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Ribosomal Protein S14 Is Altered by Two-Step Emetine Resistance Mutations in Chinese Hamster Cells†

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Four two-dimensional polyacrylamide gel electrophoresis systems were used to identify 78 Chinese hamster cell ribosomal proteins by the uniform nomenclature based on rat liver ribosomal proteins. The 40S ribosomal subunit protein affected by Chinese hamster ovary (CHO) cell one-step emetine resistance mutations is designated S14 in the standard nomenclature. To seek unambiguous genetic evidence for a cause and effect relationship between CHO cell emetine resistance and mutations in the S14 gene, we mutagenized a one-step CHO cell mutant and isolated second-step mutant clones resistant to 10-fold-higher concentrations of emetine. All of the highly resistant, two-step CHO cell mutants obtained displayed additional alterations in ribosomal protein S14. Hybridization complementation tests revealed that the two-step CHO cell emetine resistance mutants were members of the same complementation group defined by one-step CHO cell mutants, EmtB. Two-step mutants obtained from a Chinese hamster lung cell emetine-resistant clone belong to the EmtA complementation group. The two-step EmtB mutants elaborated 40S ribosomal subunits, which dissociated to 32S and 40S core particles in buffers containing 0.5 M KCl at 4°C. In contrast, 40S ribosomal subunits purified from all EmtA, one-step EmtB EmtC mutants, and wild-type CHO and lung cells were stable at this temperature in buffers containing substantially higher concentrations of salt. Thus, two-step *emtB* mutations affect the structure of S14 protein directly and the stability of the 40S ribosomal subunit indirectly.

We reported that genetic resistance to the alkaloid translation inhibitor emetine correlates with alteration of a single 40S ribosomal subunit protein structural gene in Chinese hamster ovary (CHO) cells (2, 3, 12). The protein altered in emetine-resistant CHO cells was originally (but incorrectly) designated ribosomal protein S20, based on nomenclature and a two-dimensional polyacrylamide gel electrophoresis system developed in this laboratory (2). The correlation between emetine resistance and a 40S ribosomal subunit protein agreed with the observation that emetine resistance is conferred on cell-free translation components by mutant Chinese hamster cell 40S ribosomal subunits (8). Additionally, genes encoding the altered ribosomal protein and emetine resistance cosegregated from several emetine-resistant × wild-type CHO cell hybrid clones (3); the same ribosomal protein is mutationally altered in emetine-resistant CHO cell mutants isolated in two other laboratories (15; J. Wasmuth, S. Chang, J.-J. Madjar, and D. J. Roufa, unpublished data); and emetine-resistant CHO cell clones elaborate mRNAs that encode mutant forms of the ribosomal protein in a wheat germ cell-free translation system (12). Detailed karyotype analyses of wild-type, mutant, and hybrid cell clones indicate that the CHO emetine resistance gene is located on the long arm of Chinese hamster chromosome 2 (5, 23).

Without a stringent reverse selection condition, previously we were unable to show unambiguously that a mutationally altered ribosomal protein is the product of the emetine resistance gene in CHO cells by analyzing revertants. As an alternative, we decided to isolate mutants resistant to a 10-fold-higher concentration of emetine and to determine whether these mutations affected the ribosomal protein further. Also, as we have yet to discern an altered ribosomal structural component in Chinese hamster lung (CHL) cell emetine-resistant mutants, we reasoned that two-step CHL cell mutants might exhibit more obvious, identifiable alterations in the ribosomal component responsible for their genetic drug resistance.

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In this report we describe four two-dimensional electrophoretic systems (10) with which we analyzed the ribosomal proteins purified from wild-type and emetine-resistant CHO and CHL cell mutants. These electrophoretic systems allowed us to identify each Chinese hamster ribosomal protein by the proposed standard nomenclature for mammalian ribosomal proteins (14). The ribosomal protein responsible for emetine resistance in CHO cells now is identified as protein S14 by the standard nomenclature. Somatic cell hybridization-complementation tests indicated that CHL and CHO cell two-step emetine-resistant mutants constitute two genetic classes, correlating with the EmtA and EmtB complementation groups described for one-step mutants by others (20, 21). As first reported by Wejksnora and Warner (22; Eur. J. Biochem., in press), some of our EmtB mutants elaborate 40S ribosomal subunits that are sensitive to buffers containing 0.5 M KCl. In our studies only two-step EmtB 40S ribosomal subunits are sensitive to 0.5 M KCl at 4°C. One-step EmtB and all EmtA mutants yielded 40S ribosomal subunits that were stable in buffers containing as much as 0.75 M KCl at 4°C. 40S ribosomal subunits from two-step EmtB mutants released eight ribosomal proteins, including S14, and became susceptible to proteolysis affecting most of the remaining subunit proteins when treated with 0.5 M KCl in the cold.

