Monoclonal Antibodies against Eucaryotic Ribosomes

USE TO CHARACTERIZE A RIBOSOMAL PROTEIN NOT PREVIOUSLY IDENTIFIED AND ANTIGENICALLY RELATED TO THE ACIDIC PHOSPHOPROTEINS P1/P2

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Mice were immunized against chick ribosomes with the use of various protocols and immunogen preparations. Hybridomas were prepared, clones screened, and specific antibodies identified by reversible protein staining followed by immunoperoxidase staining on nitrocellulose blots. Clones were obtained which secreted specific antibodies against ribosomal proteins S6, L7, L18a, P1/P2, and also against ribosomal RNA. Antibodies were typed by means of a dot-binding assay with typing antibodies immobilized on a solid support of nitrocellulose, and also characterized by their species cross-reactivities. The common determinant on proteins P1 and P2 cross-reacted with proteins of similar molecular weight in all eucaryotes tested, and with a determinant in a previously uncharacterized 38,000-dalton protein of the large ribosomal subunit. We designate this protein P0. The determinant of P0 was also present in a protein of similar molecular weight in all eucaryotes tested. Unlike P1 and P2, P0 was not removable from ribosomes by an ethanol-NH4Cl washing procedure. No evidence for a precursor-product relationship between P0 and P1/P2 was found. P0, P1, and P2 were found in active polysomes and in the nucleolus. The molecular weights of the nucleolar forms were not identical with those of the cytoplasmic forms, suggesting some processing during ribosomal assembly and/or transport.

The analysis of the structure and function of bacterial ribosomes has been greatly aided by the existence of a library of specific antisera against individual ribosomal proteins (for recent reviews see Refs. 1 and 2). Similar work with eucaryotic ribosomes has been hampered by the greater difficulty in obtaining sufficiently large amounts of starting material for protein purification, greater difficulty in purification, a larger number of proteins, and less immunogenicity (reviewed in Ref. 3). Monoclonal antibody techniques (4) have the potential of elimination of some of these difficulties. The problem of protein purification is replaced by the problem of screening supernatants from hybridoma clones. We developed a screening procedure based on immunoperoxidase staining of electrophoretic blots of polyacrylamide gel electrophoretograms (5). This method also permits the identification of the protein(s) containing the antigenic determinants. Here we demonstrate the possibility of obtaining monoclional antibodies against individual eucaryotic ribosomal proteins. We show characterization of such antibodies and the use of one as a probe in the study of a previously undescribed 38,000-dalton protein of the large ribosomal subunit.

EXPERIMENTAL PROCEDURES

Cell Fusion—Balb/C mice were immunized by various procedures as outlined in Table I. Spleen cells from the immunized mice were fused with myeloma cells (4), using polyethylene glycol and a 1:1 ratio of spleen cells to myeloma cells. Except for fusion 1 in Table I, the myeloma cell line P0 (obtained from Dr. T. Staehelin, F. Hoffmann-La Roche & Co., Basel) was used. Fusion 1 was with GK0. The myeloma and hybridoma cells were cultivated in Iscove’s modified Dulbecco medium, except that RPMI 1640 was used for the GK0. Mouse spleen (from C57BL/6) was obtained from Drs. J. Zinker (8) and E. coli ribosomes according to Gordon (9). Total ribosomal protein was extracted by the method of Hardy et al. (10), and ethanol-ammonium chloride extractions were done according to Hame et al. (11). Protein S6 was purified from total chick liver 40 S ribosomal subunits by a modification (12) of the method of Collatz et al. (13), through the carboxymethylcel lulose chromatography step. The 0.3 M LiCl eluate from the carboxymethylcel lulose was subjected to chromatography on sulfopropyl-Sephadex (C20) with a gradient of 0.2 to 0.5 M LiCl, pH adjusted to 4 with citric acid. The fractions emerging at 0.4 M LiCl contained S6. The S6 was finally purified by two steps of chromatography on Sephadex G-75. Nucleoli were prepared from rat liver nuclei according to Phillips and McConkey (14).

Pure ribosomal proteins L6, L7, L7a, P1, P2 of rat liver were generous gifts of Dr. I. Wool (University of Chicago), and esL7 and esL12 of Xenopus oocytes or Artemia were generous gifts from Drs. Kalthoff and Richter (University of Hamburg).

