Isolation of Chinese Hamster Ovary Ribosomal Mutants Differentially Resistant to Ricin, Abrin, and Modeccin*

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The molecular action of ricin A chain involves cleavage of the N-glycosidic bond between ribose and the adenine 4324 nucleotides from the 5' end of mammalian 28 S rRNA (Endo, Y., and Tsurugi, K. (1987) J. Biol. Chem. 262, 8128–8130). In this paper, four ricin- and abrin-resistant Chinese hamster ovary cell mutants that possess ribosomes resistant to this N-glycosidase action are described. Three of the mutant phenotypes, Lec26, Lec27, and Lec28, were recessive in somatic cell hybrids and define at least two new lectin-resistant complementation groups. The most extensively characterized mutant type, LEC17, was dominant in such hybrids. None of the mutants were cross-resistant to modeccin. Post-mitochondrial supernatants from each of the four mutants were resistant to inhibition of cell-free protein synthesis by ricin, ricin A chain, and abrin. In addition, polysomes isolated from mutant cells were resistant to cleavage of the adenine-ribose N-glycosidic bond by ricin A chain or abrin, as assayed by the release of an ~470-nucleotide fragment following aniline treatment of ribosomal RNA extracted from toxin-treated polysomes. The unique lectin-resistance properties of the different mutants suggests that the accessibility of adenine 4324 to each toxin differs. It seems likely that the recessive Chinese hamster ovary ribosomal mutants reflect structural changes in different ribosomal proteins while the dominant phenotype may be due to the modification of protein(s) or rRNA involved in toxin-ribosome interaction. Further analysis of these cell lines should provide new insights into the structure/function relationships of eukaryotic ribosomes.

Ricin and its structural and functional analogues abrin and modeccin are potent cytotoxins that consist of two, distinct, disulfide-linked peptides (A and B), each of ~30 kDa. The B chain binds to Gal or GalNAc residues present on glycoconjugates at the surface of eukaryotic cells (1) and facilitates the internalization and transport of the heterodimer (3). Within the cytoplasm, the A chain catalytically inactivates the 60 S ribosomal subunit by cleaving the N-glycosidic bond between the ribose and adenine 4324 nucleotides from the 5' end of 28 S rRNA (4). The molecular events that follow the depurination of the rRNA and functionally inactivate the ribosome for protein synthesis, as well as the structural details of toxin-ribosome interactions, have yet to be elucidated. However, the importance of higher order structure of the ribosomal domain recognized by ricin A chain is apparent from the finding that intact ribosomes are nearly 10,000-fold more sensitive to depurination of 28 S rRNA than naked rRNA (5, 6). It is of considerable interest, therefore, to identify the ribosomal proteins in the ricin-binding domain and to characterize the role of these proteins in toxin sensitivity.

In this paper, we describe four new CHO cell mutants that should prove valuable in elucidating the structure-function relationships of ribosomal proteins located near the adenine cleaved by the N-glycosidase activity of ricin, abrin, and modeccin. The mutants were isolated in four independent selections for toxin resistance. Three behave recessively in somatic cell hybrids with parental cells whereas the fourth acts dominantly in hybrids. Protein synthesis in post-mitochondrial (S10) supernatants of all four mutant lines was inhibited to a lesser extent than in S10 extracts from parent cells when incubated with ricin, ricin A chain, or abrin. Similarly, intact polysomes from mutant lines were less sensitive than those from parent cells to depurination of 28 S rRNA at a site located ~470 nucleotides from the 3' end of the RNA when treated, in vitro, with ricin or abrin. Because of their unique patterns of cross-resistance to ricin and abrin, but not to other carbohydrate-binding proteins, it seems likely that these mutants have identified four distinct gene products affecting the accessibility of adenine 4324 in 28 S rRNA to the toxins. In addition, these mutants provide evidence that the three highly homologous A chains of ricin, abrin, and modeccin interact with different microenvironments of the 60 S ribosomal subunit to exert their N-glycosidase activity.

