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Mutants of CHO Cells Resistant to the Protein Synthesis Inhibitors, Cryptopleurine and Tylocrebrine: Genetic and Biochemical Evidence for Common Site of Action of Emetine, Cryptopleurine, Tylocrebrine, and Tubulosine†

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ABSTRACT: Stable mutants resistant to the protein synthesis inhibitors cryptopleurine and tylocrebrine can be isolated in Chinese hamster ovary (CHO) cells, in a single step. The frequency of occurrence of cryptopleurine (Cry^R) and tylocrebrine (Tyl^R) resistant mutants in normal and mutagenized cell populations is similar to that observed for emetine resistant (Emt^R) mutants. The Cry^R, Tyl^R, and Emt^R mutants exhibit strikingly similar cross-resistance to the three drugs used for selection, to tubulosine and also to two emetine derivatives cephaeline and dehydroemetine, based both on assays of in vivo cytotoxicity and on assays of protein synthesis in cell-free extracts. The identity of cross-resistance patterns of the Cry^R, Tyl^R, and Emt^R mutants indicates that the resistance to all these compounds results from the same primary lesion, which in the case of Emt^R cells has been shown to affect the 40S ribosomal subunit. This conclusion is strongly supported by the failure of Emt^R, Tyl^R, and Cry^R mutants to complement each other in somatic cell hybrids. Based on these results it is suggested that the above group of compounds possesses common structural determinants which are responsible for their activity. The above mutants, however, do not show any cross-resistance to other inhibitors of protein synthesis such as cycloheximide, trichodermin, anisomycin, pactamycin, and sparsomycin, either in vivo or in vitro, indicating that the site of action of these inhibitors is different from that of the emetine-like compounds.

In bacteria, mutants resistant to various inhibitors of protein synthesis have been very useful in providing specific probes for the study of ribosome structure and function (Jaskunas et al., 1974). Unfortunately, studies on eukaryotic ribosomes have been hampered by the absence of similar appropriate mutations (Nomura et al., 1974). We have reported recently that stable mutants resistant to the protein synthesis inhibitor emetine can be isolated in a single step in Chinese hamster ovary (CHO)[1] cells (Gupta and Siminovitch, 1976). Protein synthesis in extracts of these mutant cells is resistant to the inhibitory action of emetine. In subsequent studies the emetine resistant (Emt^R) phenotype was shown to behave recessively in cell hybrids and was found to be due to an alteration in the 40S ribosomal subunit (Gupta and Siminovitch, 1977).

The successful isolation of Emt^R mutants indicated that it might be possible to obtain a spectrum of mutations involving a number of different ribosomal alterations. This approach was particularly attractive since a number of compounds are known which seem to act specifically as inhibitors of protein synthesis in yeast and mammalian cells (Pestka, 1971; Schindler and Davies, 1975; Battaner and Vazquez, 1971; Vazquez, 1974).

In this paper we report on the isolation of mutants of CHO cells resistant to the protein synthesis inhibitors cryptopleurine and tylocrebrine, two alkaloids of the phenanthrene group. However, detailed examination of the properties of these mutants as described here has revealed that these mutants are identical with the Emt^R mutants reported earlier (Gupta and Siminovitch, 1976, 1977) and that the protein synthesis inhibitors, emetine, tylocrebrine, cryptopleurine, and tubulosine, all act at the same site.

Experimental Procedures

Materials. Chemicals and antibiotics were obtained as follows: Emetine-HCl, cephaeline-HCl, and cycloheximide, Sigma Chemical Co., St. Louis, Mo.; cryptopleurine, Chemsea Manufacturing Pty, New South Wales, Australia; tylocrebrine and anisomycin, Dr. Nathan Belcher, Pfizer Inc., Groton, Conn.; pactamycin, Dr. C. P. Stanners, The Ontario Cancer Institute, Toronto, Canada; trichodermin, Dr. W. O. Godfredsen, Leo Pharmaceutical Products, Ballerup, Denmark; O-methylpsychotrine, Dr. H. T. Openshaw, Wellcome Research Laboratories, Kent, England; tubulosine, Dr. V. Deulofeu, Buenos Aires, Argentina; dehydroemetine, Roche Chemicals, Basel, Switzerland; sparsomycin, Dr. J. D. Douros, National Institutes of Health.

