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Role of DNA-Dependent RNA Polymerase III in the Transcription of the tRNA and 5S RNA Genes

(mouse myeloma cells/isolated nuclei/α-amanitin)

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ABSTRACT **Mouse myeloma cells have previously been shown (L. B. Schwartz, V. E. F. Sklar, J. A. Jaehning, R. Weinmann & R. G. Roeder, submitted for publication) to contain two chromatographically distinct forms of RNA polymerase III (designated III_A and III_B). The enzymes are unaffected by low α-amanitin concentrations which completely inhibit RNA polymerase II, but they exhibit characteristic inhibition curves (identical for III_A and III_B) at higher toxin concentrations. RNA polymerase I was unaffected at all α-amanitin concentrations tested. Myeloma RNA polymerases II, III_A, and III_B appear to be inhibited by the same mechanism, since the toxin rapidly blocks chain elongation by each enzyme. The characteristic α-amanitin sensitivity of RNA polymerase III has been employed in studies of the function(s) of the class III RNA polymerases.**

Isolated myeloma nuclei and nucleoli continue to synthesize RNA via the endogenous RNA polymerases when incubated *in vitro*. With nuclei, newly synthesized 4S precursor (pre-4S) and 5S RNA species were detected by electrophoretic analysis either of the total nuclear RNA or of the RNA released into the supernatant during incubation. The synthesis of both pre-4S and 5S RNA species was inhibited by α-amanitin, but only at high concentrations; and the α-amanitin inhibition curves for these RNAs were identical to those obtained for solubilized RNA polymerases III_A and III_B. In control experiments it was shown that the endogenous RNA polymerase II activity of isolated nuclei was inhibited by α-amanitin concentrations similar to those required to inhibit purified enzyme II. However, 40–50% of the endogenous activity of nuclei and 100% of the endogenous activity of purified nucleoli was completely resistant to the high α-amanitin concentrations necessary to inhibit the RNA polymerase III activities. These experiments rule out nonspecific inhibitory effects in the endogenous systems.

These results unequivocally demonstrate the role of RNA polymerase III (III_A and/or III_B) in the synthesis of (pre) 4S RNAs and a 5S RNA species.

Implicit in the existence of multiple forms of DNA-dependent RNA polymerase (RNA nucleotidyltransferase, EC 2.7.7.6) (1–3) in higher organisms is the likelihood that each of the enzyme forms has a specialized role in the transcription of genetic information. The available evidence supports this hypothesis. RNA polymerase I is presumed to transcribe the rRNA genes because of its nucleolar localization (4) and the insensitivity of rRNA synthesis in isolated nuclei to low concentrations of α-amanitin (5–7) that inhibit polymerase II but not RNA polymerases I and III (8, 9). Nucleoplasmic RNA polymerase II (4) is responsible for the synthesis of heterogeneous nuclear (DNA-like) RNA (5–7), from which mRNA is presumably derived.

RNA polymerase III, usually a minor enzyme component, has been found in a variety of tissues, including sea urchin (1, 2), rat liver (1, 9), yeast (1, 10), amphibian oocytes and embryos (11, 12), and human KB cells (13), although its function has remained a matter of speculation. Mouse myeloma cells have recently been found to contain relatively high levels of RNA polymerase III activity (up to 25% of the total) and two chromatographic forms (designated III_A and III_B) have been partially purified and characterized (14). For the purpose of the present experiments the most significant finding was that, while these enzymes are not inhibited by low doses of α-amanitin that completely inhibit RNA polymerase II, they are completely inhibited by higher concentrations of the toxin. No effects on RNA polymerase I were observed. The differential sensitivities of the class I, II, and III polymerases thus offered a means by which the function of the class III enzymes could be tested.

