Rifampin Susceptibility of Ribonucleic Acid Synthesis in a Fragile Saccharomyces cerevisiae Mutant

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Ribonucleic acid (RNA) synthesis in the sorbitol-dependent, fragile yeast mutant VY1160 (Venkov et al., 1974) is rapidly inhibited by rifampin. The growth of the mutant cells and protein synthesis are more slowly affected by the antibiotic, apparently as secondary phenomena. Lower doses of rifampin (50 to 100 μg/ml) preferentially inhibit ribosomal RNA synthesis in comparison to that of messenger RNA and transfer RNA. Transcription and translation of messenger RNA continues in the presence of low doses of rifampin, as evidenced by the unimpaired induction of α-glucosidase. Partially purified RNA polymerase II from this mutant, in contrast to that from the parental strain, is strongly inhibited by low concentrations (1 μg/ml) of rifampin, whereas RNA polymerase I from the two strains is similar in behavior. The mutant may be useful for the study of regulatory mechanisms of transcription in eukaryotes.

Rifampin inhibits the initiation of transcription and has been widely used in the studies on the mechanism of ribonucleic acid (RNA) synthesis in bacteria (see reference 10). It is known that wild-type yeast cells are resistant to this antibiotic. Recently, we isolated and partly characterized several sorbitol-dependent fragile mutants of Saccharomyces cerevisiae, which grow exponentially in the presence of sorbitol but break open and release up to 90% of their RNA when resuspended in low-salt buffers (13). One of these fragile mutants, VY1160, derived from the parental S288 wild strain, showed a higher sensitivity to rifampin.

Here we report results on the synthesis of different RNA species in the VY1160 fragile yeast mutant in the presence of rifampin. High concentrations of rifampin cause an early arrest in the synthesis of all types of RNA, followed by an inhibition of protein synthesis. Lower concentrations of rifampin inhibit preferentially the synthesis of ribosomal RNA (rRNA) in comparison to that of messenger RNA (mRNA) and transport RNA (tRNA). Partially purified yeast RNA polymerase II from this mutant is inhibited by low concentrations of rifampin.

MATERIALS AND METHODS

The fragile yeast mutant VY1160 was used. Media and conditions of cultivation have been described (13). RNA and protein synthesis in whole cells were determined by labeling cultures with either [3H]uracil or a 14C-labeled amino acids mixture and measuring 5% trichloroacetic acid-precipitable radioactivity. All samples were filtered through Whatman GF/C glass-fiber disks, washed with cold 5% trichloroacetic acid, dried, and counted with a toluene-2,5-diphenyloxazole (PPO)-1,4-bis-(4-methyl-5-phenyloxazole-2-ylbenzene) (dimethyl-POPOP) phosphor (toluene, 1 liter, PPO, 5 g, dimethyl-POPOP, 0.25 g) in a Packard Tri-Carb 3320 spectrometer.

Isolation of total RNA. The cells were harvested and suspended in one-fifth of the culture volume of 0.01 M NaCl, 0.01 M ethylenediaminetetraacetate (pH 7.0), 1% sodium dodecyl sulfate. The RNA was extracted with an equal volume of phenol saturated with 0.14 M NaCl (pH 6.0) for 15 min at 60 C. After cooling and centrifugation for 15 min at 5,000 × g, the water phase was deproteinized with phenol containing 10% chloroform for 15 min at 25 C. The RNA from the final water phase was precipitated with 2 volumes of 95% ethanol, containing 1% potassium acetate, at −10 C. The yield was about 1 absorbancy unit at 260 nm of RNA per 5 × 106 cells. The RNA was analyzed by agar-gel electrophoresis (11). The absorbancy of the dried agar-gel plates was recorded at 260 nm with a recording spectrophotometer. The dried agar-gel plates were cut in 2-mm slices, and RNA was hydrolyzed with 5% NH4OH for 18 h at 37 C and counted with a toluene-PPO-dimethyl-POPOP phosphor (as above) containing 30% Triton X-100. Polyadenyllic acid (poly(A))-containing mRNA was separated from total RNA by the use of an oligod(T)-cellulose column (5).

