Ultrastructural and biochemical evidence for the presence of mature steroidogenic acute regulatory protein (StAR) in the cytoplasm of human luteal cells

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Abstract

The distribution of the steroidogenic acute regulatory protein (StAR) inside thecal and granulosa-lutein cells of human corpus luteum (CL) was assessed by immunoelectron microscopy. We found greater levels of StAR immunolabeling in steroidogenic cells from early- and mid-than in late luteal phase CL and lower levels in cells from women treated with a GnRH antagonist in the mid-luteal phase. Immunoelectron microscopy revealed significant levels of StAR antigen in the mitochondria and in the cytoplasm of luteal cells. The 30 kDa mature StAR protein was present in both mitochondria and cytosol (post-mitochondrial) fractions from homogenates of CL at different ages, whereas cytochrome *c* and mitochondrial HSP70 were detected only in the mitochondrial fraction. Therefore, we hypothesized that either appreciable processing of StAR 37 kDa pre-protein occurs outside the mitochondria, or mature StAR protein is selectively released into the cytoplasm after mitochondrial processing. The presence of mature StAR in the cytoplasm is consonant with the notion that StAR acts on the outer mitochondrial membrane to effect sterol import, and that StAR may interact with other cytoplasmic proteins involved in cholesterol metabolism, including hormone sensitive lipase.

Keywords: Human corpus luteum; Ultrastructure; StAR; Immunogold

1. Introduction

The human corpus luteum (CL), a temporary endocrine gland derived from the ovulated follicle, is an active producer of steroid hormones, mainly progesterone (P4). Progesterone governs the secretory transformation of the endometrium, rendering it receptive for embryo implantation and the establishment of early pregnancy (Carr, 1992). During the menstrual cycle, CL development and function are dependent on pituitary-derived LH. In the event of conception, the activity of the luteal cells is maintained for some weeks by the trophoblast-derived hCG; later the placenta assumes the production of P4 and sustains pregnancy. Steroidogenic cells take up lipoprotein-carried cholesterol and also maintain stores of esterified cholesterol, the obligate precursor for the biosynthesis of steroid hormones. Upon hormonal stimulation of steroid synthesis, cholesterol from various pools, including the intracellular esters which are hydrolyzed, is conveyed to the inner membrane of the mitochondria to serve as a substrate for pregnenolone production (Miller, 1988). In non-placental steroid-producing tissues, the translocation of cholesterol is mediated by StAR, a fast-reacting controller of steroid synthesis (Strauss et al., 1999; Bose et al., 2002). It is thought that the rate-limiting step in P4 synthesis is the movement of cholesterol from

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the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage complex is located. StAR is essential for this sterol translocation. StAR is synthesized as a 37 kDa pre-protein with a N-terminal mitochondrial targeting sequence. This sequence directs importation of the protein into the mitochondria where it is processed to the 30 kDa mature form by a metalloproteinase. It has been postulated that StAR acts on the outer mitochondrial membrane to initiate sterol translocation and that importation into the mitochondrial matrix terminates StAR's sterol translocating activity (Christenson and Strauss, 2000).

Recently, we described the change in the expression of StAR gene and proteins in the CL throughout the luteal phase (Devoto et al., 2001). Our results indicated that StAR transcripts and StAR protein were most abundant in mid-luteal CL.

The present study examined by immunoelectron microscopy the subcellular distribution of StAR in granulosa- and theca-lutein cells of the CL throughout the luteal phase and following administration of a GnRH antagonist, which causes a rapid decline in circulating LH levels. Here we show that there is a substantial amount of mature StAR protein in the cytoplasm of luteal cells. This unexpected finding may reflect previously unrecognized processing of the StAR pre-protein in the luteal cell cytoplasmic compartment or the release of mature StAR from the luteal mitochondria. These observations challenge the existing notions regarding the targeting and processing of StAR pre-protein and/or catabolism of mature StAR. They argue for an important role of StAR outside of the mitochondria

