MOLECULAR AND CELLULAR BIOLOGY, Jan. 1985, p. 46–51
0270-7306/85/010046-06$02.00/0

Vol. 5, No. 1

Concerted Evolution of Dispersed *Neurospora crassa* 5S RNA Genes: Pattern of Sequence Conservation Between Allelic and Nonallelic Genes

EWA MORZYCKA-WROBLEWSKA, ERIC U. SELKER, JUDITH N. STEVENS, AND ROBERT L. METZENBERG*

Department of Physiological Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706

Received 16 July 1984/Accepted 25 September 1984

About 100 genes coding for 5S RNA in *Neurospora crassa* are dispersed throughout the genome (Selker et al., Cell 24:815–818, 1981; R. L. Metzenberg, J. N. Stevens, E. U. Selker, and E. Morzycka-Wroblewska, manuscript in preparation). The majority of them correspond to the most abundant species (α) of 5S RNA found in the cell. Gene conversion, gene transposition, or both may be responsible for the maintenance of sequence homogeneity (concerted evolution) of α-type 5S genes. To explore these possibilities, we isolated and characterized separate 5S regions from two distantly related laboratory strains of *N. crassa*. Restriction and sequence analyses revealed no differences in molecular location of allelic 5S genes between the two strains. However, the DNA sequences around the 5S genes are ca. 10% divergent. We concluded that transposition is not frequent enough to account for the concerted evolution of *N. crassa* α-5S genes. In contrast to sequence divergence in the flanking regions between the two strains, the 5S transcribed regions are identical (with one exception), suggesting that these genes are being corrected. We have found that flanking sequences of various *N. crassa* 5S genes within each strain are largely different. Thus, if the correction mechanism is based on gene conversion, it is limited to the transcribed regions of the genes. However, we did find a short region of consensus including the sequence TATA located 25 to 30 nucleotides preceding the position of transcription initiation. This region may be involved in the transcription of *N. crassa* 5S genes.

It is difficult to imagine that natural selection could maintain homogeneity among a large number of genes of identical function. Yet numerous cases of repeated genes have been found (e.g., rDNA, histone genes, immunoglobulin genes). Repeated DNA with no known function (e.g., satellite DNA or spacer DNA) is also common (for review, see reference 12). This has prompted investigators to propose various mechanisms of so-called horizontal, coincidental, or concerted evolution (for review, see reference 1). Concerted evolution is defined as a phenomenon in multigene families in which the members of the family are changing in unison within an organism. Proposed mechanisms of concerted evolution involve either some form of repeated expansion and contraction of the multiplicity of the genes, during which variant genes are lost, or some form of correction of one sequence by another without even temporary change in the size of the family. Concerted evolution in tandemly arranged gene families probably results from unequal crossing over, gene conversion, or both (1). Unequal crossing over cannot account for the preservation of sequence homogeneity in a dispersed gene family because crossing over between nonallelic genes would result in chromosomal translocations, inversions, or deletions, depending on the location and orientation of the interacting genes.

We examined *Neurospora crassa* genes coding for 5S RNA to look for evidence of concerted evolution among dispersed genes (29). *N. crassa* 5S genes, unlike 5S genes in most organisms, are dispersed throughout the genome. We found 5S genes with very divergent transcribed regions (designated as α, β, γ, etc.), indicating that they are not evolving in concert with each other. However, most of the 5S genes (the α genes) have identical coding regions, suggesting that concerted evolution is operating among them (29). Most α 5S genes are located on one chromosome (linkage group II); however, α genes are found on at least five of the other six chromosomes as well (R. L. Metzenberg, J. N. Stevens, E. U. Selker, and E. Morzycka-Wroblewska, manuscript in preparation). Thus, clustering of the different 5S gene types is not required for their concerted evolution. We have pointed out that transposition events or any other process which results in duplication of some genes and deletion of others could in theory result in concerted evolution (29). Results presented in this paper show that transposition of 5S genes does not occur at a frequency sufficient to account for concerted evolution. We also point out that if a correction mechanism is based on gene conversion, it must be limited to the transcribed region of the genes; the flanking regions of nonallelic *N. crassa* 5S genes are very different. However, a short conserved sequence is found preceding each *N. crassa* 5S gene. We note that a similar sequence precedes 5S RNA and tRNA genes of some other organisms.

