Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 699–703, February 1988
Biochemistry

Simultaneous transfection of COS-1 cells with mitochondrial and microsomal steroid hydroxylases: Incorporation of a steroidogenic pathway into nonsteroidogenic cells

(cholesterol side-chain cleavage cytochrome P450/17α-hydroxylase cytochrome P450/adrenodoxin/pregnenolone/dehydroepiandrosterone)

MAURICIO X. ZUBER*, J. IAN MASON, EVAN R. SIMPSON, AND MICHAEL R. WATERMAN†

Departments of Biochemistry and Obstetrics and Gynecology and the Cecil H. and Ida Green Center for Reproductive Biology Sciences, University of Texas Health Science Center, Dallas, TX 75235

Communicated by Ronald W. Estabrook, September 28, 1987

ABSTRACT **Transfected, nonsteroidogenic COS-1 cells derived from monkey kidney are found to be capable of supporting the initial and rate-limiting step common to all steroidogenic pathways, the side-chain cleavage of cholesterol to produce pregnenolone. Endogenous COS-1 kidney cell renodoxin reductase and renodoxin are able to sustain low levels of this activity catalyzed by bovine cholesterol side-chain cleavage cytochrome P450 ($P450_{scc}$) whose synthesis is directed by a transfected plasmid containing $P450_{scc}$ cDNA. Double transfection with both $P450_{scc}$ and adrenodoxin plasmids leads to greater pregnenolone production and indicates that adrenodoxin plays a role as a substrate for this reaction or that bovine adrenodoxin serves as a better electron donor than the endogenous iron-sulfur protein renodoxin. Also it is found that both the bovine adrenodoxin and $P450_{scc}$ precursor proteins are proteolytically processed upon their uptake by COS-1 cell mitochondria to forms having the same electrophoretic mobility as mature bovine adrenodoxin and $P450_{scc}$. Following triple transfection of COS-1 cells with $P450_{scc}$, adrenodoxin, and 17α-hydroxylase cytochrome P450 plasmids, pregnenolone produced in mitochondria by the side-chain cleavage reaction can be further metabolized in the endoplasmic reticulum to 17α-hydroxypregnenolone and dehydroepiandrosterone. Although this functional steroidogenic pathway can be incorporated into this nonsteroidogenic cell type, it is found to be nonresponsive to cAMP, a potent activator of steroid hormone biosynthesis in adrenal cortex, testis, and ovary. Thus the cellular mechanisms necessary to support both microsomal and mitochondrial steroid hydroxylase activities appear not to be tissue specific, whereas the acute cAMP-dependent regulation of steroidogenesis is not present in transformed kidney (COS-1) cells.**

In each of the steroidogenic pathways leading to production of glucocorticoids, mineralocorticoids, or sex hormones, the initial step is the side-chain cleavage of cholesterol to yield pregnenolone, a mitochondrial reaction that is rate-limiting due to the regulation of cholesterol availability (1). Cholesterol side-chain cleavage is catalyzed by a three component enzyme complex consisting of cholesterol side-chain cleavage cytochrome P450 [$P450_{scc}$, the product of the P450XXII gene (2)], an iron-sulfur protein (adrenodoxin), and a flavoprotein (adrenodoxin reductase). In the adrenal cortex this reaction serves as the first step in the production of glucocorticoids, mineralocorticoids, and C_{19}-steroids (the so-called adrenal androgens). In the gonads, this reaction serves as the initial step in the production of the sex hormones testosterone and estrogen as well as the progesterone produced in large quantities by the corpus luteum following ovulation. In the placenta this reaction serves as the initial step in the production of progesterone, and, in some species, of estrogen. In the present study we have examined whether this tissue-specific activity can be expressed in a nonsteroidogenic cell type by transfecting COS-1 cells of monkey kidney origin (3) with pCD vectors (4) containing full-length cDNA inserts encoding bovine $P450_{scc}$ and adrenodoxin. Kidney cells are known to contain a mitochondrial P450 that catalyzes the 1α-hydroxylation of 25-hydroxyvitamin D (5) and, therefore, it was considered possible that such cells might have the capacity to support mitochondrial steroidogenesis, providing $P450_{scc}$ could be expressed in them and incorporated into the correct subcellular compartment.