Electrophoresis Procedures—The two-dimensional polyacrylamide gel system described previously (7) was used, except for the addition of an overlayer of 2-mercaptoethanol over the first-dimensional gel after polymerization of the second-dimension gel. Slab gels for screening hybridoma supernatants were run with a 10-cm wide slot at the top of the gel, and transferred electrophoretically to nitrocellulose (5). Vertical strips were cut off the edges and stained with Amido black (5) to ascertain proper

* The abbreviations used are: TP, total protein from 80 S total ribosomes or 40 S or 60 S, ribosomal subunits, as specified; TBS, 0.15 M NaCl, 0.02 M Tris-Cl, pH 7.8; PBS, 0.15 M NaCl, 0.01 M NaPO4, pH 6.8.

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The monoclonal antibodies were obtained from fusions of spleens from mice immunized at four subcutaneous sites and one intraperitoneal site in those cases where Freund’s adjuvant was used. Otherwise the injection was intraperitoneal only. Immunizations were as follows: Fusion 1: 600 μg in complete Freund’s adjuvant on day 0, 1.2 mg on days 35, 57–60, 147–150, 210–213, each in PBS. Fusion 2: 25 μg in complete Freund’s adjuvant on day 0; 25 μg in incomplete Freund’s

drugvant on day 41. 450 μg in PBS on day 60, 100 μg in TBS on days

120–123. Fusion 3: 700 μg in incomplete Freund’s adjuvant on day 0, 1.4 mg in PBS on days 51–54. Fusion 4: 360 μg in complete Freund’s adjuvant on day 25, 2 mg of (40 S + 60 S) on days 132–135. The fusions were carried out on the day following the last injection. The antibody typing and ribosome binding were performed as described under “Experimental Procedures.”

Table 1

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Hybridoma</th>
<th>Designation</th>
<th>Immunogen</th>
<th>Screening procedure</th>
<th>Antibody class</th>
<th>Ribosome binding</th>
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<tbody>
<tr>
<td>1</td>
<td>XP2</td>
<td>Anti-L18a</td>
<td>40 S + 60 S</td>
<td>RIA, ELISA</td>
<td>IgG1, κ</td>
<td>-</td>
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<tr>
<td>2</td>
<td>VA2</td>
<td>Anti-86</td>
<td>S6</td>
<td>ELISA, blot</td>
<td>IgG1, κ</td>
<td>-</td>
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<tr>
<td>3</td>
<td>IIIC5</td>
<td>Anti-S6</td>
<td>60 S</td>
<td>Dot, blot</td>
<td>IgG1, κ</td>
<td>-</td>
</tr>
<tr>
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<td>IID4</td>
<td>Anti-I1</td>
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<td>Dot, blot</td>
<td>IgM, κ</td>
<td>+</td>
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<tr>
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<td>IV4A</td>
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<td>60 S</td>
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<td>IgM, κ</td>
<td>+</td>
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<td>XD2</td>
<td>Anti-P</td>
<td>TP80</td>
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* RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbant assay.

Antibody binding on sucrose gradients was carried out as follows. All steps were all carried out in 100 mM KCl, 10 mM Tris-C1, 5 mM MgCl2, pH 7.5. Rat or chick ribosomes or subunits (7, 2.5 A260 units each) were incubated with 50 μl of ascitic fluid (2 ml, 0.5 mg/ml) for 30 min at 4°C. The samples were then run on analytical scale exponential sucrose gradients (26.5 ml of 5.1% in the mixing chamber, 28% sucrose in reservoir, for six 4-ml gradients). The gradients were centrifuged for 1 h at 50,000 rpm and 4°C in the Beckmann SW60 rotor. The gradients were analyzed and fractions were collected. The fractions were then assayed by the dot assay procedure (17). Fractions were bound to a prewetted nitrocellulose sheet by applying solution and binding 25-μl aliquots from each sample. The presence of bound antibody was then detected as described above for the antibody typing. Controls were run with ribosomes only and ascitic fluid only. In addition, the data was confirmed with similar gradients, but using 125I-labeled antibody, prepared as described below.