MATERIALS AND METHODS

Cell lines and tissue culture. Our methods for growing Chinese hamster cells in monolayer culture have been described previously (2, 3, 7, 9, 12, 16, 17). Large cultures of cells were grown in roller bottles. Cells were harvested by treatment with trypsin during late logarithmic growth. The Chinese hamster cell clones used in these studies are listed in Table 1.

To obtain mutants resistant to high levels of emetine, we treated clones CHO-Em^r-2, CHL-Em^r-11, and CHL-Em^r-34 with 5×10^{-3} M ethyl methane sulfonate as described previously (7). After being mutagenized, cells were harvested by trypsinization and then inoculated at several densities into replicate 100-mm culture dishes in medium containing 2×10^{-7} M emetine hydrochloride. On day 4 and again on day 10 the cultures were fed with medium containing 2×10^{-6} M emetine. By day 14 macroscopic colonies of cells resistant to the higher level of emetine were apparent. These were isolated by trypsinization with cloning rings and recloned once. Mutants resistant to 2×10^{-6} M emetine arose in ethyl methane sulfonate-treated cultures with frequencies ranging from 1 to 10 colonies per 10^6 cells plated. Throughout this report we refer to the two-step mutants resistant to high concentrations of emetine by names based on their parental clones, e.g., Em^r-2-2, Em^r-11-4. Several cloned cell lines were treated with ethyl methane sulfonate to obtain 6-thioguanine resistance (6-TG^r) and ouabain resistance (Oua^r) mutations useful for stringent hybrid selection (Table 1) (3).

Hybridization-complementation tests. The method used to fuse cells pairwise has been described previously (3, 6). Briefly, two parental clones, one of which contained 6-TG^r and Oua^r mutations, were coinoculated into a 60-mm culture dish at a density of 10^6 cells each. One day later, cultures were treated with 50% (wt/vol) polyethylene glycol 6000. On day 3, cells were harvested by trypsinization and replated at several densities (10^4 to 10^6 cells) in replicate culture dishes. These were fed with medium containing HAT components (hypoxanthine, amethopterin, and thymidine), 2 mM ouabain (3), and either 0, 2×10^{-7}, or 2×10^{-6} M emetine hydrochloride. After 14 to 16 days, macroscopic colonies of hybrid cells were picked, stained, and scored (3).

TABLE 1. Chinese hamster cell clones

Clone	Description[a]	Reference or source
CHO-M	Pro$^-$ Gly$^-$ (N = 21)	L. Thompson[b]
CHL-V79, HT-1	N = 21	This laboratory (17)
CHL-V79, 462-10	TK$^-$ (N = 18)	E. H. Y. Chu[c] (17)
Em^r-1, -2	Clones derived from CHO-M on the basis of resistance to 2×10^{-7} M emetine	2
Em^r-11, -16, -25, -29, -30, -34, -35	Clones derived from 462-10 on the basis of resistance to 2×10^{-7} M emetine	2
Em^r-2-2, -2-3, -2-5	Clones resistant to 2×10^{-6} M emetine; derived from Em^r-2	This report
Em^r-11-1, -11-3, -11-4, -34-2	Clones resistant to 2×10^{-6} M emetine; derived from Em^r-11 and Em^r-34, respectively	This report
CHO-169	Derived from Em^r-2-2; 6-TG^r Oua^r	This report
CHL-216	Derived from HT-1; Emt^s 6-TG^r Oua^r	3
CHL-UCW266	EmtA tester strain	J. Wasmuth (20, 21)
CHO-UCW16	EmtB tester strain	J. Wasmuth (20, 21)
CHP-UCW282	EmtC tester strain	J. Wasmuth (20, 21)

[a] Abbreviations: Pro, proline; Gly, glycine; N, chromosome number; TK, thymidine kinase.
[b] Lawrence Livermore Laboratories.
[c] University of Michigan.

