level in the nerve fibers. Nerves placed in media containing 35 to 125 m*M* $Ca^{2+}$ were protected against an amount of BTX adequate to block fast axoplasmic transport (Fig. 3). Similarly high concentrations of $Mg^{2+}$ did not interfere with the BTX block of fast axoplasmic transport, a result suggesting some specific role for $Ca^{2+}$.

These studies do not discriminate between an effect of BTX on some membrane component to which $Ca^{2+}$ is also bound (*14*) and an action of BTX (with which $Ca^{2+}$ interferes) on the axoplasmic transport mechanism within the nerve fiber. An action of BTX within the fiber is indicated by the small decreases of $\sim$P levels at BTX concentrations effective in blocking transport, and a block of transport on the basis of a direct action of BTX on membrane excitability is excluded in that other agents that block membrane excitability do not block axoplasmic transport (*2, 3*). However, an indirect effect of BTX on the membrane, in turn affecting the axoplasmic transport mechanism, also remains a possibility. Further studies should help resolve this point.

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15 October 1974

## Homologous Cysteine-Containing Sequences in Tryptophanyl-tRNA Synthetases from Escherichia coli and Human Placentas

Abstract. *The sequence Leu-Ala-Cys-Gly-Ile-Asx-Glx in a nonapeptide isolated from the tryptophanyl-tRNA synthetase of* Escherichia coli *B is homologous to the sequence Ile-Ala-Cys-Gly-Phe-Asx-Asx in a decapeptide isolated from the tryptophanyl-tRNA synthetase of human placenta. So far no homologies have been found between cysteine-containing peptides of aminoacyl-tRNA synthetases with different amino acid specificities.*

The aminoacyl-tRNA synthetases comprise a family of enzymes required to recognize specific amino acids and cognate transfer RNA's (tRNA's). The tRNA's are gene products uniform in size, in secondary structure, and probably in tertiary structure (*1*) over an evolutionary range from bacteria to mammals. The necessary coordinate evolution of a synthetase and its tRNA substrate should cause synthetases evolved from a common ancestor to preserve essential structural features. Although the aminoacyl-tRNA synthetases present an array of quaternary structures ($\alpha_1$, $\alpha_2$, $\alpha_4$, $\alpha\beta$, $\alpha_2\beta_2$) with protomer molecular weights ranging from 33,000 to 114,000 (*2*), those specific for the same amino acid generally have similar protomer sizes and quaternary structures.

The tryptophanyl-tRNA synthetases have been purified to homogeneity from *Bacillus stearothermophilus* (*3*), *Escherichia coli* (*4*), yeast (*5*), beef pancreas (*6*), water buffalo brain (*7*), and human placenta (*8*). With one possible exception (*7*), they consist of two identical subunits, which range from a low molecular weight of 35,000 for the enzyme from *B. stearothermophilus* to a higher molecular weight of 58,000 for both the bovine (*6*) and human enzymes (*8*). Not surprisingly, the bovine and human tryptophanyl-tRNA synthetases are also similar in amino acid compositions, tryptic peptide maps (*9*), and interactions with tryptophan tRNA ($tRNA^{Trp}$) of yeast and *E. coli* (*10*), whereas the *E. coli* tryptophanyl-tRNA synthetase, molecular weight 74,000, has a different amino acid composition (*4*) and pattern of interaction with $tRNA^{Trp}$ of yeast and of *E. coli* (*8*). However, the bovine enzyme can be cleaved to an active $\alpha_2$ structure of molecular weight 82,000 (*11*), which approaches more closely the composition of the *E. coli* enzyme. Therefore, it is likely that a portion of the bovine (or human) enzyme resembles the *E. coli* enzyme more closely than is ap-

Table 1. Recoveries, $NH_2$-terminal amino acids, and compositions of three thiol peptides purified from *Escherichia coli* and two thiol peptides from human placental tryptophanyl-tRNA synthetases. The values in parentheses are the whole numbers assigned.

