Association of Methionyl-tRNA Synthetase with Detergent-insoluble Components of the Rough Endoplasmic Reticulum

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ABSTRACT Using fluorescent antibody staining, we have established the association of methionyl-tRNA synthetase with the endoplasmic reticulum in PtK_2 cells. After Triton X-100 extraction, 70% of the recovered aminoacyl-tRNA synthetase activity was found in the detergent-insoluble fraction. This fraction of the enzyme remained localized with insoluble endoplasmic reticulum antigens and with ribosomes, which were stained with acridine orange. By both fluorescence microscopy and electron microscopy the organization of the detergent-insoluble residue was found to depend on the composition of the extracting solution. After extraction with a microtubule-stabilizing buffer containing EGTA, Triton X-100, and polyethylene glycol (Osburn, M., and K. Weber, 1977, *Cell*, 12:561-571) the ribosomes were aggregated in large clusters with remnants of membranes. After extraction with a buffer containing Triton X-100, sucrose, and $CaCl_2$ (Fulton, A. B., K. M. Wang, and S. Penman, 1980, *Cell*, 20:849-857), the ribosomes were in small clusters and there were few morphologically recognizable membranes. In both cases the methionyl-tRNA synthetase and some endoplasmic reticulum antigens retained approximately their normal distribution in the cell. Double fluorochrome staining showed no morphological association of methionyl-tRNA synthetase with the microtubule, actin, or cytokeratin fiber systems of PtK_2 cells. These observations demonstrate that detergent-insoluble cellular components, sometimes referred to as "cytoskeletal" preparations, contain significant amounts of nonfilamentous material including ribosomes, and membrane residue. Caution is required in speculating about intermolecular associations in such a complex cell fraction.

In eucaryotic cells some of the components required for protein biosynthesis are bound to membranes of the endoplasmic reticulum and some are free in the cytoplasm (34). Recently, it has been suggested that the "free" ribosomes and mRNA are actually bound to a detergent-resistant "cytoskeleton" (4–6, 12, 23, 24, 38, 40, 43) or "microtrabeculae" (45). We have studied the intracellular localization of an aminoacyl-tRNA synthetase in a variety of vertebrate cells, because it provides another type of marker for the organization of the protein biosynthesis system. Eucaryotic aminoacyl-tRNA synthetases, which catalyze the formation of aminoacyl-tRNAs, occur as high molecular weight complexes (2, 7–9, 17–20, 30, 32, 33, 42) in contrast to the procaryotic counterparts (9, 33). The aminoacyl-tRNA synthetases have been shown in vitro to be associated with ribosomes (30, 41, 42), microsomes (15, 27, 41), and other protein biosynthesis components (35).

Using fluorescent antibody staining we found that methionyl-tRNA synthetase (Met-RS) is associated with the endoplasmic reticulum in cultured cells. We also demonstrate that the majority of methionyl-tRNA synthetase, which is frequently found in high molecular weight complexes (8, 9), is associated with the detergent-insoluble matrix of the rough endoplasmic reticulum, not with intermediate filaments, actin stress fibers, or microtubules which are also present in the detergent-insoluble fraction of the cells. This demonstrates that the detergent-resistant "cytoskeleton" preparation contains significant amounts of nonfilamentous material including membrane residue, and that caution is required in speculating about intermolecular associations in such a complex cell fraction.

MATERIALS AND METHODS

Materials

Materials for the preparation of rat liver aminoacyl-tRNA synthetases were obtained from sources described previously (8, 19). Rhodamine- and fluorescein-conjugated goat antibodies to rabbit immunoglobulins were obtained from BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, MD. NBD-phallacidin was purchased from Molecular Probes Inc., Plano, TX. Triton X-100 is a product of Sigma Chemical Co., St. Louis, MO. Polyethylene glycol (PEG)

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6000 was obtained from J. T. Baker Chemical Co., Phillipsburg, NJ. Other chemicals were reagent grade.

Aminoacyl-tRNA Synthetase Preparations and Assay

The rat liver 18S aminoacyl-tRNA synthetase complex containing the activities specific for Arg, Ile, Leu, Lys, and Met was purified as described (19). Leu-tRNA synthetase assays of Triton X-100 extracted cells grown on coverslips were performed as follows. The assay solution contained final concentrations of 100 mM Tris-HCl (pH 7.5 at 25°C), 6 mM $MgCl_2$, 4 mM dithiothreitol, 2.5 mM ATP, 1 mg/ml BSA, 5 mg/ml yeast tRNA, and 30 μM [^{14}C]leucine (353 mCi/mmol). Cells (2×10^5 per coverslip) grown on 18 × 18 mm coverslips were extracted with Triton X-100 according to Osborn and Weber (28) as described below. The coverslips were inverted on 500 μl of assay mix in a 60-mm petri dish and incubated at 20°C. Aliquots of 50 μl were spotted on 3-MM filter paper squares and processed as described (8, 19). To determine the amount of Leu-tRNA synthetase extracted, the Triton X-100 (0.5%) extract was assayed under conditions identical to those used for the coverslip; the assay mixture contained a final Triton X-100 concentration of 0.2%. 1 U of aminoacyl-tRNA synthetase activity is that amount which catalyzes the formation of 1 nmol of aminoacyl-tRNA in 1 min at 37°C.

Preparation of Antigen and Antibodies

Band IV (104 kdaltons) of the 18S rat liver aminoacyl-tRNA synthetase complex was extracted from preparative slab SDS PAGE gels (18, 19). 75 μg of Band IV with Freund's complete adjuvant was injected subcutaneously into New Zealand white rabbits. Boosts with 50 μg of Band IV in Freund's incomplete adjuvant were performed 3 wk after the initial injection and at 2-wk intervals with bleedings between injections. Preparations of IgG fraction and conjugation with fluorescein were as described (16). The labeled anti-Met RS IgG had 1.4 mol of fluorescein per mol IgG. Purified antibody was obtained from immune Ig by affinity chromatography on a column of synthetase complex conjugated to Sepharose 4B at a concentration of 3 mg/ml (19). Bound antibody was eluted with acid (16).

Rabbit antikeratin antibody and affinity purified rhodamine-conjugated antitubulin antibody were generous gifts from Dr. T.-T. Sun (37) and Dr. D. B. Murphy (28), respectively. The antiserum to ribosome-stripped, dog pancreas rough endoplasmic reticulum membranes was a gift from Dr. Daniel Louvard (European Molecular Biology Laboratory, Heidelberg) and has been characterized previously (25, 26).

