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Cell-Free Translation of Immunoglobulin Messenger RNA from MOPC-315 Plasmacytoma and MOPC-315 NR, a Variant Synthesizing Only Light Chain

[protein synthesis/oligo(dT)–cellulose/precursor immunoglobulin chains/ immunoglobulin synthesis/poly(A)-containing mRNA]

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ABSTRACT **Total poly(A)-containing mRNA was isolated from the MOPC-315 and MOPC-315 NR plasmacytomas. The RNA was further fractionated on sodium dodecyl sulfate–sucrose gradients. The MOPC-315 mRNA fractions directed the synthesis of both the heavy chain and light chain precursor of the MOPC-315 IgA protein in a cell-free extract of Ehrlich ascites tumor cells. None of the MOPC-315 NR mRNA fractions tested programmed the synthesis of the heavy chain in this system. Analysis of cell-free products by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and by immunoprecipitation demonstrated that no translatable heavy chain mRNA could be extracted from the MOPC-315 NR variant plasmacytoma.**

Variants of plasmacytoma cell lines with respect to immunoglobulin production have been identified and characterized in several laboratories (1–7). Such studies have potential value for a better understanding of the synthesis, assembly, secretion, and diversity of immunoglobulin molecules. Plasmacytoma variants have also been reported in studies with solid tumors maintained in mice (8–12). In the course of studies with MOPC-315, a plasmacytoma producing an immunoglobulin of the IgA class with high affinity for dinitrophenyl (Dnp) and trinitrophenyl (Tnp) groups, Hannestad *et al.* (12) reported the isolation of a variant tumor line lacking cell-bound myeloma protein capable of binding 2,4,6-trinitrophenylated sheep erythrocytes. This variant cell line, MOPC-315 NR (12), secreted only the light chain of the MOPC-315 IgA molecule.

MOPC-315 cells synthesize both heavy (H^{315}) and light (L^{315}) chains (13). The inability of the variant MOPC-315 NR to secrete H^{315} may reside in an altered DNA, a defective, deficient, or absent mRNA, or possibly a specific translational or post-translational lesion. Our studies were designed to localize the level and nature of the defect. Towards this end, this report describes the cell-free translation of the mRNAs obtained from both MOPC-315 and MOPC-315 NR.

MATERIALS AND METHODS

Reagents. DNase (electrophoretically purified, RNase-free) was obtained from Worthington. [^{3}H]Leucine (53 Ci/mmol), [^{14}C]leucine (348 mCi/mmol), [^{3}H]valine (39 Ci/mmol), and [^{3}H]alanine (42 Ci/mmol) were obtained from Amersham/Searle. [^{35}S]Methionine (395 Ci/mmol) was obtained from New England Nuclear Corp. Oligo(dT)–cellulose was prepared by the method of Gilham (14) and was the generous gift of Dr. S. Kerwar.

Tumors. MOPC-315 plasmacytoma was obtained from both Dr. M. Scharff and Dr. H. Eisen. MOPC-315 NR plasmacytoma was obtained from Dr. H. Eisen and L. Bailey. The plasmacytomas were maintained as solid tumors in Balb/c mice by serial subcutaneous passage of 0.2–0.3 ml of minced tumor fragments at intervals of 10–15 days.

Labeling of Intact Cells. Preparation and labeling of tumor cell suspensions and analysis of culture media and cell contents were performed by minor modifications of published procedures (15). The discontinuous sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gels described by Laemmli (16) were used to analyze the labeled proteins electrophoretically. [^{14}C]-Leucine was used to label MOPC-315 cell suspensions. The proteins secreted into the culture medium were precipitated with 10% trichloroacetic acid in the presence of a final concentration of 10 μg/ml of bovine serum albumin added as a carrier. These proteins were used as the source of authentic H^{315} and L^{315} radioactive markers.

Preparation of mRNA. Total nucleic acids were extracted from tumors essentially according to the procedure of Bhoopalam *et al.* (17). The nucleic acid preparation was then treated briefly with DNase (10 μg/ml for 10 min at 23°). Phenol extraction of the treated nucleic acids (18), chromatography of the RNA on oligo(dT)–cellulose (18), and fractionation of the total poly(A)-containing mRNA in $NaDodSO_4$–sucrose gradients (19) were performed with slight modifications of published procedures. A detailed description of these methods is in preparation*.

