# PROTEIN COMPOSITION OF LIVER NUCLEAR RIBONUCLEOPROTEIN PARTICLES OF RATS FED CARCINOGENIC AMINOAZO DYES

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SUMMARY: The feeding of carcinogenic 3'-methyl-4-dimethylaminoazobenzene to rats alters the protein composition of liver nuclear 30S ribonucleoprotein particles which are proposed to be involved in the processing and transport of the newly synthesized RNA. After 10 weeks of feeding of the carcinogenic aminoazo dye, one of the major proteins is missing from these particles but not from the particles isolated from liver of animals fed with noncarcinogenic 4-aminoazobenzene. In all the groups of rats studied, the RNA associated with the isolated particles was of high specific activity.

In hepatomas induced by carcinogenic aminoazo dyes and in the liver of animals fed with these carcinogens, RNA which in normal liver cells is restricted to the cell nucleus is released into the cytoplasm (1,2,3). This effect is not observed in animals fed with noncarcinogenic aminoazo dyes. The extent of the disruption of the selective release of RNA from the nucleus is illustrated by the fact that even the nonhistone chromosomal proteins associated small molecular weight nuclear RNA which is transcribed from the middle repetitive DNA sequences and in nontransformed liver cells is found only in the nuclei, is found also in the cytoplasm of hepatoma cells, as part of large RNA molecules (4).

It is assumed that the proteins which are associated with the newly synthesized DNA-like RNA in the form of nuclear RNP particles, are involved in the processing of the newly synthesized RNA and in the selective transport of mRNA into the cytoplasm (5,6,7). Therefore, we have analyzed

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Abbreviations used: RNP, ribonucleoprotein; 3'-MeDAB, 3'-methyl-4-dimethylaminoazobenzene; AB, p-aminoazobenzene; SDS, sodium dodecylsulfate; STM buffer, 0.1 M sodium chloride, 0.01 M Tris and 0.001 M $MgCl_2$.

the composition of the protein moiety of the nuclear RNP particles isolated from livers of animals fed carcinogenic 3'-MeDAB and have compared it with those isolated from the livers of animals fed noncarcinogenic AB and from animals which did not receive aminoazo dyes.

MATERIALS AND METHODS:: Male albino rats derived from Hdtzmann strain (purchased from Texas Inbred Mice Co., Houston, Texas) of 150 g were given for 10 weeks standard pellet food (Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.) mixed with olive oil (100 ml/kg dry food) containing 0.06% (w/v) 3'-MeDAB or AB (both Eastman Kodak Co., Rochester, N. Y.). Another group of rats was fed with the same food mixed with olive oil (100 ml/kg dry food) containing no dye.

After 10 weeks of feeding with aminoazo dyes, the rats were injected 30 min before their sacrifice with 10 μCi of [$^3$H]-orotic acid (Spec. activity 17.8 Ci/mmole, New England Nuclear, Boston, Mass.) for each 100 g of body weight. After the sacrifice of the animals, the livers were perfused with cold 0.14 M NaCl containing 0.01 M sodium citrate, excised and the nuclei prepared by a modification of the Chauveau procedure (8). The nuclear fraction containing the nuclear RNP particles was extracted by a modification of a procedure of Samarina, et al. (9). After the removal of the nuclear sap proteins by the extraction of the nuclei with STM buffer, pH 7.2 (STM pH 7.2 extract), the RNP particles were extracted by gentle stirring for 4 h with the same STM buffer adjusted to pH 8.0 (STM pH 8.0 extract). To separate the 30S RNP particles from other extracted components, the STM pH 8.0 extract was passed through 2.6 cm x 90 cm column of Bio-Gel A-50m and eluted with STM buffer pH 8.0. The extract separated into three fractions absorbing light at 260 nm. The second fraction which contained the RNP particles were concentrated by ultrafiltration through an Amicon UM 2 membrane and purified by centrifugation through 15-30% linear sucrose density gradient (Spinco, SW 27 rotor, 24,000 rpm for 14 h). The fractions containing the 30S RNP particles were pooled and the particles were pelleted by centrifugation at 41,000 rpm for 15 h (Spinco, rotor 50). Aliquots of each fraction were taken for RNA (10) or protein (11) determination and for the measurement of radioactivity. The samples were counted in a dioxan-based scintillation mixture (12) in a Beckman liquid scintillation counter Model LS 230. The buoyant density of the isolated particles was determined as described by Hamilton (13).

