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Identification and Characterization of a Third Complementation Group of Emetine-Resistant Chinese Hamster Cell Mutants

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We have isolated emetine-resistant cell lines from Chinese hamster peritoneal fibroblasts and have shown that they represent a third distinct class or complementation group of emetine-resistant mutants, as determined by three different criteria. These mutants, like those belonging to the two other complementation groups we have previously defined, which were isolated from Chinese hamster lung and Chinese hamster ovary cells, have alterations that directly affect the protein biosynthetic machinery. So far, there is absolute cell line specificity with respect to the three complementation groups, in that all the emetine-resistant mutants we have isolated from Chinese hamster lung cells belong to one complementation group, all those we have isolated from Chinese hamster ovary cells belong to a second complementation group, and all those isolated from Chinese hamster peritoneal cells belong to a third complementation group. Thus, in cultured Chinese hamster cells, mutations in at least three different loci, designated *emtA*, *emtB*, and *emtC*, encoding for different components of the protein biosynthetic machinery, can give rise to the emetine-resistant phenotype.

The ipecac alkaloid, emetine, is a potent inhibitor of protein synthesis in eucaryotic cells and has been used as a selective agent to isolate Chinese hamster ovary (CHO) cell mutants resistant to its cytotoxic effects (7, 14). In all the emetine-resistant CHO mutants which have been examined in enough detail, the lesions could be linked to the 40S ribosomal subunit (8). In two such mutants, the same 40S ribosomal protein has been shown to be electrophoretically altered (1, 12). In addition, there is considerable evidence that CHO cells are haploid for one locus that can be altered to give rise to the emetine-resistant phenotype (3, 6). As far as one can tell from the available data, all the CHO emetine-resistant mutants appear to belong to the same complementation group (2, 9, 10). However, we have recently reported the isolation and characterization of emetine-resistant Chinese hamster lung (CHL) cell mutants which clearly belong to a different complementation group from emetine-resistant CHO mutants isolated in this laboratory (14). Thus, three emetine-resistant mutants we isolated from CHL cells complement at least two different emetine-resistant mutants that we have isolated from CHO cells. We have designated the locus defined by the CHL mutants as *emtA* and the locus defined by the CHO mutants as *emtB* (14). The alterations in both types of mutant affect the protein synthetic machinery directly. In addition, for reasons discussed below, it seems very likely that the CHO emetine-resistant (*emtB*) mutants we isolated have alterations in the same 40S ribosomal protein that is altered in the CHO mutants examined by others. Preliminary evidence in our laboratory suggests that the CHL emetine-resistant (*emtA*) mutants also have an alteration affecting the ribosome, but if so, it must certainly be different from the alteration in the *emtB* mutants.

Because of the fact that the two different cell lines, CHL and CHO, seemed to give rise to different types of emetine-resistant mutants, we decided to examine a third Chinese hamster cell line. We therefore selected emetine-resistant mutants from a cell line derived from Chinese hamster peritoneal (CHP) fibroblasts. As will be shown in this report, these mutants clearly belong to yet a third complementation group as determined by three different criteria. We have designated the locus defined by this new complementation group as *emtC*. The alterations in the CHP emetine-resistant (*emtC*) mutants also affect protein synthesis directly, since protein synthesis in cell-free extracts from these mutants is much more resistant to emetine inhibition than that in extracts from the emetine-sensitive parent. Thus, at least three different gene products which are involved in protein synthesis can be altered to give rise to the emetine-resistant phenotype in Chinese hamster cells.

As also shown in this report, extensive complementation analyses among various emetine-resistant mutants have demonstrated absolute cell line specificity with respect to the three complementation groups. That is, *emtA* mutants can be isolated only from CHL cells, *emtB* mutants can be isolated only from CHO cells, and *emtC* mutants can be isolated only from CHP cells.

MATERIALS AND METHODS

Materials. Tissue culture medium and other cell culture reagents were purchased from GIBCO (Grand Island, N.Y.) and Irvine Scientific. Fetal bovine serum and dialyzed fetal bovine serum were obtained from Irvine Scientific. Tritium-labeled amino acids were purchased from Amersham and New England Nuclear Corp. Other supplies were purchased from sources previously indicated (14).