| Amino acid residue | Composition (moles of amino acid per mole of peptide) | | | | |
|---|---|---|---|---|---|
| | *Escherichia coli* | | | Human placenta | |
| | IIIA2 191 nmole* | IA91 428 nmole* | IA131 126 nmole* | TC2 180 nmole† | TC3 90 nmole† |
| Lysine | 0.0 | 1.0 (1) | 0.2 | 1.2 (1) | 1.1 (1) |
| Histidine | 0.2 | 0.1 | 0.8 (1) | 0.0 | 0.0 |
| Arginine | 0.9 (1) | 0.1 | 0.9 (1) | 0.2 | 0.2 |
| *S*-Carboxymethyl-cysteine | 0.5 (1) | 0.8 (1) | 0.7 (1)‡ | 0.4 (1) | 0.4 (1) |
| Aspartic acid or asparagine | 1.0 (1) | 1.1 (1) | 1.1 (1) | 2.9 (3)‡ | 2.2 (2) |
| Threonine | 0.9 (1) | 0.2 | 0.9 (1) | 0.1 | 1.1 (1) |
| Serine | 1.0 (1) | 0.3 | 0.3 | 0.2 | 1.1 (1) |
| Glutamic acid or glutamine | 1.2 (1) | 1.2 (1) | 1.1 (1) | 0.2 | 0.3 |
| Proline | 0.0 | 1.0 (1) | 0.0 | 0.0 | 0.0 |
| Glycine | 1.2 (1) | 1.1 (1) | 0.4 | 1.2 (1) | 3.0 (3)‡ |
| Alanine | 1.0 (1)‡ | 0.9 (1) | 1.0 (1) | 1.2 (1) | 0.2 |
| Valine | 0.2 | 0.3 | 1.6 (2) | 0.1 | 0.2 |
| Methionine | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Isoleucine | 0.2 | 0.9 (1) | 1.4 (2) | 2.1 (2) | 2.0 (2) |
| Leucine | 1.8 (2) | 1.0 (1)‡ | 0.1 | 0.0 | 0.0 |
| Tyrosine | 1.5 (2) | 0.0 | 0.0 | 0.0 | 0.0 |
| Phenylalanine | 0.8 (1) | 0.1 | 0.0 | 1.0 (1) | 2.0 (2) |

* The recovery from 1350 nmole of peptide. † The recovery from 500 nmole of peptide. ‡ The amino acid at the $NH_2$-terminal of the peptide.

parent from native molecular weights and compositions. Homologous sequences in the *E. coli* and human tryptophanyl-tRNA synthetases would be particularly significant because of the wide evolutionary range they represent.

Because thiol reagents inactivate both enzymes, thiols have been implicated in substrate binding, catalysis, or structural requirements; therefore we decided to sequence cysteine-containing peptides. The *E. coli* tryptophanyl-tRNA synthetase has three or four cysteine residues per subunit and no disulfide bonds (*12*), and the human tryptophanyl-tRNA synthetase has six cysteine residues per subunit.

By methods (*4, 8*) modified slightly for larger amounts of starting material, 70 mg of *E. coli* B tryptophanyl-tRNA synthetase and 46 mg of human placental tryptophanyl-tRNA synthetase were prepared. Human tryptophanyl-tRNA synthetase (35 mg) and *E. coli* tryptophanyl-tRNA synthetase (50 mg) were denatured in 8*M* urea and 5.3*M* guanidine hydrochloride, respectively, reduced with dithiothreitol, and carboxymethylated (*13*) with [$^{14}$C]iodoacetic acid (New England Nuclear; 2,800 to 10,000 count min$^{-1}$ nmole$^{-1}$). After dialysis and freeze-drying, the carboxymethylated proteins (representing 1350 nmole of *E. coli* tryptophanyl-tRNA synthetase subunit and 500 nmole of human tryptophanyl-tRNA synthetase subunit) were dissolved and digested with trypsin, the final ratio of trypsin to substrate being for *E. coli* 1 : 25 (by weight), or for humans 1 : 10; the digestion was effected in 4 hours at *p*H 8.4, at 23° or 37°C with trypsin (Worthington) treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone to inhibit contaminant chymotryptic activity (*14*). The digests were applied to Sephadex G-25 superfine (Pharmacia) columns equilibrated with 0.1*M* ammonium bicarbonate buffer, *p*H 8.1. Appropriate radioactive fractions were pooled, concentrated, and applied to a column (0.9 by 23 cm) of Aminex A-5 (Bio-Rad) resin and developed at 55°C, at 200 to 300 pounds per square inch, with a *p*H (2.5 to 5.0) and concentration gradient of pyridine acetate buffer (0.05 to 2*M* in acetate) (*15*). Appropriate radioactive fractions were pooled and concentrated. Three peptides from *E. coli* tryptophanyl-tRNA synthetase and peptide TC2 from human tryptophanyl-tRNA synthetase were pure enough to be sequenced, as shown by $NH_2$-terminal analysis (*16*) and by composition (*17*) (Table 1). Peptide TC3 from the human enzyme was further purified by ascending chromatography on a cellulose thin layer developed with a butanol, pyridine, acetic acid, water system (15 : 10 : 3 : 12) to give the purity shown in Table 1.