Analysis of polysomes. To 10 ml of a log-phase culture, 0.1 μCi of [3H]uracil per ml was added to label stable RNA. After 4 h of cultivation, 2 μCi of [3H]uracil per ml was added, and the culture was incubated further for 15 min. The cells were collected and suspended in 1 ml of 10 mM tris(hydroxy-methyl)aminomethane (Tris)-hydrochloride (pH 7.8)
from the Radiochemical Centre, Amersham, England. p-Nitrophenyl-α-D-glucopyranoside A.R., PPO, and dimethyl-POPOP were from Koch-Light Laboratories, Ltd., Colnbrook, England. Oligo (dT)-cellulose was a product of Collaborative Research Inc., Waltham, Mass. Rifampin was a generous gift of L. Silvestri, Gruppo LePetit Spa, Milan, Italy, and α-amanitin was a gift of Th. Wieland, Institute of Medical Chemistry, The University, Heidelberg, BrD. Calf thymus deoxyribonucleic acid type I was from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Response of the VY1160 mutant to rifampin. Rifampin at concentrations between 50 and 200 μg per ml of culture (5.10^6 cells) inhibits the incorporation of [3H]uracil to a plateau level at about 60% of the control (Fig. 1). Higher concentrations of the antibiotic cause additional, essentially complete inhibition of [3H]uracil incorporation. Figure 2 shows the time dependence of rifampin action on RNA and protein synthesis in VY1160. The inhibitory effect is observed as early as 5 min after addition of the antibiotic. Protein synthesis is also inhibited, but only after a lag period of more than 10 min. It should be noted that, even at high concentrations of rifampin that block RNA synthesis completely, proteins continue to be labeled at a decreasing rate for about 60 min.

Effect of rifampin on the synthesis of different RNA species. The observation of a plateau in the action of increasing concentrations of rifampin on RNA synthesis (see Fig. 1), and the rapid effect of the antibiotic on RNA synthesis followed by a relatively slow inhibition of pro-

![Fig. 1. Response of the fragile yeast mutant VY1160 to different concentrations of rifampin. Aliquots of exponentially growing VY1160 cells were labeled for 30 min with 2 μCi of [3H]uracil per ml in the absence of different concentrations of rifampin. Rifampin and [3H]uracil were added simultaneously. One-milliliter samples were precipitated with an equal volume of cold 10% trichloroacetic acid, stored at 4 C for 4 h, filtered through glass-fiber filters, and counted as described in Materials and Methods.](http://aac.asm.org/)

**Materials.** Analytical grade reagents were used throughout. [3H]uracil (specific radioactivity, 23 Ci/mmole), [3H]uridine (specific radioactivity, 52 mCi/m mole), and [14C]-labeled amino acid mixture (specific radioactivity, 50 mCi/m mole) were obtained from the Radiochemical Centre, Amersham, England. p-Nitrophenyl-α-D-glucopyranoside A.R., PPO, and dimethyl-POPOP were from Koch-Light Laboratories, Ltd., Colnbrook, England. Oligo (dT)-cellulose was a product of Collaborative Research Inc., Waltham, Mass. Rifampin was a generous gift of L. Silvestri, Gruppo LePetit Spa, Milan, Italy, and α-amanitin was a gift of Th. Wieland, Institute of Medical Chemistry, The University, Heidelberg, BrD. Calf thymus deoxyribonucleic acid type I was from Sigma Chemical Co., St. Louis, Mo.

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Effect of rifampin on the synthesis of different RNA species. The observation of a plateau in the action of increasing concentrations of rifampin on RNA synthesis (see Fig. 1), and the rapid effect of the antibiotic on RNA synthesis followed by a relatively slow inhibition of pro-
protein synthesis, suggested a selective in vivo action of rifampin on the synthesis of a particular RNA species. The inhibition of the synthesis of different RNA species by increasing concentrations of rifampin is shown in Table 1. Concentrations higher than 300 μg/ml of culture (5.10⁶ cells) caused an almost complete inhibition of all three RNA species. However, rifampin concentrations of 50 to 200 μg/ml inhibited preferentially the synthesis of rRNA as compared to that of mRNA and tRNA (Table 1).