We have shown (6) that the microsomal steroid hydroxylase 17α-hydroxylase cytochrome P450 [$P450_{17\alpha}$, the product of the P450XVII gene (2)] is functional when expressed in COS-1 cells. In the present study we have established that these cells also are capable of supporting mitochondrial steroidogenic reactions. Furthermore, it is found that both mitochondrial and microsomal steroid hydroxylases can function together to produce a steroidogenic pathway in these nonsteroidogenic cells to convert C_{27} steroids to C_{19} steroids. This system would appear to be ideal for site-directed mutagenesis aimed at elucidation of structure–function relationships of both mitochondrial and microsomal steroid hydroxylases. In addition it is evident from these studies that whereas nonsteroidogenic transformed cells have the capacity to support steroidogenic reactions, they lack the cAMP-dependent factors that are key elements in normal regulation of steroidogenesis.

MATERIALS AND METHODS

Cloning, identification, and characterization of pCD vectors containing cDNA inserts specific for bovine $P450_{scc}$ (7), $P450_{17\alpha}$ (8), and adrenodoxin (9) have been described. The $P450_{scc}$ vector used in this study contains a full-length cDNA insert that was identified and characterized by David Light (Genentech) with the partial-length clone (7) supplied by this laboratory. The amino acid coding sequence deduced from this clone is identical to that described by Morohashi *et al.*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: $P450_{scc}$, cholesterol side-chain cleavage cytochrome P450; $P450_{17\alpha}$, 17α-hydroxylase cytochrome P450; SCP_2, sterol carrier protein 2; Bt_2cAMP, N^6,$O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate.

*Present address: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

†To whom reprint requests should be addressed at: Department of Biochemistry, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235.

(10). COS-1 cells were obtained from Y. Gluzman (Cold Spring Harbor Laboratory) and were cultured and passaged as described (6). Transfection of COS-1 cells with a single plasmid was also performed as described (6), whereas simultaneous transfection with more than one plasmid utilized the same procedure except that the plasmids were mixed together before transfection.

Immunofluorescence studies of transfected COS-1 cells were carried out as described (6) with antibodies specific to bovine adrenodoxin and $P450_{scc}$ prepared in this laboratory (11). Antibodies specific for F_1-ATPase were kindly provided by Gail Breen (University of Texas at Dallas). Immunoblot analysis of adrenodoxin and $P450_{scc}$ was carried out as described (12) by using ^{125}I-labeled goat anti-rabbit IgG kindly provided by Carole Mendelson (University of Texas Health Sciences Center, Dallas). Immunoblot analysis of sterol carrier protein 2 (SCP_2) was carried out by the alkaline phosphatase procedure (13) using an antibody prepared against rat liver SCP_2 (14) and kindly provided by Terry Scallen (University of New Mexico School of Medicine). $N^6,O^{2'}$-Dibutyryladenosine 3',5'-cyclic monophosphate (Bt_2cAMP) was purchased from Sigma as was (22*R*)-hydroxycholesterol. (22*R*)-Hydroxycholesterol was used as exogenous substrate for the cholesterol side-chain cleavage reaction as it readily penetrates cellular membranes and in steroidogenic cells supports a high rate of pregnenolone production. Determination of steroid contents of medium by radioimmunoassay was carried out with highly specific antisera for pregnenolone (Chemicon International, El Segundo, CA), 17α-hydroxypregnenolone (Endocrine Sciences, Tarzana, CA), and dehydroepiandrosterone [a generous gift of John France (Postgraduate School of Obstetrics and Gynaecology, University of Auckland, New Zealand) that was raised against dehydroepiandrosterone-7-carboxymethyloxime-bovine serum albumin conjugate] and reagents and methods as described (15).

RESULTS AND DISCUSSION

Double immunofluorescence of COS-1 cells transfected with the bovine $P450_{scc}$ plasmid indicates that the bovine $P450_{scc}$ and the endogenous mitochondrial F_1-ATPase are localized in the same subcellular compartment (Fig. 1), presumably the mitochondrion. This punctate distribution of immunofluorescence is quite distinct from the network pattern observed for microsomal enzymes in COS-1 cells (6), such as bovine $P450_{17\alpha}$ resulting from transfection or endogenous NADPH-cytochrome P450 reductase. Transfection of COS-1 cells with the bovine adrenodoxin plasmid also indicates mitochondrial localization of adrenodoxin (data not shown).