Cell-Free Protein Synthesis. Ehrlich ascites S-30 extract was prepared by described procedures (20). The 33–68% $(NH_4)_2SO_4$ fraction of the 0.5 M KCl wash of rabbit reticulocyte polysomes was obtained by the method of Shafritz *et al.* (21) and used to supplement the Ehrlich ascites extract. The cell-free incubations were performed according to previously described methods with minor modifications. A detailed description of the incubation conditions for labeling with either [^{35}S]methionine or a mixture of [^{3}H]leucine, [^{3}H]alanine, and [^{3}H]valine is in preparation*.

Abbreviations: MOPC, mineral oil-induced plasmacytoma; H^{315}, heavy chain of MOPC-315 protein; L^{315}, light chain of MOPC-315 protein; $NaDodSO_4$, sodium dodecyl sulfate.

* M. Green, T. Zehavi-Willner, P. Graves, J. McInnes, and S. Pestka, in preparation.

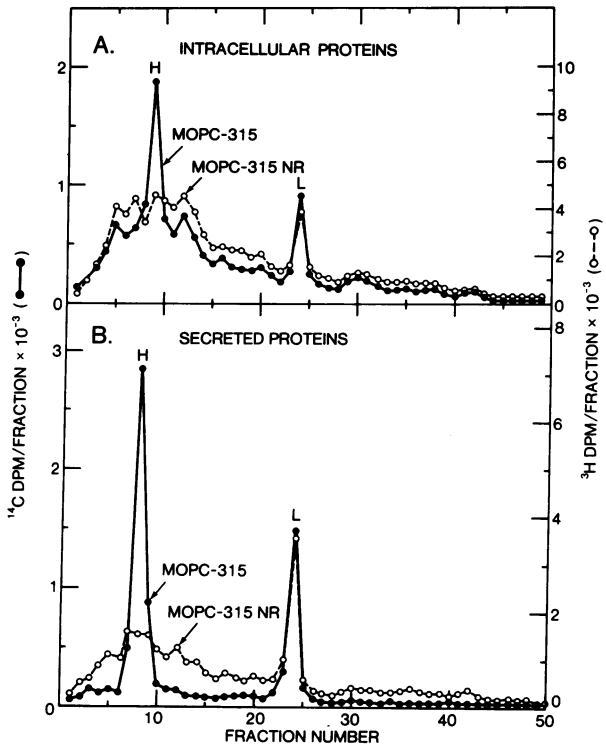

FIG. 1. NaDodSO$_4$–polyacrylamide gel electrophoretic analyses of proteins synthesized by MOPC-315 and MOPC-315 NR tumor cell suspensions. MOPC-315 and MOPC-315 NR cells were labeled with [^{14}C]leucine and [^{3}H]leucine, respectively. The cell cytoplasm and the culture media from the two lines were mixed before electrophoresis. (A) Intracellular proteins after a 30-min period of labeling; (B) secreted proteins after a 3-hr labeling period.

Analysis of Cell-Free Product. Cell-free products labeled with [^{35}S]methionine were analyzed on 12.5% discontinuous, NaDodSO$_4$–polyacrylamide slab gels based on the procedure described by Laemmli (16, *). Gels were fixed, stained, and dried for autoradiography. Cell-free products labeled with [^{3}H]leucine, [^{3}H]alanine, and [^{3}H]valine were used for immunochemical analysis by two procedures. In the first method, immunoprecipitates were obtained from the total reaction mixture by indirect immunoprecipitation as described (15, *). The precipitates were redissolved and analyzed on continuous, cylindrical NaDodSO$_4$–polyacrylamide gels (15, *). The gels were mechanically fractionated (Gel Fractionator, Gilson Medical Electronics, Inc.) into scintillation vials and radioactivity was determined. Alternatively, the labeled products were first subjected to electrophoresis on the continuous, cylindrical NaDodSO$_4$–polyacrylamide gels. The gels were mechanically fractionated, and the radioactivity eluted from each gel slice was tested for its ability to be precipitated by normal, anti-H^{315}, or anti-L^{315} rabbit serum. Indirect immunoprecipitations were performed as described (15, *). Anti-H^{315} serum was devoid of any detectable anti-L^{315} activity estimated by double diffusion and by precipitation of authentic radioactive L^{315} under conditions comparable to those given in the legends of Figs. 4 and 5.

