STIMULATION OF PROTEIN SYNTHESIS "IN VITRO" BY TRANSCRIPTS OF MOUSE MYELOMA CHROMATIN

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SUMMARY: Wheat germ RNA polymerase II has been used to synthesize RNA's from mouse myeloma chromatin. The newly synthesized RNA has been shown to stimulate amino acid incorporation in a cell-free protein synthesizing system from wheat embryos. RNA extracted from transcription mixtures lacking RNA polymerase did not stimulate amino acid incorporation. Analysis of translation products by gel electrophoresis indicated the synthesis of new peptides under the direction of the chromatin transcripts and analysis of tryptic digests of "in vitro" translation products revealed the presence of a peptide which comigrated with an authentic myeloma light immunoglobulin chain tryptic peptide.

Cell-free systems for coupled transcription and translation were developed some years ago using sub-cellular components from prokaryotic organisms (1,2). Several recent reports describe the transcription of eukaryotic viral genomes and the coupled translation of these transcripts (3,4). However, there has been only one report, to date, of the transcription of mammalian chromatin with coupled translation of these transcripts (5). The development of such coupled systems from eukaryotic cells would be of great value in assessing the role of various putative control elements (histones, nonhistones, chromosomal RNA's) in the regulation of mRNA synthesis. The present report describes recent studies on the cell-free synthesis of RNA's with messenger activity using mouse myeloma chromatin as a template. It has not yet been possible to demonstrate myeloma chromatin dependent transcription and translation in a single reaction mixture. However, it has been possible to synthesize RNA's from the chromatin template which, after appropriate purification, stimulate protein synthesis in a cell-free system from wheat embryos.

MATERIALS AND METHODS

Chromatin was prepared from solid myeloma tumors (RPC-20, a λ immunoglobulin chain producer) by the procedure of Murphy, et al. (6) and treated with one-seventh volume of 6M urea, 0.4M guanidine-HCl, 0.1% 2-mercaptoethanol, 0.1M potassium phosphate, pH 7.4, for 30 min at 37°. Chromatin was collected by centrifugation, resuspended in 0.01 M Tris-HCl, pH 8.0 and sheared by sonication for 20 sec (in 5 sec bursts) in an MSE sonicator at a setting of

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1.5 amperes. The sheared chromatin suspension was centrifuged for 10 min at 10,000 rpm and the pellet discarded.

Wheat germ RNA polymerase II was prepared by the method of Jendrisak and Burgess (7). Reaction mixtures for RNA synthesis (2 ml) contained: Tris-HCl, pH 7.8, 0.04 M; $MgCl_2$, 10 mM; potassium EDTA, 0.1 mM; dithiothreitol, 0.1 mM; KCl, 50 mM; $MnCl_2$, 1 mM; $(NH_4)_2SO_4$, 50 mM; ATP, GTP, UTP, CTP, 0.5 mM each; creatine phosphate, 5 mM; creatine phosphokinase, 0.1 mg/ml; chromatin, 0.12 mg/ml as DNA; wheat germ RNA polymerase, 2.42 mg/ml and ^{14}C-UTP (415 mCi/mmol), 2 µCi/ml. Control reaction mixtures lacked RNA polymerase. Reaction mixtures were incubated for 2 h at 25°. Ten microliter aliquots were removed from reaction vessels at various times and analyzed by trichloroacetic acid precipitation. At the end of the incubation described above, the reaction mixtures were extracted with phenol. The aqueous phase was precipitated three times with ethanol and the redissolved precipitate was treated for 60 min at 37° with DNase. The reaction mixture was extracted once with chloroform-isoamyl alcohol and the aqueous phase was applied to a 1.2 x 95 cm column of Sephadex G-50, eluted with distilled water (8).