MATERIALS AND METHODS

(i) Biological material. *N. crassa* strains Oak Ridge 74-OR23-1A (OR) and Beadle and Tatum 25a (BT) were from the Fungal Genetics Stock Center, Humboldt, Calif. (FGSC 986 and 353, respectively). Phage lambda NM781 (20) was used as the vector in constructing an *N. crassa* genomic DNA library.

(ii) DNA preparation. High-molecular-weight *N. crassa* DNA from strain BT was extracted and purified as described previously (17, 32). Plasmid DNA was prepared by a standard procedure involving chloramphenicol amplification, lysis with sodium dodecyl sulfate and cesium chloride-ethidium bromide gradient ultracentrifugation. For small-scale plasmid DNA preparation, the procedure of Birnboim and Doly (3)

* Corresponding author.

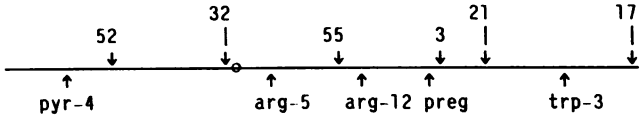


FIG. 1. Location of 5S genes and some conventional markers in linkage group II of *N. crassa* (Metzenberg et al., in preparation). Numbers above the line designate genes coding for 5S RNA. Distances are not to scale. The dot represents the centromere.

modified by Grosveld et al. (9) was used. Lambda DNA was prepared by the method of Thomas and Davis (34).

(iii) Molecular cloning. The cloning of OR 5S genes and pseudogenes was previously described (28, 29). The pool of strain BT DNA in lambda NM781 was constructed as follows. *Eco*RI-digested *N. crassa* DNA (5 μg) was ligated with 2.5 μg of *Eco*RI-cut λNM781 DNA in 100 μl of 50 mM Tris-hydrochloride buffer (pH 7.4)–10 mM $MgSO_4$–10 mM NaCl–1 mM ATP–10 mM dithiothreitol. The reaction mixture was incubated with 40 U of T4 DNA ligase (Promega-Biotec) for 2 h at 9°C and then for 18 h at 4°C. The ligated DNA was packaged in vitro into lambda particles (4), generating about 2×10^5 viable phage. This pool was then amplified in liquid culture with *Escherichia coli* K802 under conditions described by Blattner et al. (4). The resulting phage pool contained 96% hybrid phage as judged by plating on an *E. coli lacZ* strain in the presence of the indicator 5-bromo-4-chloro-3-indolyl-β,D-galactoside (6). The recombinant phage pool was screened by in situ plaque hybridization (2) after an amplification step performed as described by Woo (36). Probes consisted of OR 5S RNA gene 17, 21, or 32 and several kilobases of flanking DNA (29) cloned in pBR322 (5) and labeled by nick translation (26). Hybrid phage were purified from plaques which gave positive signals, DNA was prepared, and the *N. crassa* DNA inserts were subcloned into pBR322 (5).

(iv) Restriction mapping. Restriction endonuclease digestions were performed with commercial enzyme preparations under conditions specified by the suppliers. DNA fragments were resolved by electrophoresis through 0.7, 1.0, or 1.5% agarose gels (8) or 5% polyacrylamide gels (13). Southern hybridizations were performed by the standard procedure (31).

(v) End labeling and DNA sequencing. Cloned 5S RNA genes and adjacent flanking regions were sequenced on both strands by the method of Maxam and Gilbert (15), after 5′ end labeling with polynucleotide kinase and [γ^{32}P]ATP or 3′ end labeling with the Klenow fragment of *E. coli* DNA polymerase and [α-^{32}P]ATP. The sequencing strategy was as described in Selker et al. (29).