RESULTS

Proteins Synthesized by Intact Cells. A comparison of the intracellular and secreted proteins synthesized by cell suspensions prepared from MOPC-315 and MOPC-315 NR tumors is shown in Fig. 1. It is clear that while both cell cultures synthesize and secrete substantial amounts of L^{315}, the MOPC-315 NR cells synthesize little, if any, H^{315}. The tumors used in these experiments were representative of the tumors that were used to prepare the mRNAs.

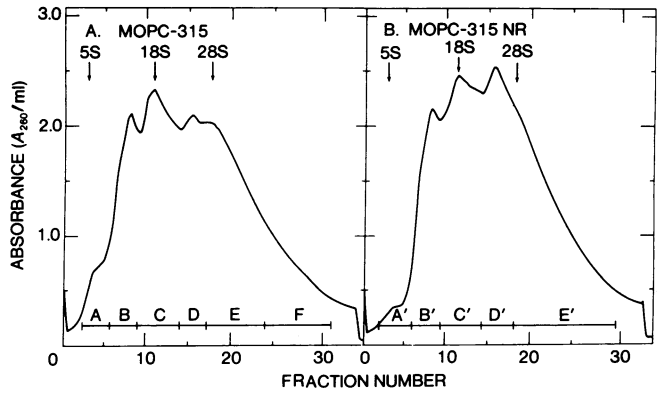

FIG. 2. NaDodSO$_4$–sucrose gradient fractionation of total poly(A)-containing mRNA from MOPC-315 and MOPC-315 NR. The gradients were 15–30% sucrose in 10 mM Tris·HCl (pH 7.4), 0.1 M NaCl, 1 mM EDTA, and 0.5% NaDodSO$_4$. Centrifugation was performed at 18° for 16 hr at 25,000 rpm in the Spinco SW 27.1 rotor. Fractions were pooled as indicated. The positions of the 5S, 18S, and 28S RNA obtained on a parallel gradient are indicated.

Isolation of mRNA. Total cellular RNA was extracted from both tumor lines as described (17, 18). The poly(A)-containing RNA was obtained by chromatography on oligo(dT)–cellulose as reported (18). The total poly(A)-containing mRNA preparations were further purified by sedimentation through sucrose gradients containing NaDodSO$_4$ (19). It can be seen that in the lighter half (18S and smaller) of the gradient the profiles are quite similar (Fig. 2). There is, however, a difference discernible in the region of the gradient containing the heavier RNA species (28S region). The two peaks of RNA present in MOPC-315 RNA have been replaced by a single larger peak in the MOPC-315 NR RNA. The fractions from the gradient were pooled as indicated in Fig. 2 and then concentrated by ethanol precipitation. The RNA pellets were resuspended in water and stored in the vapor phase of a liquid nitrogen refrigerator until used for cell-free protein synthesis. In the case of MOPC-315, the 12–14S RNA species (fraction B, Fig. 2A) coded for L^{315} and the 16–19S RNA species (fraction C, Fig. 2A) coded for H^{315} as well as L^{315}. The primary translation product of the L^{315} mRNA is a precursor longer than L^{315} (*, 22). The cell-free synthesis of immunoglobulin light chain precursor proteins has been observed in several laboratories (*, 22–26). The primary product of H^{315} mRNA translation is a protein species antigenically and structurally related to H^{315} but migrating slightly ahead of authentic H^{315} in NaDodSO$_4$–polyacrylamide gels (*, 22). It has been hypothesized that the cell-free product of immunoglobulin heavy chain mRNAs also contains a precursor segment not found in mature heavy chain, but is devoid of the carbohydrate residues contained in authentic secreted heavy chain (27, 28). These two factors would balance each other and result in similar rates of migration for the cellular and cell-free product. Furthermore, it has been demonstrated that the RNA fraction sedimenting immediately ahead of the heavy chain mRNA