Reaction mixtures for protein synthesis (50 µl) contained: Tris-HCl, pH 7.6, 20 mM, KCl, 20 mM; Mg acetate, 1 mM; 19 nonradioactive amino acids, 0.04 mM each; 2-mercaptoethanol, 2 mM; ATP, 1 mM; GTP, 0.1 mM; phosphocreatine, 5 mM; creatine phosphokinase, 0.2 mg/ml; ^{3}H-leucine (50 Ci/mmol), 200 µCi/ml; wheat embryo S30 (9), 18 A_{260} units/ml and RNA as indicated in the appropriate figures. Reaction mixtures were incubated for 60 min at 25° and processed as previously described (10).

Electrophoresis was performed on SDS-12.5% polyacrylamide gels (11). Tryptic digestion of labelled proteins was performed as described previously (12). Prior to tryptic digestion each ^{3}H-leucine labelled protein preparation (synthesized under the direction of chromatin transcripts or "control" RNA, see below) was mixed with approximately 25,000 cpm of RPC-20 λ immunoglobulin chain labelled with ^{14}C-leucine. Following digestion, tryptides were analyzed by Dowex 1 ion exchange chromatography as previously described (12).

RESULTS AND DISCUSSION

Fig. 1a shows the time course of transcription of myeloma chromatin by purified wheat germ RNA polymerase II. Based on the amount of ^{14}C-UTP incorporated, it was estimated that about 75 µg of trichloroacetic acid precipitable RNA was synthesized in 2 h in a 2 ml reaction mixture. No RNA synthesis was observed with the urea/guanidine treated chromatin in the absence of wheat germ RNA polymerase. After phenol extraction, ethanol

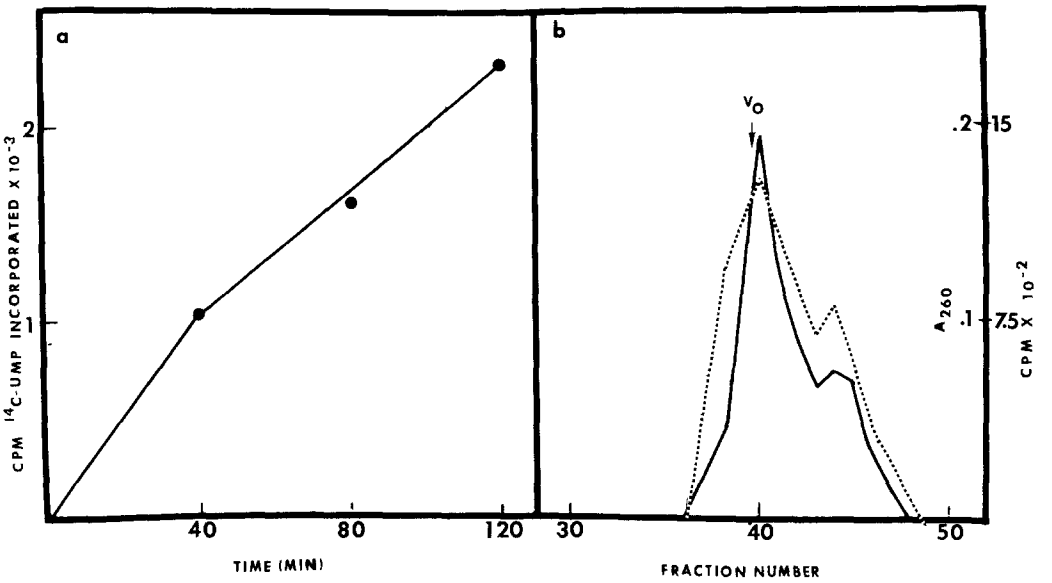

Fig. 1-(a)Time course of RNA synthesis from myeloma chromatin template. Reaction mixtures were incubated for 2 h at 25°. Ten microliter aliquots were removed at various times and analyzed for ^{14}C-UTP incorporation. Values in the figure represent cpm incorporated per ten microliters. No incorporation was observed in control mixtures lacking RNA polymerase. (b)Sephadex G-50 chromatography of control and experimental RNA's (8). The solid line line in the figure represents the radioactivity due to RNA synthesized in the experimental reaction mixture, the dashed line represents the absorbance of the RNA extracted from the control reaction mixture. In each case, fractions 38-44 were pooled, lyophilized and redissolved in distilled water.