Cell lines and cell culture. Cell lines were all grown as monolayer cultures in Dulbecco-modified Eagle medium, with additional supplements as required by various of the cell lines as described previously (14).

The CHL, CHO, and CHP cell lines used in some of these studies are listed in Table 1. Mutant cell lines isolated in this laboratory are designated by the prefix UCW. The parental CHP cell line used in these experiments, designated UCW 104, is deficient in thymidine kinase (TK^-) and does not grow in medium containing hypoxanthine, aminopterin, and thymidine. This cell line, strain CCL 14.1, was obtained from the American Type Culture Collection. The phenotypes and culture conditions for the mutants derived from the V-79 CHL and the CHO-S cell lines have previously been described in detail (14). Detailed characterizations of the CHL *emtA* mutant, UCW 181, and the CHO *emtB* mutant, UCW 10, have been described (14). The mutagenesis of UCW 104 and subsequent selection of emetine-resistant mutants were performed as previously described for the CHL and CHO cell lines (14).

Complementation analyses and isolation of hybrids. Details concerning the fusion of various cell lines with polyethylene glycol 1000 have been described (14). To perform complementation tests between the CHL mutant, UCW 181, and the CHP mutants, UCW 282 and UCW 298, cells from the fused cultures were divided into two parts. One half of the cells were placed in medium containing hypoxanthine, aminopterin, and thymidine, a combination which prevents growth of the TK^- CHP parent and the $Hprt^-$ CHL parent but allows growth of hybrids between the two. The other half of the cells were placed in the same medium with the addition of 3×10^{-7} M emetine, a concentration which would allow normal growth of either parent. All plates were incubated at 37°C. Complementation analyses between the *emtB* CHO mutant, UCW 10, and the two CHP mutants, UCW 282 and UCW 298, were performed in a similar manner, except the plates were incubated at 39°C. Hypoxanthine, aminopterin, and thymidine combined prevent growth of the CHP cell lines, and the elevated temperature prevents growth of UCW 10, because of its temperature-sensitive leucyl-transfer ribonucleic acid (RNA) synthetase. Hybrids between the different cell lines, however, will survive under these conditions. Presumptive hybrid colonies arising on plates without emetine were cloned and grown into mass culture. Karyotypic analysis was performed on all such clones to be used in subsequent biochemical experiments. All the hybrid clones isolated were near tetraploid, with modal chromosome numbers of 38 to 44.

The more extensive complementation tests, summarized in Results and in Table 3, were carried out in the same manner, using a number of genetic markers in the various parental cell lines to select for hybrids. These markers included TK^-, $Hprt^-$, resistance to ouabain (Oua^r), temperature-sensitive leucyl-, asparagyl- and methionyl-transfer RNA synthetases, auxotrophy for proline (Pro^-), and auxotrophy for glycine, adenosine, and thymidine (Gat^-).

Effect of emetine on protein synthesis in vivo and in vitro. The procedures used to determine the effect of various concentrations of emetine on the rate

TABLE 1. *List of cell lines*

Strain no.	Phenotype/genotype[a]	Parent
Derived from CHO		
tsH1	Pro^-, *leuS*(Ts)	CHO-S
UCW 10	Pro^-, *leuS*(Ts) *emtB*	tsH1
Derived from CHL		
UCW 124	$Hprt^-$, *asnS*(Ts)	UCW 113
UCW 181	$Hprt^-$, *asnS*(Ts) *emtA*	UCW 124
Derived from CHP		
UCW 104	TK^-	
UCW 282	TK^- Emt^r	UCW 104
UCW 298	TK^- Emt^r	UCW 104

