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Biochemistry

Adrenocorticotropic hormone increases specific proteins of the mitochondrial fraction that are translated inside or outside this organelle in cultured adrenal tumor cells

(mitochondrial translation products/*in vitro* protein synthesis/cell culture/densitometry)

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ABSTRACT **In addition to its stimulatory effects on steroidogenesis, adrenocorticotropic hormone (ACTH) also has a trophic action on the adrenal cell. This is manifested in part by increases in the levels of key mitochondrial steroidogenic enzymes. The mechanism by which this trophic action of ACTH occurs has been studied in monolayer cultures of mouse adrenal cortical tumor cells. ACTH treatment of these cells stimulates the relative incorporation of amino acids into at least eight specific proteins in mitochondrial preparations. Two of these ACTH-responsive proteins are among the nine major adrenal polypeptides that fulfill the criteria of mitochondrial translation products: (*i*) their synthesis in intact cells is specifically resistant to inhibition by cycloheximide yet uniquely sensitive to chloramphenicol and (*ii*) they are synthesized *in vitro* by isolated mitochondria. The other six ACTH-responsive proteins are within the much larger category of mitochondrial proteins that are synthesized on cytoplasmic ribosomes. One of the proteins synthesized in the cytoplasm electrophoretically comigrates with purified beef adrenodoxin reductase and another with beef adrenodoxin. These findings indicate that ACTH regulates the synthesis (and turnover, or both) of specific mitochondrial proteins that are synthesized inside as well as outside the mitochondria of these adrenal cells.**

Mitochondria of the adrenal cell have the unique capacity for steroid hydroxylation essential to the steroidogenic process. Adrenocorticotropic hormone (ACTH, corticotropin) is required by these cells *in vivo* and *in vitro* for the maintenance of mitochondrial morphology, size, and number (1–3). Our laboratory has previously demonstrated that incubation of monolayer cultures of mouse adrenal cortex tumor (Y-1) cells with ACTH for several successive days results in increases in steroidogenic capacity, mitochondrial cholesterol side-chain cleavage, 11-β-hydroxylation, and the mitochondrial content of cytochrome *P*-450 and adrenodoxin (4). ACTH also increases adrenodoxin reductase activity (5). ACTH had no significant effects on other mitochondrial respiratory cytochromes or on other mitochondrial enzymes, including cytochrome oxidase and ATPase (4). However, the biogenetic origins of mitochondrial enzymes involved in adrenal steroidogenesis and the mechanisms by which ACTH affects and maintains adrenal cell differentiation are not known (1, 5).

We now report studies that characterize the products of mitochondrial protein synthesis in differentiated adrenal cell cultures and demonstrate that incubation of these cells with ACTH specifically affects the incorporation of radiolabeled amino acids into specific proteins of the mitochondrial fraction, including some of the mitochondrial translation products.

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MATERIALS AND METHODS

Chemicals. Purified beef adrenal mitochondrial adrenodoxin and adrenodoxin reductase were kindly provided by Lowell Wilson. Horse serum, fetal calf serum, and minimal essential medium containing Earle's salts were purchased from GIBCO. Bovine serum albumin was obtained from Miles; the other protein molecular weight standards, L amino acids, and chloramphenicol (CAP) were obtained from Sigma. Cycloheximide (CHX) was purchased from Mann Research Laboratories, Orangeburg, New York. ACTH (Cortrosyn) was obtained from Organon and the L-[^{35}S]methionine was purchased from Amersham. Sodium dodecyl sulfate ($NaDodSO_4$) was obtained from BDH Laboratories, Poole, England. All other gel electrophoresis chemicals were purchased from Bio-Rad.

