Isolation of the Nuclear Gene Encoding a Subunit of the Yeast Mitochondrial RNA Polymerase*

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Jeffrey L. Kelly†, Arno L. Greenleaf‡, and I. R. Lehman
From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Antisera directed against the purified 145,000-dalton subunit of the Saccharomyces cerevisiae mitochondrial RNA polymerase have been used to immunoscreen a library of yeast genomic inserts constructed in the fusion protein expression vector, λgt11. A 4-kilobase pair yeast DNA fragment inserted into one of the recombinant bacteriophages appears to contain most or all of the gene that encodes the 145,000-dalton subunit.

In most instances, the role of a eukaryotic enzyme in vivo must be inferred from its activity in vitro. However, in the lower eukaryote, Saccharomyces cerevisiae, it is experimentally straightforward to obtain direct genetic support for the function of an enzyme. Thus, antisera directed against the purified enzyme can be used to clone the genes encoding the polypeptides recognized by the antisera, thereby allowing construction of haploid mutants that have been disrupted in the gene(s) of interest (1-4). The phenotype of such mutants can then be examined directly.

We have been investigating a yeast mitochondrial RNA polymerase activity (5). The near homogeneous enzyme consists of a single 145-kDa polypeptide. We have prepared antisera directed against the enzyme that recognize the 145-kDa polypeptide and inhibit the RNA polymerase activity. This paper describes our use of these antisera to clone the gene encoding the 145-kDa polypeptide from a yeast genomic library constructed in the expression vector λgt11 (1, 6).

EXPERIMENTAL PROCEDURES†

RESULTS AND DISCUSSION

Immunoscreening of the λgt11 Library—A rabbit antisem directed against the 145-kDa RNA polymerase activity (5) was used to probe a λgt11 library consisting of 2 × 10⁶ recombinant phage containing yeast genomic DNA inserts. After screening 10⁶ recombinants, 14 independent clones were isolated, each of which produced strong signals. Twelve of these were selected for further analysis. DNA was prepared from each and analyzed by restriction enzyme mapping and Southern hybridization (8). Of the 12 clones, 5 recombinants appeared to have identical inserts, and these shared DNA sequences with two others (Fig. 1). Four clones appeared to have unique inserts, and the last produced no EcoRI fragments of ≤10 kilobase pairs. Phage 102 was chosen as a representative of the abundant class of clones producing strong signals.

Phage 102-induced Proteins Are Antigenically Related to the 145-kDa Polypeptide—As a rapid means of identifying which of the clones isolated in the λgt11 library screen carried inserts encoding antigenic determinants related to the purified 145-kDa RNA polymerase, representative phage clones were used to affinity-purify antibodies from the polyclonal rabbit serum, and the purified antibodies were used to probe immunoblots of the purified 145-kDa protein. When phage 102 was used to affinity-purify antibodies, the antibodies recognized a polypeptide migrating at 145 kDa in both highly purified and less purified fractions of the RNA polymerase.

Analysis of the Cloned RNA Polymerase Gene—To investigate further the yeast DNA-encoded protein whose synthesis was induced in Escherichia coli by phage 102, strains lysogenic for several of the phages were constructed. Extracts of the induced lysogenic strains were then prepared and analyzed by electrophoresis in NaDodSO₄-polyacrylamide slab gels followed by immunoblotting. Antiserum against the purified 145-

† The abbreviations used are: NaDodSO₄, sodium dodecyl sulfate; kb, kilobase pair; IPTG, isopropyl-β-d-thiogalactoside.

FIG. 1. Relationship of yeast inserts in clones selected by immunoscreening. Phage DNA was prepared from 102 recombinant clones selected by immunoscreening and analyzed by EcoRI digestion and gel blot/hybridization with isolated insert fragments as described under "Experimental Procedures." The yeast inserts are not all present in the same orientation in relation to the β-galactosidase sequence of the 102 vector.

1 The "Experimental Procedures" are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 85M-4108, cite the authors, and include a check or money order for $1.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
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10349

AB

I

97

-11

45

67

205

205-

116

Lane A, enzyme (0.5 µg of protein) from the glycerol gradient step of the purification (Fraction VIII, peak fraction) (5); Lane C is a less pure enzyme sample (1.5 µg of protein of a side fraction from the glycerol gradient). The nitrocellulose was then reacted with the affinity-purified antibodies directed against phage 102-encoded protein (II; Fig. 2 and “Experimental Procedures”), then with 125I-Protein A, and autoradiographed. Lanes R and D are the (overexposed) autoradiographs of the stained nitrocellulose strips in Lanes A and C, respectively. The apparent “bands” below 65 kDa in Lanes A and C are staining artifacts.