The inhibition of [methyl-³H]methionine labeling of rRNA (Table 1) might have been due to an inhibition of methylation rather than to a decreased rRNA synthesis. To obtain direct evidence for the preferential inhibition of rRNA synthesis by rifampin, two types of experiments were carried out.

First, the polysome profile of VY1160 cells, short-term labeled with [³H]uracil in the presence or absence of rifampin (200 μg/ml), was analyzed (Fig. 3). Newly synthesized RNA from the control appears in polysomes, monosomes, free ribosomal subparticles, and slower sedimenting tRNA. However, RNA made in the presence of rifampin mainly enters the polysome fraction, whereas very little radioactivity is seen in the region of monosomes and free subparticles.

In the second type of experiments, growing VY1160 cells were labeled with [³H]uracil for 15 min with or without 200 μg of rifampin per ml. Total RNA was isolated from both cultures and analyzed by agar-gel electrophoresis (Fig. 4). The labeling profile of total RNA from the control shows the three main peaks of stable RNA, i.e., 25S, 18S, and tRNA. A rapidly labeled, heterogeneous RNA fraction (retained by oligo(dT)-cellulose) overlaps with the two rRNA peaks. This heterogeneous RNA is the main species labeled in the presence of rifampin (Fig. 4B). The bulk of this heterogeneous RNA, labeled in the presence of rifampin, is retained by oligo(dT)-cellulose and therefore represents poly(A)-containing mRNA (data not shown). The label in tRNA from rifampintreated cells is inhibited to about 50%, which is in good agreement with data obtained by [methyl-³H]methionine labeling (Table 1). The two types of experiments described above provide conclusive evidence that the synthesis of rRNA in vivo in the VY1160 yeast mutant is more susceptible to rifampin as compared to the synthesis of mRNA and tRNA.

**Induction of α-glucosidase in the presence of rifampin.** Enzyme induction requires the de novo synthesis of mRNA. As shown above, 200 μg of rifampin per ml blocks rRNA synthesis, whereas mRNA continues to be made at about one-half the control rate. The synthesis of functional mRNA in the presence of rifampin was studied by following the induction of α-

**Table 1. Rifampin inhibition of the synthesis of different RNA species in the fragile yeast mutant VY1160**

<table>
<thead>
<tr>
<th>Rifampin concn (μg/ml of culture)</th>
<th>rRNA (counts/min per A260 per unit)</th>
<th>tRNA (counts/min per A260 per unit)</th>
<th>poly(A)-mRNA (counts/min per 5 A260 units of total RNA)</th>
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<td>100</td>
<td>1,190</td>
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* Aliquots of a growing VY1160 culture were labeled for 15 min with 2.5 μCi of [methyl-³H]methionine per ml for rRNA and tRNA or with 4 μCi of [³H]uracil per ml for total RNA (including mRNA). The total RNA from the samples was isolated as described in Materials and Methods. rRNA was separated from tRNA by precipitation with 2 M NaCl. The poly(A)-containing mRNA fraction from total [³H]uracil-labeled RNA was obtained from 5 absorbancy units at 260 nm (A260) of total RNA by the use of an oligo(dT)-cellulose column. The figures are mean values from four independent experiments.
on the concentration of rifampin used. Very high concentrations of rifampin inhibit almost completely the induction of α-glucosidase. However, a good correlation exists between the percentage of inhibition of α-glucosidase induction and the synthesis of poly(A)-containing mRNA. These data indicate that functional mRNA continues to be made in yeast cells in the presence of rifampin under conditions that completely arrested rRNA synthesis. They show further that rifampin has little if any effect on the translation of mRNA in vivo.