Cell Growth and Labeling. Adrenal tumors were grown as transplants in LAF-1 hybrid male mice. Finely minced tumor tissue or cultured cells were suspended in isotonic saline and injected subcutaneously into one thigh of a mouse. After 3 or 4 weeks, the tumors were excised, minced, trypsinized, and centrifuged as described (6). The cells were resuspended, plated in plastic culture dishes (60 × 15 mm), and grown in 3 ml of minimal essential medium containing 12.5% horse serum and 2.5% fetal calf serum at 37°C in 5% CO_2/95% air. Except where noted, cells were grown to confluence prior to the start of each experiment. ACTH was added as indicated in fresh medium at 24-hr intervals. High levels were used to overcome possible ACTH degradation during the lengthy incubation period. Control cultures received fresh medium without ACTH. The medium used to radiolabel protein contained decreased L-methionine (0.05 μg/ml) and, when appropriate, CAP (3.0 mg/ml). The cells were preincubated for 15 min at 37°C; [^{35}S]methionine was added to 56–108 μCi/ml (510 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and, when appropriate, CHX was added (1500 μg/ml). A high CHX concentration was used to ensure maximal inhibition of protein synthesis. The cultures were then incubated for 2 hr at 37°C. [^{35}S]Methionine incorporation into trichloroacetic acid-precipitatable protein is linear for up to 4 hr under these conditions. After 2 hr, the cultures were chilled on ice and excess nonradiolabeled methionine was added. All further manipulations were at 4°C.

Mitochondrial Preparation. The cells were washed with saline, removed from the plates, and rewashed in saline with centrifugation as described (7). The cells were swollen in 0.25 M mannitol/20 mM Tris·HCl/1 mM EDTA, pH 7.4, and lysed by homogenization with a tight-fitting glass homogenizer (7). Cell wall debris and nuclei were removed by centrifugation at 2500 rpm (755 × *g*) for 10 min in a Beckman J21B centrifuge.

Abbreviations: ACTH, adrenocorticotropic hormone (corticotropin); CAP, chloramphenicol; CHX, cycloheximide; U, unit.

The supernatant was then centrifuged at 10,000 × *g* for 20 min. The resultant supernatants were designated the cytoplasmic fraction. The crude mitochondrial pellets were washed once by resuspending in the mannitol/Tris/EDTA buffer and pelleted again by centrifugation. We have shown previously that this mouse adrenal tumor lacks microsomal *P*-450 and detectable cytochrome b_5, and cytoplasmic fractions lack cytochromes aa_3, *b*, and *P*-450 (4).

Labeling Isolated Mitochondria. A modification of the procedure described by Poyton and Groot (8) for labeling yeast mitochondrial protein *in vitro* was employed. We included 18 additional L amino acids (Ala, Arg, Asp, Cys, Glu, Gly, His, Tyr, Ile, Lys, Phe, Pro, Ser, Thr, Trp, Val, Gln, and Leu at 0.1 mg/ml each). Mitochondrial fractions were prepared as above and added at 1.7 mg/ml in a final volume of 0.5 ml. The reaction mixture was preincubated for 15 min at 37°C in a shaking water bath. CAP was added at 1.25 mg/ml prior to preincubation where appropriate. Labeling was initiated by the addition of [^{35}S]methionine (749 Ci/mmol) to 98.9 μCi/ml. After 60 min of incubation, the mitochondria were chilled on ice, centrifuged for 15 min at 10,000 × *g*, washed with 0.6 M mannitol and L-methionine at 0.1 mg/ml, and recentrifuged. The mitochondria were dissolved in NaOH for gel analysis as described below.

Mitochondrial Solubilization. Mitochondrial pellets were solubilized and prepared for protein, radioactivity, and NaDodSO$_4$/polyacrylamide slab gel electrophoretic analysis by two procedures utilizing either: (*i*) NaDodSO$_4$-containing buffer as described by Douglas and Butow (9); or (*ii*) NaOH by the procedure of Douglas *et al.* (10). In the latter procedure, the mitochondrial pellets were dissolved in 50 mM NaOH at 4°C for 1 hr and mixed into an equal volume of 2-fold concentrated NaDodSO$_4$ containing sample buffer to provide the appropriate concentrations of all buffer components prior to heating as described (10).