The endogenous RNA polymerase II activity in isolated nuclei was previously shown to be inhibited at concentrations of α-amanitin similar to those which inhibited the purified enzyme II (9). Thus the same should be true for the endogenous and purified RNA polymerase III activities if RNA polymerases II and III are inhibited by a similar mechanism. Furthermore, transcription of the genes for rRNA (5), tRNA, and 5S RNA (15, 16) has been shown to proceed in isolated nuclei (via endogenous RNA polymerase activities) with the same fidelity that is observed *in vivo*. Here we have utilized mouse myeloma nuclei that are active in the synthesis of these RNAs, and the differential α-amanitin sensitivity of RNA polymerase III, to show that the 4S RNA precursors (pre-4S RNAs) and a 5S RNA species are synthesized by RNA polymerase III (III_A and/or III_B).

MATERIALS AND METHODS

Isolation of Nuclei and Nucleoli. MOPC 315 solid tumors were obtained as described previously (14). Nuclei were prepared as previously described (4, 14) except that the buffer used was 50 mM Tris·HCl, pH 7.9, 24 mM KCl, 15 mM $MgCl_2$, 0.24 mM spermine, 2 mM dithioerythritol and either 0.34 M or 2.3 M sucrose. Centrifugation was for 20 min at 40,000 rpm in an SW 40 rotor. The white nuclear pellets were resuspended to approximately 2 mg of DNA per ml in buffer C [50 mM Tris·HCl, pH 7.9, 1 mM ethylenediaminetetra-

Abbreviations: 4S RNA, low-molecular-weight RNA fraction containing the tRNAs; pre-4S RNA, 4S RNA precursor; 5S RNA, low-molecular-weight ribosome-associated RNA; 7S RNA, low-molecular-weight RNA derived from the 28S rRNA after denaturation.

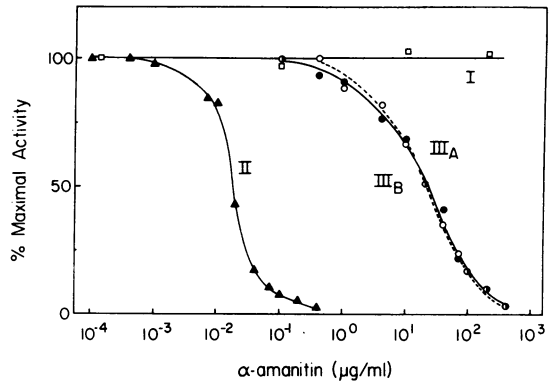

FIG. 1. Effect of α-amanitin concentration on the activity of isolated RNA polymerases. RNA polymerases I, II, III$_A$, and III$_B$ were obtained by chromatography on DEAE-Sephadex of high-ionic-strength extracts of MOPC 315 tumors as described previously (14). RNA polymerases III$_A$ and III$_B$ were further purified and concentrated by chromatography on carboxymethyl-Sephadex as described (14) and were a generous gift of Mr. V. Sklar. The activities of the partially purified RNA polymerases were measured in 25-μl reaction volumes under conditions previously described (11) with the following changes. Native MOPC 315 DNA was used as template, the Mn^{++} concentration was 1.0 mM, the ammonium sulfate concentration was 0.06 M (enzymes I, III$_A$, and III$_B$) or 0.08 M (enzyme II), and incubation was for 20 min at 20°. In all experiments, [^{3}H]-UTP (New England Nuclear Corp.) adjusted to 0.7 Ci/mmole was the sole radioactive precursor and was present at 0.05 mM. The α-amanitin concentration (premixed with substrates and template at 0° prior to enzyme addition) was varied as indicated. Maximal activity (100%) represents 85, 53, 20, and 15 pmoles of UMP incorporated per 20 min, respectively, for RNA polymerases I (□——□), II (▲——▲), III$_A$ (O- - -O), and III$_B$ (●——●).

