Detection of yeast ribosomal RNA sequences in E. coli infected with hybrid bacteriophage

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ABSTRACT

Yeast ribosomal DNA was inserted into Escherichia coli on a bacteriophage vector and the host cell RNA was then extracted and analyzed for the presence of yeast ribosomal RNA sequences. RNA complementary to yeast rDNA was detected by hybridization. The transcription of yeast rDNA was found to be independent of phage RNA synthesis and to occur on the same DNA strand as rRNA transcription in yeast. However, hybridization to restriction fragments of yeast rDNA suggested that the RNA species detected in E. coli differ somewhat from authentic yeast rRNA.

INTRODUCTION

Accurate expression of a eukaryotic gene cloned in bacteria depends upon the recognition of appropriate signals in the cloned DNA. The expression of eukaryotic DNA inserted into E. coli via plasmid or bacteriophage vectors has been monitored at the level of transcription (1-4), translation (3,5) and genetic complementation (6,7). While the expression of DNA of many types has been demonstrated by these criteria, very little information has been obtained about the specificity and fidelity of the RNA synthesis events. Translation and genetic complementation do not necessarily require a high degree of specificity at the level of RNA synthesis. Hybridization assays showing transcription of the cloned DNA also do not address the question of specificity except where asymmetric transcription has been demonstrated (2,3). Even in these cases, the RNA was found to be synthesized from the strand opposite to the one normally transcribed (2), or comparison with the in vivo transcripts was not possible (3).

The DNA coding for the ribosomal RNAs of the yeast Saccharomyces cerevisiae provides a well-defined system for examining the specificity and fidelity of transcription in E. coli of a segment of eukaryotic DNA with known transcription products. In yeast the rDNA is transcribed to yield a
35S precursor molecule which is processed in several steps resulting in
in the 25S, 18S, and 5.8S rRNAs (8,9). In addition, the map positions
and transcriptional orientations of the mature RNA species have been de-
termined (10-14), and the locations of many restriction enzyme sites have
been mapped in yeast rDNA (10-12,15,16).

Construction and selection of recombinant bacteriophage lambda (\(\lambda\))
carrying all or part of a single repeat unit of yeast rDNA (17) made pos-
sible an examination of the specificity and fidelity of transcription
of yeast rDNA in bacteria. Here we report that yeast rDNA carried into
E. coli on a phage vector is indeed transcribed with some specificity.
We have used hybridization to restriction fragments and to separated
strands of cloned yeast rDNA to study the RNA that is synthesized.

MATERIALS AND METHODS
(a) Bacteria, Phage and DNA
Growth of phage and labeling experiments were carried out in E. coli
C600. \(\lambda\)-yeast rDNA recombinant phages \(\lambda g t 40-Sc 202\) and \(\lambda g t 21-S c 302\)’, 304’,
310, and 320’ have all been reported previously (17) and are described in
Figure 1.

DNA was prepared by phenol extraction of phage that had been puri-
fied by banding in CsCl equilibrium gradients. Following extraction, the
DNA was dialyzed into 0.1 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA.