SDS PAGE AND NITROCELLULOSE SHEET BLOTTING: SDS PAGE was performed according to Gibson (14). Polypeptides were transferred to nitrocellulose sheets (39), stained with anti-methionyl-tRNA synthetase and then ^{125}I-protein A to identify immunoreactive peptides.

Anti-Met-tRNA Synthetase Antibody Specificity

The 18S synthetase complex containing ~0.05–0.1 U of the different aminoacyl-tRNA synthetases (19) was incubated with various concentrations of antiserum for 4 h at 4°C before the enzyme activities were assayed. Preimmune serum served as control. Immunodiffusion was performed in agarose (13).

Cells and Cell Culture

Cultures of PtK_2, HeLa, mouse 3T3 fibroblast, buffalo rat liver epithelial, fetal bovine aortic endothelial (10), fetal bovine smooth muscle, and rat glial C6 cells were grown on 18 × 18 mm coverslips as described (16).

Cell Fixation and Extraction

Cells grown on coverslips were fixed by several procedures. (*a*) Formaldehyde: Cells were washed in PBS for 1 min and fixed at 20°C in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4) containing 3.7% formaldehyde (1:10 vol/vol of 37% formaldehyde solution, Mallinckrodt Inc., Science Products Div., St. Louis, MO) for 4 min, followed by permeabilization with absolute methanol (−20°C), for 1 min. Cells were rehydrated in PBS before staining. (*b*) Methanol-acetone: Cells were washed in PBS for 1 min and fixed in absolute methanol (−20°C) for 5 min and acetone (−20°C) for 3 min. Rehydration in PBS was performed before staining (37). (*c*) Triton extraction in microtubule-stabilizing buffer (28): Cells were extracted with 0.5% Triton X-100 in 100 mM PIPES, pH 6.9, 1 mM EGTA, 2.5 mM GTP, and 4% PEG 6000 for 5 min at room temperature, fixed in methanol (−20°C) for 5 min, and rehydrated in PBS. (*d*) Triton extraction in CSK buffer (12): Cells were extracted with 0.5% Triton X-100, 1.0 mM $CaCl_2$, 0.3 M sucrose, 100 mM KCl, 2.5 mM $MgCl_2$, 10 mM PIPES, pH 6.8 for 1 min at 4°C, fixed in 3.7% formaldehyde in PBS for 10 min and washed with PBS (15 ml × 3) before staining.

Acridine Orange Staining

Cells were fixed by procedures described above and were incubated at 20°C for 40 min in 0.1% acridine orange solution in PBS (12). Coverslips were washed with PBS (10 ml) with 10 changes over 20 min and mounted for fluorescence microscopy.

NBD-Phallacidin Staining

Cells were fixed with acetone (−20°C) for 1–2 min and then immediately stained with NBD-phallacidin according to Barak and Yocum (3).

Fluorescent Antibody Staining and Microscopy

Coverslips were incubated with antibody 40–60 min at 37°C (16), washed in 15 ml PBS (×3) and mounted. Microscopy and photography were performed as described (16).

Staining with fluorescein-conjugated anti-Band IV IgG was performed at concentrations of 100–105 μg/ml. Anti-Band IV and affinity-purified anti-Band IV IgGs were used at 50–100 μg/ml for indirect staining. Antikeratin antiserum with 1:30 dilution and anti-ER IgG at 200–400 μg/ml were used in indirect staining experiments. Rhodamine-conjugated, affinity purified antitubulin IgG was used at 100–150 μg/ml. Fluorescein-and rhodamine-conjugated goat anti-rabbit-Ig IgG were used at concentrations of 100–200 μg/ml.

Double staining with anti-Band IV and antitubulin IgG was performed by incubation of fixed cells with a mixture of directly labeled antibodies for 60 min. Double staining with anti-ER or antikeratin antibody with fluorescein-anti-Band IV IgG was as follows. Cells on coverslips were treated and fixed as described above and then incubated with antikeratin or anti-ER IgG for 40 min. Coverslips were thoroughly washed with PBS, (15 ml, three times over 15 min) and incubated with rhodamine-conjugated goat anti-rabbit IgG for 40 min. After washing with PBS, the cells were incubated with fluorescein-conjugated anti-Band IV IgG for 40 min. The cells were washed with PBS and mounted for microscopy. When PtK_2 cells were incubated in excess rabbit preimmune IgG (500 μg/ml) after incubations with rabbit anti-ER and rhodamine-goat-anti-rabbit IgG, there was no blocking of subsequent fluorescein-conjugated anti-Band IV IgG staining. This control experiment showed no detectable binding of anti-Band IV IgG to the goat anti-rabbit-Ig in double-labeling experiments.

RESULTS

Characterization of Antibodies to Methionyl-tRNA Synthetase

The antisera to methionyl-tRNA synthetase reacted almost exclusively with the 104,000 mol wt Band IV among the rat liver 18S aminoacyl-tRNA synthetase complex (Fig. 1). There was also minor reaction with Bands I and II. The absence of reactivity with Lys-tRNA synthetase, Band VI (69 kdaltons), (18, 19), and polypeptides of other synthetases (20) indicates the high specificity of the immune IgG, because cross-reaction between enzymes of the same family might be expected.

Anti-Band IV antiserum reacted with purified 18S complex and crude postmitochondrial rat liver extract (19) with complete fusion of precipitin bands (Fig. 1). The preimmune serum showed no significant precipitation with crude liver extract or with the 18S complex (Fig. 1). This demonstrated that the antibody reacted with native methionyl-tRNA synthetase in the 18S complex and in crude liver extract without cross-reaction with other antigens.