Antibody Purification—Hybridomas were propagated intraperitoneally, and the ascitic fluid used where indicated. IgM antibodies were purified when used for 125I-labeling. Ascitic fluid (0.25 ml) was loaded on to sucrose gradients as described above for the ribosome binding, and run at 50,000 rpm for 4.5 h at 4°C. The 19 S peaks were pooled, diluted 4-fold in TBS, and concentrated by pelleting in the SW60 rotor at 60,000 rpm for 4 h at 20°C. Labeling with 125I was by a modification of the chloroamine-T method as described previously (5). The antibody designated anti-L18a (see “Results”) was affinity purified on chick TP80 coupled to CNBr Sepharose (Pharmacia, Uppsala, Sweden), as described by the manufacturer, but in the presence of 6 M urea. The column was then washed with 6 M guanidinium chloride in TBS. Ascitic fluid (3 ml) was diluted 4-fold in TBS, centrifuged at low speed, and passed through the column (2.5 ml bed volume). The column was washed with TBS and then 1 M LiCl, 20 mM Tris-C1, pH 7.4. The antibody was eluted with 0.1 M glycine-HCl, pH 3. The eluate was immediately neutralized and dialyzed against 1 mM sodium phosphate buffer, pH 7.2, and then lyophilized.

RESULTS

Characterization of the Monoclonal Antibodies against Ribosomal Proteins—Mice were immunized by several protocols as summarized in Table I. Those with sera showing antibodies against ribosomal proteins were used for the preparation of hybridomas. The earliest fusions were screened with the use of radioimmune or enzyme-linked immunosorbant assays. Later the electrophoretic blotting procedure for ribosomal proteins, and the dot immuno-binding assay with intact ribosomes, were used in parallel (see under “Experimental Procedures”). The monoclonal antibodies were characterized as summarized in Table I. The identification was performed in stages. (a) The subunit specificity was determined from data of one-dimensional electrophoreograms, with the large and small subunits run separately. This was in the acidic pH system corresponding to the second dimension of the two-dimensional ribosomal protein system (see under “Experimental Procedures”). (b) The pro-

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A. Matus, personal communication.
proteins were classified as acidic or basic by electrophoresis at alkaline pH (the first dimension of the two-dimensional ribosomal protein electrophoresis system) with separate runs at both polarities. (c) The protein containing the antigenic determinant was identified by means of its position in a two-dimensional gel electrophoretogram. The total protein pattern was first obtained by means of a reversible staining procedure with heparin and toluidine blue as described under "Experimental Procedures." The protein positions were marked and compared with the subsequent immunoperoxidase staining. In cases of ambiguity, the identification was facilitated by the use of blots of pure proteins, generously provided by Dr. I. Wool. For convenience, each antibody was designated according to the nomenclature of the ribosomal protein containing the corresponding antigenic determinant (see Table I).

The identification of the antibody specific for S6 was straightforward as the pure protein had been used for the immunization. The antibody designated anti-S6 showed cross-reaction in enzyme-linked immunosorbent assays with the pure S6 preparation. Also, the immunoperoxidase staining coincided with the protein band in blots from all three gel electrophoresis systems used in this work. The position was also readily recognizable as that of S6 in two-dimensional electrophoreograms. The identification was further confirmed by the shift in mobility of the immunoperoxidase-stainable spot corresponding to differences in the degree of phosphorylation either in two-dimensional (not shown) or in one-dimensional urea-sodium dodecyl sulfate gels. This is detailed in a separate publication (37).

A second hybridoma clone was obtained from fusion 2 (Table I), which reacted exclusively with the S6 preparation used as immunogen. The antibody was denoted anti-S6*. It did not react with freshly prepared ribosomes or ribosomal proteins, and the immunoperoxidase-stainable protein had a slightly lower mobility than the native S6. Since the pure S6 preparation had been prepared several years previously (12), it is possible that this determinant was generated by degradation of S6. Curiously, the anti-S6* cross-reacted with a protein in ribosomes from yeast and E. coli (Table II), but not with any other species tested.

The identification of the antibodies designated anti-L7 and anti-L18a is shown in blots of two-dimensional electrophoreograms in Fig. 1, A and B. The identification follows the international nomenclature (18), and was facilitated by comparison with our own earlier two-dimensional fingerprints (7). The insets show the corresponding positions for neighboring proteins in the electrophoreograms. In the case of protein L18a, the immunoperoxidase stain centered over the spot of the corresponding protein and was sufficiently well resolved from the neighboring proteins (Fig. 1B). Protein L7 is in a more crowded region of the gel (Fig. 1A), and is not completely resolved from L6 and L7a. However, blots of sodium dodecyl sulfate gels of pure proteins permitted the unambiguous assignment as L7. There was no reactivity with proteins L6 or L7a.