[a] The abbreviations are as follows: Pro^-, auxotrophy for proline; *leuS*(Ts), temperature-sensitive leucyl-transfer RNA synthetase; *asnS*(Ts), temperature-sensitive asparagyl-transfer RNA synthetase; $Hprt^-$, deficiency in hypoxanthine-guanine phosphoribosyl transferase; TK^-, deficiency in thymidine kinase; Emt^r, resistance to emetine (*A* and *B* serve to distinguish the different complementation groups). More detailed description of the cell lines are provided in the text, along with references concerning the origins of the cell lines.

of ^{3}H-amino acid incorporation into protein in vivo have been described (14). The effect of emetine on the rate of polyuridylic acid-dependent polyphenylalanine synthesis in vitro was assayed by using the microtechnique developed in this laboratory as previously described in detail (14). These assays utilized monolayer cultures grown in the 16-mm wells of 24-well culture dishes. The cells were lysed in situ and treated with micrococcal nuclease, and then extracts were supplied with polyuridylic acid as a source of translatable messenger RNA. The polyuridylic acid was present in the reaction mixtures in considerable (two- to threefold) excess.

RESULTS

Mutants resistant to 3×10^{-7} M emetine were selected from ethyl methane sulfonate-mutagenized cultures of UCW 104, as described in Materials and Methods. Four mutants were recovered from 7.5×10^6 mutagenized cells in one experiment, and two were isolated from the same number of cells in a second experiment. All six mutants have maintained resistance to emetine after 3 months of continuous culture in the absence of the drug, and the emetine-resistant phenotype of all the mutants is recessive. Two of these mutants, UCW 282 and UCW 298, were chosen for more detailed study and are listed in Table 1 along with information concerning their phenotypes and lineage. Both UCW 282 and UCW 298 grow at very near normal rates in emetine concentrations up to 4×10^{-7} M, whereas the parental cell line, UCW 104, will not grow at concentrations above 8×10^{-8} M. Since we have previously demonstrated the existence of two loci, *emtA* and *emtB*, that could be altered to give rise to emetine resistance in CHL and CHO cells, respectively (14), we were interested to determine whether the emetine-resistant mutants isolated from UCW 104 belong to either of the two complementation groups defined by the *emtA* and *emtB* mutants. Complementation tests were therefore done, as described in Materials and Methods. Both UCW 282 and UCW 298 were fused to an *emtA* mutant, UCW 181, and to an *emtB* mutant, UCW 10. After fusion of the various cell lines with polyethylene glycol 1000 and incubation in nonselective medium overnight, each of the fused populations was divided into two parts. Half the cells from each fusion were placed in medium selective for hybrids, as described in Materials and Methods, and the other half were placed in the same medium also containing 3×10^{-7} M emetine (a concentration of the drug at which all the parental cell lines grow well). After 10 to 14 days, the number of colonies on the various culture dishes was determined (Table 2). If the mutations in the two fused cell lines are allelic, hybrid colonies should appear in medium with and without emetine. However, if the mutations in the two cell lines are in different genes, hybrids should appear only in medium without emetine, since in all of these cell lines the emetine-resistant phenotypes are recessive. In all of the crosses, at least 150 hybrid colonies were observed on plates without emetine. However, in medium with a concentration of emetine that allows growth of all the parental cell lines, no hybrid colonies were observed in any of the fused cultures. These data strongly suggest that the two CHP mutants, UCW 282 and UCW 298, represent a third complementation group of emetine-resistant mutants. To further examine this possibility, we isolated several hybrid clones from each of the following crosses: UCW 181 (*emtA*) × UCW 282, UCW 181 × UCW 298, UCW 10 (*emtB*) × UCW 282, and UCW 10 × UCW 298. The hybrids were grown into mass culture and examined in more detail in experiments described below. Karyotypic analyses on the hybrids confirmed that all were near tetraploid.

TABLE 2. *Complementation analysis of CHP emetine-resistant mutants*[a]

Cross	No. of colonies in selective medium	
	Without emetine	With emetine
UCW 181 (*emtA*) × UCW 282	292	0
UCW 181 × UCW 298	220	0
UCW 10 (*emtB*) × UCW 282	217	0
UCW 10 × UCW 298	166	0

[a] The fusion procedure and the selective media for hybrids from the different crosses are described in the text. Emetine, when present, was at a concentration of 3×10^{-7} M.