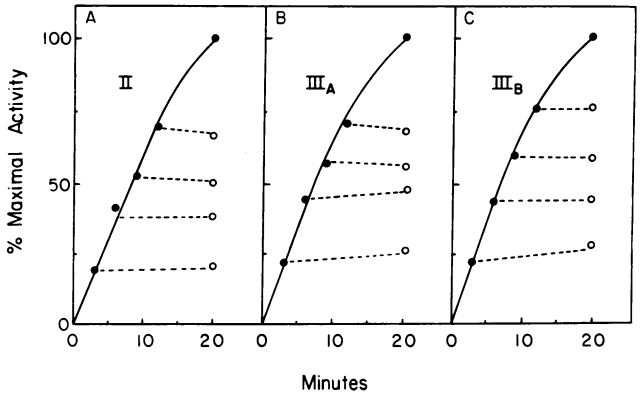

FIG. 2. Effect of α-amanitin on the activity of RNA polymerases in preinitiated transcription complexes. RNA Polymerases II, III$_A$, and III$_B$ were assayed as described in Fig. 1. For each enzyme two parallel series of reactions were initiated simultaneously. One series was employed for determination of the normal course of the reaction (●——●). Alpha-amanitin was added to samples in the other series at the times indicated by the intersection of the *dashed lines* with the *solid line* (3, 6, 9, and 12 min), and incubation continued up to 20 min (O- - -O). The final α-amanitin concentrations used were 8 μg/ml for II (*A*), 200 μg/ml for III$_A$ (*B*), and 200 μg/ml for III$_B$ (*C*). Values represent the averages of duplicate determinations.

acetate (EDTA), 5 mM $MgCl_2$, 2 mM dithioerythritol, 25% (v/v) glycerol]. Nucleoli were prepared from nuclei isolated in the absence of Mg^{++} (14). All procedures were performed at 0–4°. The conditions for RNA synthesis and the methods used for extraction and electrophoretic analysis of RNA are described in the appropriate figure *legends*.

RESULTS

Activity of Purified RNA Polymerases. Previous studies indicated that the class III RNA polymerases are inhibited by α-amanitin but only at much higher concentrations than those required to inhibit RNA polymerase II (14). Since the assay conditions used previously (calf-thymus DNA template, higher Mn^{++} concentration, 17° higher incubation temperature) differed considerably from those found to be optimal for RNA synthesis in isolated nuclei (*below*), the experiments were repeated using the latter conditions. Similar results were obtained and as shown in Fig. 1 the levels of α-amanitin which gave 50% inhibition are about 0.02 μg/ml for enzyme II and 20 μg/ml for enzymes III$_A$ and III$_B$. The latter two enzymes show identical inhibition curves. Furthermore, the data show that at 0.5 μg/ml of α-amanitin RNA polymerase II is completely inhibited, while RNA polymerases III$_A$, III$_B$, and I are nearly completely resistant. However, at about 200 μg/ml (or greater) of α-amanitin RNA polymerases III$_A$ and III$_B$ are almost completely inhibited while RNA polymerase I remains unaffected. Thus the activity of each RNA polymerase class (I, II, and III) can be readily distinguished in situations where the activities cannot otherwise be separated.

For later studies with isolated nuclei in which RNA synthesis proceeds primarily via elongation of preinitiated RNA chains, it was important to ascertain that α-amanitin could inhibit RNA polymerase III already in the act of transcription (i.e., in transcription complexes) as well as free (unbound) enzyme (Fig. 1). As indicated in Fig. 2 for enzymes II, III$_A$, and III$_B$, the addition of α-amanitin to an incubation mixture at any time during the linear period of RNA synthesis immediately halts the activity of the enzymes. Thus, as proposed previously for RNA polymerase II (8, 9), α-amanitin appears to block elongation by RNA polymerases III$_A$ and III$_B$ as well.