In steroidogenic cells, $P450_{scc}$ and adrenodoxin are mitochondrial enzymes encoded by nuclear genes. Both proteins are synthesized in the cytoplasm as higher molecular weight precursors that are proteolytically processed to their mature forms upon uptake by mitochondria (16, 17). By using *in vitro* assays consisting of isolated mitochondria and proteins synthesized in a cell-free rabbit reticulocyte lysate translation system, it has been shown that mitochondria from bovine steroidogenic tissues (adrenal cortex and corpus

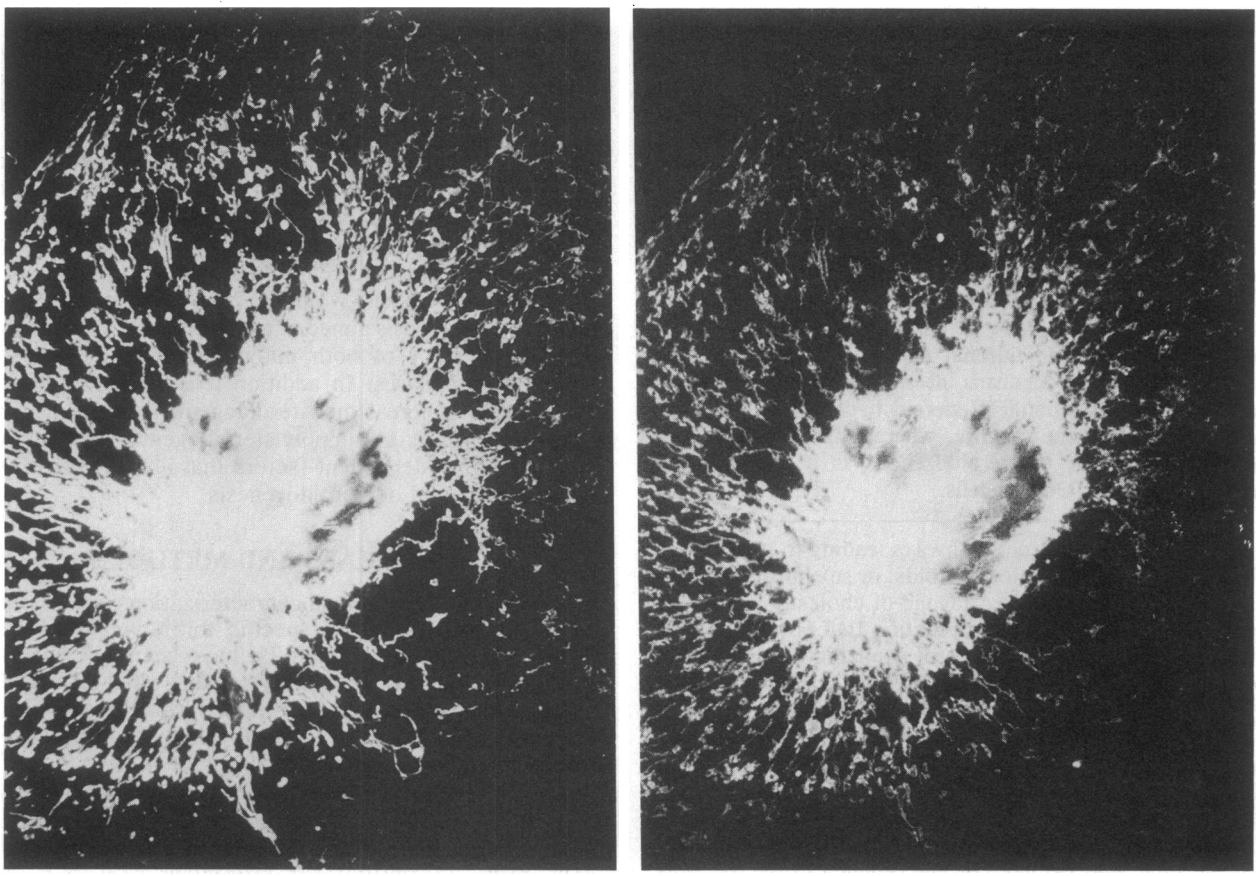

FIG. 1. Immunofluorescence of bovine $P450_{scc}$ (*Left*) and endogenous F_1-ATPase (*Right*) in the same transfected COS-1 cell. Transfection was carried out on coverslips in multiwell dishes (6) by using 10 μg of pCDP-450-10A, a plasmid containing the complete amino acid coding sequence of the bovine $P450_{scc}$ precursor. Immunofluorescence of $P450_{scc}$ was performed by using rabbit IgG specific for bovine adrenal $P450_{scc}$ as primary antibody and rhodamine-conjugated goat antibody to rabbit IgG as second antibody. Immunofluorescence of F_1-ATPase was performed by using rabbit IgG specific for bovine heart F_1-ATPase as primary antibody and fluorescein-conjugated goat antibody to rabbit IgG as the second antibody.