Cell Culture and Cell Lines. The techniques used in this laboratory for culturing CHO cells and for selecting various drug-resistance CHO lines have been described in detail earlier

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[1] Abbreviations used: CHO, Chinese hamster ovary cells; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

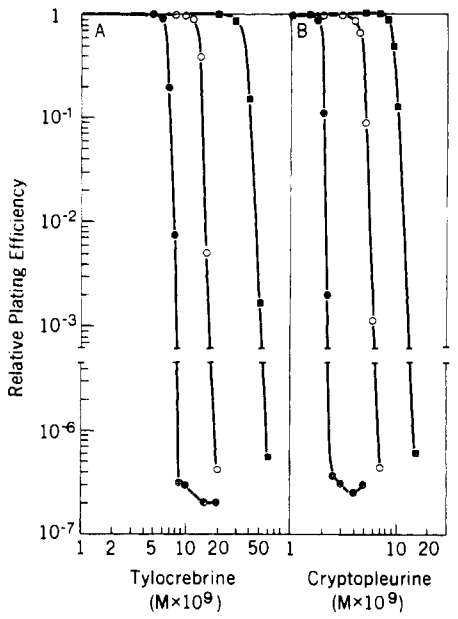

FIGURE 1: Survival curves for the wild-type cells and for Cry^R and Tyl^R mutants in presence of cryptopleurine and tylocrebrine. (A) (●—●) Pro^- (WT); (○—○) Pro^-Tyl^R1; (■—■) Pro^-Tyl^R7. (B) (●—●) Pro^- (WT); (○—○) Pro^-Cry^R4; (■—■) Pro^-Cry^R1.

TABLE I: Frequencies of Mutants Resistant to the Protein Synthesis Inhibitors Emetine, Tylocrebrine, and Cryptopleurine.

Selective agent	Selective concn (M)	Mutation frequency/10^7 cells −EMS	+EMS[a]	Representative cell lines
Emetine	1×10^{-7}	2	70	Pro^-Emt^R76 Pro^-Emt^R41-4
Tylocrebrine	1.5×10^{-8}	2.5	60	Gat^-Tyl^R1 Gat^-Tyl^R7
Cryptopleurine	5×10^{-9}	1.5	55	Pro^-Cry^R1 Pro^-Cry^R4

[a] Cells were mutagenized with 300 μg/mL of ethylmethanesulfonate for 20 h at 37 °C (45–50% survival). After mutagenesis, the cells were grown for 3 days in nonselective medium before carrying out the selection.

(Gupta and Siminovitch, 1976, 1977; Thompson and Baker, 1973). Cells were routinely maintained in suspension culture at 37 °C in complete α medium supplemented with 10% fetal calf serum (FCS, Flow Laboratories, Rockville, Ill.). The two parental CHO lines, Pro^- (proline requiring) and Gat^-Pro^+ (glycine, adenosine, and thymidine requiring), employed in this work for mutant selection and in hybridization experiments were originally obtained from The Ontario Cancer Institute, Toronto. Pro^-Emt^R76 and Pro^-Emt^R41-4 are two emetine resistant CHO cell lines which have been described earlier (Gupta and Siminovitch, 1976).

Plating Efficiencies. Relative plating efficiencies (RPE's) were determined either by complete dose-response curves or by plating 50 and 500 cells at various drug concentrations in 24-well Linbro trays (Thompson and Baker, 1973). For complete dose-response curves, 1-mL volumes of the drug and the cells were added in that order to 60-mm (10^2 to 10^4 cells) or 100-mm (10^5 to 10^6 cells) Falcon plastic tissue culture dishes containing 4 or 16 mL of α medium plus 10% FCS, respectively. For a 24-well Linbro test, the drug and cells were added in 0.5 mL volume in that order to the individual well. After 7 days of incubation at 37 °C, the plates were stained with 1% methylene blue in 70% 2-propanol and aggregates of 25 or more cells were counted as colonies in the determination of RPE's.

