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EFFECT OF SARKOSYL ON THE FIDELITY OF PRERIBOSOMAL RNA SYNTHESIS IN ISOLATED NUCLEOLI

Babrubahan Samal, N. Raghuveera Ballal, Yong C. Choi, and Harris Busch

Department of Pharmacology, Baylor College of Medicine

Houston, Texas 77030

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SUMMARY

When nucleoli isolated from Novikoff hepatoma ascites cells were treated with 2% Sarkosyl, 95% of the nucleolar proteins were extracted. In addition to RNA polymerase I, a heterogeneous group of proteins remained bound to DNA. The homochromatography fingerprinting analysis of the transcripts of the Sarkosyl pellet had virtually all of the marker oligonucleotides that are found in transcripts from whole nucleoli. In addition, seven discrete spots were present which were not found either in transcripts from intact nucleoli or in 45S pre-rRNA labeled in vivo. These results suggest that the endogenous RNA polymerase I present in the Sarkosyl pellet may have transcribed sequences juxtaposed to pre-rRNA coding region in addition to faithfully transcribing the ribosomal genes. It is possible that Sarkosyl extracted factor(s) necessary for proper termination or processing of pre-rRNA.

INTRODUCTION

It is well established that isolated nucleoli retain the faithful expression of pre-ribosomal RNA in vitro (1,2). This specific expression is also retained in isolated nucleolar chromatin and in chromatin extracted with 0.35 M NaCl (3,4). Sequential NaCl extraction revealed that the specificity of transcription was lost at the approximate salt concentration, at which the endogenous RNA polymerase was dissociated from DNA. The specificity observed was probably due to the completion of the in vivo initiated RNA chains, as added RNA polymerase did not produce faithful transcripts (1).

To investigate the contribution of nuclear proteins other than the endogenously bound RNA polymerase I for the specificity

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of in vitro transcription, the nucleolar preparations were treated with Sarkosyl. Recently, it has been reported that Sarkosyl extracts most of the nuclear proteins except the initiated RNA polymerases (5,6). This report shows that Sarkosyl extracts about 95% of the nucleolar proteins without affecting the RNA synthesizing capacity of the residual nucleoprotein complex. The RNA synthesized in vitro contains most of the T_1 ribonuclease digestion fragments characteristic of 45S pre-rRNA. In addition, discrete extra spots are also present which might reflect continued transcription beyond the termination point or a loss of processing of the primary transcript.

MATERIALS AND METHODS

Materials - ^{3}H-labeled ribonucleotides were obtained from Schwarz/Mann. [α^{32}-P]- labeled ribonucleotides (specific activity 100-200 Ci/mmol) were purchased from International Chemical and Nuclear Corporation. Sarkosyl NL-97 was a product of Ciba-Geigy.

Isolation of Nucleoli and Sarkosyl Treatment - Nucleoli were isolated from Novikoff hepatoma ascites tumor cells as described before (1). The nucleoli were suspended in TPD (10 mM Tris HCl, pH 7.9, 0.1 mM PMSF, 0.2 mM dithiothreitol) and 20% Sarkosyl was added slowly to a final concentration of 2%. The residual nucleoprotein complex was separated from the dissociated proteins by centrifugation through a cushion of 25% glycerol at 170,000 x g for 2 hr. The pellet was suspended in TPD and transcribed within 30 min.

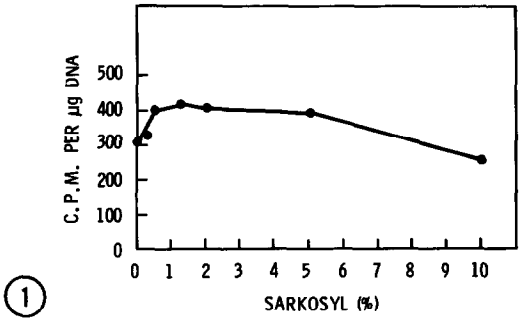
RNA Synthesis in vitro - In vitro transcription and processing for homochromatography fingerprinting was carried out as described earlier (1).

DNA and Protein Estimation - DNA was estimated by the diphenylamine method (7). The amount of protein was determined by the Bradford method (8) using Bio-rad supplied reagents and standard.

Polyacrylamide Gel Electrophoresis - Electrophoresis was carried out in 8.7% polyacrylamide gels containing SDS according to Laemmli (9). The Sarkosyl pellet was digested with DNase I (10 μg/ml) and the proteins were then solubilized in the SDS buffer.