The most sensitive test we have of emetine resistance or sensitivity is to determine the effect of various concentrations of the drug on the rate of protein synthesis in vivo. Therefore, we compared the effect of various concentrations of emetine on the rate of ^{3}H-amino acid incorporation into protein in the emetine-sensitive parental cell lines, emetine-resistant cell lines, and hybrids derived from the four different crosses mentioned above. These experiments were performed as previously described (14). In Fig. 1A are shown the results obtained from hybrids between an *emtA* mutant, UCW 181, and the two new CHP mutants, UCW 282 and UCW 298. All three of the emetine-resistant cell lines, UCW 181, UCW 282, and UCW 298, were much more resistant to emetine inhibition than their respective emetine-sensitive parents, UCW 124 and UCW 104. However, hybrids derived from fusing UCW 181 with either UCW 282 or UCW 298 were very sensitive to emetine inhibition, almost as sensitive as the diploid emetine-sen-

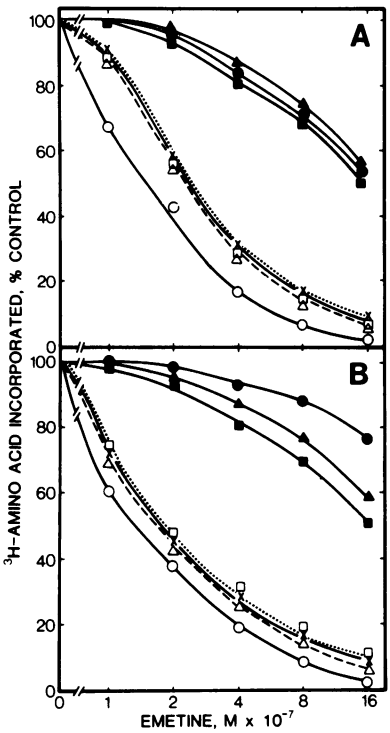

FIG. 1. *Effect of emetine on the rate of protein synthesis in vivo in emetine-sensitive cell lines, emetine-resistant cell lines, and emetine-resistant × emetine-resistant hybrids. The effect of different concentrations of emetine on the rate of protein synthesis is expressed as the rate of ^{3}H-amino acids incorporated into trichloroacetic acid-insoluble material linearly with time at a given concentration of the drug divided by the rate in the absence of the drug (100%). The 100% values in counts per minute are given in parentheses below for each cell line. See reference 14 for details. (A)* (○) *UCW 104 (156,539);* (●) *UCW 181 (107,611);* (■) *UCW 282 (42,193);* (▲) *UCW 298 (30,922);* (□) *UCW 181 × UCW 282 clone 2 (113,235); (X) UCW 181 × UCW 282 clone 3 (161,923);* (△) *UCW 181 × UCW 298 clone 1 (184,419). The emetine sensitivity of UCW 124, the emetine-sensitive parent of UCW 181, was identical to that of UCW 104 (data not shown). (B)* (○) *tsH1 (90,287);* (●) *UCW 10 (55,920);* (■) *UCW 282 [replotted from (A)];* (▲) *UCW 298 [replotted from (A)];* (□) *UCW 10 × UCW 282 clone 3 (57,565); (X) UCW 10 × UCW 282 clone 4 (38,197);* (△) *UCW 10 × UCW 298 clone 1 (91,421).*

sitive cell lines. As can be seen, the emetine sensitivity of all three hybrids tested was virtually identical. These results demonstrate that UCW 282 and UCW 298 represent a different complementation group than UCW 181. The results of similar experiments with hybrids derived from fusing an *emtB* mutant, UCW 10, with the two new emetine-resistant mutants, UCW 282 and UCW 298, are shown in Fig. 1B. In all three hybrid cell lines tested, protein synthesis was much more sensitive to emetine inhibition than in either emetine-resistant parental cell line. Again, the results with all three hybrids were virtually identical, and the hybrids were only slightly more resistant to emetine than the emetine-sensitive diploid cell lines, tsH1 and UCW 104. These results, along with those shown in Fig. 1A, demonstrate unequivocally that the two emetine-resistant CHP mutants, UCW 282 and UCW 298, complement both the *emtA* and *emtB* mutants. In addition, as shown below, we have determined that these two CHP mutants, as well as all the others, fail to complement one another. These two cell lines, therefore, represent a third complementation group of emetine-resistant mutants. We have designated the locus defined by these two mutants as *emtC*.