Cell-Free Protein Synthesis. Protein synthesis in extracts of CHO cells was carried out as has been described earlier (Gupta and Siminovitch, 1976, 1977). Reactions were usually performed in a final volume of 50 μL and contained per mL: 0.6 mL of S-30 extract, 0.9 μmol of ATP, 0.18 μmol of GTP, 10.8 μmol of creatine phosphate, 1.2 mg of creatine phosphokinase, 30 μmol of Hepes, pH 7.5, 10 μmol of dithiothreitol, 89 μmol of KCl, 1.5 μmol of $MgAc_2$, 19 unlabeled amino acids, 0.12 μmol of each, 50 μCi of [^{3}H]leucine (specific activity, 38 Ci/mmol), and the drugs at the indicated concentrations. Under these conditions, protein synthesis even without any exogenously added mRNA was linear up to at least 45 min. After 40-min incubation at 34 °C, the reactions were terminated by the addition of 0.5 mL of 0.1 N NaOH and further incubated for 15 min to hydrolyze any peptidyl-tRNA. The tubes containing reaction mixtures were then chilled in ice and 0.5 mL of 3% casamino acids and 1 mL of a 15% Cl_3CCOOH solution were added in turn. After about 15 min in ice, the precipitates were filtered on glass fiber filters, washed with cold 5% Cl_3CCOOH, dried and counted.

Cell Hybridization. Cell hybrids were formed by incubating about 5×10^5 cells from each of two pseudo-diploid lines (of the Gat^+Pro^- and Gat^-Pro^+ phenotypes, respectively) in a single well of a 24-well Linbro tray (Linbro Chemical Co., No. FB16-24TC) with β-propiolactone-inactivated Sendai virus (CFF titer ≥ 20 000, lot 1; Connaught Laboratories, Canada) as described by Baker et al. (1974). The cells were subsequently plated in medium supplemented with 10% dialyzed fetal calf serum (DFCS) and lacking proline, glycine, adenosine, and thymidine. Only hybrids containing the complementary auxotrophic markers from the two parents survive and proliferate under these conditions. Appropriate controls were included in each cross to determine the reversion frequency of the auxotrophic markers and the frequency of spontaneous fusion. Colonies which arose in selective medium were picked and subsequently cultured in the same medium. At least ten colonies from each cross were examined for their karyotype and for their resistance to various drugs by measurement of plating efficiencies. Karyotypes were determined as described earlier (Gupta and Siminovitch, 1976).

Results

Isolation of Mutants Resistant to Tylocrebrine and Cryptopleurine. Studies in yeast and HeLa cells have shown that the phenanthrene alkaloids cryptopleurine and tylocrebrine are very effective in inhibiting protein synthesis at very low concentrations (Donaldson et al., 1968; Huang and Grollman, 1972). In agreement with such studies we have observed that, in CHO cells, cryptopleurine and tylocrebrine inhibit protein synthesis by more than 90% at concentrations as low as 4.0×10^{-8} and 1×10^{-7} M, respectively (Gupta and Siminovitch, unpublished observations).

To isolate mutants resistant to these compounds, about 1–2 $\times 10^6$ CHO cells were plated in 100-mm tissue culture dishes containing either 5×10^{-9} M cryptopleurine or 1.5×10^{-8} M tylocrebrine. After about 10–12 days incubation at 37 °C, the resistant clones which survived were picked, grown in medium without the selective agent, and then tested for their resistance to the respective alkaloids.

Figures 1A and 1B show the detailed dose-response curves

TABLE II: Cross Resistance Patterns of Various Mutant Cell Lines.

Cell line	D_{10}[a] values for various inhibitors of protein synthesis (M)					
	Emetine $\times 10^{-8}$	Tylocrebrine $\times 10^{-9}$	Cryptopleurine $\times 10^{-9}$	Dehydroemetine $\times 10^{-8}$	Tubulosine $\times 10^{-7}$	Cephaeline $\times 10^{-8}$
Gat$^-$Emt^S	3.2	7.0	2.0	4.0	1.5	2.0
Pro$^-$Emt^S	3.5	7.5	2.0	4.1	1.4	2.2
Pro$^-$Emt^{R}76	41.0	15.0	5.0	29.0	3.5	12.0
Pro$^-$Emt^{R}41-4	200.0	45.0	9.2	68.0	40.0	60.0
Gat$^-$Tyl^{R}1	35.0	13.0	4.5	30.0	3.5	13.0
Gat$^-$Tyl^{R}7	180.0	40.0	10.0	70.0	38.0	55.0
Pro$^-$Cry^{R}4	40.0	14.0	5.0	30.0	4.0	13.0
Pro$^-$Cry^{R}1	160.0	45.0	10.0	70.0	45.0	60.0

