Selective Synthesis of Mitochondrial Proteins by Chinese Hamster Ovary Cells Severely Starved for Various Amino Acids

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ABSTRACT Chinese hamster ovary (CHO) cells were subjected to severe amino acid starvation for histidine, leucine, methionine, asparagine, tyrosine, glutamine, valine, and lysine, using amino acid analogs or mutations in specific aminoacyl-tRNA synthetases. At protein synthetic rates of <5%, in all cases, the newly synthesized proteins were found on two-dimensional electrophoretic gels to consist of a few intensely labeled spots, with the exception of lysine. This pattern could also be produced by strong inhibition of cytoplasmic protein synthesis with cycloheximide, and was abolished by preincubation with the mitochondrial protein synthesis inhibitor chloramphenicol. It appears therefore that the spots represent mitochondrial protein synthesis and that animal cells must have separate aminoacyl-tRNA synthetases for mitochondrial tRNAs corresponding to all these amino acids except, possibly, for lysine.

The protein synthetic machinery of mitochondria in animal cells is known to be markedly different in nature from that of the cellular cytoplasm. The DNA of these organelles has been shown to determine smaller procaryotic-like ribosomes and a complement of distinct transfer RNA (tRNA) species sufficient for translation of all mitochondrial messenger RNA (mRNA) (1). The limited size of the mitochondrial genome precludes inclusion of genes for all of the elements of the mitochondrial protein synthetic machinery, however, and evidence has been presented for the existence of host nuclear genes specific for some of these elements. Wallace et al. (2), for example, have shown that Chinese hamster ovary (CHO) cells have a distinct, presumably nuclear-coded, leucyl-tRNA synthetase for mitochondria. We present evidence that this is also the case for histidyl-, methionyl-, asparagyl-, tyrosyl-, glutamyl-, and valyl-tRNA synthetase, but not for lysyl-tRNA synthetase.

MATERIALS AND METHODS
Wild-type CHO cells (Pro CHOwt) and temperature-sensitive (ts) aminoacyl-tRNA synthetase mutants of these cells for leucine (L1 H1), asparagine (Asn7), glutamine (Gln1), and methionine (Met1) were cultured in suspension as described previously (3), while those for valine (S81), lysine (Lys101), and histidine (D10-5) were cultured on plastic surfaces (4). The origins of the cell lines are given in Thompson et al. (5, 6). Wild-type CHO cells were starved for histidine (or tyrosine) by incubation at 34°C for 30 min in medium lacking histidine (or tyrosine) and containing the analog histidinol at 5 mM (or tyrosinol at 500 µg/ml), while the mutants were starved for their amino acids by incubation at 34°C for 30 min in media lacking the relevant amino acid followed by 30-min incubation at nonpermissive temperatures (42°C for 5 min, then 39.5°C for 25 min). Details of these procedures have been given previously (3). All these media also lacked methionine and, after the above preincubation, [35S]methionine (Amersham Corp., Arlington Heights, IL; >500 Ci/mM) was added to 300 µCi/ml and the incubation continued for a further 30 min. Rates of protein synthesis were measured by the incorporation of a mixture of [3H]-phenylalanine, tyrosine, and threonine using parallel cultures treated identically, as described previously (3). After labeling with [35S]methionine, about 10⁶ cells were cooled, centrifuged, and resuspended in 100 µl of sonication buffer (7) containing DNAse (50 pg/ml) and RNAse (50 µg/ml). After sonication, the samples were placed on ice for 5 min, then prepared for two-dimensional (2-D) electrophoretic gel analysis (7). Electrophoresis in the first dimension was carried out over a pH range of 4.2-6.8 for 7,400 volt-hours, while size separation in the second dimension involved a 10% polyacrylamide slab gel. Newly synthesized proteins were detected by exposure to Kodak XR-1 x-ray film.