(b) Preparation of Labeled RNA
Overnight cultures of E. coli were grown at 37°C with shaking in MOPS
medium (18,19) supplemented with 0.2% low-phosphate casamino acids (20),
0.2% maltose and 1.3 mM KH\(_2\)PO\(_4\). The cells were then diluted 1:100 in the
same medium, except the [PO\(_4\)] was lowered to 0.46 mM, and grown with
shaking at 37°C to an absorbance at 600 nm of 0.4. For each phage tested,
10 ml of cells were pelleted by centrifugation at 6,000 RPM for 10 min,
resuspended in 1 ml of 10 mM Tris-HCl, pH 7.5, 10 mM MgSO\(_4\) and infected
with phage at a m.o.i. of 3 at 2°C. After 15 min the infected cells
were added to 10 ml of prewarmed MOPS medium with 0.2% maltose and 0.1
to 0.5 mCi of \(^{32}\)PO\(_4\) (Carrier-free, New England Nuclear). Labeling was
carried out at 37°C for 20 or 40 min, at which time the cells were poured
over about 5 ml of crushed ice and 0.1 ml of 2 M sodium azide. They
were then pelleted, resuspended in 1 ml of cold 25% sucrose, 50 mM
Tris-HCl, pH 8, 1 mM EDTA and treated for 5 min with 0.1 ml of freshly
prepared lysozyme (1 mg/ml in the same buffer). Spheroplasts were
collected by centrifugation and lysed by vortexing in 1% sodium dodecyl
sulfate (SDS) in TE buffer (10 mM Tris-Cl, pH 7.5, 1 mM EDTA). The
lysate was extracted at room temperature twice with phenol saturated
with TE, once with phenol:chloroform:iso-amyl alcohol (25:25:1) and once
with chloroform:iso-amyl alcohol (24:1). One tenth volume of 2.5 M sodium
acetate and 2.5 volumes of ethanol were added to the aqueous phase which
was then placed at -20°C for at least 2 hr or at -70°C for 20 min. After
centrifugation the pellet was washed with ethanol, dried and resuspended
in 0.5 ml of TE. Single-stranded nucleic acid was selectively precipitated
by addition of an equal volume of 4M LiCl, incubation at 4°C for 16 hr and
centrifugation. The pellet was resuspended in 0.25 ml of TE, precipitated
with ethanol as above, dried and dissolved in 1 ml of H2O. Undissolved
material was removed by passage through a millipore filter in a Swinnex
holder and the samples were stored at -20°C.

(c) Restriction Enzyme Digestions

EcoRI and HindIII restriction enzymes were purchased from Boehringer-
Mannheim Biochemicals and SstI was from Bethesda Research Labs. About 0.5
µg of DNA was digested at 37°C for 2-3 hr with an excess of enzyme. The
digestions were stopped by addition of EDTA to 12.5 mM and heating to 65°
for 3 min.

(d) Gel Electrophoresis

Restriction fragments of DNA were separated by electrophoresis in
horizontal agarose slab gels (21) run in Tris/borate buffer (22). After
electrophoresis, the gels were stained in 0.5 µg/ml ethidium bromide and
photographed under long-wave UV light. DNA bands were denatured in the
gel and transferred directly to nitrocellulose filters (23) for subsequent
hybridization.

For DNA strand separations, restricted DNA was heated to 100°C for
5 min in the presence of 4 M urea, then immediately loaded onto the gel.
After electrophoresis and staining, the DNA could be transferred onto the
nitrocellulose filters without further treatment.

(e) Hybridizations

Hybridizations with the appropriate RNA probe were carried out at 40°C
in 1-2 ml of 50% formamide, 0.6 M NaCl, 0.2 M Tris-Cl, pH 8.1, 20 mM EDTA,
0.5% SDS with unlabeled *E. coli* tRNA added to 50 µg/ml. After 20-24 hr,
filters were washed in 50% formamide, 4XSSC (SSC = 0.15 M NaCl, 0.015 M
sodium citrate) at 40°C for 30 min and two times in 2XSSC at room temper-
ature. The filters were then treated with RNase A (20 µg/ml in 2XSSC) for
RESULTS

(a) Detection of Yeast rRNA Sequences in E. coli

Infection of E. coli at 37°C with the λ-yeast recombinant phages used in the following experiments, and described in Figure 1, results in lytic growth of the phages due to a deletion in the cl (repressor) gene of the λgt21 phages constructed from phage no. 554 of Murray and Murray (24) and to the temperature sensitive cI857 gene of λgt40 recombinants constructed from λgt-λβ (25). To allow labeling times longer than that of the λ lytic cycle, the S7 amber mutation in the λ lysis function was introduced into λgt21-Sc310 and λgt21-Sc320 ̄ and the labeling was carried out in a host that does not suppress this mutation.