RESULTS AND DISCUSSION

To be able to address the question of whether 5S sequences transpose frequently, we must assume that the flanking sequences in which we find them imbedded do not transpose. A large body of data with genes 17, 21, and 32 and other 5S genes indicates that this assumption is correct (unpublished data). The flanking sequences behave as alleles; that is, each progeny of a cross contains a sequence characteristic of one parent or the other, not of both or of neither. To test the hypothesis that transposition is involved in the apparent concerted evolution of 5S genes in *N. crassa*, we compared the position of 5S genes in laboratory strains OR and BT. These strains show substantial DNA restriction fragment length polymorphism (data not shown), suggesting that at least the regions being probed do not have a recent common ancestor. Thus, if 5S gene transposition does occur frequently, the 5S genes would be expected to occupy different positions in these two strains. To look into this possibility, we cloned and sequenced 5S genes from three

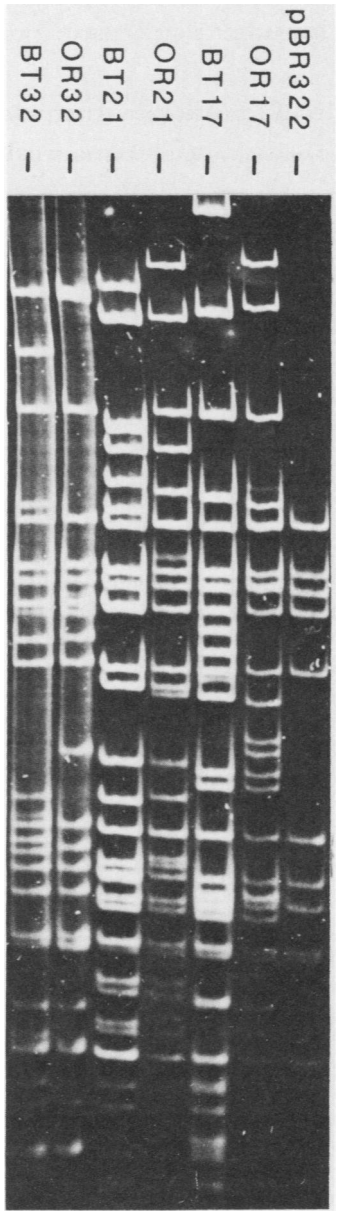
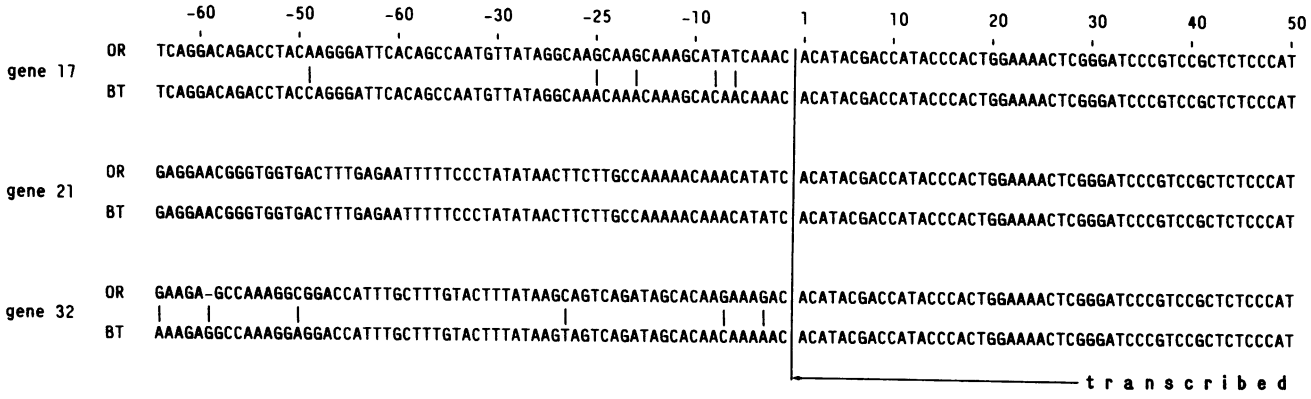