Inhibition of VY1160 yeast RNA polymerases by rifampin. The effect of rifampin on RNA polymerases was studied with crude cell lysates and purified enzymes from VY1160 cells. RNA polymerases I and II in crude cell lysates were distinguished by their susceptibility to α-amanitin. In agreement with previous authors (4, 14), both RNA polymerases from the parental s288 wild-type strain were resistant to rifampicin, tested at concentrations up to 100 μg/ml. In contrast, the RNA polymerase II activity from lysates of the VY1160 mutant was strongly inhibited by rifampin, the effect of the drug being fully displayed at 1 μg/ml. The results presented in Table 3 show that purified RNA polymerase II is markedly more

![Fig. 3. Sucrose density gradient analysis of polysomes from VY1160 cells treated with rifampin. The fragile yeast cells growing exponentially were labeled for 4 h with 0.1 μCi of [14C]uracil per ml. The culture was divided in two parts, each one receiving 2 μCi of [3H]uracil per ml with or without 200 μg of rifampin per ml. After 15 min the cells were chilled and lysed, and the crude post-mitochondrial lysates (9,000 × g supernatant fluid) was layered over a 10 to 30% sucrose density gradient as described in Materials and Methods. The [3H] and [14C] counts precipitated with cold 5% trichloroacetic acid were determined. The [3H] counts in each gradient fraction from the culture treated with rifampin were normalized against the [14C] counts of the corresponding fraction from the culture without rifampin. The [14C] (△) and [3H] (●) counts from the control culture and the [3H] (○) counts from the rifampin-treated culture are plotted.](http://aac.asm.org/)
susceptible to rifampin than RNA polymerase I. In a series of independent experiments, purified RNA polymerase II was inhibited to about 15 to 30% residual activity by 1 μg of rifampin per ml. On the other hand, α-amanitin-resistant RNA polymerase I was inhibited only to 80 to 90% residual activity by this concentration of rifampin. Experiments with crude cell lysates yielded essentially the same results. These results show that the partially purified RNA polymerase II from the VY1160 mutant strain is inhibited by rifampin at concentrations similar to those inhibiting Escherichia coli RNA polymerase (10). The possibility of cross-contamination of polymerases I and II does not permit one to conclude whether the observed slight inhibition of RNA polymerase I is significant or not. In any case, the inhibition of RNA polymerase II by rifampin is markedly more pronounced than that of RNA polymerase I.

## DISCUSSION

The in vivo synthesis of RNA in yeast cells appears to be susceptible to rifampin, as shown by amphotericin B-potentiated permeability of wild-type yeast (8). These results are confirmed in our present work, but the osmotic-susceptible mutant VY1160 is several-fold more susceptible to rifampin without the synergetic action of amphotericin B. The mutant VY1160 was isolated as a rifampin-susceptible colony on an enriched media plate containing 10% sorbitol (13), which might explain its higher susceptibility to rifampin. Thus, the action of rifampin may be explained by an increased cell wall permeability and/or alterations in RNA polymerases conferring rifampin susceptibility to these enzymes. Our results show that partly purified RNA polymerase II from the VY1160 mutant is inhibited by rifampin at concentrations comparable to those reported for E. coli (10). These results were unexpected in view of the reported unsusceptibility of purified yeast RNA polymerases I and II to rifampin (4, 14). At present, we cannot decide whether the RNA polymerase II of VY1160 mutant is itself mutated to rifampin susceptibility, or, alternatively, whether the RNA polymerase preparations obtained by the authors (4, 14) have lost some factor required for rifampin susceptibility of the enzyme during purification. The second possibility remains open since VY1160 mutant is lysed under milder conditions, with a minimal release of nuclease or protease activities.

The in vivo action of rifampin at lower doses displays a preferential inhibition of rRNA synthesis as compared to that of mRNA and tRNA. Even at complete inhibition of rRNA synthesis, protein synthesis continues and functional mRNA is made, as evidenced by the possibility of α-glucosidase induction. Therefore, rifampin does not interfere directly with cellular protein synthesis. The higher in vivo susceptibility of rRNA synthesis in the VY1160 mutant cannot be correlated with the in vitro effect of rifampin as a stronger inhibitor of RNA polymerase II. Consequently, the in vivo action of rifampin may involve additional mechanisms participating in the transcription of rRNA genes or ribosome biogenesis. It should be noted that a similar difference in the in vivo and in vitro action of the rifamycin derivative AF013 (and other related derivatives) was reported also with HeLa cells (2).

## LITERATURE CITED

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