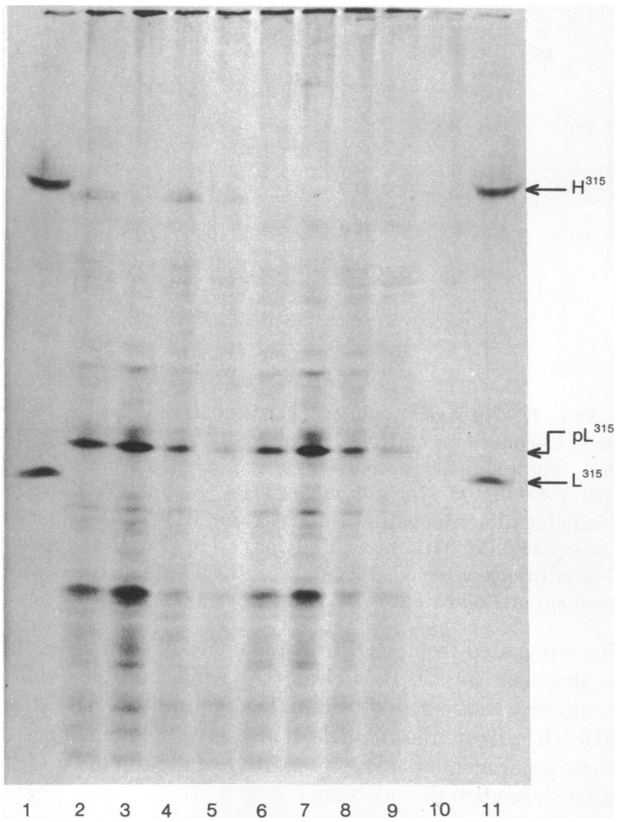

FIG. 3. Autoradiograms of [^{35}S]methionine-labeled cell-free products analyzed on NaDodSO$_4$–polyacrylamide gel slabs. Products were synthesized in the presence of the following RNA fractions: (*1*) ^{14}C-labeled marker proteins; (*2*) MOPC-315 total mRNA; (*3*) MOPC-315 fraction B (Fig. 2A); (*4*) MOPC-315 fraction C (Fig. 2A); (*5*) MOPC-315 fraction D (Fig. 2A); (*6*) MOPC-315 NR total mRNA; (*7*) MOPC-315 NR fraction B′ (Fig. 2B); (*8*) MOPC-315 NR fraction C′ (Fig. 2B); (*9*) MOPC-315 NR fraction D′ (Fig. 2B); (*10*) no RNA; (*11*) ^{14}C-labeled marker proteins. The positions of authentic H^{315} and L^{315}, as well as the position of the L^{315} precursor (pL^{315}), are indicated.

(fraction D, Fig. 2A) contains both light and heavy chain mRNA activity, possibly in the form of aggregates*.

Cell-Free Protein Synthesis and Analyses of Products. The total poly(A)-containing mRNA preparations from both MOPC-315 and MOPC-315 NR and the more purified fractions obtained from the NaDodSO$_4$–sucrose gradients were tested for their ability to serve as templates for the synthesis of polypeptide chains in a cell-free system derived from Ehrlich ascites tumor cells. The products synthesized in response to either MOPC-315 or MOPC-315 NR mRNA were compared on NaDodSO$_4$–polyacrylamide slab gels (Fig. 3). It can be seen that substantial synthesis of both H^{315} and L^{315} precursors occurred in the presence of the relevant RNA fractions from the MOPC-315 plasmacytoma. It is clear, however, that no detectable H^{315} was synthesized in the presence of any of the mRNA fractions obtained from the variant MOPC-315 NR plasmacytoma. Mixing experiments (data not shown) designed to test for the presence of an inhibitor of H^{315} mRNA translation in the mRNA obtained from MOPC-315 NR were also performed. It was observed that H^{315} mRNA from MOPC-315 could still be successfully translated in the presence of equal amounts of either the total poly(A)-containing mRNA or the 16–19S mRNA (fraction C′, Fig. 2B) from MOPC-315 NR.