FIG. 2. Restriction fragment length polymorphism between pairs of plasmids 17, 21, and 32 derived from *N. crassa* strains OR and BT. Plasmid DNA (4 μg) was cut with *Hae*III, and the resulting DNA fragments were resolved by electrophoresis through a 5% polyacrylamide gel. For every pair of plasmids, the proportion of fragments remaining the same was determined (0.74, 0.76, and 0.53 for pairs OR17-BT17, OR21-BT21, and OR32-BT32, respectively). The fraction of nucleotide differences ($1 - p$) for each region was roughly estimated as follows: let p = proportion of nucleotides remaining the same. Thus, the likelihood that both of two four-nucleotide restriction sites that define a fragment will be unchanged is p^8. For OR17-BT17, $p^8 = 0.74$; therefore, $p = 0.963$ and $1 - p = 0.037$. For OR21-BT21, $p^8 = 0.76$; therefore, $p = 0.966$ and $1 - p = 0.034$. For OR32-BT32, $p^8 = 0.53$; therefore, $p = 0.924$ and $1 - p = 0.076$.



different regions of linkage group II (Fig. 1) from strain BT. These regions had been previously cloned from strain OR on the basis of containing 5S genes located in distinct flanking sequences (29). Each of these genes is identical or very similar to the canonical α-sequence (see below). We designated them genes 17, 21, and 32 following the plasmid designations (pJS17, pDW21 and pJS32, respectively). The corresponding regions from strain BT were identified in a recombinant DNA pool in lambda NM781 phage, with plasmids carrying genes 17, 21, or 32 from strain OR as hybridization probes. These plasmids contain ca. 9, 10, and 6 kilobase pairs, respectively, of unique *N. crassa* DNA flanking the 5S genes. These long stretches of DNA, rather than the relatively short (120-base-pair) 5S regions, were responsible for the hybridization observed. The cloned *Eco*RI-generated DNA fragments were recloned intact into pBR322 and designated pEM17, pEM21, and pEM32. The cloned fragments from strain BT are of the same sizes as those from strain OR. The 5S regions were identified by Southern hybridizations with labeled 5S RNA as a probe (data not shown).

Comparison of the allelic pairs from OR and BT by restriction analysis with *Hpa*II, *Hae*III, *Hha*I, or *Alu*I (sample data with *Hae*III in Fig. 2) showed that about half of the restriction fragments are of different sizes in the digest of plasmids 32 and one-third are different between pairs of plasmids 17 and 21. Based on the proportion of restriction fragments which are of identical size, we estimate 7% nucleotide sequence difference between the OR and BT DNA on plasmids pJS32 and pEM32. Similarly, the OR and BT DNA on the two other pairs of plasmids are ca. 3.5% different (see the legend to Fig. 2).

Most significant, sequence analysis shows that all three pairs of plasmids tested contain 5S genes at exactly the same positions within their flanking sequence in the two strains (Fig. 3). The level of sequence divergence very near the genes (flanking DNA shown in Fig. 3) is similar to that detected as restriction site polymorphism in the plasmids generally. In contrast to sequence divergence in the flanking DNA (Fig. 3), transcribed regions of each pair of genes are identical, with the exception of a single nucleotide in BT 21 (Fig. 3 and 4). The transcribed regions of genes OR32, BT32, and OR21 are invariant and match the 5S transcribed region of our standard α gene OR52 (29). The 5S coding regions of genes 17 from strains OR and BT are identical, but they have two differences relative to the standard α sequence: positions 70 and 107 are thymine and adenine, respectively, rather than adenine and thymine. Interestingly, these differences are complementary, perserving the base pairing in the secondary structure characteristic for 5S RNA (Fig. 4). The single substitution in BT21 (position 69) relative to OR21 changes a G · C pair in the secondary structure to a G · U pair (Fig. 3 and 4). Genes OR17, BT17, and BT21 are transcribed in vitro in an *N. crassa* RNA polymerase III system (unpublished data). Therefore, it seems likely that they are functional variants of α-type genes rather than pseudogenes.

The fact that 5S genes are found at exactly the same positions in two apparently distantly related strains suggests that transposition is not frequent relative to mutation and therefore cannot account for the maintenance of sequence homogeneity of the multiple, α-type 5S genes in *N. crassa*.

It is often assumed that gene conversion is responsible for the concerted evolution of dispersed members of multigene families (1). However, if gene conversion between nonallelic 5S genes occurs, it must be limited to the transcribed regions because the sequences flanking nonallelic 5S genes are very different (see Fig. 5).