[a] The D_{10} value represents the concentration of drug which reduces plating efficiency of a cell line to 10% of its original value. Such data were obtained from complete survival curves such as those shown in Figures 1 and 3.

of the wild-type cells and of two representative mutants of each type against tylocrebrine and cryptopleurine. As may be seen, the mutants are resistant to even higher concentrations of the selective agents than those used for selection. For each drug more than 50 independent resistant mutants, spontaneous and mutagen induced, have been obtained and they seem to fall into two classes: (i) those which are 2- to 3-fold more resistant and (ii) those which are 4- to 7-fold more resistant than the parental cells. These mutant clones have maintained their resistant character after more than 3 months of growth in the absence of the selective agents.

The frequencies with which tylocrebrine and cryptopleurine resistant mutants are obtained in normal and mutagenized populations are shown in Table I. For the sake of comparison, Table I also gives the frequencies with which emetine resistant mutants are obtained in similar cell populations. It is evident from the data given in Table I that mutants resistant to emetine, tylocrebrine, and cryptopleurine occur spontaneously at a similar but low frequency ($1-3 \times 10^{-7}$). Further, treatment with mutagen leads to a similar (25- to 35-fold) increase in mutation frequencies for resistance to all three drugs.

Cross-Resistance of Emt^R, Tyl^R, and Cry^R Mutants. It was next of interest to determine the relationship, if any, between these three types of mutations. Our previous experiments indicated that the 40S ribosomal subunit was modified in Emt^R cells (Gupta and Siminovitch, 1977), and studies in yeast resulted in a similar conclusion for cryptopleurine and tylocrebrine resistant mutants (Skogerson et al., 1973; Grant et al., 1974). Initially, therefore, we compared the cross-resistance of these mutants, in vivo, with the three drugs used for selection, and also to tubulosine, dehydroemetine, and cephaeline. The latter two compounds are emetine derivatives which are also effective inhibitors of protein synthesis (Grollman, 1966; Openshaw, 1970). For such studies, two representative cell lines from each selection (listed in the last column, Table I) were treated with emetine, tylocrebrine, cryptopleurine, tubulosine, dehydroemetine, and cephaeline, and survival curves were determined. From these curves, a D_{10} value (dose of drug resulting in 10% survival) was obtained, and these values are shown in Table II.

As may be expected, the Emt^R mutants are cross-resistant to the two emetine derivatives, dehydroemetine and cephaeline, but in addition the mutants are also cross-resistant to cryptopleurine, tylocrebrine, and tubulosine, which are structurally distinct from emetine. Furthermore, the extent of cross-resistance is related to the level of emetine resistance of the original mutant (cf. lines 3 and 4, Table II). The mutants resistant to tylocrebrine and cryptopleurine also exhibit cross-resistance to all the other drugs listed in Table II (lines 4 to 8). However, perhaps the most striking observation is that the levels of resistance to each drug are essentially characteristic of the drug, rather than whether the mutant is Emt^R, Tyl^R, or Cry^R. For example, comparison of one set of Emt^R, Tyl^R, and Cry^R mutants (lines 4, 6, and 8, Table II) shows that the level of resistance of *all* of them is 50- to 60-fold for emetine, 6- to 7-fold for tylocrebrine, 4- to 5-fold for cryptopleurine, 16- to 18-fold for dehydroemetine, 25- to 30-fold for tubulosine, and about 30-fold for cephaeline.

This striking similarity in the behavior of these mutants indicated that Emt^R, Tyl^R, and Cry^R mutants all probably carried the same primary lesion, and that the drugs were all acting in a similar way. The cross-resistance pattern also suggests that, if mutants resistant to tubulosine, cephaeline, or dehydroemetine were selected, they would also behave identically.