To prepare labeled RNA, cells were grown in low-phosphate medium, infected with the appropriate recombinant phage and added to medium containing 32P. After extraction of the RNA, yeast-specific RNA was detected by hybridization to restriction fragments of DNA from λ-yeast rDNA recombinant phage. Figure 2 shows the hybridization of RNA extracted from cells infected with λgt21-Sc320 ̄ or λgt21-Sc310, which carry the entire yeast rDNA repeat unit in opposite orientations on two HindIII restriction fragments (see Fig. 1), to a HindIII restriction digest of λgt21-Sc320 ̄. The RNA hybridized to both yeast rDNA restriction fragments as well as to the three λ fragments. λgt40-Sc202, which has the single SstI fragment of yeast rDNA, gave similar results (not shown). These results indicate that the yeast DNA was transcribed in E. coli but does not provide information about the specificity or fidelity of the RNA synthesis.

While the RNA detected by hybridization could arise from correct transcription and processing of the yeast rRNA sequences, it also might be synthesized from λ transcription "reading-through" into the inserted yeast DNA or from random transcription of the yeast DNA due to spurious initiation events. The data shown in Figure 2 indicate that there was significantly more hybridization to the HindIII B fragment by the RNA made in E. coli than by the authentic yeast RNA. In fact, the hybridization of yeast RNA to the HindIII B fragment could be detected only after a longer exposure of the film than that shown in Figure 2. This suggests some difference between authentic yeast rRNA and that made in E. coli (see below).

(b) Specificity of Transcription of Yeast DNA in E. coli

To begin to examine the specificity of transcription of the yeast
Figure 1. Maps of recombinant bacteriophage λ carrying yeast rDNA. HindIII and SstI restriction fragments of yeast rDNA were inserted into λ as described (17). The locations and directions of transcription of the mature rRNAs and the regions covered by the three largest EcoRI fragments are shown (10–16). The map sites of the λ N gene, P1 promoter and attachment (att) site (not present in λgt40 phage) are given as reference points. The HindIII fragment carrying these sites has been inverted relative to wild-type λ in λgt21-Sc304' (17).

rDNA in E. coli, the direction of transcription was determined. This was accomplished by hybridizing RNA from infected cells, as well as RNA from yeast, to separated strands of yeast rDNA. Figure 2 shows that the single yeast SstI fragment in λgt40-Sc202 migrated as two separated DNA strands when denatured before loading on an agarose gel and that RNA extracted from cells infected with λgt21-Sc320' or λgt40-Sc202 hybridized to only one of these strands. Hybridization of yeast RNA and of yeast RNA mixed with RNA from infected cells showed that the same strand of yeast rDNA is transcribed in E. coli as in yeast. RNA from cells infected with λgt21-Sc310 phage gave similar results (not shown). Since the yeast DNA is inserted into λ in both possible orientations (see Fig. 1), these results suggest that there is some specificity in the recognition of the yeast DNA by the E. coli RNA polymerase, although synthesis of RNA from
Figure 2. Detection of yeast-specific transcripts in E. coli. DNA from λgt21-Sc320' was cut with HindIII restriction enzyme and subjected to electrophoresis in an 0.7% agarose gel. The resulting DNA bands (three from λ and two from the yeast rDNA) were denatured and transferred to nitrocellulose filters (23). Hybridization of 32P-labeled RNA from cells infected with a) λgt21-Sc310 or b) λgt21-Sc320' or from c) yeast is shown. To determine the direction of transcription, DNA from λgt40-Sc202 was cleaved with SstI and heat denatured immediately prior to loading on a 0.7% agarose gel. The yeast SstI A fragment migrated as two separated DNA strands which could be transferred to nitrocellulose filters without further treatment. The filters were then hybridized with 32P-labeled RNA from cells infected with d) λgt21-Sc320' or e) λgt40-Sc202 or from f) yeast. Apparently the λ SstI fragments do not transfer well without denaturing in the gel as in a and b and, therefore, do not appear as strong bands in the autoradiograph.

the opposite strand followed by specific degradation cannot be ruled out. Also, because the asymmetry of the yeast-specific RNA synthesis is independent of the orientation of the yeast DNA, it is probably not due to "readthrough" of λ transcription. Finally, the hybridization to the HindIII B fragment must represent little or no 5S RNA which is synthesized from the opposite strand of DNA.