The antibody designated anti-P also presented difficulties,
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Because P1 and P2 run much more diffusely than other acidic ribosomal proteins in the standard ribosomal protein two-dimensional gel electrophoresis system, we investigated their mobility in another system, namely the nonequilibrium electrophoresis system of O'Farrell et al. (16). The immunoperoxidase-stained blot of such an electrophoretogram is shown in Fig. 2. Here, P1 and P2 run as compact spots. It was surprising to see a third protein of considerably higher molecular weight (38,000) in the immunoperoxidase-stained blot. This protein may possibly correspond to one already described by others (see under "Discussion"). Because it is stained by the antibody designated anti-P, we designate this protein as P0.

Characteristics of Proteins containing the Determinant for Anti-P—The proteins designated P1/P2 are likely to be the same as a highly conserved acidic phosphoprotein doublet, referred to by various terminologies by various authors (see under "Discussion"). We also found that the determinant in a 13,500-dalton doublet was present in all eucaryotes tested, and was also more conserved than the determinants for our other monoclonal antiribosomal protein antibodies (see Table II).

In addition, the immunoperoxidase-stained electrophoretogram of chick liver TP80 with nonequilibrium focussing in the first dimension (I) and sodium dodecyl sulfate in the second (II). The blot was immunoperoxidase-stained with anti-P as described for Fig. 1.

![Fig. 2](image-url) Immunoperoxidase-stained blot of a two-dimensional gel electrophoretogram of chick liver TP80 with nonequilibrium focussing in the first dimension (I) and sodium dodecyl sulfate in the second (II). The blot was immunoperoxidase-stained with anti-P as described for Fig. 1.

![Fig. 3](image-url) Cross-reactivity of anti-P with ribosomal proteins from various species. From left to right, each slot contained the equivalent of 25 µg of total protein from ribosomes of human placenta, rat liver, chick liver, trout liver, Drosophila, Xenopus oocytes, and yeast (see under "Experimental Procedures"). After the sodium dodecyl sulfate electrophoresis, the gels were blotted and immunoperoxidase-stained with anti-P. The arrows indicate positions of molecular weight standards run in the same gel: phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,000), and α-lactalbumin (14,700).

![Fig. 4](image-url) Analysis of subunit distribution of the proteins cross-reacting with the anti-P antibody. Rat liver 80 S ribosomes (13 A260 units) were dissociated into subunits and separated on isokinetic sucrose gradients as described under "Experimental Procedures," in 20 mM Tris-HCl, 500 mM KCl, 3 mM MgCl2, 1 mM dithiothreitol. Centrifugation was in the Beckmann SW60 rotor at 50,000 rpm for 60 min at 20 °C. The gradient was analyzed and fractions collected. The subunits were precipitated with 10% trichloroacetic acid, dissolved in the sample buffer of the sodium dodecyl sulfate gel system, pH adjusted to neutrality with NH4OH, analyzed on a 15% polyacrylamide-sodium dodecyl sulfate gel, blotted on to nitrocellulose, and immunoperoxidase-stained. The staining pattern is shown beneath the corresponding fractions in the optical density profile.
ogram of Fig. 3 shows that there is a conserved determinant in a protein with approximately 38,000 molecular weight, corresponding to that designated P0. The yeast 13,500-dalton doublet was also present, but too weak to appear in the photograph (channel 7). In separate experiments, purified proteins eL7 and eL12 (20) from both Xenopus and Artemia also showed corresponding immunoperoxidase-stained doublets at 13,500 daltons. This further supports the idea of the evolutionary conservativeness of these proteins and suggests a basis for a uniform nomenclature (see under "Discussion").

We performed a more detailed analysis of the ribosomal subunit distribution of the proteins reactive with the anti-P monoclonal antibody in order to verify that P0 is a bona fide ribosomal protein. Individual fractions from a sucrose gradient were concentrated, subjected to electrophoresis on sodium dodecyl sulfate gels, and immunoperoxidase-stained with the anti-P antibody. The results in Fig. 4 show that both P0 and P1/P2 co-sedimented with the 60 S ribosomal subunit. As an additional internal control for the alignment of the fractions with the sucrose gradient optical profile, the same blot was subjected to a second round of antibody binding, and immunoperoxidase staining with the anti-S6 antibody (not shown). This co-sedimented with the 40 S subunit peak. We would also like to draw attention to the possibility of reutilization of blots in this way, a point made independently by Legocki and Verma (21).