In our earlier work we showed that the lesion in Emt^R cells could also be demonstrated in vitro since protein synthesis in extracts of such cells was resistant to the inhibitory action of the drug (Gupta and Siminovitch, 1976, 1977). It was, therefore, of interest to determine whether extracts made from mutants resistant to one drug showed any cross-resistance to the other drugs. This was done using two Emt^R mutants (listed in Table I) of different degrees of resistance, and measuring the comparative effects of drugs such as, emetine, cryptopleurine, tylocrebrine, and tubulosine, as well as several other drugs which are known to affect protein synthesis in eukaryotes. In order to relate these data to the effects of the drugs in vivo, we also simultaneously carried out dose-response curves for all the drugs. The in vivo and in vitro data for five of these drugs are shown in Figures 2 and 3.

It is clear from these results that protein synthesis in extracts of Emt^R cells is relatively resistant to cryptopleurine, tylocrebrine, and tubulosine, as well as emetine, but is not resistant to cycloheximide. Furthermore, the degree of resistance in vitro (Figure 2) is related to the degree of resistance in vivo (Figure 3), although the concentrations required for an equivalent effect in vivo and in vitro are somewhat different. This latter difference may be due to different degrees of accumulation of these drugs inside the cell (Grollman, 1968). The results from Figures 2 and 3, plus additional data for other drugs, are compiled in Table III for one of the Emt^R mutants, Pro$^-$Emt^{R}41-4. The in vitro resistance is expressed by an I_{50}

TABLE III: Resistance of Wild-Type and Emt^R Mutant Cell Lines to Various Inhibitors of Protein Synthesis, in Vivo and in Vitro.

Inhibitors	In vivo resistance D_{10} values (M)		Degree of resistance	Resistance in extracts I_{50} values (M)		Degree of resistance
	Wild type	Emt^R mutant		Wild type	Emt^R mutant	
Emetine	3.1×10^{-8}	2.0×10^{-6}	64.0	2.4×10^{-6}	4.5×10^{-5}	19.0
Tylocrebrine	7.5×10^{-9}	4.5×10^{-8}	6.0	4.0×10^{-7}	1.8×10^{-6}	4.5
Cryptopleurine	2.1×10^{-9}	9.0×10^{-9}	4.3	3.3×10^{-7}	6.6×10^{-7}	2.0
Tubulosine	1.4×10^{-7}	4.2×10^{-6}	30.0	4.5×10^{-7}	4.4×10^{-6}	10.0
Cephaeline	2.2×10^{-8}	6.0×10^{-7}	27.0	1.4×10^{-6}	2.0×10^{-5}	14.0
Dehydroemetine	4.1×10^{-8}	7.0×10^{-7}	17.0	1.3×10^{-6}	4.0×10^{-5}	13.3
Pactamycin	2.0×10^{-7}	1.9×10^{-7}	1.0	1.5×10^{-7}	1.2×10^{-7}	0.8
Cycloheximide	4.5×10^{-7}	4.5×10^{-7}	1.0	3.5×10^{-6}	3.8×10^{-6}	1.1
Trichodermin	3.5×10^{-7}	3.6×10^{-7}	1.0	9.0×10^{-7}	1.0×10^{-6}	1.0
Anisomycin	6.0×10^{-8}	3.0×10^{-8}	0.5	4.0×10^{-7}	2.2×10^{-7}	0.55
Sparsomycin	1.0×10^{-6}	8.0×10^{-7}	0.8	5.0×10^{-6}	3.6×10^{-6}	0.70