Despite the high degree of divergence in the 5′ flanking sequences of *N. crassa* 5S genes, a short region of striking homology does exist. The sequence TATA is found exactly 26 to 29 nucleotides upstream from transcription initiation sites of *N. crassa* 5S genes (Fig. 5). The sole exception is gene 52, in which case CATA is found. The TATA box is always flanked by a thymine or an adenine at position −30 and a guanine or an adenine at position −25. The nontranscribed pseudogene N5spl (28; Fig. 5, ψ4) is not preceded by a TATA box. The function of this short conserved sequence in front of *N. crassa* 5S genes is not known. Similar or identical sequences occur in the same location upstream of many genes transcribed by RNA polymerase III in several organisms, including *Schizosaccharomyces pombe* (14, 33), *Saccharomyces cerevisiae* (16, 23, 35), *Lupinus luteus* (25), *Drosophila melanogaster* (10, 24), and *Bombyx mori* (11, 18). This sequence is not associated with polymerase III-specific genes of vertebrates. A TATA box has been known to be involved in transcription by RNA polymerase II (21). In contrast, it had been thought that the sequences required for transcription of polymerase III-specific genes are limited to the transcribed regions (27). However, at least some polymerase III-specific genes also require external regulatory signals for their transcription in vitro (7, 11, 18) and in vivo

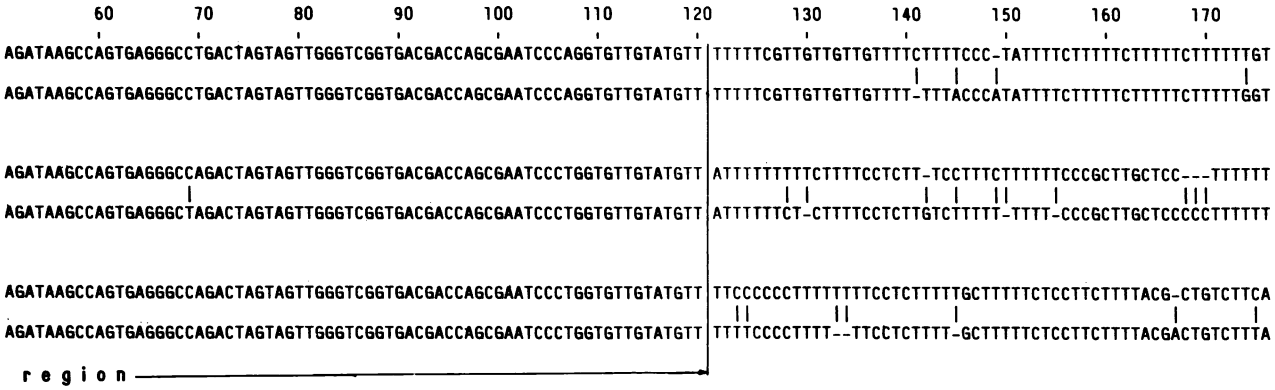


FIG. 3. Nucleotide sequence of 5S RNA structural regions and flanking sequences of plasmids 17, 21, and 32 from OR and BT. The positions of differences between OR and BT are indicated. The noncoding DNA strand is shown.

(30). In the case of *B. mori*, it has been recently shown that the TATA or TACTA box is a part of a sequence necessary for transcription of both tRNA and 5S RNA genes (11, 18a). It seems reasonable to suppose that this sequence is also involved in transcription of *N. crassa* 5S genes.

We have shown that transposition of *N. crassa* 5S RNA genes is not frequent enough to account for their concerted evolution. The sequence divergence between strains BT and OR tells us that the multiple identical genes are not the result of recent gene amplification. Most likely, a correction mechanism checks and repairs 5S genes of a given isotype. Coexistence of several very different 5S isotypes indicates that information transfer between pairs of substantially different isotypes of 5S genes does not occur (29). This poses a question similar to that of speciation at the level of organisms: how different must two genes be before they define noninteracting isotypes? For example, does gene 17, which has two differences relative to the standard sequence,