For SDS-polyacrylamide disc gel electrophoresis, the pelleted particles were dissolved overnight at 25°C in 10 mM sodium phosphate buffer, pH 7.0 containing 1% SDS, 1% β-mercaptoethanol and 8 M urea, and the solution was directly applied onto 10% polyacrylamide gels. The electrophoresis was performed as described previously (12) with 0.1 M sodium phosphate buffer, pH 7.0 (containing 0.1% SDS) as the electrolyte. The gels were fixed overnight in 10% trichloroacetic acid, stained in Coomassie brilliant blue and scanned in a Gilford spectrophotometer at 600 nm.

The protein moiety of the isolated particles was analyzed by two dimensional polyacrylamide gel electrophoresis as described by Orrick, et al. (14). The particles were dissolved in a sample buffer (8 M urea, 0.9 M acetic acid, 10% β-mercaptoethanol and 10% glycerol) by incubation at 25° for 16 h. In the first dimension, the proteins were separated by electrophoresis in 10% polyacrylamide gel containing 4.5 M urea with 1.8 M acetic acid as the electrolyte. In the second dimension, the proteins were separated

Table 1. Association of newly synthesized RNA with purified liver RNP particles in control rats and in rats fed AB or 3'-MeDAB

| Source of RNA | Specific Activity of RNA (dpm/μg) Control Animals | Animals Fed AB | Animals Fed 3'-MeDAB |
|---|---|---|---|
| Total nuclei | 66.5 | 72.1 | 49.4 |
| STM pH 7.2 extract | 30.4 | 29.9 | 61.2 |
| STM pH 8.0 extract | | | |
| First fraction eluted from Bio-Gel A-50m | 1.7 | 1.9 | 0.5 |
| Nuclear ribonucleoprotein purified from second fraction eluted from Bio-Gel A-50m | 146.1 | 139.2 | 142.5 |
| Third fraction eluted from Bio-Gel A-50m | 41.7 | 10.6 | 27.4 |

An aliquot from each fraction was precipitated with equal volume of cold 20% trichloroacetic acid. The precipitate was washed twice with 70% ethanol, dissolved in appropriate volume of 0.1 M sodium hydroxide and counted in a dioxan based scintillation cocktail.

on 12% polyacrylamide slab gel (containing 0.1% SDS and 6 M urea) in an Ortec electrophoresis apparatus (Ortec, In., Oak Ridge, Tenn.) using 0.1 M phosphate buffer, pH 7.1 (containing 0.1% SDS) as the electrolyte solution. The gels were stained in Coomassie brilliant blue.

RESULTS: The nuclear 30S RNP particles isolated from the rat livers have very reproducible composition containing 20.8 $\pm$ 1.3% RNA and the rest proteins. This composition agrees with that determined from isopycnic banding in CsCl gradient of the formaldehyde fixed RNP particles ($\rho$ = 1.397 $gcm^{-3}$). No significant difference was found in the composition of the particles isolated from the livers of the 3 different experimental groups of rats. The results presented in Table I show that the RNA in these particles has higher specific activity than the RNA recovered in any other nuclear fraction after 30 min labeling *in vivo* with [$^3$H]-orotic acid. The particles were prepared by a