2. Materials and methods

2.1. Subjects

All women gave signed informed consent to participate in this study. The internal review board of Hospital Clínico San Borja-Arriarán approved the experimental protocol. CL was enucleated at the time of minilaparotomy from women undergoing tubal ligation, as described previously. The surgery was conducted at Hospital Clínico San Borja-Arriarán, National Health Service-University of Chile (Santiago, Chile). Subjects aged 30–39 years, with normal body mass index, regular menstrual cycles, and had not received any form of hormonal treatment in the last 3 months before participating in the study. Fifteen women were scheduled for surgery at different times of the luteal phase and two of these women were given 2 mg *sc* of GnRH antagonist (Cetrotide®, Serono), during mid-luteal phase for 48 h before surgery. Pieces of luteal tissue were used for immunoelectron microscopic studies of StAR and for morphological analysis. Samples of luteal tissue were also used for immunoblot analyses of StAR, MLN64 and cytochrome *c*. Blood was collected before surgery for steroid determinations.

2.2. Dating of the CL

CL were dated on the basis of the presumptive day of ovulation (d = 0), which was determined by serial urinary LH measurements (Clearplan® Unipath, Bedford, UK) and serial vaginal ultrasound images of the ovaries. Additionally, plasma E2 and P4 levels and the histological features of each CL were used to confirm tissue dating as described previously (Devoto et al., 2001). The CL was classified as early (4 days post-ovulation), mid (5–10 days post-ovulation), and late (>11 days from ovulation).

The entire CL was enucleated from the ovary and immediately transported under sterile conditions to the laboratory. The gland was washed with cold NaCl (0.9%) solution to remove blood clots and immediately divided into radial blocks. Small pieces of tissue for histology were fixed in 4% buffered paraformaldehyde and embedded in paraffin wax.

2.3. Immunochemicals

The following antibodies were used in our studies:

- Rabbit anti-StAR polyclonal antibody raised against the human StAR C-terminus (START) domain (Pollack et al., 1997).
- Rabbit anti-MLN64 polyclonal antibody raised against the START domain of the recombinant protein (Zhang et al., 2002).
- Rabbit anti-ELIP polyclonal antibody raised against the protein from barley leaves (gently donated by Prof. Dr. Klaus Kloppstech, Botanical Institute, University of Hanover, FRG).
- Clone JG1 mouse monoclonal antibody raised against human mitochondrial heat sckock protein 70, obtained from Affinity Bioreagents Inc., CO, USA.
- Clone 7H8.2C12 mouse monoclonal antibody raised against denatured cytochrome *c*, purchased from Zymed Laboratories Inc., CA, USA.
- Clone 6H2 mouse monoclonal antibody raised against full-length, native human cytochrome *c*, obtained from Santa Cruz Biotechnology, Inc., CA, USA.
- Goat anti-rabbit IgG labeled with 15 nm gold particles, purchased from British BioCell, Cardiff, UK.

2.4. Immunoelectron microscopy

Fragments of CL, approximately 1 mm^3 in size, were fixed by immersion in 4% (w/v) freshly de-polymerized paraformaldehyde prepared in 0.1 M phosphate buffer pH 7.4 and containing 0.5% (w/v) glutaraldehyde. In order to preserve antigen reactivity in the tissue, a post-fixation with osmium tetroxide was avoided. After fixation, the samples were washed with phosphate buffer, dehydrated and embedded in LR-Gold as described (Sierralta, 2001). From the embedded tissues, ultrathin sections (70 nm thickness) were cut with a Reichert-Jung ultramicrotome; the sections were