RESULTS

When the transcriptional activity of Novikoff hepatoma nucleoli was tested in the presence of increasing concentrations



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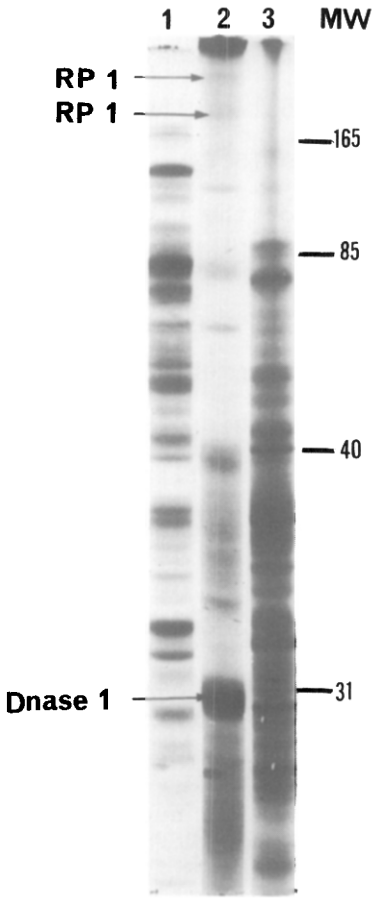

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Figure 1. ^{3}H-GTP incorporation by nucleoli in the presence of increasing concentration of sarkosyl. Assay conditions were as described in Materials and Methods.

Figure 2. SDS-Polyacrylamide gel electrophoresis of proteins from (1) nucleoli, (2) sarkosyl pellet and (3) sarkosyl supernatant. Electrophoresis was done as described in Materials and Methods. RP1 indicate the two large subunits of RNA polymerase I. Numbers on the right are the molecular weight markers x 10^{-3}.

of Sarkosyl, the incorporation of ^{3}H-GTP was increased 50-60% above the control and the transcriptional activity was not significantly inhibited even at a Sarkosyl concentration of 5% (Fig. 1). Centrifugation of the Sarkosyl-treated nucleoli through a cushion of 25% glycerol separated the dissociated pro-

TABLE 1

Effect of sarkosyl on the nucleolar activity

	Activity*	Protein/DNA
Nucleoli	300	3.47
Nucleoli + Sarkosyl (2%)	420	-
Sarkosyl Pellet	476	0.19

* c.p.m. of ^{3}H-GTP incorporated per μg DNA

teins from the residual nucleoprotein pellet. This pellet contained the endogenous RNA polymerase I activity of the nucleoli, although 95% of the nucleolar proteins were extracted (Table 1). A second extraction resulted in complete loss of activity.

SDS-polyacrylamide gel electrophoresis revealed 15-20 bands in case of the proteins from the residual complex, compared to nearly 40 bands found in that of the supernatant (Fig. 2). Although the Sarkosyl pellet contained endogenous RNA polymerase I activity, the two polypeptides corresponding to the two large subunits of RNA polymerase I were only faintly visible on the gel indicating that the enzyme is only a minor component of the residual pellet.

The fidelity of the *in vitro* transcription of Sarkosyl-treated nucleoli was tested by homochromatography fingerprinting of the T_1 ribonuclease digestion products (1). As shown in Figure 3A, RNA transcribed from the Sarkosyl-treated nucleolar pellet contained all the marker oligonucleotide spots, except spots 4 and 10, that are found in transcripts from intact nucleoli (Fig. 3B). Furthermore, there were seven additional