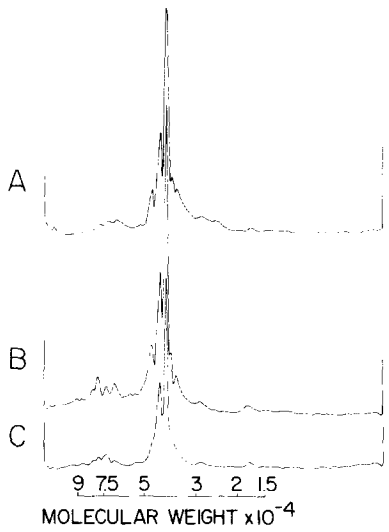
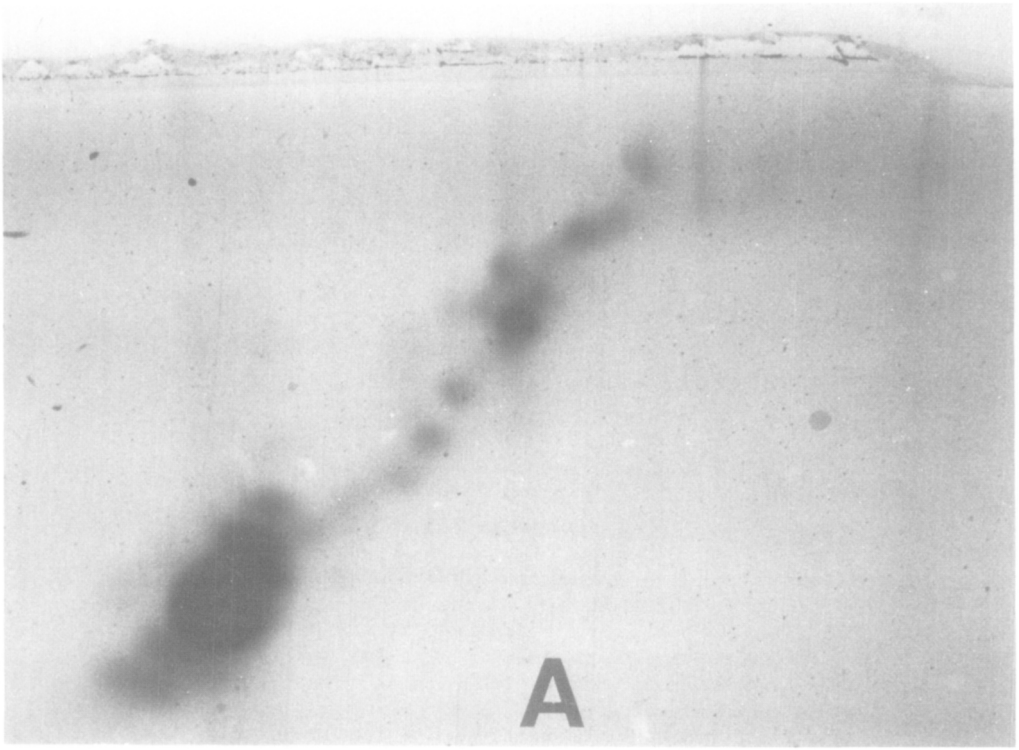
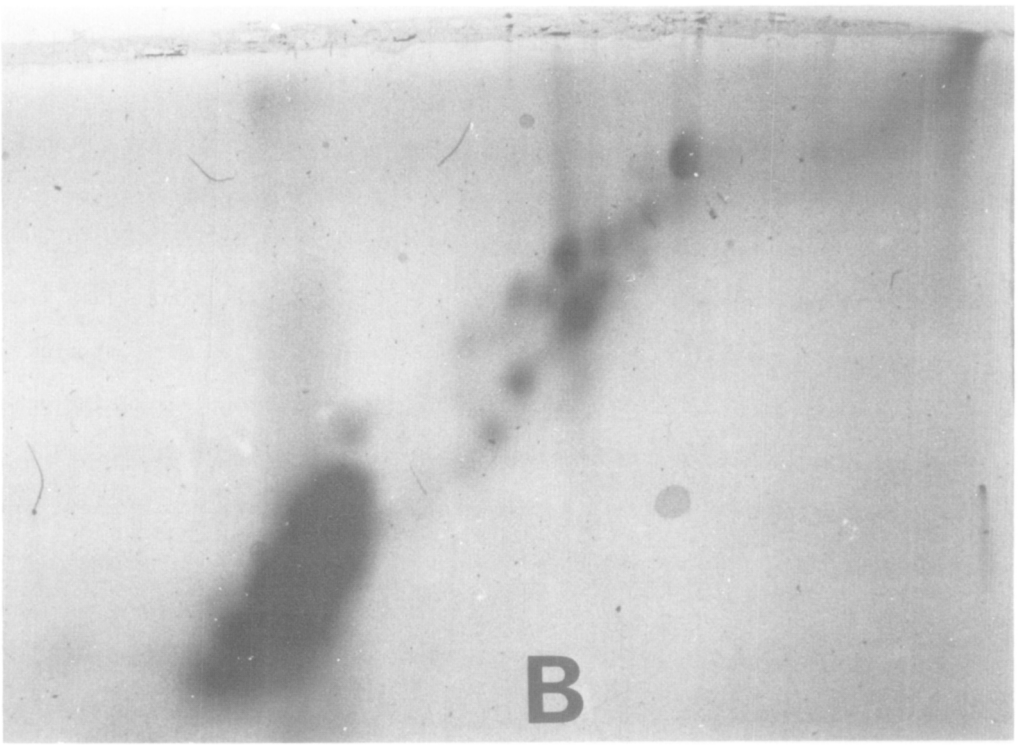