luteum) take up and process both precursors (16, 18). However, in *in vitro* assays using mitochondria from nonsteroidogenic bovine tissues (heart, liver, and kidney) only the adrenodoxin precursor is taken up and processed, while the $P450_{scc}$ precursor is refractory to such maturation events (16). As can be seen by immunoblot analysis (Fig. 2), bovine $P450_{scc}$, following transfection of COS-1 cells with the $P450_{scc}$ plasmid, is identical in size to $P450_{scc}$ observed in bovine adrenocortical cells (the mature form). The $P450_{scc}$ cDNA insert in the pCD vector used for transfection contains the nucleotide sequence encoding the 39-amino acid $P450_{scc}$ precursor sequence (10), yet only the mature form is observed in COS-1 cells. Likewise in Fig. 3 it is seen that the mature form of adrenodoxin (12 kDa) predominates in COS-1 cells transfected with the adrenodoxin plasmid, although a small amount of the precursor (19 kDa) is detectable. Thus the precursor forms of bovine $P450_{scc}$ and bovine adrenodoxin are processed *in situ* in COS-1 cells to forms that are the same size as their respective mature forms in bovine steroidogenic tissues. The inability of bovine kidney mitochondria to process the $P450_{scc}$ precursor *in vitro* (16) when monkey kidney COS-1 cell mitochondria can support this maturation event *in situ* (this study) is unexplained. In another laboratory, it had been observed (19) that the bovine $P450_{scc}$ precursor could not be processed by rat heart mitochondria but could be processed by rat liver mitochondria. Perhaps these variations in processing arise from species differences; although in the comparison of $P450_{scc}$ processing by bovine and monkey kidney mitochondria, we cannot rule out the possibility that *in vitro* processing may differ from *in situ* processing in COS-1 cells. The *in situ* studies described herein give no information concerning the efficiency of uptake of $P450_{scc}$ into kidney mitochondria. These studies utilize a single cell type (COS-1) whereas the *in vitro* studies utilized mitochondria from whole kidney cortex. It is apparent, however, as opposed to our conjecture (16), that at least some mitochondria from nonsteroidogenic tissues do contain the capacity to import and process steroidogenic precursors (ref. 19 and this study). Whether the proteolytic cleavage of the adrenodoxin and $P450_{scc}$ precursors occurs between the correct amino acids in COS-1 cells is not known, although the resultant mature forms are of similar size upon electrophoresis to those in bovine adrenocortical cells. Also it can be noted whereas no detectable $P450_{scc}$ is found in untransfected COS-1 cells (Fig. 2), two bands of ≈12 kDa are found to be endogenous in COS-1

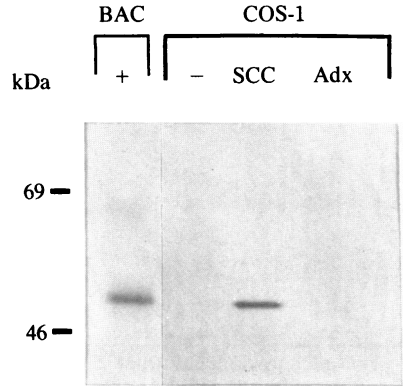

FIG. 2. Immunoblot of $P450_{scc}$ in transfected COS-1 cells. The left lane labeled BAC is the immunoblot of $P450_{scc}$ in the cell lysate (50 μg of protein) from cultured bovine adrenocortical cells treated with corticotropin (1 μM) for 24 hr. The right three lanes labeled COS-1 are immunoblots of $P450_{scc}$ in cell lysates (50 μg) from mock-transfected COS-1 cells (lane −), COS-1 cells transfected with the $P450_{scc}$ plasmid (lane SCC), and COS-1 cells transfected with the adrenodoxin plasmid (lane Adx).