Radioactivity Measurements and Gel Electrophoresis. [^{35}S]Methionine incorporation was determined as trichloroacetic acid-precipitable radioactivity on Whatman GF/C filters as described (11). Samples were analyzed in a Searle model 880 scintillation counter. Samples (approximately 45 μg) of mitochondrial protein were subjected to electrophoresis on NaDodSO$_4$/polyacrylamide slab gels containing 10%, 12.5%, or 15% acrylamide according to the procedure described by Douglas *et al.* (12). The labeled peptides were visualized by staining with Coomassie brilliant blue and autoradiography on Kodak RP-2 x-ray film (12). Molecular weight values appearing in the figures are the known values of purified standards that were electrophoretically separated in parallel channels. They include: phosphorylase *a* (92,000), bovine serum albumin (68,000), pyruvate kinase (57,000), liver glutamate dehydrogenase (53,000), fumarase (49,000), liver alcohol dehydrogenase (41,000), glyceraldehyde phosphate dehydrogenase (36,000), chymotrypsinogen (23,000), and cytochrome *c* (13,400).

Densitometry. The x-ray films were scanned with a Schimadzu ES 910 thin-layer scanner at a wavelength of 550 nm. The relative intensities of the various protein bands on the x-ray film autoradiogram were proportional to the amount of radioactivity between instrumental absorbance values of 0.02 and 0.4. The area under each band was quantified by integration above the broken base lines (Figs. 4 and 5) during each scan with a Spectra-Physics Minigrator, and the ACTH-dependent change in relative specific radioactivity of each band, *i*, as a function of ACTH treatment was calculated as follows:

$$\text{Relative specific activity of band } i = \frac{\left(\frac{\text{Area of band } i}{\text{Area of all bands}}\right)_{\text{in scan of ACTH-treated specimen}}}{\left(\frac{\text{Area of band } i}{\text{Area of all bands}}\right)_{\text{in scan of control specimen}}}$$

Thus, the relative contribution of a given band to the total radioactivity in the ACTH-treated samples is compared to the relative contribution of the corresponding band to the total radioactivity in the control sample. These values reflect the *minimal* change in each protein, because the integration was performed from the broken base lines and does not attempt to correct for the presence of any less dominant bands that might have comigrated with the proteins of interest.

Protein and Steroid Determinations. Protein in cellular fractions or total cell protein per culture dish was measured by a modification of Lowry's procedure (13, 14). For whole dish determinations, cells were washed twice with saline solution, removed from dishes with a rubber policeman, and suspended in 10% trichloroacetic acid prior to assay. Steroids in the growth media were measured fluorometrically after extraction with methylene chloride as described (6).

RESULTS

Identification of Mitochondrial Translation Products. The effects of CHX, a specific inhibitor of cytoplasmic protein synthesis (9, 15, 16), were compared to those of CAP, a mitochondria-specific translation inhibitor (9, 15–18), during the incorporation of [^{35}S]methionine by cell cultures (Fig. 1 *Left*). CHX was found to inhibit total incorporation by 95% ± 1% (mean ± SD, n = 3). Autoradiograms of dried NaDodSO$_4$/polyacrylamide slab gels after electrophoresis revealed three major proteins labeled in the presence of CHX and, as ascertained from longer exposures of the x-ray films, six or seven