Endogenous RNA Polymerase Activity in Nuclei and Nucleoli. Previous studies indicated that the endogenous RNA polymerase II activity of isolated nuclei exhibits the same sensitivity to α-amanitin as does the purified enzyme on exogenous DNA (9). Similar results are found in the present system (Fig. 3) under conditions employed later to study the synthesis of specific RNA species. Nuclei were incubated with increasing concentrations of α-amanitin for 20 min at 20°. At two different salt concentrations, where endogenous RNA polymerase II activity is high, about the same α-amanitin concentrations were necessary for 50% inhibition of the total sensitive activity (0.022 μg/ml at 0.04 M ammonium sulfate and 0.030 μg/ml at 0.20 M ammonium sulfate, as indicated by the *arrows* in Fig. 3). These values were comparable to that obtained with the purified enzyme on exogenous DNA (Fig. 1). A small 5–10% reduction in total RNA synthesis was occasionally observed at α-amanitin concentrations comparable to those required to inhibit purified RNA polymerase III, but whether this can be totally attributed to endogenous RNA polymerase III activity is not yet certain.

In contrast to the results obtained with intact nuclei, the endogenous RNA polymerase activity in isolated nucleoli was completely refractory to concentrations of α-amanitin that completely inhibit RNA polymerase III activity (Fig. 3). Furthermore, the sizes and amounts of the RNAs synthesized

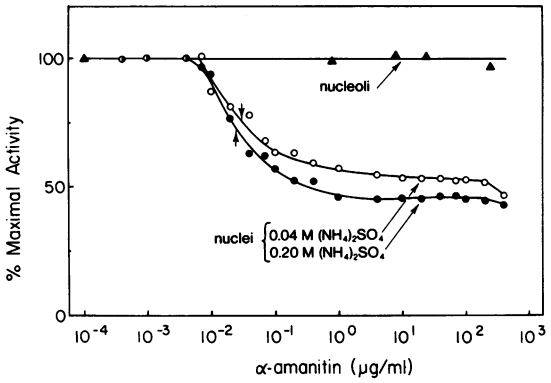

FIG. 3. Effect of α-amanitin concentration on endogenous RNA polymerase activity in isolated nuclei and nucleoli. Nuclei and nucleoli were obtained as described in *Materials and Methods*. The endogenous RNA polymerase activity in nuclei or nucleoli was measured under conditions similar to those in Fig. 1 except for the following changes. Exogenous DNA was omitted and phosphoenol pyruvate and pyruvate kinase (Sigma, Type IV) were added, respectively, to final concentrations of 4 mM and 7 units/ml. Reactions were terminated by addition of DNase I (Worthington, electrophoretically purified) to 800 μg/ml and further incubation for 10 min at 0°. The digests were spotted on DEAE paper discs, washed, and dried, and radioactivity in RNA was determined as described (11). Maximal activity (100%) represents 48, 60, and 189 pmoles of UMP incorporated, respectively, in nuclei assayed at 0.04 M ammonium sulfate (O——O) or nuclei assayed at 0.20 M ammonium sulfate (●——●), and nucleoli assayed at 0.06 M ammonium sulfate (▲——▲). The *vertical arrows* indicate the points at which that activity which is ultimately sensitive to α-amanitin is inhibited by 50%.

by nucleoli in the presence and absence of 120 μg/ml of α-amanitin were identical, as determined by sucrose density gradient analysis of high-molecular-weight RNAs (the predominant species) and polyacrylamide gel electrophoretic analysis of low-molecular-weight RNAs (data not shown). These data are consistent with those demonstrating that nucleoli synthesize rRNA (7) and that they contain predominantly, if not exclusively, RNA polymerase I (4, 14). In addition, the high-molecular-weight RNAs (>10 S) synthesized by isolated nuclei in the presence of low (1 μg/ml) and high (200 μg/ml) concentrations of α-amanitin were also quantitatively and qualitatively the same (data not shown). Since isolated somatic cell nuclei synthesize almost exclusively rRNA at low concentrations of α-amanitin (where RNA polymerase II is inhibited) (5, 7), these observations suggest that the endogenous RNA polymerase I activity remains completely refractory to high concentrations of α-amanitin even when measured in isolated nuclei, and provide further evidence for the specificity of the toxin.