Preparation of Chinese hamster cell ribosomes and ribosomal subunits. Ribosomes were purified from freshly harvested or frozen Chinese hamster cell pellets as previously described (2, 9, 12). Ribosomes were dissociated into their component subunits (40S and 60S) in buffer containing puromycin. For screening purposes, 0.5×10^7 to 1.0×10^7 cells were harvested from monolayer cultures and suspended in 100 μl of 0.03 M Tris-hydrochloride–0.125 M KCl–0.005 M magnesium acetate–0.01 M 2-mercaptoethanol–0.02% (vol/vol) Nonidet P-40 (pH 7.4). After 15 min at 4°C, the cell debris was sedimented by centrifugation at 12,000 × *g* for 8 min in an Eppendorf microcentrifuge. Supernatant fractions were treated with puromycin and KCl (0.1 mM and either 0.5 or 0.625 M, respectively). After 15 min at 0°C and 10 min at 37°C (6), the solutions were clarified by centrifugation for 4 min at 12,000 × *g*. Samples of the supernatant fractions (3 units as measured by absorbance at 260 nm [A_{260} units]) were layered on preformed 5 to 20% (wt/wt) sucrose gradients containing 0.05 M Tris-hydrochloride, 0.5 M KCl, and 0.005 M $MgCl_2$ (pH 7.4). After centrifugation at 45,000 rpm for 2 h in a Beckman SW50.1 rotor maintained at 4°C, the gradients were harvested with an Instrumentation Specialties Co. fractionator (model no. 640) and UV analyzer (model no. UA-5).

The above procedure was scaled up 50-fold to analyze the proteins contained within isolated ribosomal subunits. After treatment with puromycin and KCl, 150 A_{260} units of the soluble supernatant fractions were layered on 15 to 30% (wt/wt) sucrose gradients and centrifuged for 22 h at 24,000 rpm in an SW27 rotor (Beckman) maintained at 4°C. The gradients were harvested, and the fractions of interest were pooled. Their macromolecular contents were precipitated by adding trichloroacetic acid to 10% (wt/vol). The precipitates were collected by centrifugation for 30 min at 10,000 × *g*, and proteins were extracted from the pellets by the methods indicated below.

TABLE 2. Differences among the electrophoretic mobilities of Chinese hamster cell and rat liver ribosomal proteins[a]

Subunit protein	Comparison[b]
40S	
S6	CH larger than RL
S7	CH larger than RL
S10	CH smaller and more basic than RL
S14	CH more basic than RL
S17	CH larger and more basic than RL
S19	CH smaller and more basic than RL
S27a	CH larger and more basic than RL
60S	
L6	CH more acidic than RL
L7a	CH smaller than RL
L10	CH larger than RL
L13	CH larger and more basic than RL
L19	CH more basic than RL
L26	CH larger and more basic than RL
L29	CH more acidic than RL

[a] Ribosomal subunit proteins are designated by the uniform nomenclature for mammalian ribosomal proteins (14) as deduced from four two-dimensional polyacrylamide gel systems (Fig. 1).

[b] CH, Chinese hamster; RL, rat liver.

Preparation and analysis of ribosomal proteins. Proteins were extracted from 80S ribosomes or ribosomal subunits and core particles in 67% (vol/vol) acetic acid containing 0.2 M magnesium acetate (19). They were alkylated by treatment with iodoacetamide (13) and then analyzed by four complementary two-dimensional polyacrylamide gel electrophoresis systems, described previously (10, 11, 13). In all four electrophoretic systems, the first-dimension tube gels were composed of 4% (wt/vol) acrylamide and contained 8 M urea. The second-dimension slab gels contained either 12.5% (systems I and II) or 18% (systems III and IV) polyacrylamide. System I involved an acid urea first-dimension gel buffer (pH 5.5) and a neutral sodium dodecyl sulfate (SDS) second-dimension gel buffer. System II used a basic urea first dimension (pH 8.6) and neutral SDS second dimension. System III used the basic urea first dimension and then an acid urea (pH 4.5) second dimension. System IV used two acid urea buffers: a pH 5.5 first dimension and a pH 4.5 second dimension. Individual ribosomal proteins were identified by their migration in all four electrophoretic systems by the proposed uniform nomenclature for mammalian ribosomal proteins (14), as later amended (13).