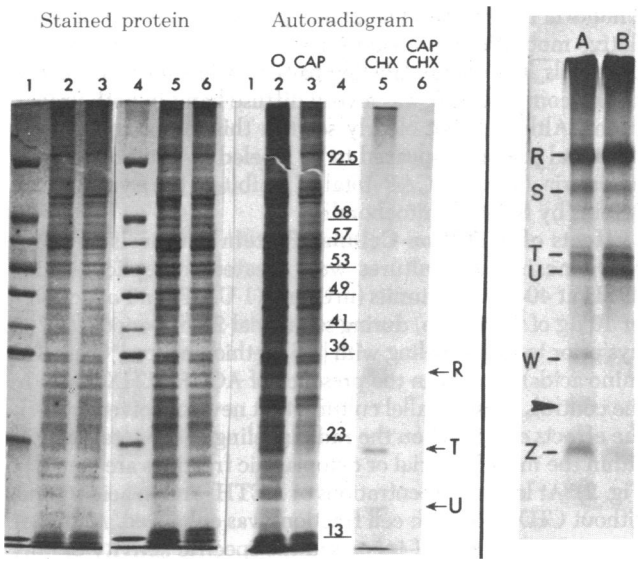

FIG. 1. (*Left*) Influence of specific inhibitors of protein synthesis. Cells were radiolabeled for 2 hr with [^{35}S]methionine in the absence or presence of CHX or CAP and mitochondrial fractions were dissolved in NaDodSO$_4$ prior to electrophoresis in a 10% polyacrylamide slab gel. Stained protein: Coomassie blue stained gel, channels 1 and 4 = 1 μg of each of the molecular weight standards, channel 2 = 0 (no drugs), channel 3 = CAP, channel 5 = CHX, channel 6 = CAP and CHX. For the autoradiogram, the stained slab gel was dried and exposed to x-ray film for: channels 2 and 3, four days; channels 5 and 6, seven days. Channel 4 indicates marker molecular weights × 10^{-3}. (*Right*) Proteins synthesized *in vitro* by isolated mitochondria. Mitochondria were isolated from cultured cells and radiolabeled 1 hr with [^{35}S]methionine. The mitochondria were analyzed by electrophoresis in a 15% polyacrylamide slab gel (channel A, 35,700 cpm, 32 μg of protein). For comparison, mitochondria labeled with [^{35}S]methionine for 2 hr in culture in the presence of CHX were analyzed in the same gel (channel B, 36,000 cpm, 17 μg of protein). This autoradiogram is from a 4-day exposure of the gel.

proteins with lower levels of incorporation (compare Fig. 1 *Left* with Fig. 3). CAP, added with CHX, inhibited total methionine incorporation by 99.3% ± 0.1% (n = 3) and no label appeared in gel patterns even with longer exposure times. CAP added alone decreased total protein synthesis by 30–40%. Similar decreases have been reported in other cell types (19, 20) and are believed to be due to an alteration in the rate of labeling of amino acid pools (19). CAP alone did not specifically affect any of the CHX-sensitive proteins; however, if did inhibit those proteins not sensitive to inhibition by CHX (compare components T and U in channel 2 to T and U in channel 3). None of the CHX-resistant proteins were detectable in the cytoplasmic fractions of cells radiolabeled with CHX present.

Differences in the gel profiles of CHX-resistant proteins were observed between those mitochondrial pellets that were first dissolved in NaOH and those dissolved in $NaDodSO_4$ without NaOH. The alkaline treatment reduced the amount of high molecular weight material at the top of gels. Fig. 1 *Left* illustrates the samples dissolved in $NaDodSO_4$ buffer alone and Fig. 3 shows samples dissolved in NaOH. Similar effects of this treatment with base on the gel electrophoretic profiles of mitochondrial proteins in yeast have been observed by others (10, 21).

Protein Synthesis by Isolated Mitochondria. To further substantiate that CHX-resistant mitochondrial proteins are synthesized intrinsically on mitochondrial ribosomes, mitochondrial fractions were prepared from adrenal tumor cell cultures and incubated with [^{35}S]methionine *in vitro*. Proteins labeled *in vitro* by isolated mitochondria were analyzed by electrophoresis in slab gels along with proteins labeled in intact cells in the presence of CHX (Fig. 1 *Right*). The isolated mitochondria incorporated label into proteins that migrate with relative mobilities identical to those of proteins labeled in the whole cells. The overall labeling patterns were also remarkably similar; component R was a very diffuse band in both preparations. Although not clearly seen in this reproduction, one additional protein appeared to be labeled by the isolated mitochondria (arrow). CAP totally inhibited the synthesis of proteins by isolated mitochondria.