The immune serum specifically inhibited the activity of rat liver methionyl-tRNA synthetase without inhibition of synthetases specific for Ile, Lys, Leu, and Arg (Fig. 2). The same concentration of preimmune IgG did not inhibit Arg-, Ile-, Leu-, Lys-, or Met-tRNA synthetase activities. These results confirm the identification of the Met-tRNA synthetase from sheep and rabbit as polypeptides of M_r = 103,000 and 108,000, respectively (20). The rabbit and rat liver complexes have

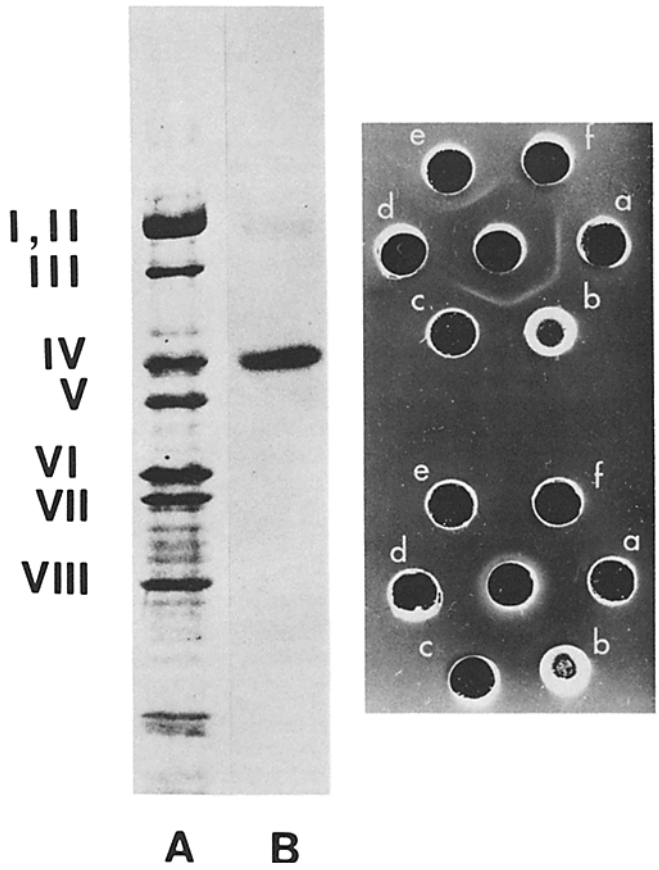


FIGURE 1 Characterization of antibodies raised against Band IV. *Left:* Polypeptides of the rat liver 18S aminoacyl-tRNA synthetase complex were separated by gel electrophoresis and stained with Coomassie Blue (lane *A*) or transferred to nitrocellulose paper and stained with IgG raised against Band IV (lane *B*). Band VI, previously identified as Lys-tRNA synthetase, showed no cross-reaction. *Right:* Immunodiffusion of antiserum (22 μl, upper center well) and the rat liver 18S complex (22 μl, wells *a*, *c*, and *e*) and rat liver postmitochondrial fraction (22 μl, well *b*) shows precipitin lines of identity between the 18S complex and postmitochondrial fractions. Preimmune serum (22 μl, lower center well) with identical amounts of antigens in surrounding wells show no significant precipitin lines. Wells *d* and *f* contained 22 μl of PBS.

essentially identical polypeptides (Yang, D. C. H., unpublished result).

Localization of Methionyl-tRNA Synthetase in PtK_2 Cells

After formaldehyde fixation and permeabilization with acetone/methanol, there was extensive coincidence of the staining patterns with antibodies to methionyl-tRNA synthetase (antisynthetase) (Fig. 3*a*) and endoplasmic reticulum (anti-ER) (Fig. 3*b*). Staining with purified antibodies to methionyl-tRNA synthetase gave the same result as immune Igs. The anti-ER staining pattern in PtK_2 was similar to that observed in normal rat kidney cells with the same antiserum (25). With both antibodies the fluorescence intensity was highest in the perinuclear region. In thinner peripheral regions where there was less superimposition, it could be seen that both antibodies stained a reticular structure that extended about halfway to the cell margins. When focusing through the cell, it was clear that the stained structure was also reticular in the thicker perinuclear region. The concurrence of the two antibody staining patterns established that at least part of the methionyl-tRNA synthetase is associated with the endoplasmic reticulum. The antisynthetase and anti-ER staining patterns were not identical, however (Fig. 3 *a* and *b*). The antisynthetase stained some parts of the reticulum relatively more intensely than did the anti-ER. Furthermore, there was low intensity staining of the cytoplasmic matrix and intense staining of the nucleolus with antisynthetase that was not present with anti-ER.

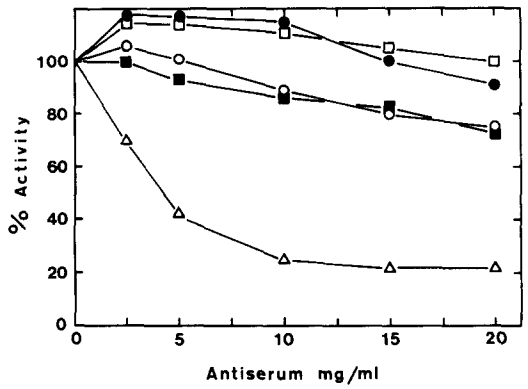


FIGURE 2 Effect of anti-Band IV on aminoacyl synthetase activity. Anti-Band IV immune serum specifically inhibited rat liver methionyl-tRNA synthetase (△) activity but not synthetases specific for Arg (○), Ile (●), Lys (■), and Leu (□). Preimmune serum did not inhibit the synthetase activities.

Fixation with methanol/acetone without formaldehyde gave similar results (not illustrated), but the reticular staining pattern in the peripheral cytoplasm was less distinct. Instead the stained structures were more punctate. Direct and indirect staining methods with synthetase antibodies gave the same results.

Association of Methionyl-tRNA Synthetase, ER Antigens, and Ribosomes with the Detergent-insoluble Fraction

When PtK_2 cells were extracted with Triton X-100, both antisynthetase (Fig. 3*c*) and anti-ER (Fig. 3*d*) stained the insoluble cell remnant that remained attached to the coverslip. The staining patterns were identical to each other and differed from unextracted cells (Fig. 3 *a* and *b*) in that the reticulum was coarser and more homogeneous. Also, the staining of the nucleolus by antisynthetase was more intense in detergent-extracted cells. The staining pattern was the same after 5–15-min extractions and corresponded closely with a phase-dense perinuclear reticulum (Fig. 4 *a* and *b*). In addition to synthetase and ER antigens, this detergent-insoluble reticulum was stained with acridine orange (Fig. 4*c*), indicating that it contained RNA.