RESULTS

Analysis of Chinese hamster cell ribosomal proteins. The four complementary two-dimensional electrophoretic systems used in these studies resolve ribosomal proteins derived from a variety of eucaryotic organisms. Because of the resolution inherent in this combination of electrophoretic systems, it was not necessary to resolve the 40S and 60S ribosomal subunits before extraction of proteins and electrophoretic analyses. Thus, artifacts that arise during ribosomal subunit dissociation and isolation were avoided (2). Furthermore, as the now-standard nomenclature for mammalian ribosomal proteins is based on the identities of rat liver ribosomal proteins (14), the electrophoretic systems described permitted accurate and detailed comparisons among ribosomal proteins from several animal species, in particular between those of the Chinese hamster and rat liver.

Patterns of wild-type CHO cell ribosomal proteins observed in all four electrophoretic systems are shown in Fig. 1. In combination the four systems resolved 78 Chinese hamster ribosomal proteins: 45 attributed to the 60S ribosomal subunit and 33 to the 40S subunit. Sixty-four of these displayed electrophoretic mobilities identical to rat liver ribosomal proteins and were identified on this basis. The remaining 14 ribosomal proteins, including the one correlated with emetine resistance mutations in CHO cells (see below), migrated with mobilities that differed slightly from those of rat liver ribosomal proteins in at least one gel system. These pro-