EXPERIMENTAL PROCEDURES

Materials—Sodium [251]iodide (100 mCi/ml), L-[4,5-3H]leucine (120–190 Ci/mmol, 1 mCi/ml), human placental ribonuclease inhibitor were purchased from Amer sham Corp. BSA (fraction V), the lectins from Pttilota plumosa, Robinia pseudoacacia, Viscum album, and Triticum vulgaris (WGA), were obtained from Sigma; ricin, ricin A chain, the lectins from Erythrina cristagalli, LCA, L-PHA, E-PHA, Ricinus communis agglutinin-agarose and ricin-agarose were from Vector (Burlingame, CA); EMS was obtained from Eastman Kodak (Rochester, NY); Pronase and RNA molecular weight markers (III) were from Boehringer Mannheim; ConA-Sepharose and Staphylococcus aureus micrococcal nuclease were from Pharmacia LKB Biotechnologies Inc.; rabbit β-globin mRNA was from Bethesda Research Laboratories; abrin from EY Laboratories (San Mateo, CA); aniline was from Aldrich and merrin was from Behring Diagnostics. Alpha

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1 The abbreviations used are: CHO, Chinese hamster ovary; BSA, bovine serum albumin; FCS, fetal calf serum; L-PHA, leukophytohemagglutinin; E-PHA, erythrophytohemagglutinin; WGA, wheat germ agglutinin; LCA, Lens culinaris agglutinin; ConA, concanavalin A; EMS, ethylmethane sulfonate; Hepes, 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid; PBS, phosphate-buffered saline; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid.

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(a) medium was purchased from Gibco; horse serum was obtained from Hazleton Biologics (Lenexa, KS), and FCS was from FLOW Laboratories (McLean, VA). Pseudomonas toxin and modeccin were generously donated by Dr. April Robbins (National Institutes of Health, Bethesda, MD); pactamycin was a gift from Upjohn.

**Cell Lines**—The derivations of parent CHO cell lines Pro 5, Gat 2, and the glycosylation mutant Pro LEC10.3C (LEC10) were previously described; cells were grown on or on 100-mm tissue culture dishes in an atmosphere of 5% CO2 in air at 37°C in a medium containing 10% (v/v) FCS (al0%FCS) or 10% (v/v) horse serum with 1% (v/v) FCS. No mycoplasma contamination was detected by Hoechst staining of indicator 3T6 cells grown in the presence of test cells for 3 ± days (9).

The selection schemes to obtain Lec26, Lec27, and Lec28 were described (11, 12). The extracts were assayed for their ability to translate exogenously added, rabbit globin mRNA and 14.4 ng/ml [125I]-ricin. Following a 15 min incubation at 4°C, cells were pelleted, supernatants were prepared from exponentially growing CHO cells as described (11, 12). The extracts were assayed for their ability to incorporate [3H]leucine into trichloroacetic acid-precipitable cpm in the absence or presence of ricin, ricin A chain, abrin, or modeccin. The in vitro translation mixtures consisted of 100 nCi ml−1 of [3H]leucine, 25 mm K HEPES, pH 7.6, 110 mm KCl, 1 mm magnesium acetate, 1 mm ATP, 0.25 mm GTP, 0.4 mm spermidine, 4% (v/v) glycerol, 5 mm creatine phosphate, 180 µg/ml creatine kinase, 8 µm β-mercaptoethanol, and 5 µCi of [3H]leucine and water or toxin in a final reaction volume of 100 µl. After incubation for various times at 30°C, 10 µl aliquots were removed in duplicate at different times, mixed with 30 µl of 5% (w/v) trichloroacetic acid, and 1.0 ml 20% (w/v) trichloroacetic acid. The trichloroacetic acid precipitates were collected on pre-soaked GF-C glass fiber filters, dried, and counted in a toluene-based liquid scintillator.