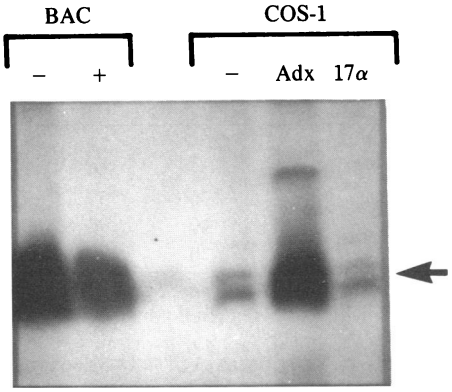

FIG. 3. Immunoblot of adrenodoxin in transfected COS-1 cells. The left lanes labeled BAC are the immunoblot of adrenodoxin in cell lysates from cultured bovine adrenocortical cells untreated (−, 50 μg of cell lysate) or treated (+, 10 μg of cell lysate) with 1 μM corticotropin for 24 hr. The right three lanes labeled COS-1 are immunoblots of adrenodoxin in cell lysates (50 μg of protein) from mock-transfected COS-1 cells (lane −), COS-1 cells transfected with the adrenodoxin plasmid (lane Adx), and COS-1 cells transfected with the $P450_{17\alpha}$ plasmid (lane 17α). The arrow indicates the position of migration (12 kDa) of purified bovine adrenodoxin on the same gel.

cells using the antibody to bovine adrenodoxin (Fig. 3). This endogenous pattern is identical to that observed in bovine kidney tissue and the lower band of this doublet has been postulated to be the endogenous kidney renodoxin, which is closely related structurally to adrenodoxin (20).

Not only were the precursor forms of $P450_{scc}$ and adrenodoxin processed *in situ* in COS-1 cells but they were also found to be enzymatically active. Using COS-1 cells transfected with the $P450_{scc}$ pCD vector alone, pregnenolone production was detectable after a 10-hr incubation with (22*R*)-hydroxycholesterol as substrate (data not shown) and reached a level ≈7 times above background (nontransfected cells) after a 24-hr incubation (Table 1). This modest cholesterol side-chain cleavage activity is apparently supported by endogenous COS-1 renodoxin reductase and renodoxin, proteins that normally support 1α-hydroxylation of 25-hydroxyvitamin D in kidney mitochondria. The ability of this kidney electron transport system to support adrenal steroid hydroxylation ($P450_{scc}$ or 11β-hydroxylase cytochrome P450) has not been studied in detail. In contrast pregnenolone production is much higher in COS-1 cells transfected with both $P450_{scc}$ and adrenodoxin pCD vectors (Table 1). Also the more adrenodoxin plasmid used, the greater the side-chain cleavage activity, up to 10 μg of plasmid. Perhaps after double transfection with 10 μg of each plasmid, the limiting component of this heterologous cholesterol side-chain cleavage complex is the endogenous flavoprotein

Table 1. Pregnenolone production in double-transfected COS-1 cells

Adx, μg of DNA*	$P450_{scc}$, μg of DNA†	Pregnenolone, pmol/ml per 24 hr‡
−	−	21
−	10	153
0.1	10	230
1	10	470
10	10	550

Adx, adrenodoxin; scc, side-chain cleavage.
*Adrenodoxin plasmid DNA was used in transfection.
†$P450_{scc}$ plasmid DNA was used in transfection.
‡Pregnenolone produced from (22*R*)-hydroxycholesterol at 2 nmol/ml; each value is an average of two measurements on each sample of medium.

Table 2. Steroids produced in triple-transfected COS-1 cells

Adx, μg of DNA*	$P450_{scc}$, μg of DNA†	$P450_{17\alpha}$, μg of DNA‡	pmol/ml per 24 hr§ Preg	17αOH-Preg	DHEA
–	–	–	21	<6	<1.6
10	10	–	550	<6	<1.6
–	10	30	53	21	19
0.1	10	30	94	90	55
10	10	30	190	136	54

Adx, adrenodoxin; scc, side-chain cleavage; Preg, pregnenolone; 17αOH-Preg, 17α-hydroxypregnenolone; DHEA, dehydroepiandrosterone.

*Adrenodoxin plasmid DNA was used in transfection.

†$P450_{scc}$ plasmid DNA was used in transfection.

‡$P450_{17\alpha}$ plasmid DNA was used in transfection.