RESULTS AND DISCUSSION
As discussed previously (4), effective starvation of animal cells for amino acids requires the use of amino acid analogs or of mutations in aminoacyl-tRNA synthetases. These are now available for a variety of amino acids (5, 6). During studies on errors in protein synthesis induced by amino acid starva-
tion using these analogs and mutants (3, 4, 8), we observed a common residual pattern of protein synthesis on 2-D gels as the rate of protein synthesis was reduced <5% (Fig. 1). This pattern consisted of a diffuse spot with a molecular weight of about 35,000, and a complex group of three to four spots with molecular weights of about 20,000 (Fig. 1). This molecular weight pattern agrees approximately with that observed for rat hepatoma mitochondria translation products by Kolarov et al. (9). These workers obtained five prominent bands on one-dimensional SDS electrophoretic gels: a diffuse band at ~39,000 mol wt and bands at 23,000 and 21,000 mol wt corresponding to the three subunits of cytochrome oxidase; a band at 21,000 mol wt corresponding to a subunit of the ATPase; a band at 32,000 mol wt corresponding to a subunit of the cytochrome b-c1 complex, and an unassigned band of relatively high molecular weight. Our failure to detect the latter two bands could possibly be due to their having isoelectric points out of the pH range of our gel system. Stronger evidence for the mitochondrial nature of these spots was obtained by the use of specific inhibitors: cycloheximide for protein synthesis by cytoplasmic ribosomes, and chloramphenicol for protein synthesis by mitochondrial ribosomes. Cycloheximide treatment of wild-type CHO cells gave the same pattern (Fig. 1 C) and chloramphenicol treatment of cultures showing the pattern abolished it, leaving blank gel patterns (Fig. 1 G). Also, Wallace et al. (2) showed that, for the is leucyl-tRNA synthetase mutant used here, proteins synthesized under extreme nonpermissive conditions were found in isolated mitochondria, and protein synthesis by isolated mitochondria was not inhibited by these conditions. We conclude that the pattern represents proteins synthesized by mitochondrial ribosomes.

When the ratio of radioactivity applied to the gels was the same as the ratio of the rates of protein synthesis, the mitochondrial spots were more intense than any spots seen in the same positions on the control gels (Fig. 1). Also, these spots became even more intense as the starvation was tightened (Fig. 1 H). Thus, if [35S]methionine incorporation into mito-
Chondria can be equated to rates of protein synthesis, this would indicate that amino acid starvation results in an increased rate of mitochondrial protein synthesis.

This same mitochondrial pattern was obtained when protein synthesis was inhibited using aminoacyl-tRNA synthetase mutants for leucine (Fig. 1E), glutamine (Fig. 1D), methionine (not shown), valine (not shown), histidine (not shown), and asparagine (not shown). The mutant for lysine, on the other hand, gave an attenuated but normal pattern of newly synthesized proteins at all levels of inhibition (Fig. 1B). Since the aminoacyl-tRNA pools in mitochondria are, by calculation, sufficient for less than a minute of protein synthesis at normal rates, the mitochondria must have some means of maintaining normal aminoacyl-tRNA levels under starvation conditions. We interpret these results as evidence for the existence of separate genes, presumably nuclear, coding for non-ts mitochondrial enzymes for all of these amino acids, except for lysine. The mitochondrial pattern of residual protein synthesis was also observed when protein synthesis was inhibited with the amino acid analogs histidinol (Fig. 1F) and tyrosinol (not shown). This could be due to different affinities of the mitochondrial enzymes relative to the cytoplasmic enzymes for the analogs or to failure of the analogs to penetrate the mitochondrial membrane.

These results are consistent with the view that the distinct mitochondrial tRNA species coded for by the mitochondrial genome require aminoacyl-tRNA synthetases with distinct specificities. Another viewpoint is that mitochondrial specificity is dictated by the necessity for selective intracellular transport (10); both requirements could, of course, be operative. It will be interesting to see whether lysyl-tRNA synthetase is the only exception to this rule, as new animal cell mutants for further aminoacyl-tRNA synthetases become available. How nuclear genes evolved for what appears to be a symbiotic organelle remains a fascinating problem.

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