We used a biochemical assay for leucyl-tRNA synthetase, which is tightly associated with methionyl-tRNA synthetase (8, 19, 20), to determine quantitatively what fraction of the enzyme was retained in the detergent-insoluble cell remnant attached to the coverslip. As in the case of purified rat liver 18S complex, 0.2% Triton X-100 inhibited synthetase activity by ~40%. This concentration of Triton was included in the assay mixtures to make the different fractions comparable. Of the remaining synthetase activity 30% was extracted and 70% was bound to the coverslip. Thus, both quantitatively and morphologically a majority of the synthetase is associated with detergent-insoluble components of the endoplasmic reticulum.

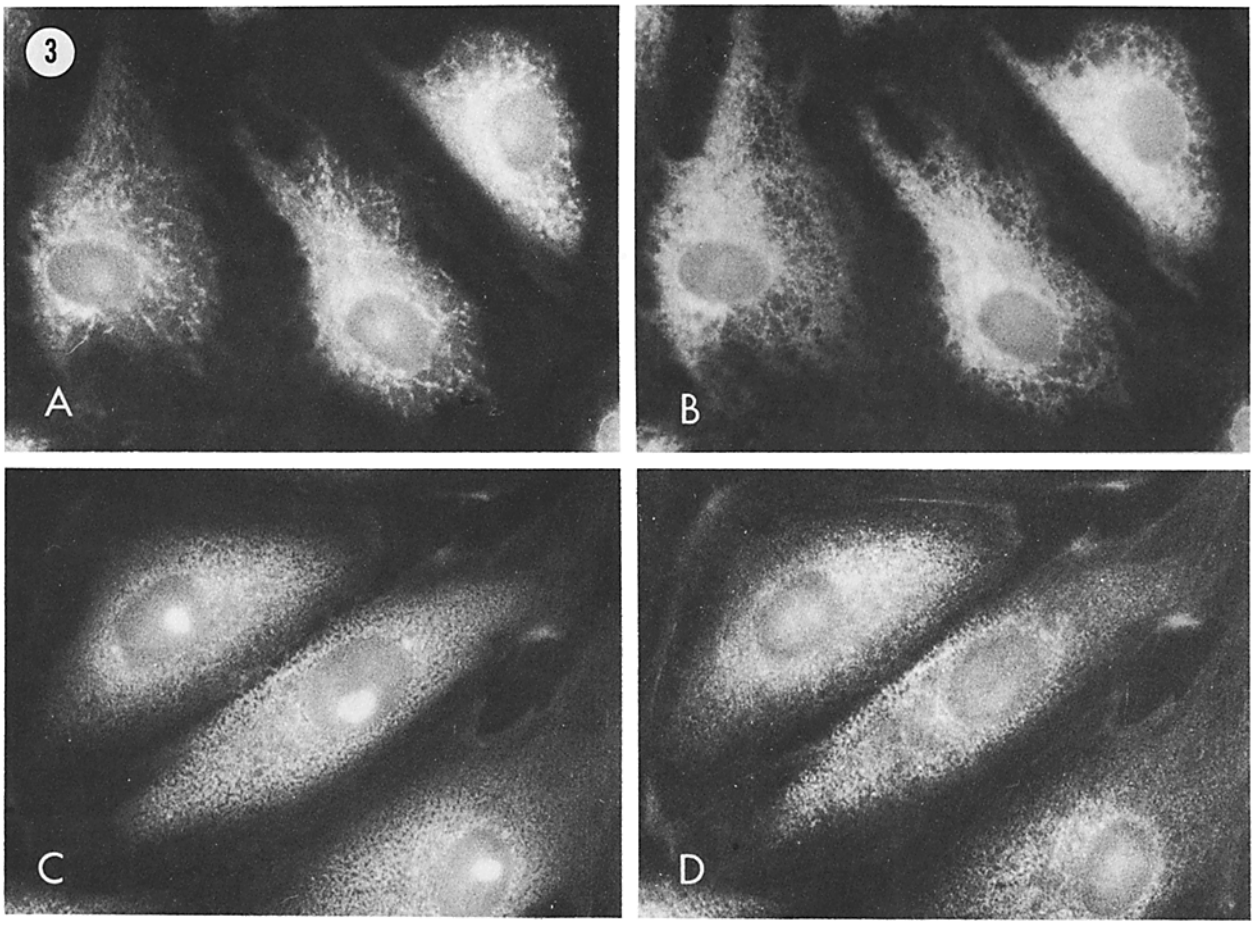


FIGURE 3 Double fluorescent-antibody staining of PtK_2 cells. (*A* and *B*) Formaldehyde fixed. (*C* and *D*) Extracted with microtubule stabilizing buffer with 0.5% Triton X-100. (*A* and *C*) Stained with fluorescein-conjugated anti-synthetase. (*B* and *D*) Stained indirectly with anti-ER and rhodamine-labeled goat anti-rabbit Ig. Note the coincidence of the fluorescent patterns, especially in the detergent-extracted cells.

Because mammalian Met-tRNA synthetase frequently occurs in large complexes that are precipitable with 2–5% PEG 6000 (20), we tested an extraction solution with 30% glycerol replacing the 4% PEG. Nuclei stained more intensely in glycerol than in PEG, but the reticular staining pattern with antisynthetase was the same as in Fig. 4 *b*.

After extraction with Triton X-100 in CSK buffer (12) antisynthetase (Fig. 4 *d*), anti-ER (Fig. 4 *e*), and acridine orange (Fig. 4 *f*) all give similar cytoplasmic staining patterns, which differed from that in cells extracted with Triton in the microtubule-stabilizing buffer (Fig. 4 *a–c*). The perinuclear cytoplasmic staining was more punctate than reticular and both antibodies stained the nucleus more intensely. This latter difference was due, in part, to weaker cytoplasmic staining with both antibodies in CSK buffer compared with the microtubule-stabilizing buffer.

Electron Microscopy

The differences in antibody staining patterns were explained by electron microscopy. In control cells (Fig. 5 *a*, *b*, and *c*) the rough endoplasmic reticulum was distributed throughout the perinuclear cytoplasm. There was also a large number of polyribosomes, not associated with membranes, which were seen particularly well in very thin sections (Fig. 5 *c*). When viewed from above the concentration of ER appeared highest in the immediate perinuclear region because the cell was thickest there. In the peripheral regions, PtK_2 cells are exceptionally thin and there is little or no ER. These ultrastructural features accounted fully for the intensity and patterns of staining with antisynthetase (Fig. 3 *a*) and anti-ER (Fig. 3 *b*).