§Produced from (22*R*)-hydroxycholesterol at 2 nmol/ml; each number is an average of two measurements on each sample of medium.

renodoxin reductase. From these results, it appears that the small amount of endogenous renodoxin in monkey kidney cells (Fig. 3) is limiting and that adrenodoxin itself probably acts as a substrate in the side-chain cleavage reaction with respect to its interaction with $P450_{scc}$. Of course, it is also possible that the interaction of $P450_{scc}$ with renodoxin is not as efficient as with adrenodoxin. The results in Table 1 also show that transfection with two vectors leads to incorporation of two plasmids into the same COS-1 cells and that monkey kidney renodoxin can transfer electrons to bovine $P450_{scc}$ as can monkey kidney renodoxin reductase to bovine adrenodoxin. Finally, it can be concluded that monkey kidney cells are able to supply sufficient mitochondrial NADPH to support the observed level of cholesterol side-chain cleavage activity and that they can also insert the iron-sulfur center into adrenodoxin and the heme group into $P450_{scc}$, both being required for this activity.

Following transfection of COS-1 cells with 10 μg of adrenodoxin plasmid and 10 μg of $P450_{scc}$ plasmid and incubation for 24 hr with (22*R*)-hydroxycholesterol, the culture medium was incubated with *Pseudomonas testeronii* 3β-hydroxysteroid dehydrogenase (21), and the products were analyzed by HPLC. Greater than 95% of the product was progesterone indicating little or no subsequent metabolism of pregnenolone in these COS-1 cells. However, when triple transfection was carried out with the $P450_{scc}$, adrenodoxin, and $P450_{17\alpha}$ pCD vectors, 17α-hydroxypregnenolone and dehydroepiandrosterone were observed as products in addition to pregnenolone (Table 2). This result indicates that pregnenolone produced in mitochondria of COS-1 cells can translocate to the endoplasmic reticulum where it is further metabolized by $P450_{17\alpha}$. We have reported (6) that bovine $P450_{17\alpha}$ in transfected COS-1 cells can catalyze steroid 17α-hydroxylase and steroid C-17,20-lyase activities leading to the production of 17α-hydroxypregnenolone and dehydroepiandrosterone, respectively, from exogenous pregnenolone (6). From the present studies it is apparent that pregnenolone produced endogenously within transfected COS-1 cells can also be converted to these products. Thus the complete steroidogenic pathway for the conversion of C_{27} sterols to C_{19} steroids can be established by transfection of COS-1 cells. What is not known, even in steroidogenic tissues, is whether the transport of pregnenolone from the mitochondrion to the endoplasmic reticulum is facilitated by a transport system or is the result of simple diffusion. Whatever the case, it is clear that pregnenolone can readily move between compartments in kidney cells as well as in steroidogenic cells.

Steroidogenesis in the adrenal cortex and the gonads is regulated by cAMP in response to peptide hormones binding to specific cell-surface receptors (22). As noted in Table 3 a low level of cholesterol side-chain cleavage activity was detectable in the absence of added (22*R*)-hydroxycholesterol in cells transfected with the adrenodoxin and $P450_{scc}$ plasmids. Thus while kidney mitochondria may not contain a large reservoir of endogenous steroidogenic cholesterol in their inner mitochondrial membrane near the transfected $P450_{scc}$, clearly some inner membrane cholesterol is available to $P450_{scc}$. Addition of 1 mM cAMP, which would acutely enhance steroidogenesis (pregnenolone production) in steroidogenic cells by 10 times or more from endogenous substrate, has no effect on the pregnenolone production from endogenous cholesterol in COS-1 cells. The apparent maximal capacity for pregnenolone production in these transfected cells is also shown in Table 3 [(22*R*)-hydroxycholesterol as substrate] and, as expected, is nonresponsive to cAMP, similar to results observed with steroidogenic cells. Thus one or more cAMP-responsive elements of the steroidogenic pathway are not found in transformed kidney cells. We presume that one of the missing elements is required for facilitated cholesterol transport to the inner mitochondrial membrane that is believed to be the key regulatory step in the acute steroidogenic action of peptide hormones (23). As shown in Fig. 4, SCP_2, a protein thought to be essential for transport of cholesterol to the mitochondrion in steroidogenic tissues (24), is present in COS-1 cells. While the level of SCP_2 in COS-1 cells is much less than

Table 3. Effect of cAMP on pregnenolone production in double-transfected COS-1 cells