Immunochemical Analysis of the Cell-Free Products. Studies with the mRNAs obtained from the MOPC-315 plasmacytoma had demonstrated that the cell-free products programmed by partially purified H^{315} mRNA fractions (16–19S) resulted in the synthesis of fragments antigenically related to H^{315} in addition to complete H^{315} and L^{315}*. To determine whether the 16–19S class of MOPC-315 NR mRNA produced any portion of H^{315}, immunochemical analysis of the cell-free products was performed. The cell-free products of mRNA fractions of both MOPC-315 and MOPC-315 NR were compared with regard to their ability to be precipitated by normal rabbit serum or by rabbit antiserum to H^{315} or L^{315} (Figs. 4 and 5). It can be seen that the cell-free products of the 16–19S mRNA fraction from MOPC-315 NR still contained the L^{315} protein. However, no significant immunoprecipitable heavy chain or H^{315}-related peptides were detected (Fig. 5). Essentially identical results were obtained if the cell-free products were fractionated on NaDodSO$_4$–polyacrylamide gels and then immunoprecipitated with the antisera (see *Materials and Methods*).

DISCUSSION

The inability to produce heavy chain is a stable characteristic of the MOPC-315 NR variant tumor line. Several mechanisms could be postulated to explain this phenomenon. It is possible, for example, that the DNA segment coding for H^{315} has been lost, in whole or part. Alternatively, it is possible that the gene for H^{315} has been altered such that it can no longer be properly transcribed or yields upon transcription an mRNA that cannot be properly processed in the nucleus or transported into the cytoplasm. Possibly, a modification of the gene for H^{315} has occurred which leads to the transcription of an mRNA sequence that cannot be properly translated. This would be the case if, for example, translation of H^{315} mRNA was prematurely terminated by a nonsense codon arising either by direct base substitution or as the result of a change in the reading frame of the message. Also, it could be argued *a priori* that the loss of the ability to synthesize H^{315} may be the result of a translational defect which would specifically block H^{315} biosynthesis even if active mRNA were available. Furthermore, conceivably a specific inhibitor of H^{315} mRNA transcription or translation might be produced in the MOPC-315 NR variant. Additionally, the presence of one of many post-translational defects could account for the lack of secreted H^{315}: these include increased degradation rate, defective carbohydrate substitution, deficient precursor cleavage enzyme, as well as other alternatives. The experiments described in this report allow an examination of some of these possibilities.

No H^{315} or immunoprecipitable H^{315} fragments larger than light chain were observed in the cell-free products programmed by any of the MOPC-315 NR fractions. This observation rules out the existence of a specific defect in the translational or post-translational apparatus of this variant plasmacytoma line. A specific protein inhibitor of H^{315} translation is also excluded, for H^{315} mRNA of comparable activity to that of the MOPC-315 parent line should have been translated in the heterologous Ehrlich ascites system if it existed. To account

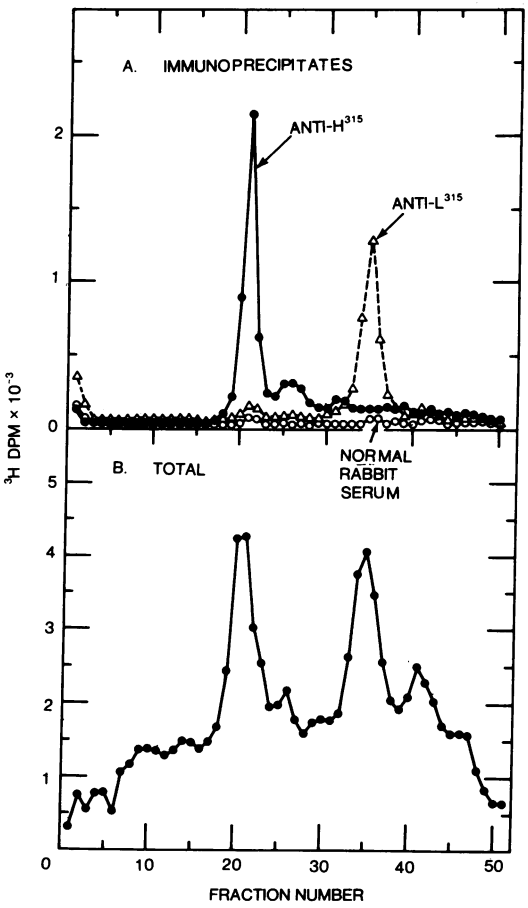

FIG. 4. Immunoprecipitates of cell-free products of MOPC-315 16–19S mRNA (fraction C, Fig. 2A) analyzed on continuous NaDodSO$_4$–polyacrylamide gels. (A) Immunoprecipitation of cell-free products by normal serum (○), anti-H^{315} serum (●), or anti-L^{315} serum (△); (B) total radioactivity in cell-free products.