Extraction of PtK_2 cells with Triton X-100 in microtubule-stabilizing buffer (28) removed most cellular membranes and amorphous components of the cytoplasmic matrix, but remarkable aggregates of ribosomes with some membrane fragments were left with microtubules and the nuclear remnant (Fig. 5 *d*, *e*, and *f*). In many, but not all, cases the ribosomes appeared to be trapped in these membrane fragments. These large clumps of membranes and ribosomes explained why the two antibodies and acridine orange gave coarse reticular staining patterns (Fig. 3 *c* and *d*, and 4 *a*, *b*, and *c*) after extraction with this buffer.

Extraction of PtK_2 cells with Triton X-100 in CSK buffer (12) removed almost all cytoplasmic membranes recognizable by electron microscopy of thin sections (Fig. 5 *g*, *h*, and *i*), but left behind large numbers of ribosomes in relatively small clusters as shown previously (12). There were occasional structures that might be fragments of rough ER (Fig. 5 *h*), but they were not common. Consequently, the ER antigens and synthetase, which were spread diffusely in small spots throughout the cytoplasm (Fig. 4 *d* and *e*), were most likely associated with the ribosome clusters (Fig. 4 *f*), not with trilaminar membrane structures.

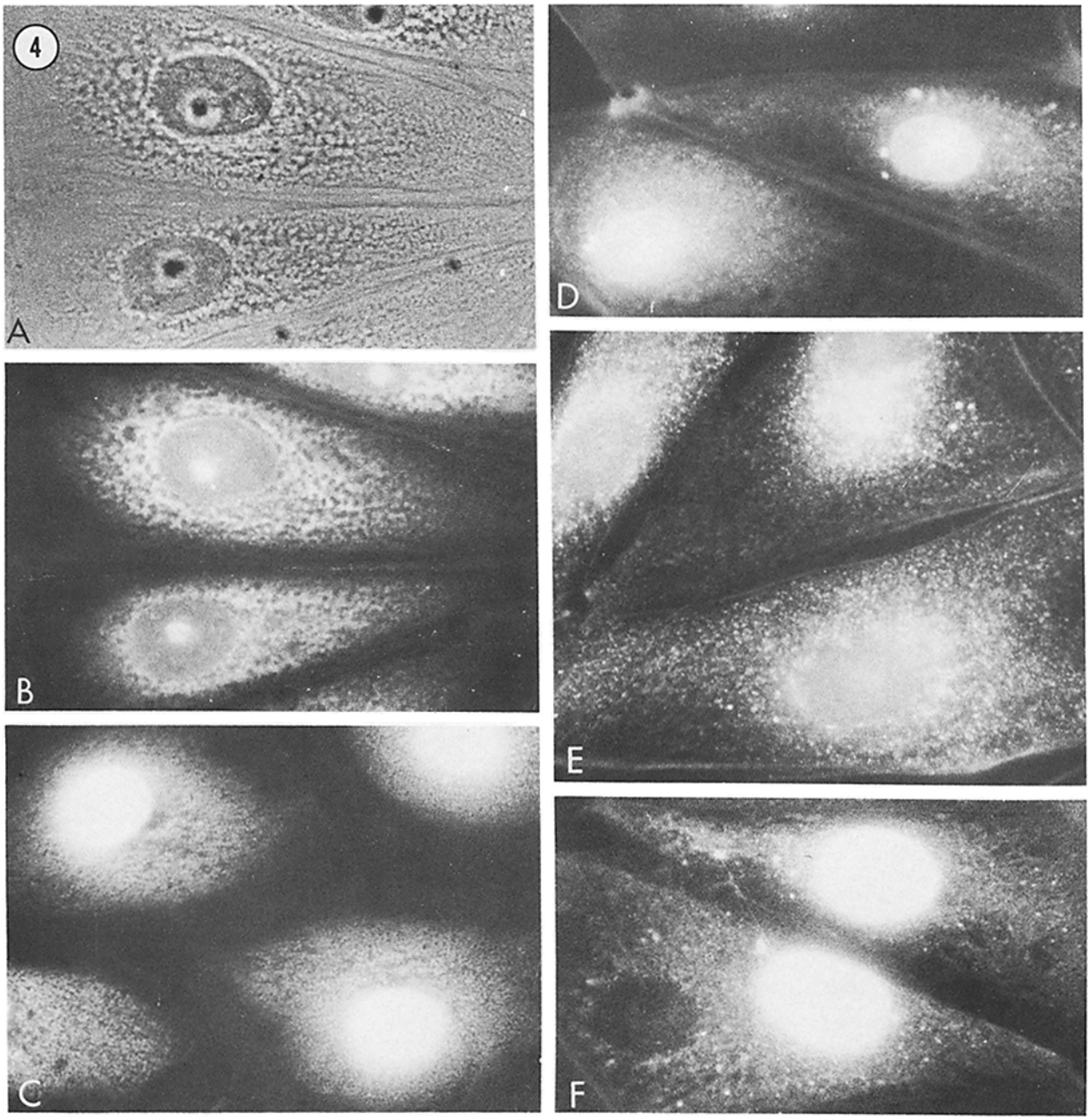


FIGURE 4 Antibody and acridine orange staining of detergent-extracted PtK_2 cells. (*A*) Phase contrast, (*B* and *D*) direct stain with fluorescein-labeled antisynthetase, (*E*) indirect stain with anti-ER, and (*C* and *F*) acridine orange stain. (*A*, *B*, and *C*) Extracted with 0.5% Triton X-100 in microtubule-stabilizing buffer and (*D*, *E*, and *F*) extracted 0.5% Triton X-100 in CSK buffer. The reticular phase-dense cytoplasmic network (*A*) corresponds to the antisynthetase staining (*B*).

Distribution of Methionyl-tRNA Synthetase in Other Cells

Antisynthetase stained Triton-extracted rat liver epithelial cells (Fig. 6*a*), 3T3 cells (Fig. 6*b*), rat glial C6 cells (Fig. 6*c*), and bovine endothelial cells (Fig. 6*d*) with the same coarse reticular pattern seen with PtK_2 cells. HeLa cells stained more diffusely and bovine smooth muscle cells had many discrete peripheral spots stained. Nucleoli were stained with varying intensities in all cell types examined.