Synthesis of 5S and pre-4S RNA in Isolated Nuclei. Others have shown that HeLa (15) and myeloma (16) nuclei synthesize 5S RNA and 4S precursor (pre-4S) RNA species and that a large fraction of this is released into the incubation medium (15). Here the effects of α-amanitin on the synthesis of these low-molecular-weight RNAs are examined. In the experiment shown in Fig. 4 only the RNA released into the incubation medium was examined, since in the present system about 80% of the total newly synthesized RNA in the 4S–5S region is released, thus permitting analysis of restricted classes of RNAs and reducing background radioactivity. With UTP as radioactive precursor little or no radioactivity in RNA is

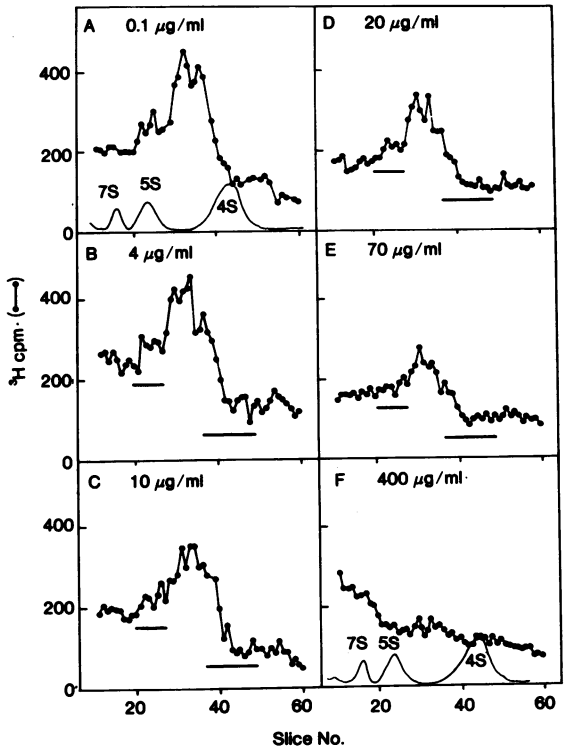

FIG. 4. Effect of α-amanitin on the synthesis of low-molecular-weight RNA released from nuclei during incubation. Isolated nuclei were incubated in the presence of 0.06 M ammonium sulfate as described in Fig. 3 except that the reaction volumes were increased to 50 μl and the [^{3}H]UTP specific activity to 36.8 Ci/mmole. Alpha-amanitin was present at concentrations of 0.1 μg/ml (*A*), 4 μg/ml (*B*), 10 μg/ml (*C*), 20 μg/ml (*D*), 70 μg/ml (*E*), and 400 μg/ml (*F*). After incubation for 10 min at 20°, reactions were terminated by cooling to 0°, and carrier ribosomal RNA was added. Nuclei were removed by centrifugation for 10 min at 1600 × *g* in a refrigerated IEC PR-6 centrifuge. RNA in the supernatant was phenol extracted and ethanol precipitated twice (22). Half of each sample (about 9000 cpm for each) was analyzed on sodium dodecyl sulfate–polyacrylamide slab gels (12 cm × 14 cm × 0.1 cm) as described (23). The gels contained 12% acrylamide and 0.4% ethylene diacrylate, and the electrophoresis buffer was 36 mM Tris·HCl (pH 7.2), 30 mM NaH_2PO_4, 1 mM ethylenediaminetetraacetate, and 0.2% dodecyl sulfate. Electrophoresis was at 20° for 5 hr at 100 V and 40 mA. A purified RNA mixture containing ribosomal 7S RNA, 5S RNA, and tRNA markers (previously denatured with formamide) was run on adjacent slots of the same slab gel. The identity of these RNAs was previously ascertained by comparison with highly purified cytoplasmic tRNA, 5S RNA from purified ribosomes, and 7S RNA from purified 28S RNA. In panels *A* and *F* the absorbance scans obtained with a Gilford Linear Transport at 260 nm are reproduced (*solid lines*), while in panels *B–D horizontal bars* denote the corresponding positions of the 5S and 4S RNA markers. Gel slices (1.1 mm) were dissolved in 0.1 ml TS-1 solubilizer (Research Products International Corp.) and 5 ml of toluene-based scintillation fluid, incubated overnight at 37° with shaking, and radioactivity was determined (no backgrounds were subtracted).