To further characterize the yeast transcripts made in E. coli, labeled RNA from cells infected with each of the recombinant phage used in

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the above experiments was hybridized to nitrocellulose filter transfers from agarose gels of the three largest EcoRI restriction fragments of yeast rDNA (See Fig. 1). EcoRI A includes most of the coding region for the 25S rRNA, the EcoRI C fragment contains most of the 18S region, and the EcoRI B fragment includes the 5S region as well as "spacer" DNA, which contains both untranscribed sequences and part of the 35S precursor sequences that are removed by processing (10-12,16). In all cases the results, shown in

![Yeast-C](a)(b)

**Figure 3. Hybridization to EcoRI fragments of yeast rDNA.** Labeled RNA from cells infected with a) λgt21-Sc320' or b) λgt60-Sc320' was hybridized to blots of λgt21-Sc320' DNA (left) and λgt60-Sc202 DNA (right) cleaved with EcoRI and separated on gels of 1% agarose. Gel bands representing yeast rDNA are indicated. Other bands represent λ DNA or DNA from λ-yeast junctions. RNA from cells infected with λgt21-Sc310 gave essentially identical results.
Figure 3 showed significant hybridization to fragments B and C, but weak hybridization to the A fragment. This does not correspond to the results obtained with yeast rRNA as a probe, where strong hybridization to the A and C fragments and weak hybridization to the B fragment is observed. Thus, it appears likely that the yeast RNA(s) synthesized in E. coli do not represent exactly the same species found in yeast. This discrepancy is not unexpected for λgt40-Sc202 where the yeast rDNA is split in the middle of the 18S coding region while for λgt21-Sc310 and λgt21-Sc320' it could be due to incorrect or incomplete transcription, or processing, or both.

(c) Independence of Yeast-Specific Transcription from λ RNA Synthesis

The independence of the yeast-specific transcription was further demonstrated using a pair of recombinant phage, λgt21-Sc302', and λgt21-Sc304' (Figure 1), which carry just the yeast rDNA HindIII A fragment in the same orientation as λgt21-Sc320'. The two phages differ in that the central HindIII fragment of λ554 (24) in λgt21-Sc304' has been reversed in orientation relative to normal λ DNA during in vitro recombination, whereas it is in the correct orientation in λgt21-Sc302' (17). Therefore, P_L-promoted transcription, which could be reading through into the yeast DNA in λgt21-Sc320' and in λgt21-Sc302', proceeds rightward away from the yeast insert in λgt21-Sc304'. Figure 4 shows that RNA from both λgt21-Sc302' and λgt21-Sc304'-infected cells hybridized strongly to the yeast rDNA HindIII A fragment and was transcribed in the same direction as the RNA from λgt21-Sc320'-infected cells. Thus, the transcription of yeast rDNA in E. coli must be initiated within the yeast DNA itself.

To examine the transcription of yeast rDNA in E. coli in the absence of transcription of the λ DNA, λgt21-Sc310, λgt21-Sc320' and λgt40-Sc202 phages were super-infected into a λ lysogen of E. coli. These cells have a λ genome integrated into the host chromosome and synthesize a single product, the λ repressor protein, which prevents transcription of the DNA.