Test for Association of Methionyl-tRNA Synthetase with Cytoplasmic Fibers

In an effort to learn why a majority of the methionyl-tRNA synthetase was associated with the detergent-insoluble fraction of PtK_2 cells, which also contained microtubules (28), intermediate filaments (44), and actin filaments (44), we compared the localization of the synthetase with these fibers using double fluorochrome staining. There was no labeling of microtubules by antisynthetase (Fig. 7*a* and *b*), although both antigens were concentrated in the perinuclear area. In cells fixed with acetone, there was no detectable stress fiber staining by antisynthetase (Fig. 7*c* and *d*). The peripheral fluorescence seen in double-labeling experiments was not observed in cells labeled with antisynthetase alone. Likewise, there was no correlation of the antisynthetase distribution with keratin fibers (Fig. 7*e* and *f*).

Controls for Fluorescence Staining

Controls for antisynthetase staining consisted of (*a*) adsorption of fluorescein-conjugated antisynthetase Ig with rat liver

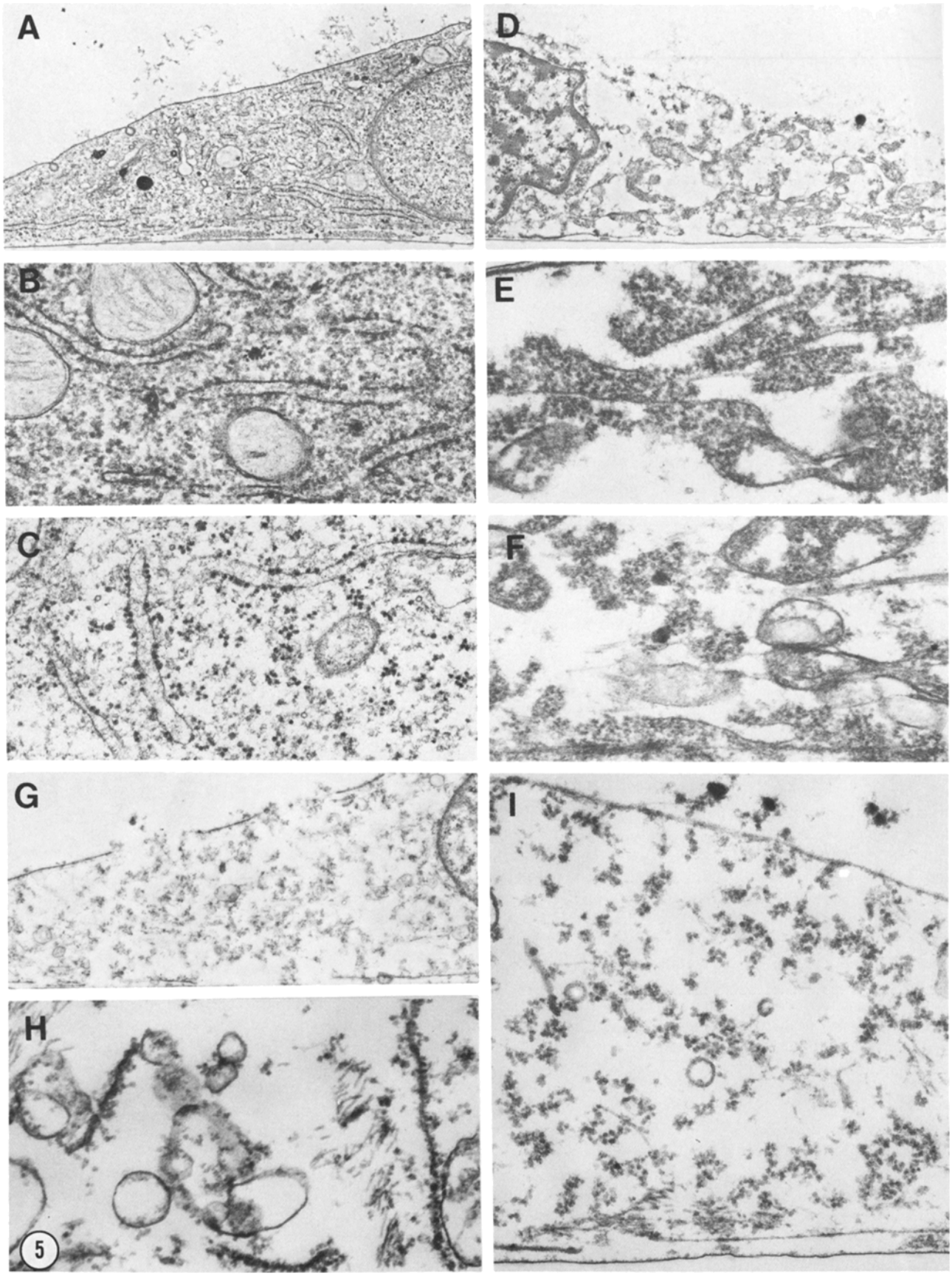


FIGURE 5 Electron microscopy of thin sections of PtK_2 cells. Control (*A, B,* and *C*) extracted with Triton X-100 in microtubule-stabilizing buffer (*D, E, F*), and extracted with Triton X-100 in CSK buffer (*G, H,* and *I*). The cells were fixed with 100 mM glutaraldehyde, 2 mg/ml tannic acid, 0.013 mg/ml saponin in 100 mM sodium phosphate, 50 mM KCl, 5 mM $MgCl_2$, pH 7.0, followed by 4 mM OsO_4 in the same buffer, pH 6.0, dehydration, and embedding. Magnifications: *A, D,* and *G*: × 11,500; *B, C, E, F, H,* and *I*: × 46,000.