apparent in the 4S RNA region, consistent with the observation that *in vivo* the 4S RNA species are synthesized as larger precursors which are subsequently processed by cytoplasmic enzymes (reviewed in ref. 17). In Fig. 4, the large radioactive peak to the left of the 4S region appears to represent 4S RNA precursors (15), since incubation of these RNAs with cyto-

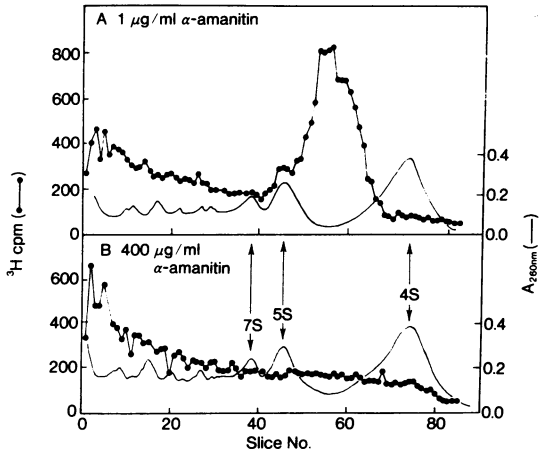

FIG. 5. Effect of α-amanitin on the synthesis of total low-molecular-weight RNA in isolated nuclei. Isolated nuclei were incubated as described in Fig. 4. Alpha-amanitin was present at a concentration of 1 μg/ml (*A*) or 400 μg/ml (*B*). Reactions were terminated by cooling to 0° and were then treated with 80 μg/ml of DNAse for 10 min at 0°. Total RNA in each reaction mixture was extracted as described in Fig. 4. Each ethanol-precipitated sample was dissolved in 100% formamide (23°) to denature the RNA and was diluted with an equal volume of 1 M sucrose prior to electrophoresis. Sodium dodecyl sulfate–polyacrylamide electrophoresis of the samples (2.5×10^5 cpm in *A* and 2.3×10^5 cpm in *B*) and of formamide-denatured marker RNAs were the same as in Fig. 4.

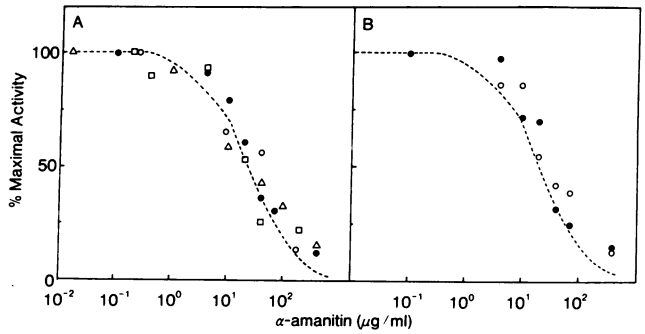

FIG. 6. Sensitivity of pre-4S RNA and 5S RNA synthesis to α-amanitin. The data are taken from experiments (analogous to those shown in Figs. 4 and 5) in which the synthesis of pre-4S RNA and 5S RNA by isolated nuclei was monitored. Data are calculated as the % radioactivity remaining in the pre-4S and/or 5S RNA peak as a function of the α-amanitin concentration present during the period of synthesis. In (*A*) the results of four separate experiments are shown. In three of these experiments (○, △, □) the total 5S plus pre-4S RNA synthesized in isolated nuclei was monitored. Data for the fourth experiment (●) is from Fig. 4, where only RNA released into the supernatant was monitored. The 100% values (with the appropriate backgrounds subtracted) were 9364 cpm (○), 9537 cpm (□), 8910 cpm (△), and 3798 cpm (●). In (*B*) the 5S RNA and pre-4S RNA data from the experiment in Fig. 4 are plotted separately. The radioactivity under the region occupied by the 5S RNA marker was scored as 5S RNA (after background subtraction) and all radioactivity to the right of the 5S RNA region and up to the rightward boundary of the tRNA marker was scored as pre-4S RNA (after background subtraction). The 100% values were 3254 cpm for the pre-4S RNA (○) and 543 cpm for the 5S RNA (●). Some additional data (results at different α-amanitin concentrations) from the same experiment, but not shown in Fig. 4, are also plotted. The *dashed line* in both (*A*) and (*B*) represents the α-amanitin inhibition curve for RNA polymerases III_A and III_B and is replotted from Fig. 1.