**RNA Isolation**—Total RNA was isolated from 1.3 ± 10° exponen-
trially growing CHO cells by the guanidinium method exactly as described (14) and stored at 50 °C as an aqueous solution.

Preparation of Polysomes—Polysomes were prepared at 4 °C from mutant and parent CHO cells by a modification of a previously described procedure (5). Exponentially growing cells (3 × 10^6) were harvested by centrifugation at 370 × g for 10 min and washed with 0.15 M NaCl. Following centrifugation at 1600 × g for 10 min, the cell pellet from 10^6 cells was resuspended by vortexing in 75 μl of 2.5% (v/v) Nonidet P-40 in 0.2 M sucrose in buffer A (20 mM Tris-Cl, pH 7.6, 5 mM magnesium acetate, 100 mM NH₄Cl, 1.0 mM dithiothreitol). The mixture was centrifuged at 2300 × g for 10 min, and the upper 2/3 of the supernatant (PMS) collected. Sodium deoxycholate (13% w/v) at 1/9 the volume of the PMS was added dropwise to this supernatant which was incubated for an additional 30 min in an ice bath with stirring. The deoxycholate-treated PMS was layered on a discontinuous sucrose gradient of 7 ml of 0.7 M sucrose in buffer A over 7 ml of 2.0 M sucrose in buffer A in Beckman SW-28 polyallomer tubes and centrifuged at 160,000 × g for 24 h. The polysome pellet was washed with an equal volume of distilled, deionized H₂O, resuspended in 1.0 ml of 0.2 M sucrose in buffer A, and stored in 100–150 μl aliquots at -70 °C. Sedimentation profiles of this preparation on continuous gradients of 15–33% (w/w) sucrose in 0.15 M NaCl, 10 mM Tris-Cl, pH 7.4, 1.5 mM MgCl₂ centrifuged at 25,000 rpm in a Beckman SW-28 rotor for 90 min, confirmed that most of the preparation consisted of polysomes.

Removal of Ethanol-soluble Ribosomal Proteins from Polysomes—Acidic ribosomal proteins were extracted from polysomes by a modification of a previously described procedure (16). All steps in the extraction protocol were carried out at 4 °C. Aliquots of 150 μl thawed polysomes were mixed with 64.3 μl of 0.7 M sucrose in buffer A containing 5.8 μl of 7.0 M KCl, 1.5 μl of 0.5 M β-mercaptoethanol, and 221.4 μl of absolute ethanol and stirred in an ice water bath for 30 min. The mixture was centrifuged at 15,000 rpm for 10 min in an SS-34 rotor, and the pellet resuspended in 150 μl of 0.2 M sucrose in buffer A. Buffer A, containing 0.7 M sucrose, 2.0 M KCl, 0.5 M β-mercaptoethanol and absolute ethanol (in the volumes given above), was added to the resuspended pellet and the mixture incubated with stirring in an ice bath for an additional 30 min. The extracted polysomes were centrifuged as above and the pellet resuspended in 110 μl of 0.2 M sucrose in buffer A for immediate treatment with ricin as described above.

Aniline Treatment and Gel Electrophoresis of Ribosomal RNA—RNA extracted from polysomes or cytoplasm (~0.6 μg) was incubated on ice for 10 min with 15.2 μl of 1.0 M aniline buffer, pH 5.1 (prepared freshly by the addition of 0.5 ml glacial acetic acid, 7.0 ml of distilled deionized water, 1.0 ml of purified aniline stored under nitrogen, and 2.5 ml of 1.0 M acetic acid, in that order). The reaction was terminated with the addition of 200 μl of distilled deionized water, and the aniline was removed by evaporation of the sample to dryness in vacuo.