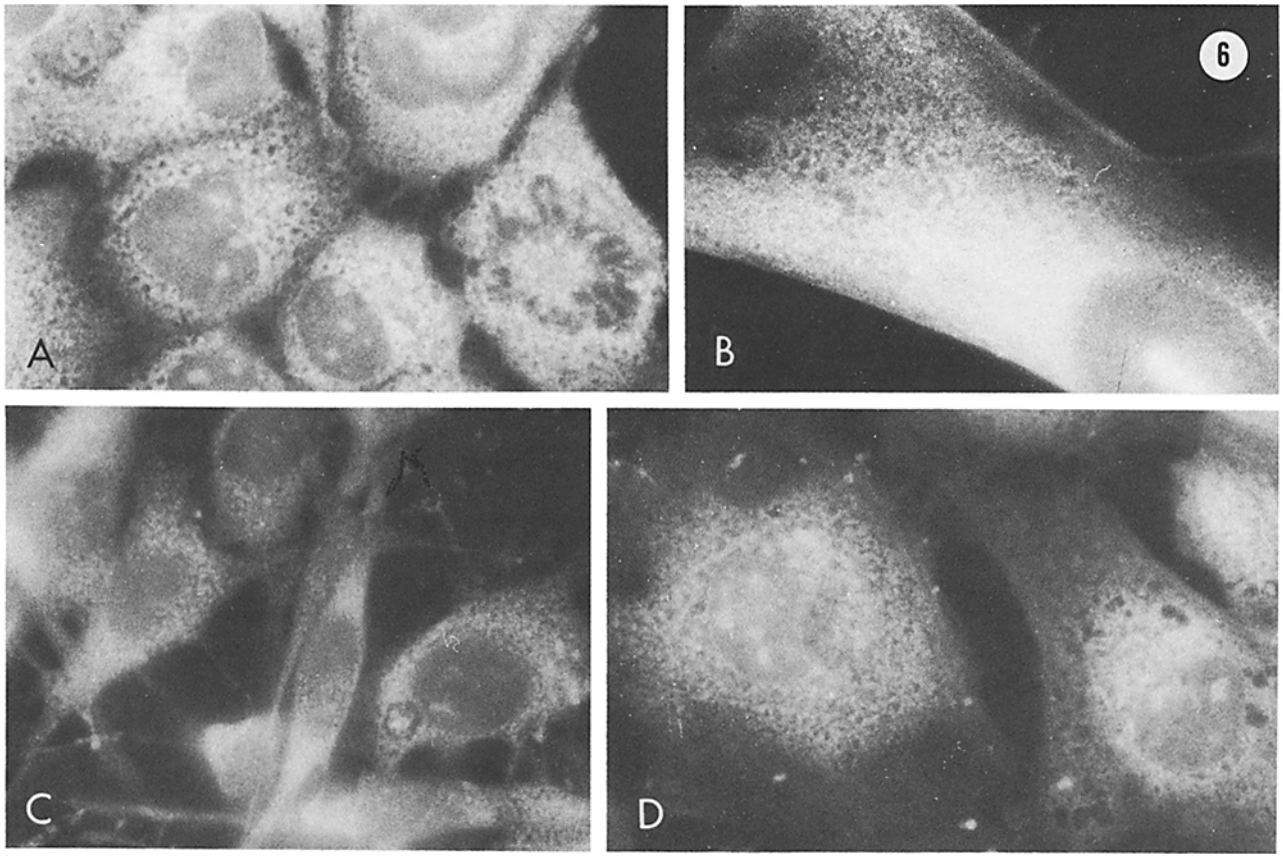


FIGURE 6 Staining of various cell types with fluorescein-conjugated antisynthetase. Cells were extracted with 0.5% Triton X-100 in microtubule-stabilizing buffer. (*A*) Buffalo rat liver epithelial cells, (*B*) mouse 3T3 fibroblasts, (*C*) rat glial C6 cells, and (*D*) bovine aortic endothelial cells. There is a reticular staining pattern in all of the cell types. Nucleolar staining varies from very intense staining in 3T3 cells to less intense staining in rat liver and rat glial cells.

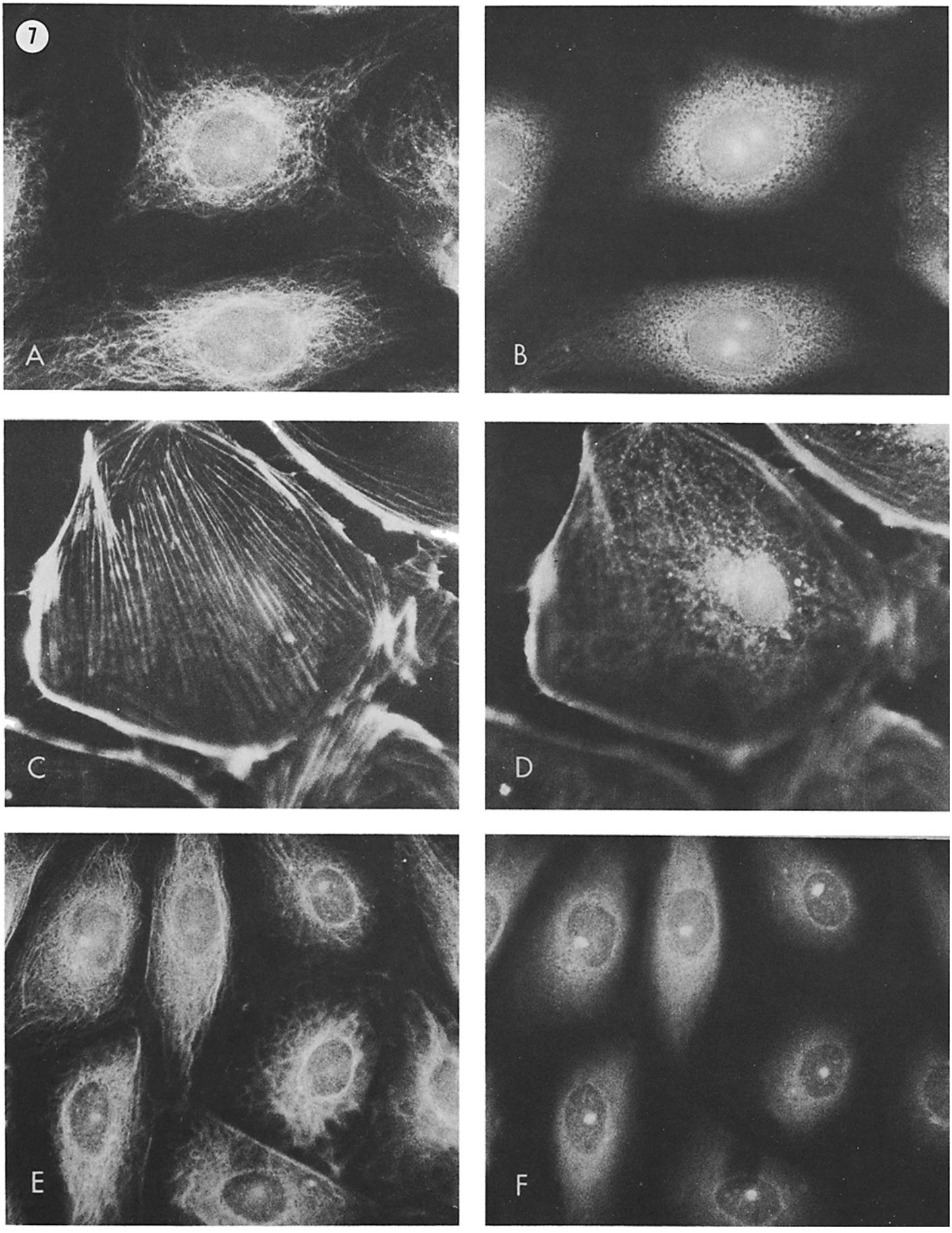
18S synthetase complex-Sepharose 4B affinity gel, (*b*) indirect staining with preimmune IgG, (*c*) indirect staining with flow-through IgG from synthetase complex affinity gel, (*d*) direct staining with the second antibody, rhodamine- or fluorescein-conjugated goat anti-rabbit IgG. There is an absence of direct staining by labeled immune-Ig after adsorption. Indirect staining with preimmune IgG at the same concentration as immune IgG showed no significant staining. The affinity-purified IgG showed indirect staining of the cytoplasm and no nucleolar staining, indicating that the IgG responsible for nucleolar staining was lost during affinity chromatography. The immune Ig, but neither the adsorbed immune Ig nor the purified antibody, stained the nucleoli. Rhodamine- and fluorescein-conjugated goat anti-rabbit IgG showed virtually no staining at the concentration used.