Effects of ACTH on Cellular Protein Synthesis and Steroidogenesis. Cell cultures were treated in replicate with ACTH at 40–800 milliunits (mU)/ml (1 U.S.P. unit of activity per 10 μg of Cortrosyn) during sequential 24-hr periods for 2–3 days prior to radiolabeling with [^{35}S]methionine (or ^{14}C-labeled amino acids) for 2-hr in the presence of ACTH, CHX, or both. The controls were parallel cultures that never received ACTH. The effects of ACTH on the radiolabeling of the total protein within the mitochondrial or cytoplasmic fractions are complex (Fig. 2). At lower concentrations of ACTH, amino acid uptake without CHX into both cell fractions was enhanced. At higher concentrations, ACTH increased the specific activity of only the cytoplasmic fraction. Similar patterns have been observed in three other experiments. With CHX added, ACTH at low concentrations *decreased* [^{35}S]methionine uptake into the mitochondrial fraction to one-half, but at higher levels, there was no effect (Fig. 2, Mito + CHX). These effects might reflect changes either in rates of total protein synthesis or in the relative specific activity of the amino acid pool. During the period prior to radiolabeling, ACTH slowed the daily increase in total cellular protein accumulation per dish to about 85% of control (Fig. 2 *Inset*).

Steroid output by these cells increased markedly between the first and second 24-hr periods of ACTH treatment, with smaller increases being seen during the third 24-hr period (4, 22). This sequential increase is not merely related to cell growth (4, 22). For example, in a representatitive experiment described in Fig. 2, the ACTH-dependent increase in steroid output was still significant when normalized per mg of cellular protein—i.e., from 12.0 ± 2.8 μg of steroid per mg of protein (mean ± SD) for the first 24 hr to 19.5 ± 2.8 μg of steroid per mg of protein for the third 24 hr. Steroid outputs at the end of the third day did not vary by more than 25% for duplicate cultures given ACTH at 40–800 mU/ml, but all outputs were at least 20-fold higher than from control cultures.

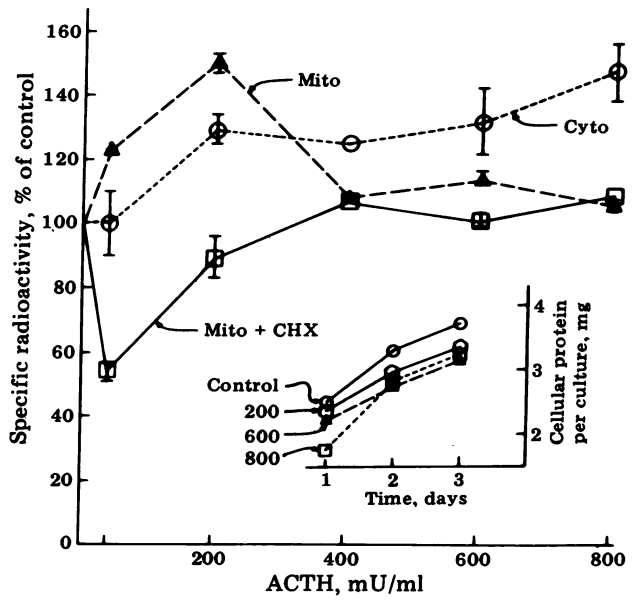

FIG. 2. ACTH effects on general apparent protein synthesis. Cultures were grown, treated with ACTH, and labeled as described in the legend to Fig. 1, except the cultures were preincubated for 40 min with ACTH, when appropriate, prior to the addition of CHX and [^{35}S]methionine. The cultures were not confluent at the time of initial addition of ACTH. Duplicate cultures were radiolabeled with [^{35}S]-methionine in the presence of the appropriate dose of ACTH. Specific radioactivity was determined from triplicate aliquots for Lowry protein and acid-precipitable cpm. The bars represent the deviation between duplicate plates. The values are plotted as percent of the specific activities of controls (labeled without ACTH), which were: mitochondrial fraction + CHX = 545 ± 59 cpm/μg of protein (□); mitochondrial fraction − CHX = 15,938 ± 2,291 cpm/μg of protein (△); cytoplasmic fraction − CHX = 15,486 ± 892 cpm/μg of protein (○); cytoplasmic fraction + CHX = 141 ± 8 cpm/μg of protein (not plotted). (*Inset*) Rate of protein synthesis. Total protein per dish was determined each day from parallel cultures of the same tumor used for the labeling experiment in this figure. The points are from duplicate plates for ACTH-treated (mU/ml indicated) and single for the control.