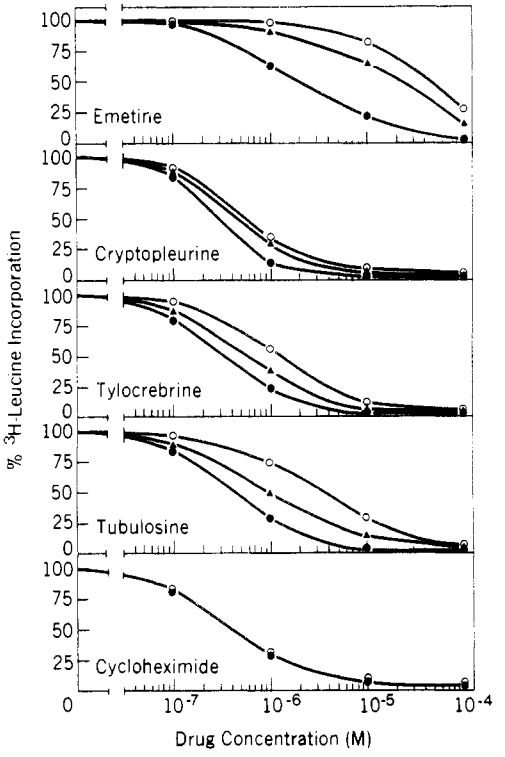

FIGURE 2: Effect of various inhibitors of protein synthesis on [^{3}H]leucine incorporation in the extracts of wild-type and Emt^R mutant cells. (●—●) Pro$^-$ (WT); (▲—▲) Pro$^-$Emt^{R}76; (○—○) Pro$^-$Emt^{R}41-4.

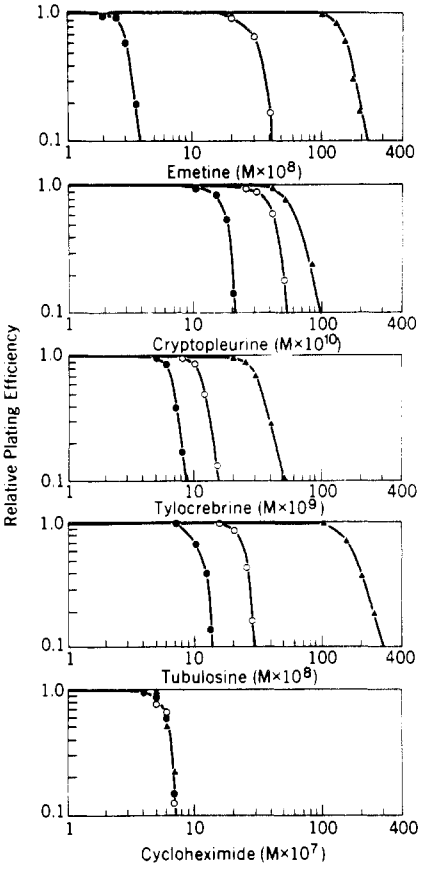

FIGURE 3: Dose-response curves of wild type cells and two Emt^R mutant cells in presence of increasing concentrations of various inhibitors of protein synthesis. Pro$^-$ (WT) (●—●); Pro$^-$Emt^{R}76 (○—○); Pro$^-$Emt^{R}41-4 (▲—▲).

value, that is, the concentration of drug required for a 50% inhibition of protein synthesis, and the in vivo resistance is given by a D_{10} value, the dose of drug required to reduce survival of cells to the 10% level, measured from a survival curve. Several conclusions can be derived from these data. (1) As indicated earlier, Emt^R mutants are cross-resistant to tylocrebrine, cryptopleurine, and tubulosine as well as to the two emetine derivatives, cephaeline and dehydroemetine, all of which are inhibitors of peptide chain elongation (Grollman and Jarkovsky, 1974; Pestka, 1971; Battaner and Vazquez, 1971). Of this group of inhibitors, emetine, cryptopleurine, and tylocrebrine are known to act on the 40S ribosomal subunit (Gupta and Siminovitch, 1977; Grant et al., 1974). (2) The fact that the cross-resistance is observed in vitro, as well as in vivo, rules out any trivial explanation for the pleiotropy, such as cell permeability (Bech-Hansen et al., 1976). (e) Cross-resistance is not observed, either in vivo or in vitro, for several other known inhibitors of protein synthesis, such as cycloheximide, anisomycin, trichodermin, sparsomycin, and pactamycin. The former four compounds are believed to act on the 60S ribosomal subunit (Rao and Grollman, 1967; Pestka, 1971; Schindler et al., 1974; Jimenez et al., 1975), whereas pactamycin acts on the 40S ribosomal subunit and is an inhibitor of peptide chain initiation (Goldberg et al., 1973).