Aniline-treated RNA was resuspended in 15 μl of gel loading buffer (100 μg/ml sucrose, 7 μl urea, 0.4 μg/ml bromophenol blue, 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3). Incubated at 90 °C for 30 s, immediately chilled on ice, and electrophoresed for 3 h at 30 mA constant current in gels of 5% (4.85% w/v) acrylamide monomer, 0.125 M Tris (pH 8.5) bisacrylamide) acrylamide (in 7 μl water) in 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3. Negatives of photographs of gels stained for 30 min with 1 μg/ml ethidium bromide were scanned with an LKB 2222-010 Ultra-Scan XL densitometer to approximate the relative amount of ~470 nucleotide fragment obtained compared with an internal standard (5 S rRNA).

RESULTS
From a selection designed to obtain highly ricin-resistant CHO cells, a colony with a novel lectin-resistance phenotype was identified. Clones of the isolate were ~35-fold more ricin-resistant and ~4-fold more abrin-resistant than CHO parent cells, but were not altered in their resistance to other carbohydrate-binding proteins such as L-PHA, ConA, WGA, and LCA (Table I). Somatic cell hybrids between a clone of the new isolate (termed LEC17) and parent CHO cells were as lectin-resistant as the mutant (Table II), thereby identifying LEC17 as a mutant with a novel, dominant Lec' phenotype (8, 17). Because L.ELC17 was cross-resistant to ricin and abrin, two lectins that recognize and bind Gal/GalNAc-terminating carbohydrates (1), and because of the known properties of CHO glycosylation mutants (17), it seemed likely that LEC17 cells might express altered carbohydrates on cell-surface glycoconjugates. However, both LEC17 and parent cell lines bound essentially the same amount of 125I-ricin at 4 °C over a toxin concentration range of 0.1–100 μg/ml (data not shown). By contrast, LEC10, a ricin-resistant glycosylation mutant with altered cell-surface carbohydrates (10) bound significantly less ricin.

TABLE I

<table>
<thead>
<tr>
<th>Lectin</th>
<th>CHO Cells</th>
<th>LEC17</th>
<th>Lec26</th>
<th>Lec27</th>
<th>Lec28</th>
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<td>0</td>
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TABLE II

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</table>

Hybrids were obtained by fusing Pro' and Gat' cell lines and selecting in deficient medium as described (19). Hybrid colonies were picked, cultured, and tested for lectin resistance against ricin (Ric) and/or abrin (Abr). R = fold-resistance compared with parent CHO cells; (R) = <2-fold. Hybrids are usually 2-3-fold more resistant to Ric compared with diploid parent CHO cells (8). The partial lectin resistance observed with cultured hybrids was not apparent in crosses involving Lec26, Lec27, or Lec28, when the mass population of hybrids was tested by plating in the presence of lectin during selection in hybrid medium (data not shown). Thus complementation (denoted +) was concluded if the mass population of hybrids had a D₉₀ approximately equal to that of parental CHO cells and the sensitivity of isolated hybrids was markedly greater than at least one of the lines in the cross. Although the partial lectin resistance of cultured hybrids may indicate a codominant effect, it is more likely due to the growth advantage of diploid Pro' revertants that arose at a frequency of 10^-4 to 10^-5. Many separate crosses were performed between other recessive CHO ricin-resistant mutants and Lec26 (4), Lec27 (4), or Lec28 (6) mutants and all these hybrids exhibited complementation (data not shown). ND = not determined.
ricin-resistant Ribosomal Mutants of CHO Cells