ACTH Effects on Cytoplasmic Translation Products. The labeling of six proteins in the mitochondrial fraction of nuclear–cytoplasmic origin (CHX-sensitive/CAP-insensitive) is specifically enhanced by ACTH (Fig. 3, bands B, C, E, F, G, and X). These relative increases were determined from densitometric scans of autoradiographs, like those illustrated in Fig. 4. Bands B, C, E, F, G, and X increase up to 2.4-, 1.7-, 1.4-, 3.3-, 1.8-, and 2.1-fold in this experiment, respectively. The apparent molecular weights of these bands are: B = 75,000; C = 73,000; E = 52,000; F = 30,000; G = 25,000; and X = 12,000. Further purification of mitochondria by sucrose density gradient centrifugation (11) prior to electrophoresis did not decrease the relative amounts of any of these ACTH-responsive proteins from these patterns (not shown). Purified beef adrenal mitochondrial adrenodoxin reductase electrophoretically comigrates with band E and purified beef adrenal mitochondrial adrenodoxin with band X. A similar pattern of ACTH-dependent changes in labeling was obtained with a mixture of 14 ^{14}C-

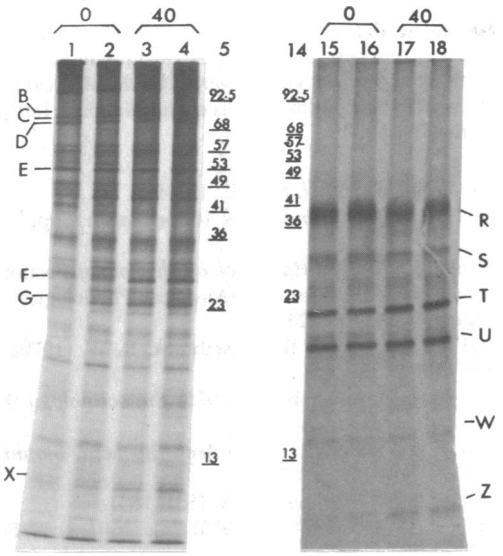

FIG. 3. Influence of ACTH on labeling of mitochondrial proteins. Autoradiograms. Portions (1/20th) of the mitochondrial fractions obtained from individual culture plates in the experiment described in the legend to Fig. 2 were dissolved in NaOH and analyzed by electrophoresis. This figure is a composite of autoradiograms of two different exposure times from two 12% polyacrylamide slab gels. Channels 1–4: proteins labeled in the absence of CHX (approximately 30,000 cpm per channel) visualized from an 8-day exposure to the dried gel. Channels 15–18: proteins labeled in the presence of CHX (2400 cpm per channel) visualized from a 28-day exposure. The duplicate channels for each ACTH treatment (0 or 40 mU/ml) are from duplicate mitochondrial preparations prepared in parallel from duplicate tissue culture plates. Channels 5 and 14 contained molecular weight standards.