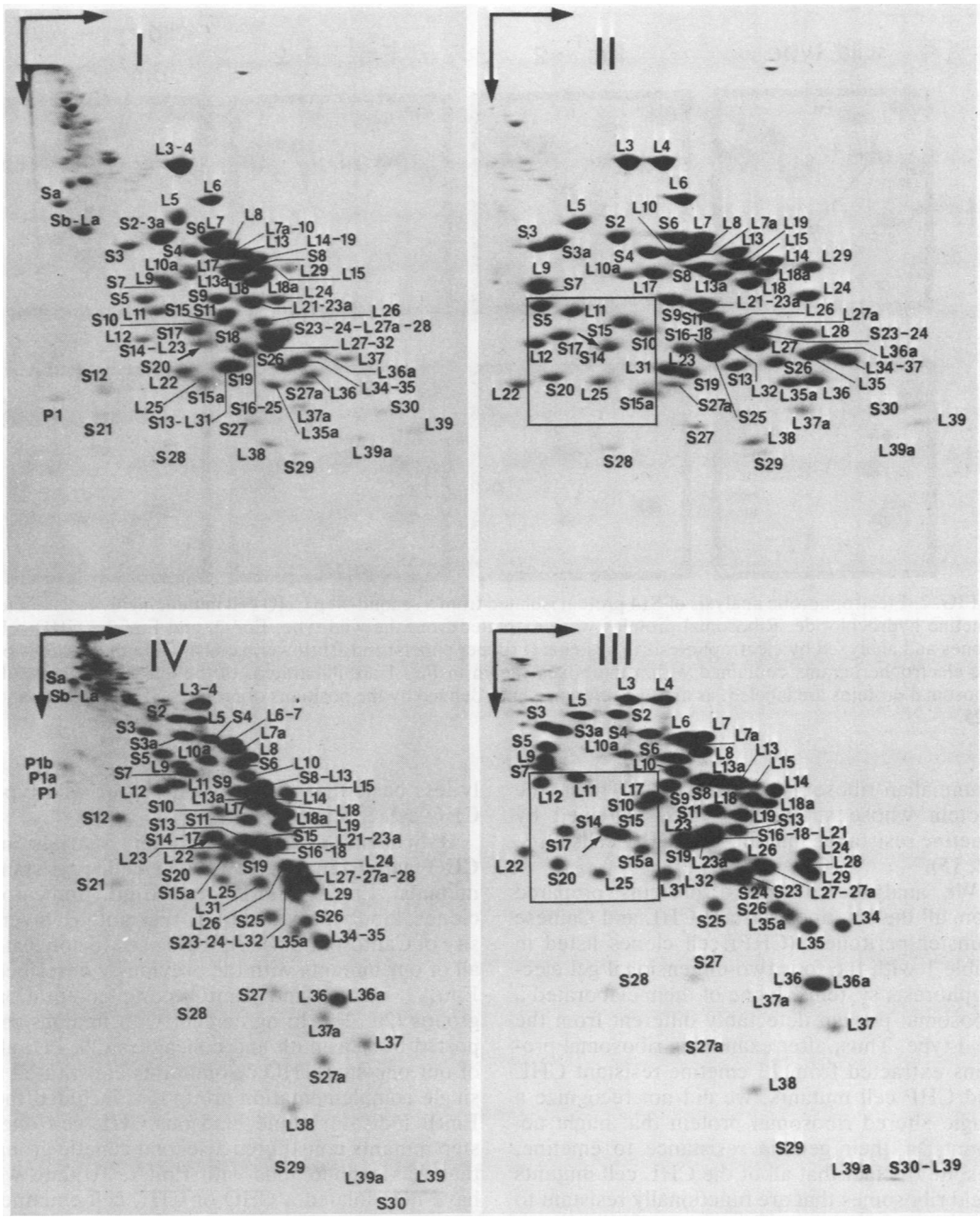

FIG. 1. Two-dimensional polyacrylamide gel electrophoresis of CHO cell ribosomal proteins. Four electrophoretic systems were used to analyze the proteins extracted from 5-A_{260}-unit samples of Chinese hamster cell 80S ribosomes. Details of the electrophoretic conditions and methods have been described before (10–12). As discussed in the text, each protein spot, visualized by staining with Coomassie blue, was assigned its standard name (14) by comparison with similar patterns of rat liver ribosomal proteins. Proteins designated S derive from the 40S ribosomal subunit and those designated L derive from the 60S ribosomal subunit. Acidic ribosomal proteins (Sa, Sb, S12, S21, La, and P1) were observed only in systems I and IV, both of which include acidic first-dimension gels. The insets in II and III are explained in the legend to Fig. 2.

teins and their distinguishing electrophoretic properties are given in Table 2. Confirmation of the indentities of the 14 proteins was based on tryptic and chymotryptic fingerprints after radioiodination (J.-J. Madjar and D. J. Roufa, unpublished data). The ribosomal protein referred to previously as S20 (2, 3) is designated S14 in the currently accepted nomenclature for

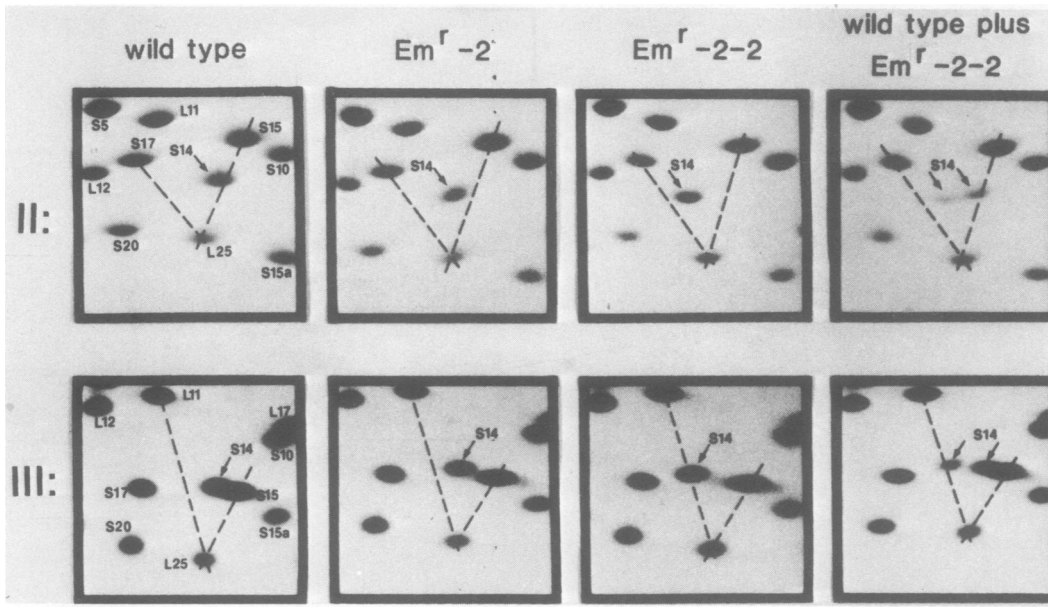

FIG. 2. Electrophoretic analysis of S14 protein isolated from a second-step CHO cell mutant highly resistant to emetine hydrochloride. Ribosomal proteins were extracted from the wild-type, Em^r-2, and Em^r-2-2 CHO cell clones and analyzed by electrophoresis in systems II (upper panels) and III (lower panels). Only the portions of the electropherograms contained within the boxes shown in Fig. 1 are illustrated. In the left-most panels, all ribosomal proteins are labeled, as are two reference lines defined by the positions of proteins S17, L11, S15, and L25.