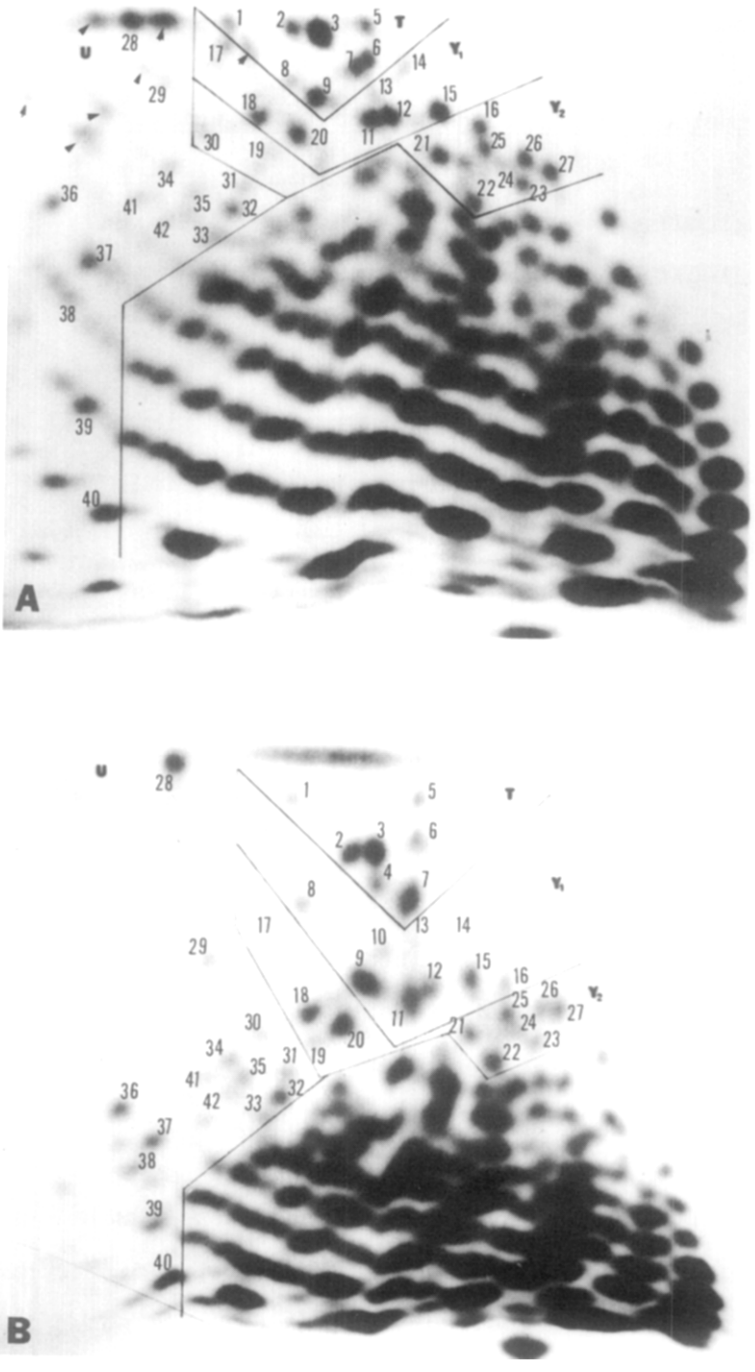

Figure 3. Homochromatography fingerprinting of the T_1 ribonuclease digestion products of RNA transcribed from (A) sarkosyl pellet and (B) intact nucleoli. Pointers in A indicate spots that are not found in B.

spots (pointers) in the upper U and T regions of the map. These spots were not found in the *in vitro* transcripts of nucleoli or in 45S pre-rRNA labeled *in vivo* (1).

DISCUSSION

It is now generally accepted that transcription of pre-rRNA *in vitro* in isolated nuclei or nucleoli is by elongation by endogenous RNA polymerase I of rRNA chains initiated *in vivo*. The endogenous activity is resistant to salt extraction and is not inhibited by reagents such as heparin or rifampicin AF/013 which inhibit the activity of solubilized RNA polymerase on deproteinized DNA templates (10,11). Recently, the nonionic detergent Sarkosyl has been used to dissociate nuclear proteins for biochemical (5) and electron microscopic studies (6). Sarkosyl treatment was reported to enhance RNA polymerase II activity approximately by 10-fold while polymerase I activity increased only slightly (5). Electron microscopic studies have shown that this detergent dissociates most of the chromatin associated proteins, leaving behind DNA bound RNA polymerase complexes (6).

However, it had not been shown whether the transcription of the residual nucleoprotein complex exhibited the specificity characteristics of intact nuclei or nucloli. Results presented in this report indicate that the endogenous RNA polymerase I molecules present in Sarkosyl-treated nucleolar pellet transcribe ribosomal RNA faithfully. The appearance of discrete oligonucleotide spots that are not part of 45S pre-rRNA pattern suggests that there is either impairment of termination or of processing. It is remarkable that the engaged polymerase molecules transcribe mainly the rDNA and to a limited extent, additional regions of the non-transcribed spacer regions. This re-

sult suggests that some protein factors analogous to bacterial rho factor (12) may be essential for proper termination.

Although more than 95% of the proteins are dissociated by Sarkosyl treatment, the residual pellet still contains about 20 polypeptides. Comparison with the subunit pattern of highly purified RNA polymerase I preparation from Novikoff hepatoma indicated that the two high molecular weight polypeptides are not the major component of the residual complex. These results suggest that the polymerase-DNA complexes seen on electron micrographs (6) contain a number of proteins besides RNA polymerase I. Further studies are necessary to determine the function of proteins other than RNA polymerase I in the residue and the nature of the possible termination defect.

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