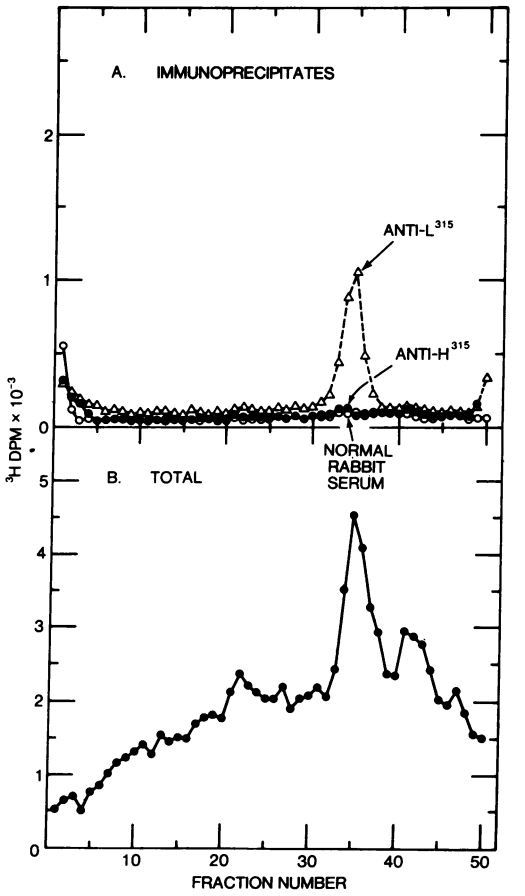

FIG. 5. Immunoprecipitates of cell-free products of MOPC-315 NR 16–19S mRNA (fraction C′, Fig. 2B) analyzed on continuous NaDodSO$_4$–polyacrylamide gels. (A) Immunoprecipitation of cell-free products by normal serum (○), anti-H^{315} serum (●), or anti-L^{315} serum (△); (B) total radioactivity in cell-free products.

for the observed results, any inhibitor protein would have to bind to the poly(A)-containing mRNA tightly enough to survive the rigorous extraction procedures used to prepare the mRNA. It must be noted that, possibly, small amounts of fragments of H^{315} that are not recognized and, therefore, not efficiently precipitated by anti-H^{315} serum may be synthesized.

The addition of MOPC-315 NR mRNA fractions failed to block the cell-free translation of H^{315} directed by MOPC-315 mRNA fractions. Thus, the existence of an inhibitor RNA species is unlikely. Indeed, since none of the MOPC-315 NR mRNAs directed H^{315} synthesis, such a postulated inhibitor RNA would have to purify with total poly(A)-containing mRNA and be distributed throughout the NaDodSO$_4$–sucrose gradient used for mRNA purification. We conclude, therefore, that no translatable poly(A)-containing mRNA capable of directing the synthesis of intact H^{315} can be extracted from the MOPC-315 NR variant plasmacytoma.

The present report is limited to translational analysis of the mRNAs. Whether the defect is the result of an alteration of DNA or mRNA requires further study. It should be possible, however, to resolve this question by a direct measurement of H^{315} DNA and mRNA by hybridization studies. Highly labeled H^{315} mRNA or a DNA complementary to H^{315} mRNA can be used to probe the MOPC-315 NR DNA for the presence of H^{315} nucleotide sequences. Additionally, complementary DNA could be used to estimate the amount of H^{315} RNA sequences in the total cellular RNA.

The data obtained in studies as described in this report should help elucidate the coordination between the genetic information and the cellular machinery for macromolecular biosynthesis and processing that is necessary for the production of functional immunoglobulin molecules. With this information, it should be possible to develop a cell-free system that will produce an active antibody molecule when programmed by suitable templates. Furthermore, many variant plasmacytoma cells of different types have been found and studied (1–11). The methods outlined in this report should permit the elucidation of their molecular basis.

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