Figure 4. Independence of yeast-specific transcription from λ RNA synthesis. Labeled RNA from cells infected with a) λgt21-Sc302' or b) λgt21-Sc304' or from a λ lysogen infected with c) no phage, d) λgt21-Sc320', e) λgt21-Sc310 or f) λgt40-Sc202 was analyzed as in Figure 2. The top panel is the HindIII digest of λgt21-Sc320' DNA and the bottom λgt40-Sc202 DNA cut with SstI and denatured before electrophoresis. The small amount of hybridization to the faster migrating strand of yeast rDNA by the λgt21-Sc304' RNA is also occasionally seen for RNA prepared with the other phage in very long exposures of the autoradiogram as shown here. This apparently represents a low level of transcription from this strand which was not further characterized.
of the integrated phage and of the super-infecting phage. Labeled RNA was extracted from the cells and hybridized as in the previous experiments. The results are shown in Figure 4. While little $\lambda$ transcription was detected (compare Fig. 2a,b and Fig. 4d,e), hybridization to the yeast rDNA HindIII and EcoRI fragments and to the separated strands was very similar to results obtained from the lytic infections. These findings confirm the independence of the yeast rDNA transcription from $\lambda$ RNA synthesis.

**DISCUSSION**

We have shown that infection of E. coli with bacteriophage $\lambda$ carrying the coding region for the rRNAs of yeast results in the synthesis of RNA sequences which hybridize to yeast rDNA and that this transcription does not depend on $\lambda$ RNA synthesis. Hybridization to separated strands of yeast rDNA indicated that the transcription proceeds in the same direction as normal yeast rRNA synthesis. However, some yeast rRNA sequences appear to be under-represented (25S) while other regions of the yeast rDNA, not normally seen as stable RNA species in yeast, are present. The lack of significant RNA from the 25S RNA coding region may reflect premature termination of the transcription in E. coli or preferential degradation of these sequences. The sequences that hybridize to the "spacer" region are apparently mostly not 5S RNA and may represent under-processed rRNA precursor or the presence of additional initiation sites in this region which are recognized by the E. coli RNA polymerase.

The fact that yeast rDNA can be transcribed in the correct direction in E. coli indicates that the E. coli RNA polymerase can recognize a specific sequence or sequences, although these sequences are not necessarily identical to those recognized by the yeast RNA polymerase I which synthesizes the 35S rRNA precursor (26,27). Since the 5' terminal sequence of the yeast primary rRNA transcript has not been determined, it will be difficult at this time to determine if the recognition is correct.

Our results, however, do indicate that the transcription might start in approximately the same region of rDNA as normal yeast transcription and that several initiation sites may be used. The fact that the yeast HindIII A rDNA fragment by itself is transcribed independently in Agt21-Sc304' indicates that there is an initiation site for E. coli RNA polymerase in this fragment. In addition, the detection of strong hybridization of RNA from cells infected with Agt21-Sc310 to the EcoRI B and C fragments together with the finding of little hybridization to the A fragment sug-
gests that transcription starts not only in the HindIII A fragment but also in the HindIII B / EcoRI B region (see Fig. 1). Thus, transcription of yeast rDNA in E. coli may start at several sites located approximately between the region coding for the 3' end of 25S RNA and the 5' end of the 18S RNA region. Indeed, E. coli RNA polymerase binds to several distinct sites on the EcoRI B fragment in vitro (F. Farrelly and J. Cramer, personal communication). If unique yeast-specific RNAs can be isolated from E. coli infected with these recombinant phages, mapping of the RNAs and examination of the 5' ends should reveal whether one or more initiation sites are used.

We conclude that while yeast rDNA inserted into E. coli is transcribed with some specificity, i.e., in the correct direction and possibly starting at or near the normal initiation site, the fidelity of transcription, processing or both is decreased. Further analysis of the yeast-specific RNAs produced in E. coli should determine at what step of RNA synthesis fidelity is lost and, consequently, which steps are carried out correctly. Examination of the nucleic acid structure of any yeast sites found to be correctly recognized in E. coli should reveal regulatory features conserved between the two organisms.

REFERENCES

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