Substrate	Bt_2cAMP, 1 mM*	Mock transfection†	Double transfection‡	Pregnenolone, pmol/ml per 24 hr
Endogenous cholesterol	–	+	–	<6
	+	+	–	<6
	–	–	+	80
	+	–	+	90
(22*R*)-Hydroxycholesterol (20 μM)	–	+	–	<6
	+	+	–	<6
	–	–	+	1840
	+	–	+	1800

For mock transfections, all transfection procedures were followed but no plasmid was added. Double transfection procedures used 10 μg of adrenodoxin and 10 μg of $P450_{scc}$ plasmid DNA. Three days after transfection the medium was changed; the fresh medium contained 1 mM Bt_2cAMP where indicated. After 24 hr, the medium was removed and its pregnenolone content was determined as a measure of metabolism of endogenous cholesterol. Fresh medium containing Bt_2cAMP and (22*R*)-hydroxycholesterol where indicated was added to the same dishes, and after a 24-hr incubation the experiment was terminated, and pregnenolone was determined in the medium to measure apparent maximal side-chain cleavage activity.

*Bt_2cAMP was added (+) or was not (–).

†Cells were (+) or were not (–) mock-transfected.

‡Cells were (+) or were not (–) double-transfected.

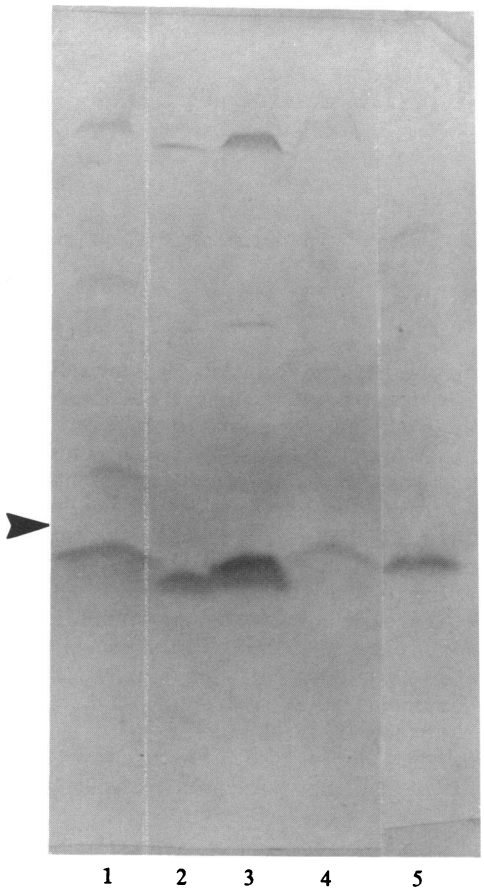

FIG. 4. Immunoblot of SCP_2 in COS-1 cells. Lanes: 1, 200 μg of protein from total cell lysate from cultured, untransfected COS-1 cells; 2, 20 μg of total cell lysate from cultured bovine adrenocortical cells; 3, 200 μg of sample in lane 2; 4, 200 μg of total cell lysate from untransfected COS-1 cells (different culture than in lane 1); 5, 200 μg of total homogenate from human fetal adrenal. The 12-kDa SCP_2 bands are seen below the arrow that indicates the position of migration of the 14-kDa marker (lactalbumin) on the same gel. The higher molecular weight bands on this gel are of unknown origin.

found in bovine adrenocortical cells, it is not very different from that found in human fetal adrenal cells, a very active steroidogenic tissue. Therefore, we imagine that the missing cAMP-responsive elements in monkey kidney cells act prior to and/or subsequent to the transport of cholesterol to the mitochondria. Potential candidates for such factors missing in nonsteroidogenic cells would include cholesterol ester hydrolase (25) and the so-called "cycloheximide-sensitive factor" (26). Clearly the cAMP-mediated mobilization of cholesterol to the inner mitochondrial membrane is tissue-specific and serves a key regulatory role in steroidogenesis; for example, in adrenal cells leading to the rapid production of glucocorticoids in response to stress, the "fight or flight" response.

In summary, by transfection of nonsteroidogenic COS-1 cells with plasmids containing cDNA sequences for steroid hydroxylases we have been able to model several steps, both mitochondrial and microsomal, in the steroidogenic pathway leading from C_{27} steroids to C_{19} steroids. Thus, a number of steps required in the processing of these transcripts, including cleavage of precursor sequences, insertion into mitochondria or endoplasmic reticulum, introduction of heme and iron-sulfur prosthetic groups, can be accomplished by these nonsteroidogenic cells. By contrast, these studies indicate that the acute mobilization of cholesterol in steroidogenesis is a tissue-specific event as is, of course, the expression of steroid hydroxylase genes. Also it is apparent from these studies that site-directed mutagenesis of both mitochondrial and microsomal steroid hydroxylases can be carried out with COS-1 cells as an expression system for evaluation of alterations in enzymatic activities.