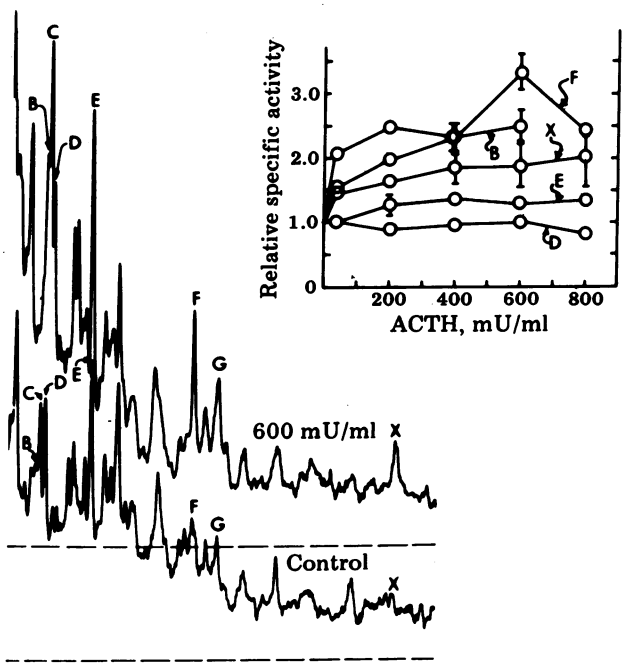

FIG. 4. Total mitochondrial proteins radiolabeled in the absence of CHX. The densitometric traces of an autoradiogram produced by a 4-day exposure of the polyacrylamide gels described in Fig. 3 are shown. The lower trace is the control; the upper trace is the sample treated with 600 mU of ACTH per ml. The area under each peak was quantified as described in *Materials and Methods*. The bars in the *Inset* represent the deviation in quantification of the duplicate channels for duplicate cultures for each ACTH treatment; the deviations not shown do not extend beyond the confines of the symbols.

labeled L amino acids. Quantification of the ACTH concentration effects on some of the bands is illustrated in the *Inset* in Fig. 4. The patterns shown are representative of seven separate experiments.

ACTH Effects on Mitochondrial Translation Products. Three proteins of mitochondrial origin (CHX-insensitive/CAP-sensitive) are also affected by ACTH (Fig. 3). Fig. 5 illustrates these changes in microdensitometric scans. While R decreases maximally to 70% of control, T and Z increase to 160% and 280% of control, respectively. The changes are maximal at ACTH concentrations of 40–200 mU/ml. Bands U and W do not vary significantly. The apparent molecular weights of these bands are: R = 36,500; S = 28,000; T = 22,600; U = 19,500; W = 14,000; and Z = 10,700. None of the adrenal mitochondrial translation products comigrate with purified beef adrenal mitochondrial adrenodoxin reductase or adrenodoxin. Similar ACTH-dependent patterns were observed in control versus ACTH-stimulated cells labeled with a mixture of 14 ^{14}C-labeled L amino acids (not shown). These patterns are representative of five similar experiments.

DISCUSSION

These studies indicate that stimulation of adrenal cells with ACTH in culture for extended periods of time affects the synthesis (or turnover) of specific proteins in the mitochondrial fraction, including some translated on mitochondrial ribosomes. In most studies of mitochondrial proteins, the protocol used to identify the mitochondrial translation products consists of labeling intact cells with a radioactive amino acid in the presence of an inhibitor of either mitochondrial or cytoplasmic protein synthesis (20, 23, 24). With CHX treatment of yeast, neurospora, *Xenopus*, or mammalian cells, only 8–10 prominent mitochondrial proteins are radiolabeled in the NaDodSO$_4$/polyacrylamide gel electrophoretic patterns (15, 16, 24). To date, the categories established by this protocol in all cell types have held up under further evaluation, including *in vitro* translation studies (15, 16) and, in yeast and neurospora, genetic and biochemical analysis of cytoplasmically inherited mutations (9, 15, 16, 25). Macino and Tzagoloff (26) have reported a close correspondence between the amino acid sequence of a proteolipid subunit of ATPase and the genetic code contained in the nucleotide sequence of a portion of yeast mitochondrial DNA. Most of the products of mitochondrial protein synthesis have been identified as the hydrophobic subunits of cytochrome oxidase, oligomycin-sensitive ATPase, or (in yeast and neurospora) cytochrome *b* (15). In yeast a mitochondrially synthesized protein, designated var 1, is involved in the assembly of the small subunit of the mitochondrial ribosome (27). The identity of mitochondrial translation products in animal cells has not been well established (20, 28). In *Xenopus* there are at least three major mitochondrially synthesized polypeptides for which no functional identity has yet been established (29).