One of the well defined characteristics of P1/P2 and the related proteins in other species, as well as of the probable corresponding proteins in E. coli, is their ethanol extractability (11, 22). We therefore investigated the ethanol extractability of P0 as well as P1/P2. The pellet and supernatant fractions from successive washes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the content of P1/P2 and P0 revealed by immunoperoxidase stain. It can be seen from the result in Fig. 5 that the bulk of the P1/P2 was removed in the first wash (channel 2), while the P0 remained associated with the core throughout the washing steps (channels 6-9). The apparent inconsistency between the stain intensity in the wash and corresponding core fractions is due to the nonlinearity of the immunoperoxidase staining process (see Ref. 17).

The existence of immunologically related higher and lower molecular weight polypeptides suggests a possible precursor-product relationship between the forms. We failed to generate cross-reacting peptides with molecular weight corresponding to P1/P2 by a variety of limited proteolysis protocols using the core fraction devoid of P1/P2, as in Fig. 5, channel 9. A precursor form is likely to be absent from active polysomes. We therefore analyzed fractions from polysome profile

![Fig. 5. Analysis of content of proteins cross-reactive with anti-P in ethanol-NH4Cl cores and wash fractions from 80 S ribosomes from chick liver. Channels 1 and 10 are control, untreated ribosomes, and channels 2-4 represent the material from supernatants of successive washes according to Hamel et al. (11). Channels 6-9 are the corresponding successive core fractions. The gels were run and processed as in Fig. 2.](image-url)
of a rat liver extract by methodology as in Fig. 4, and found no evidence for selective presence or absence in the different fractions (not shown).

A precursor form is also likely to be selectively associated with the nucleolus, where assembly of ribosomal precursors takes place (23). Furthermore, it could be anticipated that P1/P2 would be absent from the nucleolus, as they are known, at least in Xenopus oocytes, to be exchangeable in the cytoplasm, and therefore are probably late added proteins (24).

We subjected whole nucleoli and TP80 from rat liver to analysis on polyacrylamide-sodium dodecylsulfate gels followed by immunoperoxidase staining. The stained spots were scanned with a densitometer, and the results are shown in Fig. 6 (traces A and C). To our surprise, both 13,500- and 38,000-dalton peptides were present in the nucleolus. Again, this does not support the idea of a precursor-product relationship between P0 and P1/P2. However, it raises the question of whether P1/P2 are early or late assembled proteins (see under "Discussion").

More careful examination of the blots of Fig. 6 revealed discrepancies between the exact molecular weights of the various bands (see the recorder printouts of the relative positions of the peaks in Fig. 6). This was confirmed by the appearance of the scan of a mixture of the TP80 and the nucleolar preparations (Fig. 6, scan B), where the peak positions are intermediate. The nucleolar P0 was apparently slightly larger than in the cytoplasm, and the nucleolar P1/P2 showed only one band intermediate between the cytoplasmic P1/P2. The significance of this observation is not clear at this point, but suggests some processing of the P proteins during ribosomal assembly and transport.

Discussion

The results in this work reinforce the idea of the usefulness of monoclonal antibodies against ribosomal proteins. However, their usefulness as functional probes is limited by the finding that a number of the monoclonal antibodies obtained were directed against determinants not accessible in the intact ribosome (see Table I). Only the anti-P antibody was against accessible determinants, i.e., one out of five of the antibodies obtained. It is not clear whether this fraction is representative or not, because of the small number of antibodies obtained and the fact that we were exploring various protocols. It is clear, however, that the protocols used yield 1–2 positive clones/fusion. The amount of work to obtain a complete library of such antibiribosomal proteins monoclonal antibodies can be extrapolated from these results and from the total number of ribosomal proteins (about 70) (Ref. 3).

In a similar study with E. coli ribosomes, four independently selected clones gave antibodies against only two proteins (25). This is not the case for the monoclonal antibodies against eucaryotic ribosomal proteins described here.

Even when an antibody is not directed against an accessible determinant, a variety of parameters can be investigated with the electrophoretic blotting and immunoperoxidase staining techniques. Other proteins sharing the same determinants can be readily identified, although more detailed protein analysis is required for proof of the degree of relatedness of such proteins. However, preliminary conclusions can be drawn, which would have been complicated by the presence of possible impurities if polyclonal antibodies had been used. For example, this approach revealed a previously unknown ribosomal protein with unusually high molecular weight (38,000) as it shared a common determinant with P1/P2. We have designated this protein P0. The determinant was evolutionarily conservative.