To determine whether the mutations conferring emetine resistance to mutants belonging to this complementation group affect the protein synthetic machinery directly, we next examined the effect of emetine on protein synthesis in vitro in extracts derived from the various cell lines. These experiments utilized the microtechnique developed in the laboratory as previously described (14). As shown in Fig. 2A, protein synthesis in extracts derived from both *emtC* mutants, UCW 282 and UCW 298, was much more resistant to emetine inhibition than that in the emetine-sensitive parent, UCW 104. Thus, the alterations in these two mutants must affect the protein synthetic machinery directly, as we have previously demonstrated for the *emtA* and *emtB* mutants (14). Also shown in Fig. 2A is the effect of emetine on protein synthesis in extracts derived from a UCW 181 × UCW 282 hybrid and a UCW 181 × UCW 298 hybrid. For both hybrids, the sensitivity of protein synthesis to emetine in cell-free extracts was intermediate between diploid emetine-resistant cell lines (UCW 181, UCW 282, UCW 298) and emetine-sensitive cell lines (UCW 104 and UCW 124). Similar results were obtained in other independently derived hybrids from the same two crosses. As discussed previously (14), this is the expected result if the alterations in the two different emetine-resistant parents affect different components of the ribosome. Thus, a hybrid between two such cell lines would be expected to contain both emetine-sensitive and emetine-resistant ribosomes. In vivo this would result in protein synthesis being almost completely sensitive to emetine since, in the presence of the drug, sensitive ribosomes in polysomes would be "frozen" on the messenger RNA and thereby block translation by resistant ribosomes. However, in the in vitro assays with messenger RNA (polyuridylic acid) in excess, each ribosome es-

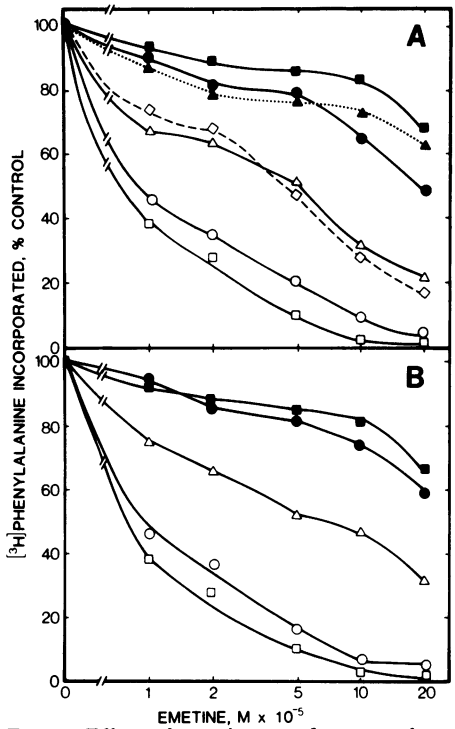

FIG. 2. *Effect of emetine on the rate of protein synthesis in vitro in cell-free extracts. The rate of the polyuridylic acid-dependent synthesis of [^{3}H]polyphenylalanine in the presence of the indicated concentrations of emetine in extracts prepared from the various cell lines was determined as described in detail in reference 14. The amount of [^{3}H]phenylalanine incorporated in the absence of the drug is defined as 100% for each cell line. The 100% values in counts per minute are given in parentheses below for each cell line. (A) (□) UCW 104 (63,106); (○) UCW 124 (71,558); (■) UCW 282 (30,969); (▲) UCW 298 (47,143); (●) UCW 181 (99,365); (△) UCW 181 × UCW 282 clone 2 (88,756); (◇) UCW 181 × UCW 298 clone 3 (47,713). (B) (○) tsH1 (71,126); (□) UCW 104 [replotted from (A)]; (●) UCW 10 (45,239); (■) UCW 282 [replotted from (A)]; (△) UCW 10 × UCW 282 clone 3 (90,085).*

sentially has its own messenger RNA, and translation by resistant ribosomes will not be hindered by emetine-blocked sensitive ribosomes.