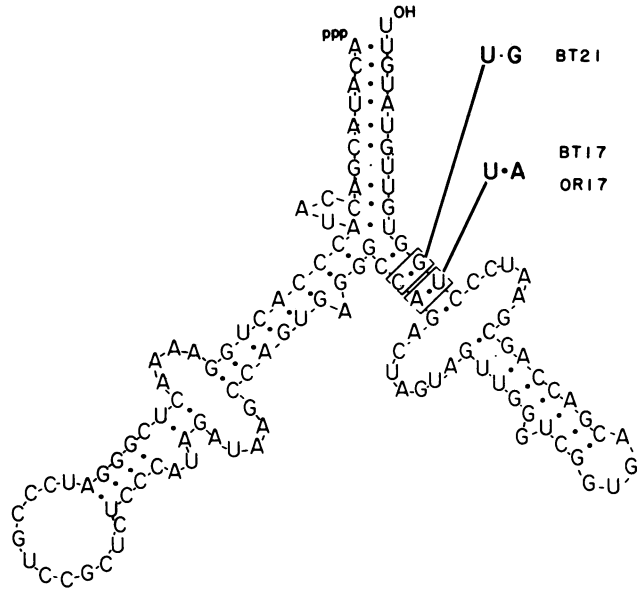


FIG. 4. Secondary structure of *N. crassa* α 5S RNA, showing location of nucleotide differences in OR17, BT17, and BT21 relative to the canonical α sequence. The secondary structure is drawn as proposed by Nishikawa and Takemura (22) for *Torulopsis utilis* 5S RNA.

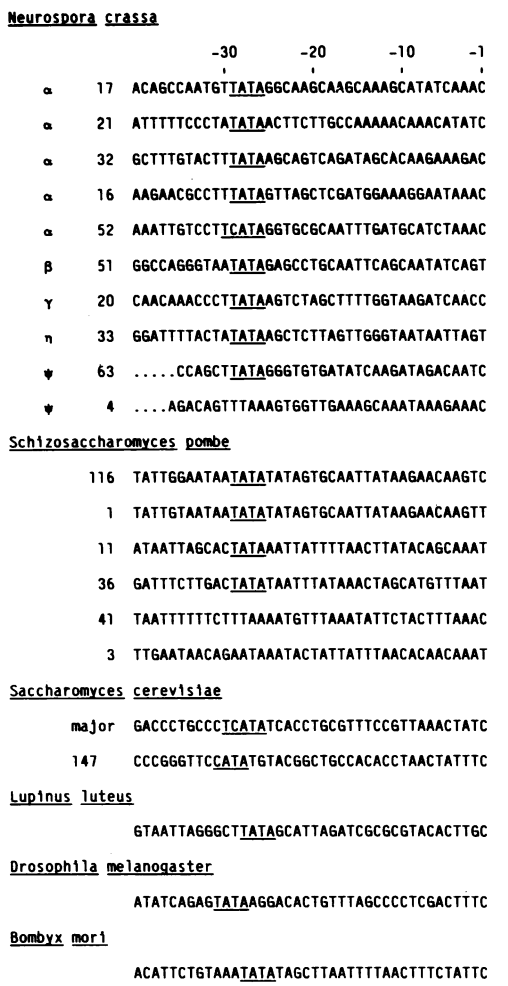


FIG. 5. Nucleotide sequences of 5′ flanking regions of 5S RNA genes. The α, β, γ, and η isotype genes have numerous differences in their coding sequences. The two ψ (pseudogenes) are very different from one another, but neither one is homologous to a full-length 5S gene. *N. crassa* sequences are from strain OR. α52, β51, ψ4, and portions of α16 and γ20 were previously published (28, 29). The other *N. crassa* sequences were determined in this work. *Schizosaccharomyces pombe* genes 1, 11, 36, and 41 are from Tabata (33), and 116 and 3 are from Mao et al. (14). The sequences of *Saccharomyces cerevisiae* (16, 23, 35), *L. luteus* (25), *D. melanogaster* (24) and *B. mori* (11) were described previously.

participate in information exchange with canonical α-5S genes?