The peptides were dansylated (*16*) and sequenced by the Edman procedure (*18*). The dansylated amino acids were chromatographed with the solvents (*19*) on polyamide thin layers, with dansylated amino acid standards (Fig. 1). The assignments of asparagine in the third position and of glutamic acid in the tenth position of peptide IIIA2 were made according to electrophoretic mobilities and molecular weights of the whole peptide and of the residual COOH-terminal tetrapeptide (*20*). All dansyl-Edman degradations were carried through to the direct determination of the COOH-terminal residue. There were no ambiguities, and the sequences (Fig. 1) agree with the compositions (Table 1). The Ile-Ile (*21*) in peptide TC2 and the Ile-Phe in peptide TC3 were partially resistant to hydrolysis, and both dansylated dipeptides were identified.

As indicated in Fig. 1, peptide TC2 from human tryptophanyl-tRNA synthetase and peptide IA91 from *E. coli* tryptophanyl-tRNA synthetase share an

| Enzyme | Peptide | Sequence |
|---|---|---|
| Human tryptophanyl-tRNA synthetase | TC3 | Gly-Ile-Phe-Gly-Phe-Thr-Asx-Ser-Asx-Cys-Ile-Gly-Lys |
| | TC2 | Asx-Ile-Ile-Ala-Cys-Gly-Phe-Asx-Asx-Lys |
| *E. coli* tryptophanyl-tRNA synthetase | IA91 | Leu-Ala-Cys-Gly-Ile-Asx-Glx-Pro-Lys |
| | IA131 | Cys-Ile-Val-Asx-Glx-His-Ala-Ile-Thr-Val-Arg |
| | IIIA2 | Ala-Leu-Asn-Cys-Tyr-Thr-Tyr-Phe-Gly-Glu-Leu-Ser-Arg |
| *E. coli* methionyl-tRNA synthetase (*23*) | | Gly-Pro-Cys-Trp-Lys |
| | | Asn-Ala-Cys-Leu-Gly-Ala-Ile-Asn-Ser-Met |
| | | Asn-Leu-Cys-Asp-Lys |
| | | Ile-Leu-Val-Thr-Cys-Ala-Leu-Pro-Tyr-Ala-Arg |
| | | Gly-His-Glu-Val-Asp-Phe-Ile-Cys-Ala-Asp-Asp-Ala-His-Gly-Thr-Pro-Ile-Met |
| *E. coli* isoleucyl-tRNA synthetase (*24*) | IV/4 | Gly-Leu-Ser-Gly-Tyr-Asx-Ser(Cys-Ser) |
| | V/3 | Cys-Val-Ser-Asx-Val-Ala-Gly-Asx-Gly-Glx-Lys |
| | X/2 | Met-Glx-His-Ser-Tyr-Pro-Cys-Cys-Trp-Arg |
| | X/1 | Cys-Trp-His-Tyr-Thr-Glx-Asx-Val-Gly-Lys |
| | VI/64 | Val-Ala-Glx-His-Ala-Glx-Ile-Cys-Gly-Arg |
| | IX/8 | Ala-Val-Gly-Cys-Ala-Lys |
| | IX/9 | Cys-Pro-Arg |
| | N/56 | Ile-Glu-Ser-Met-Val-Ala-Asp-Arg-Pro-Asn-Trp-Cys-Ile-Ser-Arg |

Fig. 1. Known thiol peptide sequences in aminoacyl-tRNA synthetases.