Using the same type of labeling protocols applied to yeast and animal cells, we have shown the capability to study the two classes of proteins in the mitochondrial fraction of mouse adrenal cell cultures. The validity of our classification of proteins as mitochondrial translation products was substantiated by the similarity between the electrophoretic patterns of mitochondrial proteins radiolabeled with [^{35}S]methionine in intact cells with added CHX and proteins radiolabeled by isolated mitochondria. The patterns of ACTH-dependent changes are complex and appear to be quantitatively influenced by hormone concentration. Other factors may be involved in this process, because cyclic AMP mimics ACTH action under these conditions (ref. 30 and unpublished results).

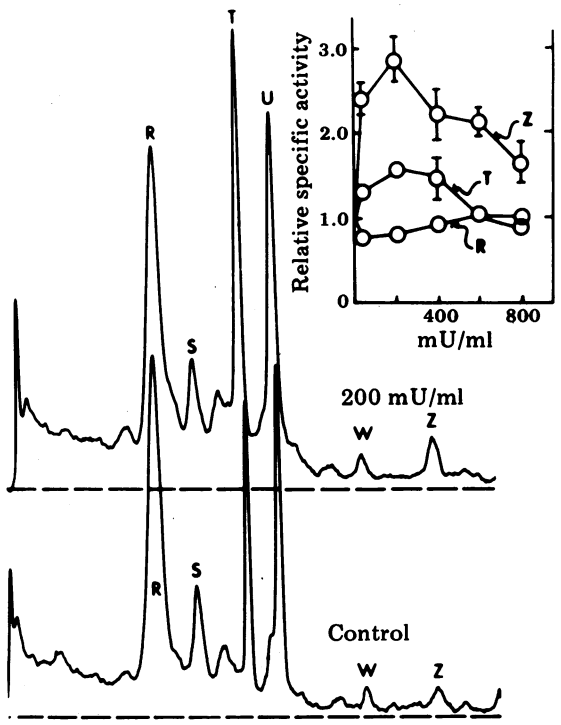

FIG. 5. Mitochondrial proteins radiolabeled in the presence of CHX. The densitometric traces of an autoradiogram from a 50-day exposure of the polyacrylamide gel described in Fig. 3 are shown. The bottom trace is the control; the upper one is the sample treated with ACTH at 200 mU/ml, scanned as in Fig. 4. The changes in relative specific activities of bands R, T, and Z as a function of ACTH concentration are shown in the *Inset*. Band U did not change significantly and is not plotted. The bars represent deviations in the quantification of the duplicate channels for duplicate cultures for each ACTH treatment.

Establishment of the functional identities of the ACTH-responsive mitochondrial translation products requires further analysis. They may be the hydrophobic anchoring subunits of some of the enzymes already known to contain mitochondrially synthesized subunits—e.g., cytochrome oxidase, ATPase, or cytochrome *b*. However, we and others have shown previously that ACTH has no significant effect on the enzymatic activities of cytochrome oxidase and ATPase or on spectrally measured cytochromes aa_3 and *b* (4, 5). They may be the catalytic portions of some of the known steroidogenic enzymes. However, adrenodoxin and adrenodoxin reductase purified from beef adrenal mitochondria are not electrophoretically similar to any of the ACTH-responsive mitochondrial translation products. Moreover, the reported molecular weights of beef mitochondrial cytochrome *P*-450s differ from those of these mitochondrially synthesized proteins (31, 32). Yet these mitochondrial translation products may prove to be important to the steroidogenic process because, as pointed out by Shatz and Mason (16) in establishing structural definitions of cytochrome oxidase and mitochondrial ATPase, it becomes difficult to determine "... where the enzymes end and the membrane begins." Therefore, some of the ACTH-responsive mitochondrial gene products may function in the adrenal cell as anchoring subunits for the proper arrangement of the electron-transferring portions of some known steroidogenic enzymes.

These effects of ACTH introduce another aspect of how this polypeptide hormone may regulate the capacity of its target cell for steroidogenesis.

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