mammalian ribosomal proteins (14). This is the protein whose structural gene is affected by emetine resistance mutations in CHO cells (2, 3, 12, 15).

We analyzed ribosomal proteins prepared from all the emetine-resistant CHL and Chinese hamster peritoneal (CHP) cell clones listed in Table 1 with the four two-dimensional gel electrophoresis systems. None of them elaborated a ribosomal protein detectably different from the wild type. Thus, after examining ribosomal proteins extracted from 13 emetine-resistant CHL and CHP cell mutants, we did not recognize a single altered ribosomal protein that might account for their genetic resistance to emetine, despite the fact that all of the CHL cell mutants yield ribosomes that are functionally resistant to emetine in cell-free assays (2).

We observed that all of the highly emetine-resistant mutants isolated from Em^r-2 elaborated S14 proteins that displayed an even more pronounced electrophoretic alteration than did the S14s purified from the original one-step CHO mutants. Moreover, all of the two-step CHO mutants examined elaborated very similar, if not identical, electrophoretic forms of the S14 protein. Electrophoretic analyses of the S14 protein extracted from Em^r-2-2 ribosomes in systems II and III are shown in Fig. 2. Note that the S14 extracted from Em^r-2-2 was substantially less basic than either the Em^r-2 or wild-type CHO cell S14 proteins.

Hybridization-complementation analysis of CHO and CHL cell two-step emetine-resistant mutants. EmtA, EmtB, and EmtC indicator clones, kindly provided by J. Wasmuth (University of California, Irvine), were used to correlate all of our mutants with the previously described EmtA, EmtB, and EmtC complementation groups (20, 21). In agreement with findings reported by Wasmuth and colleagues (20, 21), all of our one-step CHO cell mutants constituted a single complementation group that included the EmtB indicator clone; and our CHL cell one-step mutants constituted a second genetic group that was synonomous with EmtA. To date we have not isolated a CHO or CHL cell emetine-resistant mutant that does not complement the EmtC indicator clone (data not shown). All CHO and CHL cell two-step emetine resistance phenotypes are recessive to wild-type emetine sensitivity as well as to one-step emetine resistance (Table 3). Because of this, hybridization-complementation tests among the two-step mutant clones were possible. In these tests, hybrids were isolated in fully selective medium containing one of three emetine hydrochloride concentrations (0, 2×10^{-7}, or 2×10^{-6} M). All of the second-step mutations obtained in an *emtB* genetic background (Em^r-2) were also *emtB* muta-

TABLE 3. Hybridization-complementation analysis of CHO and CHL cell two-step emetine-resistant mutant clones[a]

Second-step mutant clone	Hybridization with CHL-216[b] at emetine concn (M):			Hybridization with CHO-169 (Em^r2-2) at emetine concn (M):		
	0	10^{-7}	10^{-6}	0	10^{-7}	10^{-6}
CHO						
CHO[b]	—[c]	—	—	151	0	0
UCW-16	162	0	—	36	31	0
Em^r-2	539	0	—	249	206	0
Em^r-2-2	580	0	0	39	40	28.5
Em^r-2-3	178	0	0	34.5	26	28.5
Em^r-2-5	490	0	0	106.5	109	95
CHL						
Em^r-11-1	281	0	0	57.5	0	0
Em^r-11-3	241	0	0	150	0.5	0
Em^r-11-4	—	—	—	90	0	0
Em^r-34-2	490	0	0	59	0	0

[a] Hybrid clones formed between CHL-216, CHO-169, and each of the second-step mutants listed were selected in medium containing HAT components, 2 mM ouabain, and the indicated concentrations of emetine. Each value represents the mean number of hybrid colonies that survived selection on duplicate dishes.
[b] Wild type.
[c] —, Not done.

tions, and none of the second-step mutations derived in an *emtA* background (Em^r-11) were *emtB* mutations. Thus, the cell line specificity of emetine resistance mutations in Chinese hamster cells pertains to two-step mutations as well as to one-step mutations.