precipitation and DNase treatment, the extracts of experimental (containing RNA polymerase) and control (lacking RNA polymerase) reaction mixtures were purified by chromatography on Sephadex G-50. Figure 1b (solid line) shows the pattern of radioactivity obtained for the experimental mixture. A major and minor radioactive peak were obtained, eluting near the void volume of the column. Although no labelled RNA was synthesized in the reaction lacking RNA polymerase, some material absorbing UV-light at 260 nm was recovered after phenol extraction and DNase treatment of the reaction mixture. This material behaved identically to the labelled chromatin transcripts on Sephadex G-50 (Fig. 1b, dashed line). Fractions from the columns were pooled as indicated in the figure legend and lyophilized. Based on the recoveries of A_{260} units from the chromatography of control and experimental extracts, it was estimated that 45 µg of RNA was synthesized in the "in vitro" reaction mixture. This is somewhat less than the figure obtained by direct precipitation of aliquots of the reaction mixtures

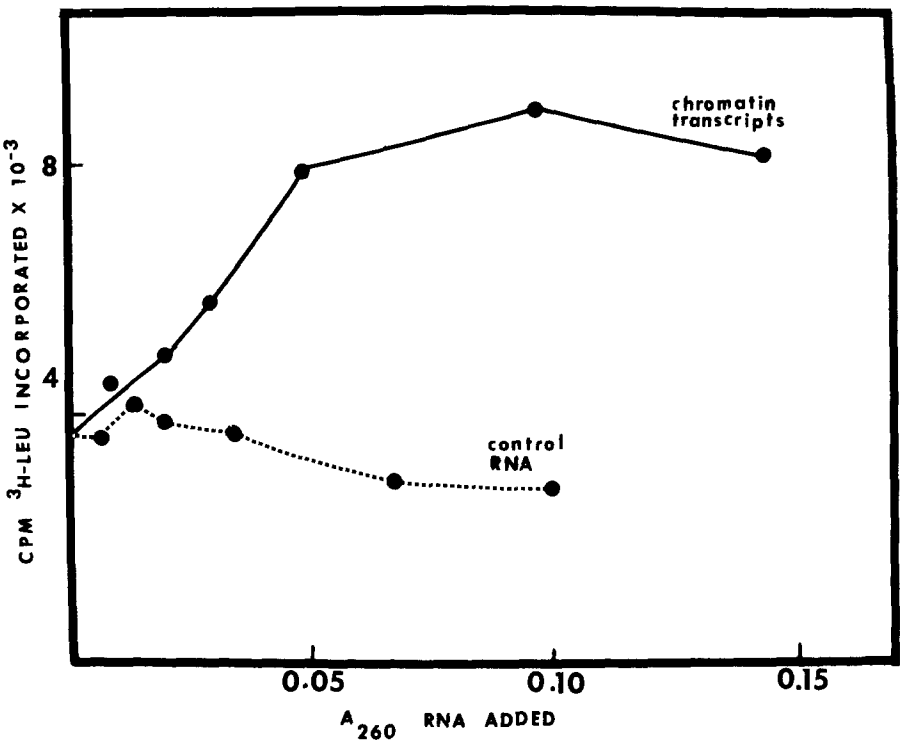

Fig. 2-Stimulation of "in vitro" protein synthesis by chromatin transcripts. Reaction mixtures for protein synthesis were described in Materials and Methods. The solid line in the figure represents the reaction mixtures containing newly transcribed RNA while the dashed line represents reaction mixtues containing control RNA.

(75 µg) and the difference may be due to losses resulting from the procedure used to purify the newly synthesized RNA. The identity of the unlabelled material extracted from the control reaction mxiture was established by RNase digestion of an aliquot of the lyophilized material obtained after Sephadex chromatography. Rechromatography of the RNase digest showed that the UV-absorbing peak had disappeared from the void volume, and now eluted at a position coressponding to that of ^{3}H-UTP (data not shown). Sucrose gradient analysis of these RNA's from control reaction mixtures indicated a major peak sedimenting at about 4S, whereas the newly synthesized RNA sedimented in a broad band between 4 and 8S (data not shown).