Figure 2B shows the results of similar experiments with a representative hybrid between UCW 282 and the *emtB* mutant UCW 10. Again, protein synthesis in extracts derived from such a hybrid was more sensitive to emetine inhibition than that in either resistant parent, but it was considerably more resistant than that in emetine-sensitive cell lines. The results of similar experiments with hybrids between UCW 10 and UCW 298 were virtually identical. The results of these in vitro assays, therefore, confirm that the CHP mutants, UCW 282 and UCW 298, define a third class of emetine-resistant mutants with alterations directly affecting protein synthesis.

One interesting aspect of the three complementation groups is the apparent cell line specificity. Thus, *emtA* mutants have been isolated only from CHL cells, *emtB* mutants have been isolated only from CHO cells (14), and *emtC* mutants have been isolated only from CHP cells. To expand upon this initial observation and determine whether we could isolate more than one class of emetine-resistant mutant from one cell line, we isolated additional emetine-resistant mutants from several different clones of CHL, CHP, and CHO cells. These mutants were then used to perform more extensive complementation analyses, as described below.

Emetine-resistant mutants were isolated from five different cloned cell lines derived from V-79 CHL cells, as previously described (14). Four of the clones had been selected in this laboratory previously and have a number of different genetic markers unrelated to the emetine-resistant phenotype. The fifth clone was our wild-type V-79 CHL stock, UCW 100. For convenience, these are referred to below as CHL clone 1, clone 2, etc. Similarly, emetine-resistant mutants were isolated from three different cloned CHO cell lines, each having a number of other genetic markers. All three CHO clones were derived, initially, from the Toronto CHO-S cell line. Four of the emetine-resistant CHP mutants described above (two each from two independent cutures) were used in these experiments, along with two isolated from a cloned derivative of our UCW 104 CHP stock. The various emetine-resistant mutants from the three cell lines already had a number of different selectable genetic markers, and in some cases new markers were selected for after isolation of the emetine-resistant mutants. These various markers, including TK$^-$, Hprt$^-$, ouabain resistance (Oua^r), various temperature-sensitive aminoacyl-transfer RNA synthetases (asparagyl, leucyl, or methionyl), auxotrophy for proline (Pro$^-$), and auxotrophy for glycine, adenosine, and thymidine (Gat$^-$), allowed us to select hybrids between any undefined emetine-resistant mutant and already defined *emtA*, *emtB*, and *emtC* mutants. Each undefined emetine-resistant mutant to be tested was fused to a known *emtA* mutant, *emtB* mutant, and *emtC* mutant by using the basic procedure outlined in Materials and Methods. Twenty-four hours after fusion, cells were plated in medium selective for hybrids between the parental cell lines, with and without emetine. Two weeks later, the number of hybrid colonies on the plates was determined. The results of these experiments are summa-

rized in Table 3. In every fusion reported, at least 100 hybrid colonies were observed from fused cultures plated without emetine. Two mutants were defined as not complementing if the numbers of hybrid colonies on plates with and without emetine were within 20% of one another. Two mutants were defined as complementing if the number of hybrid colonies on plates with emetine was at least 100-fold lower than that on plates without the drug. All 11 emetine-resistant CHL mutants, isolated from five different clones, failed to complement a known *emtA* mutant, but complemented both an *emtB* and *emtC* mutant. Similarly, all seven CHO mutants could unequivocally be assigned to the *emtB* complementation group, and all six CHP mutants could be assigned to the *emtC* complementation group. In no case did we obtain an equivocal result. Thus, we never observed a mutant which failed to complement more than one of the already defined mutants. Thus, as far as our analyses have gone, the cell line specificity for the different complementation groups remains absolute. Interestingly, we recently isolated two independent emetine-resistant mutants from a fourth Chinese hamster cell line derived from female lung tissue (Dede) and found that both belonged to the *emtB* complementation group.