Ala-Cys-Gly sequence. The homologous region becomes a heptapeptide by inclusion of the amino acid residues in the box in Fig. 1. In every case the amino acid replacements in the heptapeptides are possible by a single base change in a codon and are those found most frequently in families of homologous proteins (*22*). We were struck to discover such related peptides at the outset of our sequencing, covering only 56 residues in the two enzymes. No homology of similar extent exists among any of the other cysteine-containing peptides in Fig. 1, showing all those so far published for any aminoacyl-tRNA synthetase (*23, 24*). Since all of the cysteine-containing tryptic peptides of *E. coli* isoleucyl-tRNA synthetase are known, the lack of homology with any cysteine sequence in that enzyme is certain.

The homologous region may be more extensive. If the proline residue next to the COOH-terminus in peptide IA91 has been deleted during evolution of peptide TC2, then the COOH-terminal lysine residues of peptides IA91 and TC2 correspond, and the homologous region may extend from these lysine residues. Moreover, the homologous region may extend from the $NH_2$-terminals, since the isoleucine residue next to the $NH_2$-terminus of peptide TC2 may represent a replacement allowable by a single base change in a codon of lysine, which may be the COOH-terminal residue of the tryptic peptide preceding IA91. The ultimate extent and role of the homologous region and whether other cognate series of aminoacyl-tRNA synthetases have homologies remain to be determined.

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16 October 1974

## Chromosome-Wide Event Accompanies the Expression of Recessive Mutations in Tetraploid Cells

Abstract. *Mutants resistant to 6-thioguanine were induced in pseudotetraploid hybrid Chinese hamster cells homozygous wild-type at the locus for hypoxanthine phophoribosyl transferase but heterozygous for the linked marker glucose-6-phosphate dehydrogenase. About half of these mutants had concomitantly lost the wild-type allele for glucose-6-phosphate dehydrogenase, as expected if mutation plus chromosome segregation had occurred.*

The interpretation that variant clones of cultured animal cells arise from mutations is complicated by the fact that the cells of higher organisms can also give rise to somatically heritable phenotypic changes by epigenetic processes. The latter are presumed to underlie cellular differentiation. If mutation in cultured cells is to be studied as a phenomenon per se, and if it is to be used as a tool to illuminate cell function, it is important to be able to distinguish mutation from epigenetic processes in any particular system.

There are now several systems in which the demonstration of an altered gene product in variant cells provides strong evidence for a mutational event (*1*). In most of these systems, a constitutive and ubiquitous enzyme has been studied, which makes it less likely that variants would arise epigenetically. However, in several of the same or similar systems, predictions of variant frequencies as a function of gene dosage, made on the assumption that purely mutational events were occurring, have not always been borne out. Thus Harris (*2*) found no difference in the spontaneous mutation rate to 8-azaguanine resistance in diploid, tetraploid, and octaploid Chinese hamster cells, and Mezger-Freed (*3*) found no difference between haploid and diploid frog cells in the frequency of 5-bromodeoxyuridine–resistant variants. Resistance to each of these drugs is usually associated with a recessive loss in enzyme activity (*4–6*), although this point was not tested in the experiments cited. In the case of recessive mutations, it should be necessary to mutate each copy of the gene in question before a mutant phenotype can be expressed and recognized. If each mutation is an independent event, the mutant frequency should be an exponential function of the gene dosage; for example, the frequency with two genes should be approximately the square of the frequency in the single gene case. The failure of this prediction has led to the idea that mutation is not operating in these systems (*2, 3*).

We have previously examined this question, comparing mutation frequencies induced by ethyl methanesulfonate (EMS) in (pseudo) diploid and tetraploid Chinese hamster ovary (CHO) cells (*7*). Mutants resistant to 6-thioguanine (TG) were induced at a frequency of $2.5 \times 10^{-4}$ in diploid cells, while in tetraploids the figure was $0.9 \times 10^{-5}$. Drug resistance was shown to be recessive and associated with the loss of hypoxanthine phosphoribosyltransferase (HPRT) activity. Since the gene specifying this enzyme is X-linked in humans (*8*) and probably also in Chinese hamster cells (*9, 10*) there is presumably only one active gene in diploid cells and two active genes in tetraploids (*11*). While the 25-fold difference was highly