN and followed immediately by A; both of these are relatively conserved features of eukaryotic translation initiation sites (Kozak, 1983). The sequence CACACA has been found about 10 nucleotides upstream from the ATG start codon of several yeast messages (Dobson *et al.*, 1982) and has been postulated to have a role in translation (Stiles *et al.*, 1981). The sequence ACACCACA is centered 11 bp upstream of the first start codon in Ty3. Although there is no obvious homology between the amino acid sequence encoded in this portion of Ty3 and the TYA or TYB proteins by dot-matrix analysis, projected amino acid sequences encoded by both types of elements are rich in proline in this region (Clare and Farabaugh, 1985). Thus the internal domain of Ty3 may, like those of other retrotransposons, encode proteins which specify replication and integration functions.

*Conclusions*—Several points suggest that sigma and/or Ty3 is capable of transposition: 1) Ty3 has many of the conserved structural features of retroviruses and retrotransposons; 2) the genomic locations of Ty3 elements in five different strains

of yeast are heterogeneous and, therefore, compatible with genomic rearrangements involving transposition; 3) the internal domain and flanking sigma element(s) are transcribed, giving rise to transcripts that are potential RNA intermediates in replication; and 4) the high degree of homology within the sigma element family is consistent with their recent derivation from a limited number of actively transposing elements.

The regulation of Ty3 transcription, and hence transposition, may be more stringent than that of Ty1 and Ty2. In fact, the prezygotic pattern of Ty3 expression provides an intriguing parallel to germ-line activation of P elements in *Drosophila* (Laski *et al.*, 1986). If Ty3 transposition is pheromone-dependent, it is tempting to speculate that the cell has devised a means of avoiding gross genomic variation except when diploidization is imminent.

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*Note Added in Proof*: BRL has revised the reported size of the smallest RNA size marker from 0.33 to 0.24 kbp. The sizes of the 0.96-, 0.88-, 0.67-, 0.61-, and 0.46-kbp transcripts which hybridize to sigma are accordingly, 1.0, 0.92, 0.65, 0.58, and 0.40 kbp, respectively.

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