plasmic extracts produces maturation to 4S RNA species (refs. 17, and 19, and unpublished observations). When CTP was employed as radioactive precursor, considerable radioactivity in the 4S region was observed (data not shown), presumably as a result of end labeling of tRNA via contaminating cytoplasmic enzymes present even in highly purified nuclear preparations.

Two small radioactivity peaks are apparent in the 5S RNA region in Fig. 4. Similar conformational isomers of 5S RNA have previously been described in native 5S RNA from mouse myeloma cells (16) and in 5S RNA from other sources (18). The 5S RNA marker in Fig. 4 does not display this electrophoretic heterogeneity because of prior denaturation in formamide, and in subsequent experiments similar denaturation of newly synthesized RNA resulted in a single radioactive 5S RNA species (see Fig. 5, below). The mobility of the radioactive 5S RNA species is not affected by prior incubation with cytoplasmic extracts (unpublished observations) as described in other systems (19).

The data in Fig. 4 show that increasing concentrations of α-amanitin result in inhibition of the synthesis of the 5S and pre-4S RNA species. To exclude the possibility that the toxin simply inhibits the release of the RNA species from nuclei instead of their synthesis, total RNA from nuclei incubated under similar conditions was analyzed as shown in Fig. 5. Both 5S RNA and pre-4S RNAs are synthesized in the presence of 1 μg/ml of α-amanitin (panel *A*) while neither is synthesized in the presence of 400 μg/ml of α-amanitin (panel *B*), in agreement with the observations in Fig. 4.

In order to compare directly the α-amanitin sensitivity of 5S and pre-4S RNA synthesis with the α-amanitin sensitivity of RNA polymerases III_A and III_B, the data from several experiments are summarized in Fig. 6. The percentages of radioactivity incorporated into the combined 5S and pre-4S RNA's or separately into 5S RNA and pre-4S RNA are shown, respectively, in Fig. 6*A* and *B*. Fig. 6*A* includes data from three experiments in which whole nuclear RNA was examined and data from one experiment in which only RNA released into the incubation medium was examined. In Fig. 6*B* the data from a single experiment (that shown in Fig. 4) are shown. The α-amanitin inhibition curve for RNA polymerases III_A and III_B (Fig. 1) is reproduced in both panels (*dashed line*). In all cases the α-amanitin sensitivity of nuclear 5S and pre-4S RNA synthesis corresponds closely to the α-amanitin sensitivity of purified RNA polymerases III_A and III_B.

DISCUSSION

The activities of the class I, II, and III RNA polymerases can readily be distinguished on the basis of their differential sensitivities to α-amanitin (ref. 14 and Fig. 1). In the present experiments α-amanitin was utilized in conjunction with an *in vitro* transcription system (isolated nuclei) to demonstrate the function of RNA polymerase III (III_A and/or III_B) in the synthesis of pre-4S and 5S RNA species. This combined approach was necessary for several reasons. The purified RNA polymerases (including RNA polymerase III) have not yet been demonstrated to selectively transcribe specific genes when presented with natural DNA templates *in vitro*. Second, intact cells, unlike isolated nuclei (9), are not freely permeable to α-amanitin, making it impractical to determine intracellular

concentrations of toxin. Moreover secondary effects of the toxin are apparent in intact cells (20).