It was of interest to examine the ability of these RNA's to stimulate protein synthesis in the wheat embryo S30 cell-free system (9). In the experiments of Fig. 2, the ability of control and newly transcribed

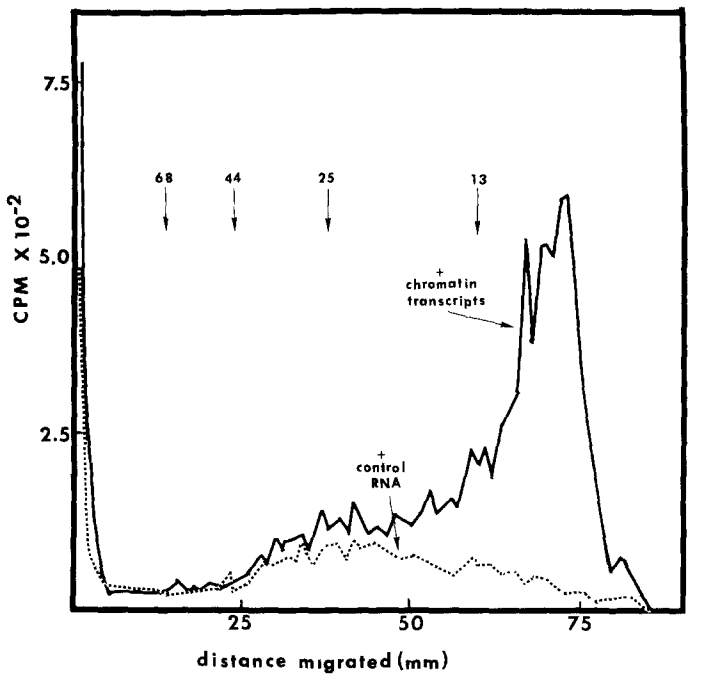

Fig. 3-Electrophoretic analysis of proteins synthesized under the direction of chromatin transcripts. Large scale reaction mixtures were prepared as described in Materials and Methods. Reaction mixtures containing control and experimental RNA were combined, washed with trichloroacetic acid, then ethanol-ether, and analyzed on SDS-polyacrylamide gels. Gels were sliced and examined by liquid scintillation counting. In the experiments presented, 14,300 cpm of ^{3}H-leucine labelled protein and 7,000 cpm of ^{14}C-labelled protein were applied to the gel.

RNA's to stimulate ^{3}H-leucine incorporation is depicted. It can be seen that the control RNA is very mildly stimulatory at low concentrations (about 0.2 A_{260}/ml) and actually inhibits protein synthesis at concentrations of 2 A_{260}/ml. In contrast, newly synthesized RNA stimulated protein synthesis at concentrations up to 2 A_{260}/ml, and, at that point, the level of incorporation was nearly three times that observed in reaction mixtures containing control RNA. In order to examine the products synthesized in the presence of newly transcribed and control RNA's, products of large scale reaction mixtures containing ^{3}H-leucine and "in vitro" chromatin transcripts (1.93 A_{260}/ml), or ^{14}C-leucine and control RNA (1.37 A_{260}/ml) were combined and analyzed by SDS-polyacrylamide gel electrophoresis (11). The data of Fig. 3 indicate that several proteins of molecular weight 10,000 or less, were synthesized in the presence of the "in vitro" transcripts. In contrast, these protein peaks are completely absent from the electrophoretic profile for proteins synthesized in the presence of control RNA. Indeed, the latter profile was identical to that obtained for endogenous ^{14}C-leucine