Ricin-resistant Ribosomal Mutants of CHO Cells

The possibility that the LEC17 mutant might be altered in the intracellular target of ricin, the 60 S ribosomal subunit, was explored in a cell-free in vitro translation system. Post-mitochondrial (S10) supernatants were prepared from LEC17 and parent CHO cells and assayed for their ability to incorporate [3H]leucine into trichloroacetic acid-precipitable cpm at 30 °C in the presence or absence of various concentrations of ricin, ricin A chain, abrin, or modeccin. As shown in Fig. 1, protein synthesis proceeded essentially linearly as a function of time for up to 20 min after transfer of translation mixtures to 30 °C. However, protein synthesis in parent and LEC17 cell extracts was inhibited to different extents in the presence of ricin, ricin A chain, or abrin. As expected from their lack of cross-resistance to modeccin (Table I), LEC17 and parent extracts were equally sensitive to this toxin in vitro. Plots of log toxin concentrations versus % trichloroacetic acid-precipitable cpm obtained after 40 min of incubation at 30 °C were linear, and therefore were used to compare the concentration of toxin necessary to reduce incorporation of trichloroacetic acid-precipitable cpm by 50% (I50) in LEC17 and parent cell extracts (Table IV). It is apparent that LEC17 extracts were significantly more resistant to the inhibitory action of ricin, abrin, and ricin A chain on protein synthesis compared with parental CHO S10 extracts (22-, 9-, and 56-fold, respectively) yet were as sensitive as the latter to inhibition by modeccin.

As ricin has been shown to inhibit the elongation step of

<table>
<thead>
<tr>
<th>Toxin</th>
<th>D10</th>
<th>D10/</th>
<th>D10/</th>
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<tbody>
<tr>
<td></td>
<td>Monensin</td>
<td>Monensin</td>
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<tr>
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<tr>
<td>LEC17</td>
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<td>10</td>
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</table>

Fig. 1. Post-mitochondrial (S10) supernatants from parent (○) or LEC17 (□) cells were incubated at 30 °C in the absence (open symbols) or presence (closed symbols), respectively, of 3 µg/ml ricin (Ric), 50 ng/ml ricin A chain (Ric A), 100 ng/ml abrin (Abr), or 300 ng/ml modeccin (Mod) as described under "Experimental Procedures." The percent trichloroacetic acid-precipitable cpm in duplicate 10-µl aliquots of reaction mixtures taken at the indicated incubation times were plotted relative to the cpm obtained after 40 min of incubation in mixtures lacking toxins. The trichloroacetic acid-precipitable cpm in 10-µl aliquots of the latter samples ranged from 10,900 to 22,700. Each panel shows results obtained from one experiment.
It was precluded with parent CHO cells. Cell-free in vitro translation of exogenous rabbit globin mRNA in micrococcal nuclease-treated parent SlO extracts, translation of endogenous proteins (4, 25-30), are enzymes that specifically hydrolyze the adenine-associated ribose susceptible to cleavage (via a β-elimination reaction) following treatment with aniline at acidic pH. Aniline treatment of depurinated 28 S rRNA of rat liver and rabbit reticulocytes. Removal of the adenine from the sugar-phosphate backbone of the nucleic acid renders the phosphodiester bonds located on either side of the adenine-associated ribose susceptible to cleavage (via a β-elimination reaction) following treatment with aniline at acidic pH. Aniline treatment of depurinated 28 S rRNA results in the release of a fragment consisting of the 3' most 470 nucleotides of this RNA (5). To determine whether CHO ribosomes were similarly acted on by ricin, total cellular RNA was isolated from exponentially growing parent and LEC17 cells that had been cultured for 3 h in the absence or presence of 100 ng/ml or 300 ng/ml ricin or 25 ng/ml abrin. The generation of an ~470 nucleotide fragment was observed for each cell population (Fig. 3). A significantly greater amount of fragment was released from parent RNAs compared with LEC17 RNAs, consistent with the different sensitivity of LEC17 cells to the two toxins (Table I).