FIG. 4 (right). Phage 102-encoded protein is not a fusion protein with β-galactosidase. Extracts induced from lysogens of either λgt11 (A) or phage 102 (B) were analyzed by NaDodSO₄-polyacrylamide gel electrophoresis and immunoblotting (see “Experimental Procedures”). The rabbit antiserum used was directed against E. coli β-galactosidase (at 1:1000 dilution). Lane S contained molecular weight markers including β-galactosidase (116,000), which is marked by the arrow (the other standard proteins were myosin, phosphorylase b, bovine albumin, egg albumin, and carbonic anhydrase (from Sigma); approximately 100–300 µg of each standard protein was applied to the gel). Note that the β-galactosidase band in the phage 102 extract (B) is very slightly larger than bona fide β-galactosidase in the λgt11 control extract (A).

kDa protein detected several polypeptides in the extract of the phage 102 lysogen that were absent from the λgt11 control lysate (Fig. 2). In addition to the general background smear not seen in the control, distinct bands were observed in the 120–140-kDa range (I) and at about 50 kDa (II).

To test whether these phage 102-encoded proteins were immunologically related to the 145-kDa RNA polymerase subunit, they were used to affinity-purify the cognate IgGs from the anti-polymerase serum. The proteins in a phage 102 lysate were transferred electrophoretically from a preparative NaDodSO₄-polyacrylamide gel to nitrocellulose paper, and strips of the nitrocellulose carrying the 120–140-kDa polypeptide (I) and the 50-kDa polypeptide (II) were used as affinity matrices to purify IgGs from the antiserum. When the affinity-purified antibodies were, in turn, reacted with a nitrocellulose blot of the purified enzyme, they detected uniquely the 145-kDa polypeptide (Fig. 3). In a less highly purified polymerase preparation, the affinity-purified antibodies still reacted uniquely with the 145-kDa polypeptide (Fig. 3). Furthermore, both affinity-purified IgG preparations I and II gave virtually identical results, indicating that the prominent protein bands encoded by phage 102 are related to the 145-kDa RNA polym-
erase species, and that the smaller polypeptides are presumably metastable degradation products of the largest (approximately 140 kDa) polypeptide synthesized in E. coli. These results demonstrate that the yeast DNA insert in phase 102 encodes a protein product strongly related antigenically to the purified yeast RNA polymerase subunit.

If the phase 102-encoded proteins consist of a yeast DNA-encoded sequence fused with the large N-terminal fragment of β-galactosidase, as expected from the structure of λgt11 recombinants (13), they should also react with antibodies directed against β-galactosidase. However, in contrast to this expectation, the anti-β-galactosidase serum did not react with the 120-140-kDa bands present in the phage 102 lysate carry little if any β-galactosidase amino acid sequence; either they are encoded by a transcript initiated independently of the β-galactosidase promoter, or they represent products of translation re-start or protein processing. Whatever the mechanism of their origin, the size of the largest proteins (approximately 140 kDa) suggests that most of the 4-kb yeast DNA insert carried by phase 102 represents a protein-coding sequence.

These data are consistent with the conclusion that the yeast DNA insert in phase 102 encodes a large polypeptide that represents most of the 145-kDa yeast RNA polymerase. Therefore, it was to be expected that the IgGs that were affinity purified using the proteins synthesized in E. coli (above) should inhibit the highly purified yeast RNA polymerase. As shown in Fig. 5, the affinity-purified antibodies directed against the 120-140-kDa bands (I) inhibited the rate of the RNA polymerase reaction. The affinity-purified antibodies directed against the 50-kDa bands (II) were less inhibitory.

Isolation of the Genomic Region around the Phage 102 Insert—To obtain clones that contained larger regions of the yeast genome and included the insert in phase 102, a library constructed of approximately 15-kb yeast genomic sequences inserted into the vector pBR322 (9) was screened using the yeast insert present in phase 102 as a probe. In a screen of 15,000 recombinants, 15 independent clones were isolated, of which 12 were selected for analysis. The restriction enzyme map of the genomic insert contained in a representative phage is shown in Fig. 6. It should be noted that the EcoRI sites at both ends of the insert in phase 102 correspond to genomic EcoRI sites.

In summary, our results indicate that we have cloned the gene for the 145-kDa subunit of the yeast mitochondrial RNA polymerase. The availability of this clone has already permitted genetic tests of its role in vivo (14).

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M. Snyder, personal communication.

Experimental Procedures

Methodology: Yeast strains (GenBank accession number YAP213-7651) were obtained from M. Snyder's laboratory. The mitochondrial RNA polymerase (M. Snyder, personal communication) was isolated from S. cerevisiae strain W303 (MATa his3-11,15 leu2-3,112 trp1-289 ura3-52). The enzyme was purified by a modification of the method of Snyder and coworkers (1).

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