TABLE 3. *Complementation analysis of emetine-resistant CHL, CHO, and CHP mutants*[a]

Parent 2	Parent 1		
	emtA	*emtB*	*emtC*
CHL			
Clone 1			
Mutant 1	−	+	ND
Mutant 2	−	+	+
Clone 2			
Mutant 1	−	+	+
Mutant 2	−	ND	+
Clone 3			
Mutant 1	−	+	ND
Mutant 2	−	+	+
Clone 4			
Mutant 1	−	+	+
Mutant 2	−	ND	+
Clone 5			
Mutant 1	−	+	+
Mutant 2	−	+	ND
Mutant 3	−	+	+
CHO			
Clone 1			
Mutant 1	+	−	+
Mutant 2	+	−	+
Clone 2			
Mutant 1	+	−	+
Mutant 2	+	−	+
Clone 3			
Mutant 1	+	−	+
Mutant 2	+	−	+
Mutant 3	+	−	+
CHP			
Clone 1			
Mutant 1	+	ND	−
Mutant 2	+	+	−
Mutant 3	+	ND	−
Mutant 4	+	+	−
Clone 2			
Mutant 1	+	+	−
Mutant 2	+	+	−

[a] The experiments were performed as described in the text. +, Complementation (no or few hybrids in selective medium with emetine); −, lack of complementation (approximately equal numbers of hybrids in medium with and without emetine); ND, not determined.

DISCUSSION

The evidence presented in this report demonstrates that emetine-resistant cell lines derived from CHP cells define a third complementation class of emetine-resistant mutants. We have designated the locus defined by these mutants as *emtC*. The mutations in these cell lines affect the protein synthetic machinery directly, since protein synthesis in cell-free extracts derived from these mutants was quite resistant to emetine. We have previously defined two other complementation groups among emetine-resistant mutants: *emtA* mutants, all of which are derived from CHL cells, and *emtB* mutants, all of which are derived from CHO cells (14). We have also shown that the alterations in both the *emtA* and *emtB* mutants affect the protein synthetic machinery. Thus, in Chinese hamster cells at least three different gene products, all of which are involved in protein synthesis, can be altered to give rise to the emetine-resistant phenotype.

It is very likely that the mutations in our CHO *emtB* mutants are allelic to those in emetine-reistant CHO mutants described by others (7, 9, 12) and that the locus involved encodes for a 40S ribosomal protein. These assumptions are based upon the following observations. (i) CHO cells appear to be haploid or hemizygous for one locus that can be altered to give rise to the emetine-resistant phenotype (4, 6, 16). Thus, the vast majority of emetine-resistant mutants isolated from this cell line would have alterations in this locus if other loci that could be altered to give rise to the emetine-resistant phenotype (i.e., *emtA* or *emtC*) are dizygous, since the phenotype of emetine resistance is recessive. (ii) All of the emetine-resistant CHO mutants tested thus far belong to the same complementation group (2, 9, 10). In at least two CHO mutants the same 40S ribosomal protein has been shown to be

electrophoretically altered (1, 12). (iii) Campbell and Worton (4) have shown that the locus they have designated as *emt* in CHO cells is linked to a gene, *chr,* defined by mutants resistant to chromate. We have recently determined that the *emtB* locus in our CHO mutants is also linked to the *chr* locus (15).