The elucidation of the molecular site of action of ricin A chain by Endo et al. (4, 5) permitted a more detailed investigation of the nature of the mutations in LEC17, Lec26, Lec27, and Lec28 cells. The A chains of ricin, abrin, and modecacin, as well as a number of ribosome-inactivating proteins including gelonin, saporin, and the family of pokeweed antiviral proteins (4, 25-30), are enzymes that specifically hydrolyze the N-glycosidic bond of adenine located in an evolutionarily conserved region 4324 nucleotides from the 5' end of 28 S rRNA of rat liver and rabbit reticulocytes. Removal of the adenine from the sugar-phosphate backbone of the nucleic acid renders the phosphodiester bonds located on either side of the adenine-associated ribose susceptible to cleavage (via a β-elimination reaction) following treatment with aniline at acidic pH. Aniline treatment of depurinated 28 S rRNA results in the release of a fragment consisting of the 3' most 470 nucleotides of this RNA (5). To determine whether CHO ribosomes were similarly acted on by ricin, total cellular RNA was isolated from exponentially growing parent and LEC17 cells that had been cultured for 3 h in the absence or presence of 100 ng/ml or 300 ng/ml ricin or 25 ng/ml abrin. The generation of an ~470 nucleotide fragment was observed for each cell population (Fig. 3). A significantly greater amount of fragment was released from parent RNAs compared with LEC17 RNAs, consistent with the different sensitivity of LEC17 cells to the two toxins (Table I).

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To determine whether each of the mutants was resistant to the action of ricin and/or abrin on 28 S rRNA in vitro, polysomes were isolated from mutant and parent CHO cells and incubated in the absence or presence of different concentrations of ricin or abrin at 37 °C for 15 min. Ribosomal RNAs extracted from the treated polysomes were incubated with aniline and electrophoresed on acrylamide/urea gels (Fig. 4). At a given ricin or abrin concentration, the amount of fragment obtained in each case was greater for rRNAs derived from parent compared with mutant polysomes. The differences in fragment levels were considerably smaller than expected from lectin-resistant patterns (Table II) and log_{10} values (Table IV). This may be due to non-linear relationships in either ethidium bromide staining or negative exposures or to
Ricin-resistant Ribosomal Mutants of CHO Cells

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Parent LECl7

Ric Abr Ric Abr

I oln 1 oln

gem

FIG. 3. Cleavage of 28 S rRNA in intact cells. Exponentially growing parent and LECl7 cells were incubated for 3 h at 37 °C in the absence or presence of 100 ng/ml or 300 ng/ml ricin (Ric) or 25 ng/ml abrin (Abr). Aliquots (6.7 μg) of total cellular RNAs prepared from these cells were treated with aniline at acidic pH and electrophoresed as described under "Experimental Procedures." The arrow points to the ~470-nucleotide fragment that was released following aniline treatment of rRNAs derived from toxin-treated cells.

FIG. 4. Cleavage of 28 S rRNA in polysomes. Polysomes prepared from parent and mutant cells were incubated at 37 °C for 15 min in the absence and presence of ricin or abrin. RNA was extracted, treated with aniline, and electrophoresed on acrylamide/urea gels. In panels a–c, polysomes were incubated without (A and F) or with 40 ng/ml (B and G), 160 ng/ml (C and H), 300 ng/ml (D and I), or 1000 ng/ml (E and J) ricin. In panel d, polysomes were incubated without (A and F) or with 0.1 μg/ml (B and G), 0.2 μg/ml (C and H), 1.0 μg/ml (D and I), or 2.0 μg/ml (E and J) abrin. Lanes labeled S in each panel correspond to the parent RNA sample from Fig. 3 (300 ng/ml Ric), included as a control for the efficacy of the aniline treatment. RNA molecular weight markers of 0.3–0.6 kb are shown in panel a (Std.).

FIG. 5. Cleavage of 28 S rRNA in ethanol-extracted polysomes. Polysomes prepared from parent and LECl7 CHO cells were extracted with ethanol as described under "Experimental Procedures" and incubated with ricin for 15 min at 37 °C. Extracted RNA was treated with aniline and electrophoresed on acrylamide/urea gels. Lanes A and F, RNAs extracted from intact parent and LECl7 polysomes, respectively, incubated with 10 μg/ml ricin. Lanes B–E and G–J, RNAs extracted from ethanol-treated parent and LECl7 polysomes, incubated without ricin (B and G) or in the presence of 0.2 μg/ml (C and H), 2.0 μg/ml (D and I), or 10 μg/ml (E and J) ricin. The arrow marks the 470 nucleotide fragment.

the fact that the lowest concentrations of lectin with maximal activity or parental polysomes were not determined. Nevertheless, the data in Fig. 4 show that all four mutants possess polysomes that are resistant to the action of abrin or ricin compared with parental polysomes. Thus, all four mutants appear to have acquired resistance to abrin and ricin by virtue of an alteration in a structural component of the ribosome.