Structural instability of two-step EmtB 40S ribosomal subunits. Wejksnora and Warner (22; in press) recently reported that some EmtB mutants possess 40S ribosomal subunits sensitive to buffers containing high concentrations of KCl. They observed that treatment with 0.5 M KCl promoted the loss of several proteins, including S14, from salt-sensitive EmtB 40S ribosomal subunit preparations. At 20°C the effect of KCl was enhanced over its effect at 4°C (Wejksnora and Warner, in press). We examined the effects of elevated salt concentrations (0.5 and 0.625 M KCl) on 40S ribosomal subunits purified from our one- and two-step emetine-resistant mutants to investigate the genetic basis for EmtB heterogeneity in this phenotype. Our studies confirmed the observations reported by Wejksnora and Warner and further indicated that sensitivity to KCl at 4°C is a property of two-step EmtB 40S ribosomal subunits (Fig. 3 and Table 4).

Ribosomes purified from wild-type Chinese hamster cells were dissociated to their component subunits (40S and 60S) by treatment with puromycin and centrifugation through sucrose gradients in a buffer containing 0.5 (Fig. 3A) or 0.625 M (not shown) KCl. Areas under the 40S and 60S ribosomal subunit peaks indicated that the two subunits were obtained in the expected 1:1 stoichiometric ratio from wild-type ribosomes. In contrast, when two-step EmtB ribosomes were analyzed in sucrose gradients containing 0.5 M KCl, we observed sedimentation patterns such as that shown in Fig. 3B. Note that the unimodal 40S subunit peak characteristic of wild-type CHO cell ribosomal subunits was replaced by a Y peak (32S) and a shoulder (40S).

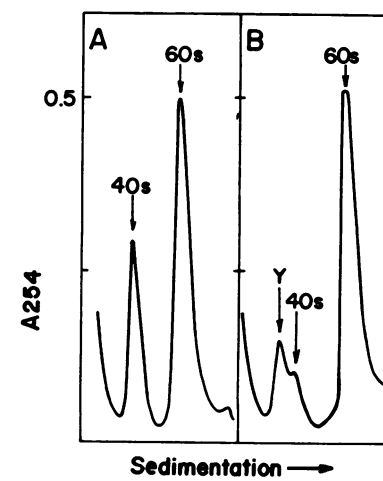

FIG. 3. Sedimentation analysis of ribosomal subunits prepared from the CHO cell wild-type (A) and Em^r-2-2 (B) clones. Ribosomes were dissociated into their component subunits by treatment with puromycin and analyzed by velocity sedimentation through sucrose gradients, as described in the text. Gradients were harvested through a continuous-flow UV spectrophotometer.

Using this test to screen all of the ribosomal subunits, we observed that only two-step EmtB highly resistant mutants (Table 1) and one two-step EmtB mutant provided by J. Wasmuth (UCW-599) contained salt-sensitive 40S ribosomal subunits. All of the one-step EmtA, EmtB, and EmtC and the two-step EmtA clones listed in Table 1 elaborated stable 40S ribosomal subunits that contained the complete complement of 40S subunit ribosomal proteins (Fig. 1) in both 0.5 and 0.625 M KCl.

To more accurately determine the effect of high salt concentrations on second-step EmtB 40S ribosomal subunits, we isolated a large amount of the 32S and 40S peaks from KCl-treated Em^r-2-3 ribosomes. We analyzed their component ribosomal proteins by two-dimensional polyacrylamide gel electrophoresis (system II) (Table 4). The 40S core particle lacked eight basic proteins characteristic of native CHO cell 40S ribosomal subunits. These included the mutationally altered S14 protein as well as S3a, S8, S10, S16, S18, S26, S27a, and S30. The Em^r-2-3 32S Y peak showed an even more dramatic loss of ribosomal proteins. This peak contained only eight bona fide basic ribosomal proteins: S3, S13, S15a, S17, S19, S20, S28, and S29. In addition, the 32S core particle contained a number of other peptides that did not migrate to positions characteristic of authentic Chinese hamster ribosomal proteins. Rather, the peptides appeared to be proteolytic degradation products derived from the ribosomal proteins that originally were part of the 40S subunit.