Several groups have described a doublet of evolutionarily conservative, ethanol-extractable acidic phosphoproteins in the large ribosomal subunit of a variety of eucaryotes (reviewed in Ref. 26), and have used a variety of terminologies. They may also be related to the acidic proteins L7/L12 of E. coli (Ref. 26). We have confirmed the evolutionary conservativeness of the determinant for the antibody designated anti-P, since it reacts with P1/P2 of rat liver from Tsarugii et al. (19), with eL7/eL12 of Xenopus and Artemia from Van Agthoven et al. (20), and with corresponding proteins from all the other eucaryotes we have tested here (Table II and Fig. 3). Vidales et al. (27) have raised conventional polyclonal antibodies against the yeast ethanol-extractable acidic phosphoproteins, variously referred to as L44/L45 (28) or L35/L36 (29). This polyclonal antibody cross-reacted with a 13,500-dalton doublet from wheat germ, Drosophila eggs, trout liver, chick liver, rat liver, chick liver, rat liver, rabbit reticulocytes, or human placenta ribosomes (27). Furthermore, the same polyclonal antibody showed reaction with a protein equivalent to that designated here as P0. Leader and Coia (30) have described a doublet of ethanol-extractable phosphoproteins from Krebs' ascites cells, which they have designated L7 and L8. Horak and Schiffmann (31) described a triplet of acidic proteins, L40a, L40b, and L40c, of which L40b and L40c were phosphorylated. Since the relatedness or identity of all of these acidic proteins either has been confirmed or can be confirmed by the antibodies described here, we propose that a uniform nomenclature should be adopted by all authors in the future.

The antibody designated here as P0 cross-reacted with the 38,000-dalton species in the other eucaryotic ribosomes tested. It appears to be of similar molecular weight to a phosphoprotein designated P1 in yeast (29) and L5 of Krebs ascites cells (33). The latter proteins have not been characterized. The protein P0, as well as P1/P2, co-migrated in two-dimensional polyacrylamide gels with radioactive spots in autoradiograms from material prepared from 3P-labeled mouse 3T3 cells.

No evidence was found for a precursor-product relationship between P0 and P1/P2, but the investigation led to the finding that both forms were in the nucleoli. This finding is controversial in view of the observation of Kalthoff and Richter (24) that the proteins eL7/eL12, when injected into Xenopus oocytes, are freely exchangeable with the bound form in mature cytoplasmic ribosomes, and from this proposed that these proteins are added late in ribosome assembly. This is not consistent with their presence in the nucleolus, as proteins added early in the assembly. A soluble pool of immunologically reacting protein has been seen in yeast (34) and Artemia (20). We have so far not been able to detect any soluble pool of protein cross-reactive with the anti-P in material from chick or rat liver. The reasons for these apparent inconsistencies are not clear, but cannot be accounted for by incorrect identification of the respective proteins, as the identification has been validated here by proteins P1/P2 and eL7/eL12 donated by the respective laboratories.

A further application of the antibodies described here is the use of the blotting and immunoperoxidase techniques as part of an assay for the phosphorylation of ribosomal protein S6, as described in detail in another publication (37). This lends itself to the study of the phosphorylation in biological material that is not easily assayed by conventional means.

Out of the series of immunizations and fusions described here, we obtained one monoclonal antibody which was reactive with ribosomal RNA of all species tested; of various

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1. I. Wool, personal communication.
3. H. Towbin and Martin-Pérez, unpublished data.
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homopolynucleotides tested, only with poly(U); and not with tRNA. Monoclonal antibodies against ribosomal RNA have also been obtained from hybridomas derived from the spleen of autoimmune mice (35, 38). ELISA (4.35) showed that the antiribosomal RNA antibody had a strong preference for poly(G,C) and poly(C,C,U). We have shown antibodies against ribosomal proteins in human autoimmune diseases (36), and have now extended this to an autoimmune strain of mice. A fruitful approach for establishing a hybridoma library for antibodies against ribosomal and other antigens might then be the preparation of hybridomas from such autoimmune strains of mice.

Acknowledgments—We would like to thank Theo Staehelin for initially introducing us to monoclonal antibody technology, to Drs. Wool and Richter for gifts of reference proteins, Drs. P. Nielsen, A. Matus, and L. Jimenez de Aua for suggestions concerning the manuscript. We would also like to thank Drs. Wool, Kalthoff, Richter, duPasquier, and Gehring for gifts of materials that were used in this work.

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