In view of the site of action of emetine, the recessive nature of the emetine-resistant phenotypes in vivo, and partial expression of the emetine-resistant phenotypes in cell-free protein-synthesizing systems prepared from hybrid cells (with messenger RNA in excess), we feel reasonably certain that the *emtA* and *emtC* mutants also have alterations affecting the ribosome. Experiments to localize emetine resistance to ribosomal or soluble fractions in reconstituted cell-free protein-synthesizing systems from the *emtA* and *emtC* mutants are in progress. However, these experiments are presenting some unexpected technical difficulties. Whereas two methods of preparing large-scale extracts from cultured cells (7, 13) suitable for fractionation yield good results with CHO cells, the same procedures result in very poor extracts from the more fibroblastic CHL and CHP cell lines. A third procedure previously reported to give good extracts from CHL cells (11) has also yielded very poor results in this laboratory.

In addition, we are currently analyzing the ribosomal proteins prepared from the various mutant and wild-type cells by using two-dimensional gel electrophoresis, to determine whether we can detect any alterations in the various emetine-resistant mutants.

In view of the complex nature of the ribosome and the interactions among its various components, it would not be surprising to find that alterations in any one of several proteins at or near the site at which emetine binds could decrease the affinity of the ribosome for the drug and lead to the emetine-resistant phenotype. Thus, the gene products of the *emtA, emtB,* and *emtC* loci may all be involved in defining the site at which emetine binds to the Chinese hamster ribosome. The isolation of mutants with alterations in these different gene products should enable us and others to make significant advances in the dissection of mammalian protein synthesis by using a combined biochemical and genetic approach. One goal of this laboratory is to use the currently available protein synthetic mutants to begin constructing a genetic map of the functionally related genes involved in protein synthesis. Toward this end, we have recently determined that the *emtB* locus is closely linked to another gene involved in protein synthesis, the structural gene for leucyl-transfer RNA synthetase (*leuS*) (15). The *emtA* locus, however, is not linked to *leuS* and is almost certainly on a different chromosome than *emtB* (15), demonstrating beyond a reasonable doubt that the *emtA* and *emtB* loci are distinct. The only information we have so far concerning *emtC* is that it, unlike *emtB* (4), is not linked to the *chr* locus, and thus is not linked to *emtB.* We have no information concerning a possible linkage relationship between *emtA* and *emtC.*

Finally, the cell line specificity of the different complementation groups poses some interesting questions. As already discussed, the preponderance of *emtB* mutants in CHO cells can most likely be explained by the fact that this locus, and a good portion of the region surrounding it on the long arm of chromosome 2, is haploid in this cell line (4, 16). Thus, it is interesting to speculate that perhaps CHL cells are haploid for the *emtA* locus and CHP cells are haploid for the *emtC* locus, explaining the apparent cell line specificity. It should be noted that all three of the different Chinese hamster cell lines have unique karyotypes. That is, when the various cell lines were established in culture, each apparently "evolved" a somewhat different karyotype that was compatible with rapid growth in culture. Although the karyotype of each is now relatively stable, they are all distinct from one another. It does not seem unreasonable, therefore, that different regions of the genome have by chance been rendered haploid in the different cell lines as a result of these karyotypic changes. In fact, the *emtA, emtB,* and *emtC* loci may help define such regions in the different cell lines. We plan to examine this possibility by using two types of experiments. The first will involve measuring more precisely the induced mutation frequencies for *emtA, emtB,* and *emtC* mutants in CHL, CHO, and CHP cells, respectively. Experiments of this type have been used by others to argue for or against hemizygosity of various loci in different Chinese hamster cell lines (3). The second type of experiment will involve determining the segregation rates for the three loci in question from appropriate hybrids between *emtA, emtB,* or *emtC* mutants and wild-type CHL, CHO, or CHP cells. This kind of analysis has also been used to examine questions concerning hemizygosity versus dizygosity of loci in Chinese hamster cells (5, 6). For a number of reasons, however, both types of experiments will be difficult to interpret for this complex system, especially since all three loci encode gene products that are almost certainly essential for cell viability. Hopefully, they will eventually enable us to determine whether hemizygosity is involved in the cell line specificity observed with respect to the *emtA* and *emtC* loci, as well as provide valuable information on more basic

questions concerning the mechanism underlying gene segregation in cultured somatic cells.

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