TABLE 4. Basic ribosomal proteins contained within ribonucleoprotein particles resulting from treatment of EmtB second-step mutant 40S ribosomal subunits with 0.5 M KCl

40S subunit protein	Present in ribonucleoprotein particle:	
	40S	32S (Y)
S2	+	−
S3	+	+
S3a	−	−
S4	+	−
S5	+	−
S6	+	−
S7	+	−
S8	−	−
S9	+	−
S10	−	−
S11	+	−
S13	+	+
S14	−	−
S15	+	−
S15a	+	+
S16	−	−
S17	+	+
S18	−	−
S19	+	+
S20	+	+
S23	+	−
S24	+	−
S25	+	−
S26	−	−
S27	+	−
S27a	−	−
S28	+	+
S29	+	+
S30	−	−

DISCUSSION

Using a combination of four two-dimensional polyacrylamide gel electrophoresis systems, we correlated 78 Chinese hamster ribosomal proteins with standard rat liver ribosomal proteins. Chinese hamster cell 60S ribosomal subunits contain 45 proteins; and Chinese hamster 40S ribosomal subunits contain 33 proteins. Four independently obtained CHO cell emetine-resistant mutants (two isolated in this laboratory and two contributed by J. Wasmuth) elaborated an identical electrophoretic variant form of the 40S ribosomal subunit protein, S14. The remaining 77 proteins extracted from the ribosomes of CHO cell mutants and all 78 proteins from the ribosomes of CHL and CHP emetine-resistant mutants appeared to be wild-type proteins.

Each of the four CHO cell two-step emetine-resistant clones had S14 proteins that differed electrophoretically from those of the one-step emetine-resistant mutants and the S14 from wild-type CHO cells. This provided compelling genetic evidence for a cause and effect relationship between emetine resistance and the structure of ribosomal protein S14 in CHO cells.

All of the S14 proteins obtained from two-step emetine-resistant CHO cell mutants had identical electrophoretic mobilities, including a two-step CHO cell mutant provided by J. Wasmuth. That emetine resistance mutations in CHO cells yielded altered S14 proteins with apparently identical structures suggests that a combination of stringent constraints influences the types of mutations obtainable in the S14 structural gene. It is possible that the constraints are both genetic and biochemical. They might reflect the structure of the CHO cell genome, the genetic code words used to specify the amino acid sequence of S14, the apparently complex protein-protein and protein-RNA interactions required for correct ribosomal subunit assembly and function, or a combination of these.

Only CHO cell two-step emetine-resistant mutations rendered 40S ribosomal subunits sensitive to high concentrations of KCl at 4°C. Thus, it appears that the S14 elaborated by EmtB two-step mutants, in contrast to those from the wild type and one-step emetine resistant mutants, imparts the structural instability to 40S ribosom-

al subunits described by Wejksnora and Warner (22; in press). The sensitivity of the 40S subunit from two-step EmtB mutants to high concentrations of KCl is probably a direct consequence of the mutation affecting the S14 protein, because ribonucleoprotein core particles derived from two-step mutant ribosomes (32S and 40S) lacked several specific ribosomal proteins, including S14. Perhaps the second-step S14 mutation affects protein-protein and protein-RNA interactions that are important for the maintenance of 40S ribosomal subunit structure. It is interesting to note that 40S ribosomal subunit sensitivity to KCl is not a direct consequence of the level of drug resistance in an EmtB mutant. Em^r-1 is a one-step, high-resistance mutant (2) whose ribosomes are as stable in 0.5 M KCl as are wild-type ribosomes.

Our hybridization-complementation studies showed that the cell line-specific nature of emetine resistance Chinese hamster cell mutations pertains not only to one-step CHO and CHL cell clones (20, 21), but to high-resistance CHO and CHL cell two-step mutants as well. On the basis of cell fusions involving indicator mutant clones obtained from J. Wasmuth (20, 21), we assigned all of our CHL cell two-step mutants to the EmtA complementation group and all of our CHO cell two-step mutants to the EmtB group. The genetic basis for cell line-specific mutations observed in this system probably resides in the genome structure of each cell line, perhaps reflecting hemizygous chromosomal regions as proposed by others (4, 5, 18, 23).

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