The technical assistance of Leticia Cortez and the editorial assistance of Susan Alexander are greatly appreciated. This work was supported by U.S. Public Health Service Grants DK28350, HD13234, and CA30253 from the National Institutes of Health and Grant I-624 from The Robert A. Welch Foundation. M.X.Z. was supported in part by U.S. Public Health Service Training Grant 5-T32HD07190.

1. Waterman, M. R. & Simpson, E. R. (1985) *Mol. Cell. Endocrinol.* **39**, 81–89.
2. Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1987) *DNA* **6**, 1–12.
3. Gluzman, Y. (1981) *Cell* **23**, 175–182.
4. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 280–289.
5. Driscoll, W. J. & Omdahl, J. L. (1986) *J. Biol. Chem.* **261**, 4122–4125.
6. Zuber, M. X., Simpson, E. R. & Waterman, M. R. (1986) *Science* **234**, 1258–1261.
7. John, M. E., John, M. C., Ashley, P., MacDonald, R. J., Simpson, E. R. & Waterman, M. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5628–5632.
8. Zuber, M. X., John, M. E., Okamura, T., Simpson, E. R. & Waterman, M. R. (1986) *J. Biol. Chem.* **261**, 2475–2482.
9. Okamura, T., John, M. E., Zuber, M. X., Simpson, E. R. & Waterman, M. R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5705–5709.
10. Morohashi, K., Fujii-Kuriyama, Y., Okada, Y., Sogawa, K., Hirose, T., Inayama, S. & Omura, T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4647–4651.
11. Kramer, R. E., DuBois, R. N., Simpson, E. R., Anderson, C. M., Kashiwagi, K., Lambeth, J. D., Jefcoate, C. R. & Waterman, M. R. (1982) *Arch. Biochem. Biophys.* **215**, 478–485.
12. Zuber, M. X., Simpson, E. R., Hall, P. F. & Waterman, M. R. (1985) *J. Biol. Chem.* **260**, 1842–1848.
13. Blake, M. S., Johnston, K. H., Russell-Jones, G. J. & Gotschlick, E. C. (1984) *Anal. Biochem.* **136**, 175–179.
14. Vahouny, G. V., Chanderbahn, R., Noland, B. J., Irwin, D., Dennis, P., Lambeth, J. D. & Scallen, T. J. (1983) *J. Biol. Chem.* **258**, 11731–11737.
15. Mason, J. I., Hemsell, P. G. & Korte, K. (1983) *J. Clin. Endocrinol. Metab.* **56**, 1057–1062.
16. Matocha, M. F. & Waterman, M. R. (1984) *J. Biol. Chem.* **259**, 8672–8678.
17. Matocha, M. F. & Waterman, M. R. (1985) *J. Biol. Chem.* **260**, 2259–2265.
18. Matocha, M. F. & Waterman, M. R. (1986) *Arch. Biochem. Biophys.* **250**, 456–460.
19. Ogishima, T., Okada, Y. & Omura, T. (1985) *J. Biochem. (Tokyo)* **98**, 781–791.
20. Bhasker, C. R., Okamura, T. & Waterman, M. R. (1987) *Eur. J. Biochem.* **164**, 21–25.
21. Talalay, P. & Dobson, M. M. (1953) *J. Biol. Chem.* **205**, 823–837.
22. Waterman, M. R. & Simpson, E. R. (1985) in *Adrenal Cortex*, eds. Anderson, D. C. & Winter, J. S. D. (Butterworths, London), pp. 57–85.
23. Privalle, C. T., Crivello, J. F. & Jefcoate, C. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 702–706.
24. Chanderbahn, R., Noland, B. J., Scallen, T. J. & Vahouney, G. V. (1982) *J. Biol. Chem.* **257**, 8928–8934.
25. Beckett, G. J. & Boyd, G. S. (1977) *Eur. J. Biochem.* **72**, 223–233.
26. Pedersen, R. C. & Brownie, A. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1882–1886.