Fig. 1. Effect of feeding carcinogenic and noncarcinogenic aminoazo dyes on the protein composition of the nuclear 30S RNP particles from rat liver. Densitometric tracings of SDS-polyacrylamide discgel electrophorograms of proteins of the RNP particles isolated (A) from livers of animals not fed aminoazo dye, (B) from livers of animals fed noncarcinogenic AB for 10 weeks and (C) from livers of animals fed carcinogenic 3'-MeDAB for 10 weeks. 30-40 µg of protein samples were loaded on each gel. For details of conditions used for electrophoresis refer to Material and Methods.

procedure used for the isolation of nuclear RNP particles which were proposed to be involved in the processing and in the selective transport into the cytoplasm of the newly synthesized nuclear DNA-like RNA and are identical in their properties with the particles described in the literature (5,6,7).

After the separation of the proteins of the nuclear 30S RNP particles by the electrophoresis in SDS-polyacrylamide disc gels about 70% of the proteins of these particles isolated from livers of the two control groups of rats are present as three bands of polypeptides with apparent molecular weights of 38,000, 40,500 and 42,000 daltons. (Fig. 1A and B). However, in the electrophoretic profile of the proteins of these particles isolated from the livers of rats fed carcinogenic 3'-MeDAB, the band with the apparent molecular weight of 42,000 daltons is missing (Fig. 1C).


A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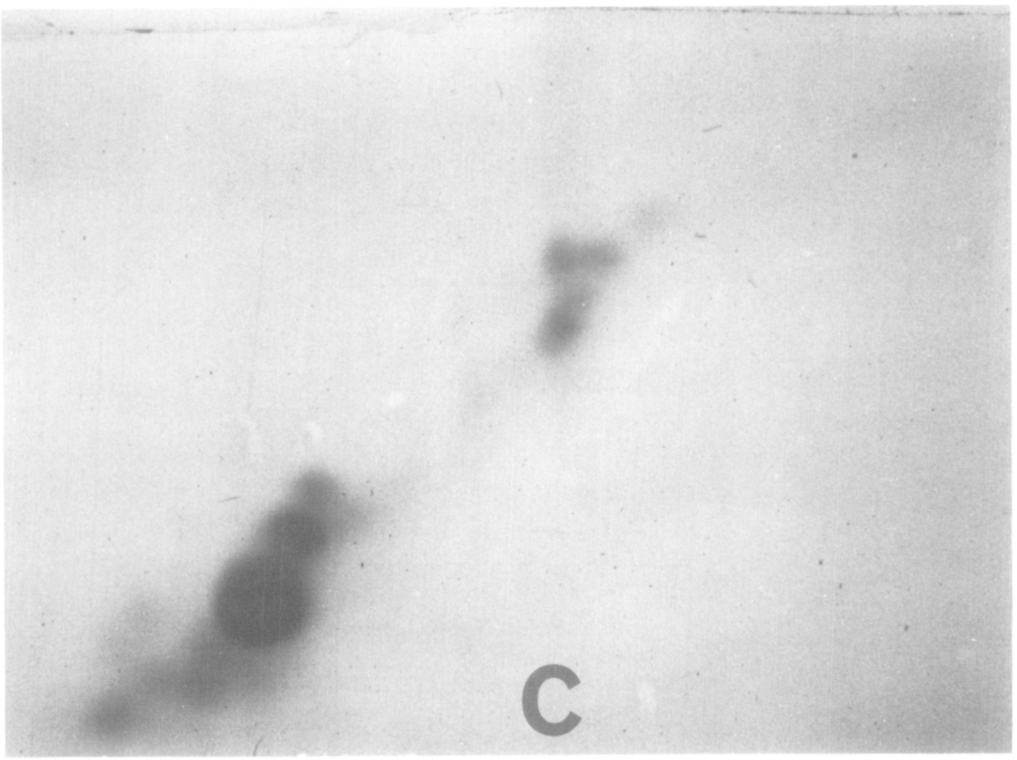