Complementation Analysis. The pattern of cross-resistance observed for emetine, cryptopleurine, tylocrebrine, tubulosine, dehydroemetine, and cephaeline indicates that all these inhibitors probably act at the same site and that Emt^R, Tyl^R, and Cry^R mutants, therefore, bear lesions in the same gene. Since

TABLE IV: Complementation Analysis of Emt^R, Tyl^R, and Cry^R Mutants in Somatic Cell Hybrids.

Cell lines[a]	D_{10} values for various inhibitors (M)		
	Emetine $\times 10^{-8}$	Cryptopleurine $\times 10^{-9}$	Tylocrebrine $\times 10^{-9}$
Pro$^-$Emt^S × Gat$^-$Emt^S	3.8	2.0	7.5
Pro$^-$Emt^{R}41-4 × Gat$^-$Emt^S	5.5	2.1	8.0
Pro$^-$Emt^{R}41-4 × Gat$^-$Tyl^{R}7	180.0	9.7	42.0
Pro$^-$Cry^{R}1 × Gat$^-$Tyl^{R}7	170.0	9.8	40.0
Pro$^-$Cry^{R}1 × Gat$^-$Emt^{R}57	170.0	9.5	42.0

[a] The resistance levels of the parental cell lines used in these crosses for emetine, tylocrebrine, and cryptopleurine are given in Table II. The cell line Gat$^-$Emt^{R}57 is similar to Pro$^-$Emt^{R}41-4 in its levels of resistance.

Emt^R cells, as well as Cry^R and Tyl^R cells, behave recessively in somatic cell hybrids (Gupta and Siminovitch, 1977; Gupta and Siminovitch, unpublished observations), it was possible to test this supposition by complementation analysis.

Hybrids were constructed between Emt^R × Cry^R, Emt^R × Tyl^R, and Tyl^R × Cry^R cells by using appropriately marked auxotrophic lines, and selecting for complementation between the latter markers. The hybrid lines were then tested for their resistance to the three drugs, the results of which are shown in Table IV. As may be seen, all the hybrids are about as resistant to the drugs as the parental lines. The fact that these mutants are unable to complement each other strongly supports the notion that mutation in each case lies in the same gene.

Discussion

The studies described in this paper indicate that several compounds, namely, emetine, cryptopleurine, tylocrebrine, tubulosine, and two emetine derivatives dehydroemetine and cephaeline, which inhibit protein synthesis in animal cells probably all act at the same site in the 40S ribosomal subunit. This conclusion is based on the identical cross-resistance patterns of independently isolated Emt^R, Tyl^R, and Cry^R mutants to the above compounds, in vivo and in vitro, and from the failure of the above mutants to complement each other in somatic cell hybrids. As might be expected, in the cases where resistant mutants have been obtained, the spontaneous and mutagen-induced frequencies of the mutations are very similar (Table I).

The genetic and biochemical data presented in this paper suggested that there should be a molecular basis for the observed similarities in the behavior of these alkaloids, and we have therefore compared their chemical structures. This examination has revealed that these seemingly dissimilar compounds, e.g., emetine, cryptopleurine, tylocrebrine, and tubulosine, do have some structural features in common (Figure 4, darkened structures). It seems likely that it is this structure which might constitute the active determinant of these compounds and is responsible for their biological activity. The mutants in each case would then be selected against the same common determinant and hence would be identical. It is, of course, possible that the structure of the remainder of the molecule might modify, sterically hinder, or even abolish the

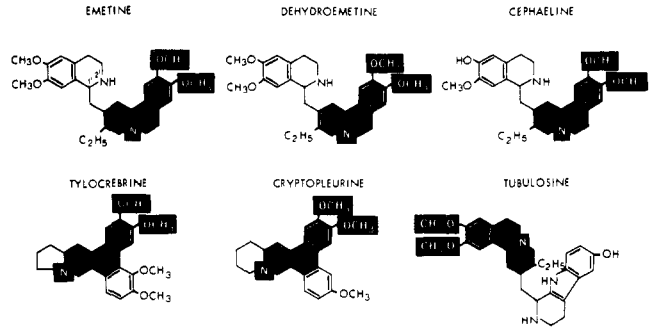

FIGURE 4: Structural formulas of emetine and emetine-like inhibitors of protein synthesis.