Whatever the correction mechanism is, it cannot be totally efficient, or else the distinct 5S isotypes would not have arisen in the first place. Furthermore, the fact that the sequences flanking the 5S regions are not identical indicates that these regions are not being corrected. Gene conversion events among nonallelic 5S genes may be responsible for the apparent concerted evolution of *N. crassa* 5S genes. Correction of *N. crassa* 5S genes may be analogous to the correction of *Schizosaccharomyces pombe* tRNA genes observed by Munz et al. (19). In both of the systems, dispersed genes transcribed by *Pol*III are corrected by a process limited to the transcribed regions. A mechanism related conceptually to ordinary DNA-mediated gene conversion but based on RNA-mediated transfer of information would be very attractive. Such a mechanism would explain why correction is limited to the transcribed region. In addition, RNA-mediated correction would have the feature that successful genes, that is, genes which are well transcribed and which make stable RNAs, would be favored.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM08995 to R.L.M. from the National Institutes of Health and by a fellowship to E.U.S. from the Helen Hay Whitney Foundation. We thank A. Becker for providing us with protein A preparation for in vitro packaging of lambda DNA and E. Lund and B. Mann for critical reading of the manuscript. We are indebted to D. G. Morton and K. U. Sprague for communication of their unpublished results.

LITERATURE CITED

1. **Arnheim, N.** 1983. Concerted evolution of multigene families, p. 38–61. *In* M. Nei and R. K. Koehn (ed.), Evolution of genes and proteins. Sinauer Associates Inc. Publishers, Sunderland, Mass.
2. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques *in situ*. Science **156**:180–182.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Blattner, F. R., A. E. Blechl, K. Denniston-Thompson, H. E. Faber, J. E. Richards, J. L. Slightom, P. W. Tucker, and O. Smithies.** 1978. Cloning human fetal γ-globin and mouse α-type globin DNA: preparation and screening of shotgun collections. Science **202**:1279–1284.
5. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
6. **Davies, J., and F. Jacob.** 1968. Genetic mapping of the regulator and operator genes of the *lac* operon. J. Mol. Biol. **36**:413–416.
7. **Dingerman, T., D. Johnson Burke, S. Sharp, J. Schaack, and D. Söll.** 1982. The 5′ flanking sequences of *Drosophila* $tRNA^{Arg}$ genes control their *in vitro* transcription in a *Drosophila* cell extract. J. Biol. Chem. **257**:14738–14744.
8. **Dugaiczyk, A., H. W. Boyer, and H. M. Goodman.** 1975. Ligation of *Eco*RI endonuclease-generated DNA fragments into linear and circular structures. J. Mol. Biol. **96**:171–184.
9. **Grosveld, F. G., H. H. M. Dahl, E. deBoer, and R. A. Flavell.** 1981. Isolation of β-globin-related genes from a human cosmid library. Gene **13**:227–237.
10. **Indik, Z. K., and K. D. Tartof.** 1982. Glutamate tRNA genes are adjacent to 5S RNA genes in *Drosophila* and reveal a conserved upstream sequence (the ACT-TA box). Nucleic Acids Res. **10**:4159–4172.
11. **Larson, D., J. Bradford-Wildox, L. S. Young, and K. U. Sprague.** 1983. A short 5′ flanking region containing conserved sequences is required for silkworm alanine tRNA gene activity. Proc. Natl. Acad. Sci. U.S.A. **80**:3416–3420.
12. **Long, E. O., and I. B. Dawid.** 1980. Repeated genes in eucaryotes. Annu. Rev. Biochem. **49**:727–764.
13. **Maniatis, T., A. Jeffrey, and H. van deSande.** 1975. Chain length determination of small double- and single-stranded DNA molecules by polyacrylamide gel electrophoresis. Biochemistry **14**:3787–3794.
14. **Mao, J., B. Appel, J. Schaack, H. Yamada, and D. Söll.** 1982. The 5S RNA genes of *Schizosaccharomyces pombe*. Nucleic Acids Res. **10**:487–500.
15. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:455–560.
16. **Maxam, A. M., R. Tizard, K. G. Skryabin, and W. Gilbert.** 1977. Promoter region for yeast 5S ribosomal RNA. Nature (London) **267**:643–645.
17. **Metzenberg, R. L., and T. J. Baisch.** 1981. An easy method for preparing Neurospora DNA. Neurospora Newsl. **28**:20–21.
18. **Morton, D. G., and K. U. Sprague.** 1982. Silkworm 5S RNA and alanine tRNA genes share highly conserved 5′ flanking and coding sequences. Mol. Cell. Biol. **2**:1524–1531.