Fig. 2. Effect of feeding carcinogenic and noncarcinogenic aminoazo dyes on the composition of protein moiety of nuclear 30S RNP particles as studied by two dimensional polyacrylamide gel electrophoresis. (A) Particles from liver of animals not fed aminoazo dye. (B) Particles from liver of animals fed noncarcinogenic AB. (B) Particles from liver of animals fed carcinogenic 3'-MeDAB. 60-80 µg of protein samples were used for each separation. Electrophoresis in the first dimension was run from right to left in acid-urea disc gel and that in the second dimension was run from top to bottom in SDS-slab gels. For details see Methods and Materials.

Analysis of the protein moiety of the nuclear 30S RNP particles by two dimensional polyacrylamide gel electrophoresis further characterized this difference. In two dimensional system, the protein moiety of the nuclear RNP particles isolated from the livers of control group of rats not fed aminoazo dye was separated into a cluster of four major spots and at least 24 minor spots (Figs. 2A and 3A). No difference in protein spots was observed when the particles isolated from the livers of rats fed noncarcino-

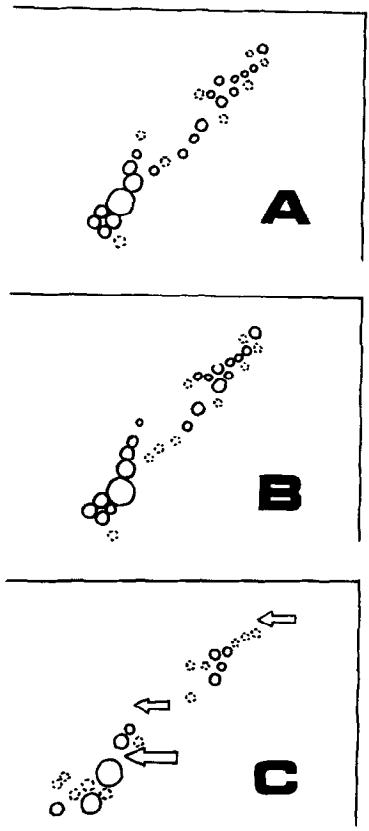


Fig. 3. Diagramatic presentation of two dimensional electrophoretic gel patterns of Fig. 2. The missing spots in case of particles isolated from livers of animals fed 3'-MeDAB are marked with arrows.

genic AB were analyzed in the same system (Figs. 2B and 3B). In contrast to these results, the two dimensional electrophoretic patterns of nuclear 30S RNP particles from livers of rats fed carcinogenic 3'-MeDAB consistently indicated the absence of one major spot and two minor spots (Figs. 2C and 3C). The missing spots are marked by arrows in Fig. 3C. Spots adjoining the cluster of four main spots of nuclear 30S RNP particles, in the left corner of the electrophorograms, vary in their intensity from preparation to preparation and are most probably due to the contamination with proteins of immature ribosomal subunits which co-sedimented with the nuclear 30S particles (15).

DISCUSSION: Our finding that the carcinogenic 3'-MeDAB changes the composition of the protein moiety of the nuclear 30S RNP particles which are proposed to be involved in the processing and transport of DNA-like RNA, suggests that the azocarcinogens could disrupt the processing and release of newly synthesized nuclear DNA-like RNA by the interference with the function of proteins of the particles involved in the processing and transport. This suggestion is further supported by the results published earlier from this laboratory. Hepatoma induced 3'-MeDAB contains in its nucleus a smaller amount of the proteins of nuclear 30S RNP particles as compared with the amount of these proteins present in the nucleus of normal liver cell (12). In other experiments, it was shown that the feeding of rats with carcinogenic 3'-MeDAB (but not noncarcinogenic AB) specifically inhibited the incorporation of [$^{14}$C]amino acids into proteins of the nuclear 30S RNP particles (16). The studies on the *in vivo* binding of [$^{3}$H]-3'-MeDAB to the various fractions of rat liver nuclear proteins have shown that the proteins of the nuclear 30S RNP particles had the highest specific activity (17).

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