α-Amanitin is a suitable reagent for analysis of the sensitivity of endogenous RNA synthesis in nuclei since it was found to block RNA synthesis instantaneously in preinitiated complexes (refs. 8, 9, and Fig. 2), and since it was shown, in the case of RNA polymerase II, to inhibit equally effectively the activity of the purified enzyme on exogenous DNA and the activity of the endogenous enzyme (presumably in preinitiated complexes) (ref. 9 and Figs. 1 and 3). Moreover, nonspecific effects of high α-amanitin concentrations appear unlikely because of the complete insensitivity of the nucleolar RNA polymerase, as well as a major fraction of the endogenous nuclear activity, to these concentrations (Fig. 3). The use of a specific enzyme inhibitor clearly avoids the ambiguities encountered in attempts to use nonspecific reagents such as salts or metal ions to discern the functions of specific enzymes in complex transcription systems (15), since in the latter case effects on nonenzymatic components associated with specific gene transcription cannot readily be eliminated.

It is inferred that the toxin α-amanitin interacts directly with the RNA polymerase III enzymes, since it does not block the activity of RNA polymerase I on any templates and since the same concentrations are required for 50% inhibition of activity with myeloma DNA, calf-thymus DNA, and poly(dA-dT) (Fig. 1, ref. 14, and unpublished observations). Furthermore, α-amanitin appears to inhibit both class II and class III RNA polymerases by blocking RNA chain elongation (Fig. 2), and in the case of RNA polymerase II has been shown to bind to one of the large subunits of this enzyme (21). Whether RNA polymerase III contains a similar α-amanitin-binding subunit remains to be established. The possibility that an impurity in the α-amanitin inhibits RNA polymerase III seems unlikely, since three different samples of α-amanitin were found to be equally effective in inhibiting RNA polymerase III activity (unpublished observations).

The present studies demonstrate that MOPC 315 nuclei synthesize pre-4S and 5S RNA via endogenous RNA polymerase, consistent with previous observations (15, 16). These RNA species were initially identified by their electrophoretic mobilities. The pre-4S RNAs were further identified by their susceptibility to conversion to 4S RNA species by cytoplasmic extracts (unpublished observation and refs. 17 and 19), and the 5S RNA peaks were further identified as true 5S RNA because of their resolution into two distinct electrophoretic peaks before (Fig. 4) but not after (Fig. 5) formamide denaturation (refs. 16, 18, and Figs. 4 and 5). The synthesis of these pre-4S and 5S RNAs shows exactly the same sensitivity to α-amanitin as do the purified class III RNA polymerases (Fig. 6). These data then provide very strong evidence for the involvement of a class III RNA polymerase in the transcription of the pre-4S and 5S genes. However, it is not yet possible to decide whether both groups of genes are transcribed by only one (III_A or III_B) or by both of the class III enzymes or whether III_A and III_B have distinct roles in the transcription of these genes. In addition, the present data do not rule out the possible involvement of an RNA polymerase III in the synthesis of other (minor) RNA species, although it is clear that other functions of the class III polymerases may be readily tested using methodology analogous to that described in this paper. It may also be noted that the present data do rule out the direct involvement of RNA polymerase III in the transcription of nucleolar (rRNA) genes in somatic cells, a possibility considered unlikely (4, 14), but not eliminated by earlier studies of the sensitivity of rRNA synthesis in isolated nuclei to low concentrations of α-amanitin (5–7).

The present data further support the idea that different genes or groups of genes are transcribed by distinct forms of RNA polymerase which differ in their template specificities. Thus, the class I, II, and III RNA polymerases transcribe, respectively, the genes for rRNA (4–7), the heterogeneous DNA sequences (4–7), and the genes for 5S RNA and the tRNAs (present data). Recent studies (14) indicate that the levels of RNA polymerases I and III show considerable variability, depending on the physiological state of the cell, and suggest that the activity of the rRNA and the tRNA and 5S RNA genes might be regulated directly via specific enzyme levels, although the need for additional factors is not ruled out. In contrast, the levels of RNA polymerase II show much less variability and suggest that the selective activity of this enzyme(s) in the transcription of specific gene sequences is probably regulated by other undefined components.

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