A preliminary attempt to localize the altered structural component in LECl7 to a specific subset of ribosomal proteins was made by comparing the ricin sensitivity of ethanol-extracted polysomes from parent and LECl7 cells. The subset of ribosomal proteins that have acidic pI values are believed to be present in >1 molar equivalent/ribosome (31) as would be required if an altered form is to have a dominant effect. They are also involved in ricin-sensitive steps of protein synthesis such as EF-1-dependent binding of aminoacyl-tRNA (32) and EF-2-dependent GTPase activity (33). In addition, most of the acidic ribosomal proteins appear to be associated with the 60 S subunit and may be situated in close proximity to the ricin-binding site (32). Therefore, the possibility that one of these proteins might be altered in LECl7 was explored.

As demonstrated by McConnell and Kaplan (16), treatment with 50% ethanol results in the selective removal of the acidic proteins from the large subunit of the 80 S ribosome of rat liver. When ethanol-treated polysomes from parent and LECl7 cells were incubated with ricin as in Fig. 4, a reduced amount of fragment was obtained from parent ribosomes and extensive degradation of 18 S rRNA was observed (Fig. 5). In addition, the amount of ~470-nucleotide fragment obtained from ethanol-treated LECl7 polysomes suggests that extraction of acidic ribosomal proteins does not abrogate the LECl7 phenotype. It is apparent from Fig. 5 that ethanol-treated polysomes are significantly more resistant to the action of ricin than intact polysomes (compare lanes E and J with lanes A and F, respectively). This result concurs with previous reports on the resistance of naked rRNAs to ricin A (5, 15) and further supports the idea that ribosomal integrity plays a crucial role in facilitating toxin activity.

DISCUSSION

Four new mutants of CHO cells that are differentially resistant to ricin, abrin, and modeccin have been isolated and partially characterized. Each of the mutants is resistant to
the inhibition of cell-free protein synthesis by ricin A chain and abrin and each possesses polypeptide resistant to the N-acetylglucosidase activity of ricin or abrin in vitro. Thus, all four cell lines, LEC17, Lec26, Lec27, and Lec28, belong to a class of Ric6 CHO mutants that exhibit alterations in the eukaryotic ribosome.

Despite extensive searching for toxin-resistant ribosomal mutants in mammalian cells (1), only one has been previously reported. Ono et al. (34) obtained Ric6 CHO cells that possessed 60 S ribosomal subunits more resistant than parental CHO ribosomes to inhibition of polyphenylalanine synthesis by ricin. With the isolation of the mutants described in this paper, it would seem possible to isolate a series of CHO ribosomal mutants. Although the frequency of the dominant mutant, LEC17, was low in CHO populations (<3.9 × 10⁻⁶),² the three recessive phenotypes Lec26, Lec27, and Lec28 were recovered in routine screening of toxin-resistant CHO mutants that arose at frequencies of ~10⁻⁶ to 10⁻³. It should be possible to obtain more eukaryotic ribosomal mutants from CHO cell populations, especially if different mutagens are used. Toxin-resistant glycosylation mutants that arise frequently from ricin selections can be selected against (or used) to isolate mutants that resist the action of modeccin. The mutants should also be useful in experiments to define the active site of the A chain of ricin, abrin, and modeccin.

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Isolation of Chinese hamster ovary ribosomal mutants differentially resistant to ricin, abrin, and modeccin.
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