18a. **Morton, D. G., and K. U. Sprague.** 1984. *In vitro* transcription of a silkworm 5S RNA gene requires an upstream signal. Proc. Natl. Acad. Sci. U.S.A. **81**:5519–5522.

19. **Munz, P., H. Amstutz, J. Kohli, and U. Leupold.** 1982. Recombination between dispersed serine tRNA genes in *Schizosaccharomyces pombe*. Nature (London) **300**:225–231.
20. **Murray, N. K., W. J. Brammar, and K. Murray.** 1977. Lambdoid phages that simplify the recovery of *in vitro* recombinants. Mol. Gen. Genet. **150**:53–61.
21. **Nevins, J. R.** 1983. The pathway of eucaryotic mRNA formation. Annu. Rev. Biochem. **52**:441–466.
22. **Nishikawa, K., and S. Takemura.** 1974. Structure and function of 5S ribosomal ribonucleic acid from *Torulopsis utilis*. II. Partial digestion with ribonucleases and derivation of the complete sequence. J. Biochem. **76**:935–947.
23. **Piper, P. H., A. Lockheart, and N. Patel.** 1984. A minor class of 5S rRNA genes in *Saccharomyces cerevisiae* X2180-1B, one member of which lies adjacent to a Ty transposable element. Nucleic Acids Res. **12**:4083–4096.
24. **Pirotta, V., and C. Tschudi.** 1978. Organisation and sequence in cloned 5S RNA genes from *Drosophila melanogaster*, p. 127–134. *In* H. W. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier/North-Holland Publishing Co., New York.
25. **Rafalski, J. A., M. Wiewiorowski, and D. Söll.** 1982. Organization and nucleotide sequence of nuclear 5S rRNA genes in yellow lupin (*Lupinus luteus*). Nucleic Acids Res. **10**:7636–7642.
26. **Rigby, P. W., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
27. **Sakonju, S., D. V. Bogenhagen, and D. D. Brown.** 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription. I. The 5′ border of the region. Cell **19**:13–25.
28. **Selker, E. U., S. J. Free, R. L. Metzenberg, and C. Yanofsky.** 1981. An isolated pseudogene related to the 5S RNA in *Neurospora crassa*. Nature (London) **234**:576–578.
29. **Selker, E. U., C. Yanofsky, K. Driftmier, R. L. Metzenberg, B. Alzner-DeWeerd, and U. L. RajBhandary.** 1981. Dispersed 5S RNA genes in *N. crassa*: structure, expression and evolution. Cell **24**:819–828.
30. **Shaw, K. J., and M. V. Olson.** 1984. Effects of altered 5′-flanking sequences on the *in vivo* expression of a *Saccharomyces cerevisiae* $tRNA^{Tyr}$ gene. Mol. Cell. Biol. **4**:657–665.
31. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–518.
32. **Stevens, J. N., and R. L. Metzenberg.** 1982. Preparing Neurospora DNA: some improvements. Neurospora Newsl. **29**:27–28.

33. **Tabata, S.** 1981. Nucleotide sequences of the 5S ribosomal RNA genes and their adjacent regions in *Schizosaccharomyces pombe*. Nucleic Acids Res. **9**:6425–6437.
34. **Thomas, M. J., and R. W. Davis.** 1975. Studies on the cleavage of bacteriophage lambda DNA with *Eco*RI restriction endonuclease. J. Mol. Biol. **91**:315–328.
35. **Valenzuela, P., G. I. Bell, F. R. Masiarz, L. J. DeGennaro, and W. J. Rutter.** 1977. Nucleotide sequence of the yeast 5S ribosomal RNA gene and adjacent putative control regions. Nature (London) **267**:641–643.
36. **Woo, S. L.** 1979. A sensitive and rapid method for recombinant phage screening. Methods Enzymol. **68**:389–395.