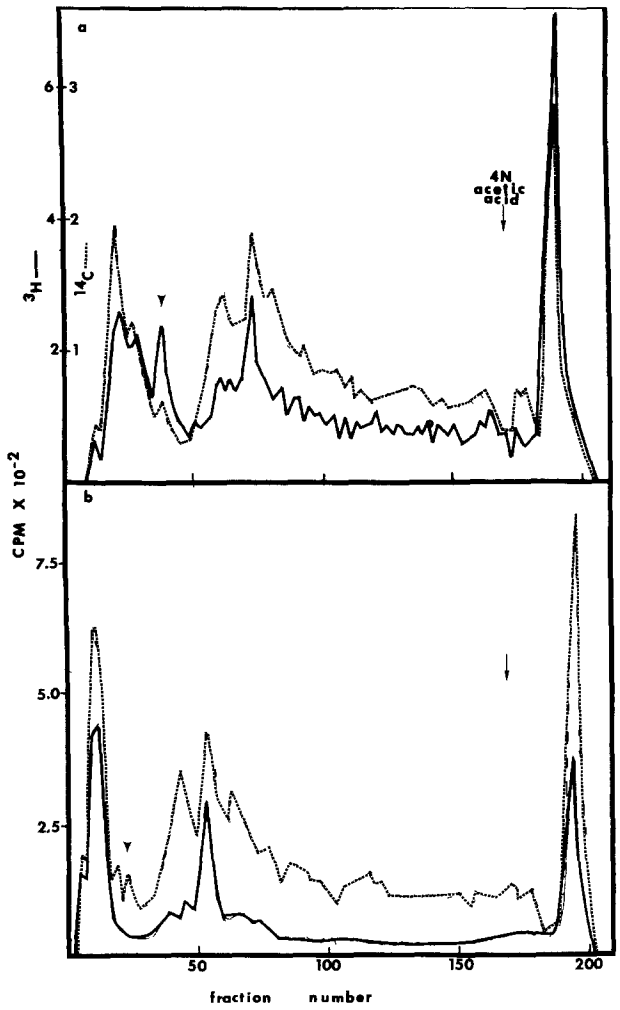

Fig. 4-Ion exchange chromatography of tryptic peptides. Tryptic peptides were prepared as described in Materials and Methods and in reference 12. (a) tryptides from reaction mixtures primed with "in vitro" chromatin transcripts; (b)tryptides from reaction mixtures primed with control RNA.

labelled proteins synthesized by the wheat germ system in the absence of added RNA.

The major protein synthesized by the RPC-20 myeloma is a λ immunoglobulin chain. It was hoped that the myeloma chromatin transcripts might contain the mRNA for this protein, however, no proteins of the size of the λ chain (25,000 daltons) were observed in the electrophoretic profile of Fig. 3. Because of the small size of the proteins synthesized in the presence of the chromatin transcripts "in vitro," it was felt that the absence of the λ chain product might simply reflect the inability of the cell-free

system to synthesize complete polypeptide chains from the chromatin transcripts. To examin the possibility that λ chain fragments (possibly resulting from translation of fragments of immunoglobulin mRNA, or from premature termination of protein synthesis) were among the products of translation in the presence of chromatin transcripts, these products were subjected to tryptic digestion. In these experiments, ^{3}H-labelled proteins, synthesized in the presence of "in vitro" chromatin transcripts or control RNA, were mixed with ^{14}C-leucine labelled RPC-20 λ chain, performic acid oxidized, digested with trypsin and the tryptic peptides separated by ion exchange chromatography. Fig. 4a shows that the tryptic peptides obtained by digestion of proteins synthesized in the presence of "in vitro" transcripts contained by one peak which co-migrated with a peptide peak from the authentic RPC-20 λ chain and which was completely absent from the peptide pattern from control reactions (Fig. 4b).

The data presented above suggest that RNA with messenger activity can be synthesized from myeloma chromatin "in vitro." The fact that the RNase sensitive material extracted from control reactions does not stimulate protein synthesis in the wheat germ S30 argues against the protection of endogenous messages by the chromatin transcripts. Further, both electrophoretic and tryptic peptide analysis of the products of protein synthesis in the presence of the chromatin transcripts suggest the synthesis of new proteins under their direction. Studies are now in progress to further characterize the "in vitro" synthesized RNA's and to analyze more fully the proteins synthesized under their direction.

ACKNOWLEDGMENT

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