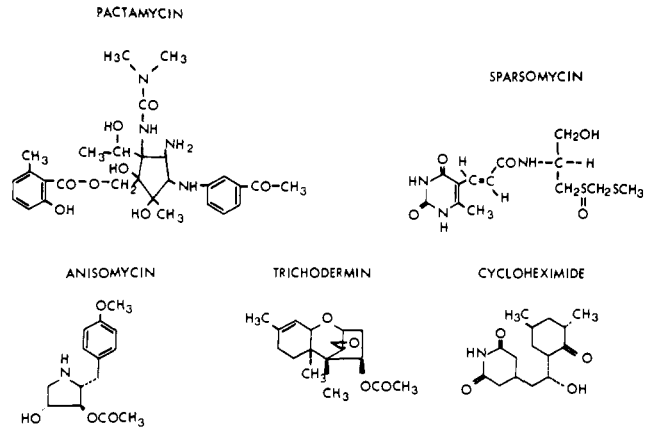

FIGURE 5: Structural formulas of various other inhibitors of protein synthesis.

action of the active moiety. Such modifications may account for the different effectiveness of the above compounds (Figures 2 and 3 and Table III), and for the inactivity of the compounds such as isoemetine, *O*-methylpsychotrine, and *N*-methylemetine as inhibitors of protein synthesis (Grollman and Jarkovsky, 1974).

It is also of interest that inhibitors of protein synthesis which do not show cross-resistance with the emetine-like compounds, and which, therefore, most probably act at different sites, show very limited structural similarities with those componds (Figure 5). Cycloheximide is a particularly interesting example, since several years ago, based on structural considerations, Grollman (1966, 1977) proposed that this compound, emetine, and tubulosine acted in the same way. However, this conclusion is clearly incorrect, based on several lines of evidence: (i) Emt^R mutants are not cross-resistant to cycloheximide either in vivo or in vitro; (ii) the structural features which appear to be important for the activity of emetine and tubulosine are not present in cycloheximide; (iii) work in yeast indicates that cycloheximide acts on the 60S ribosomal subunit, whereas emetine acts at the 40S level (Rao and Grollman, 1967; Gupta and Siminovitch, 1977); (iv) cycloheximide inhibits both peptide chain initiation and elongation steps (Baliga et al., 1969; Obrig et al., 1971; Pestka, 1971); its effect on the latter step is presumably due to inhibition of peptidyl transferase activity (Pestka et al., 1972; Schneider and Maxwell, 1973). In contrast, emetine and tubulosine specifically inhibit only the elongation step by preventing ribosome translocation along mRNA and have no effect on peptidyl transferase activity (Grollman and Jarkovsky, 1974; Vazquez, 1974; Carrasco et al., 1976); and (v) in contrast to the ease of isolation of Emt^R mutants in CHO cells, we have failed to obtain a

stable cycloheximide resistant mutant in spite of repeated attempts (Gupta and Siminovitch, unpublished observations).

Emetine and related compounds have long been used as a specific remedy for treatment of ameobic dysentery in man, and also for treatment of several viral diseases such as herpes zoster and viral hepatitis (Rogers, 1912; Del Puerto et al., 1968). Such compounds have also proved useful for the treatment of cancer in man and experimental animals (Lewisohn, 1918; Abd-Rabbo, 1969; Panettiere and Coltman, 1971; Jondorf et al., 1971). However, the chemotherapeutic usefulness of emetine is somewhat limited due to its secondary effects such as cardiotoxicity and muscle weakness (Klatskin and Friedman, 1948; Duane and Engel, 1970). It is possible that, while the therapeutic effects of emetine are due to its effect on protein synthesis (Grollman and Jarkovsky, 1974), the adverse effects of emetine might be due to other effects of the molecule. In addition to compounds described in this paper there are several other compounds, such as tylophorine, deoxytubulosine, alangicine, and dihydroprotoemetine, which probably possess the active structural moiety of emetine-like compounds and, hence, might be inhibitors of protein synthesis (Openshaw, 1970; Glasby, 1975). It seems plausible, therefore, that among these drugs some might be effective therapeutically, but may have limited or no side effects.

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