DISCUSSION

In this communication we demonstrate the association of a majority of Met-tRNA synthetase with the endoplasmic reticulum by single and double fluorochrome staining of PtK_2 cells with antisynthetase and anti-ER antibodies. The association of aminoacyl-tRNA synthetases with microsomes has been reported for rat liver (41), chicken embryo (27), and chinese hamster ovary cells (15). The mode of association of the synthetases with membranes is not known. The associations of aminoacyl-tRNA synthetases with ribosomes (30, 41, 42), high molecular weight RNA (1, 30) and lipids (2, 32) suggest both indirect and direct modes of membrane association. Reconstitution studies with purified components are necessary for a better understanding of the synthetase-membrane association.

The functional significance of ER-associated aminoacyl-tRNA synthetase is unclear presently, but the proximity of aminoacyl-tRNA synthetase to ribosomes may provide a high local concentration of aminoacyl-tRNAs for maximal efficiency of protein biosynthesis. The association of synthetase with the nucleolus is strongly suggested by the specificity of the fluorescent antibody staining and by the absence of nucleolar staining in control experiments. It cannot be totally ruled out that there may be a small fraction of non-antisynthetase antibody that specifically reacts with the nucleolus. The occurrence of nucleolar aminoacyl-tRNA synthetase (22) and

FIGURE 7 Comparison of cytoplasmic filament networks and antisynthetase staining. (*A*) Rhodamine-antitubulin IgG staining of PtK_2 cells extracted with Triton X-100 in microtubule-stabilizing buffer. (*C*) NBD-phallacidin staining of PtK_2 cells fixed with acetone. (*E*) Indirect staining with antikeratin IgG of cells extracted as in (*A*). (*B, D,* and *F*) Micrographs of the same cells stained with fluorescein-labeled antisynthetase.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elongation factor activity (29) have been reported; however, the functional significance of nucleolar protein biosynthetic factors is not understood presently.

We have provided evidence that a substantial fraction of both the Met-tRNA synthetase and the RER antigen(s) are insoluble in detergent, as is the cellular fiber system (keratin, actin/stress fibers, and microtubules), but the Met-tRNA synthetase was not associated morphologically with any of these fiber systems. Similarly, cytochrome b_5, an endoplasmic reticulum protein, has been shown by Franke et al. (11) to remain in Triton-extracted PtK_2 and 3T3 cells in a reticular cytoplasmic pattern. Penman and co-workers (6) have previously shown that 100% of polysomes remain associated with the detergent-resistant fraction of HeLa cells, while 75% of cellular proteins were extracted. They noted that membrane-bound ribosomes synthesizing viral glycoproteins were detergent-insoluble and perhaps remain bound to unextracted endoplasmic reticulum residue (6). The detergent-insoluble ribophorin framework of the endoplasmic reticulum described by Kreibich (21) may play a key role in maintaining the organization of the protein synthetic apparatus of the rough ER including the aminoacyl-tRNA synthetases. In addition to these detergent-insoluble ER components, both a lamina of the plasma membrane (4) and the nuclear lamina-pore complex are also insoluble in Triton (13). Furthermore, the cholera toxin receptor ganglioside GM_1, and ~20% of total cellular phospholipids and ganglioside remain associated with Triton-extracted 3T3 cells (36). Galactosphingolipid also appears to remain associated with microtubulelike fibers in monkey kidney epithelial cells after Triton extraction according to the method of Osborn and Weber (31).

Thus, the detergent-insoluble fraction of PtK_2 cells contains the three major cell fiber types, a nuclear remnant (13), ribosomes (12), and substantial representation from the membranes of the endoplasmic reticulum (21). After extraction these ER membrane components may or may not appear to form ultrastructurally recognizable membranes. There is no reason, *a priori*, that these various components are specifically bound to each other simply because they have similar solubilities. The widespread use of the term "cytoskeleton" to designate the detergent-insoluble residue of cells is, without question, an over simplification and is, in fact, a misnomer.

It has been suggested that polyribosomes are associated with "cytoskeleton" preparations (4–6, 12, 23, 24, 43) or specifically with actin stress fibers (38), intermediate filaments (40), or the microtrabeculae (45). These varied putative associations might be construed to indicate that they are nonspecific. One explanation has been that mRNAs are bound to the "cytoskeleton" (5, 6, 12, 24, 43) and that ribosomes are indirectly associated with the cytoskeleton by association with mRNA (6, 12). While this is possible, our observation of ER components in these detergent-extracted cell residues adds the possibility that the ribosomes could be bound to these membrane molecules, not to one of the cellular fibers. Furthermore, the available evidence cannot rule out trapping or other nonspecific associations in these heterogeneous fractions.

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