collected on Formvar-coated 200 mesh gold grids and immediately incubated as described previously (Sierralta et al., 1995). Briefly, the sections were incubated in blocking buffer (50 mM Tris, pH 7.4, 0.15 M NaCl containing 5% fetal calf serum) for 30 min at room temperature and subsequently incubated in blocking buffer containing a 1:100 dilution of rabbit anti-StAR antibody or a non-related (anti-ELIP) rabbit antibody for 2 h at room temperature. Following exhaustive washes in Tris-buffered saline, grids were incubated with 15 nm gold-labeled anti-rabbit IgG diluted 1:50 in blocking buffer. After washes, the sections were lightly stained with 5% aqueous uranyl acetate and Reynolds lead citrate. Specimens were viewed with a Philips CM100 electron microscope at 80 kV and photographed on Kodak 4489 EM film. The density of labeling was determined by counting the number of gold particles present in mitochondrial or cytoplasmic areas of cells from five sections obtained from four different blocks prepared from the tissues (Sierralta et al., 1995; Griffith, 1993). Thus, for each CL, a total of 20 randomly selected fields were analyzed; the labeling data is presented as mean values ± SEM. To control the specificity of labeling, the anti-StAR antibody was replaced either by anti-ELIP or by sera from non-immunized rabbits; less than one gold particle in 20 μm^2 of section were detected in these control sections.

2.5. Immunoblot analysis

Luteal tissues were processed as described previously (Devoto et al., 2001), with minor modifications. In brief, the tissues were homogenized in five volumes of 0.25 M sucrose in 10 mM Tris–HCl pH 7.4 containing 10 mM EDTA, 0.2 mM PMSF and 10 μg/mL aprotinin. The homogenate was first centrifuged at 600 × *g* for 10 min to sediment nuclei and cell debris; the supernatant was spun at 12,000 × *g* for 15 min. The post-mitochondrial supernatant and the mitochondrial pellet were analyzed by Western blotting under denaturating (StAR, MLN64, mHSP70, cytochrome *c*) or non-denaturating (native cytochrome *c*) conditions. Protein concentrations were determined by the dye-binding assay (Bio-Rad Laboratories Inc). For the analysis of StAR, MLN64 and mHSP70, 10 μg of protein were loaded onto 10% SDS-PAGE electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Hybridon, Millipore Corp., Bedford, MA, USA). For cytochrome *c*, 10 μg protein samples were loaded onto 12.5% SDS-PAGE electrophoresis. For the analysis of *native* cytochrome *c*, the samples were loaded onto 12.5% PAGE containing neither SDS nor thiol-reducing reagents; to ensure the release of cytochrome *c*, aliquots of the mitochondrial pellets were sonicated before loading; after electrophoresis, the bands were transferred to PVDF membranes with transfer buffer without denaturants. All the membranes were incubated with the cognate primary antibodies and developed with the ECF Western blotting kit (Amersham Bioscience, Sunnyvale, CA, USA). The fluorescent signal was quantified on a Typhoon 9200 (Amersham Bioscience, Sunnyvale, CA, USA), using ImageQuant 5.2 software (Molecular Dynamics, Inc.) to analyze the signals.

2.6. Statistics

Statistical significance of multiple comparisons was determined by ANOVA following Bonferroni's multiple comparison procedure (Pagano and Gauvreau, 2000).

3. Results

3.1. Immunoelectron microscopic studies of CL at different times throughout the luteal phase and after administration of the GnRH antagonist

Figs. 1 and 2 depicts the immunolabeling of cytoplasmic and mitochondrial StAR in granulosa and theca-lutein cells respectively. Figs. 1b and 2b show the StAR immunolabeling in mid-luteal steroidogenic cells. The density of StAR immunolabeling in the cytoplasm and mitochondria of the subpopulations of steroidogenic cells was greater during mid-luteal phase as compared with early and late CL (Figs. 1a and c, and 2a and c). StAR immunoreactivity was significantly diminished in both the cytoplasm and mitochondria of the lutein cells within late-luteal phase CL (Figs. 1c and 2c). A substantial decline of StAR immunolabeling in the steroidogenic cells of mid-luteal phase CL, following the administration of the GnRH antagonist was noted. The decrease in StAR immunoreactivity was observed in the cytoplasm and mitochondria of granulosa and theca-lutein cells (Figs. 1d and 2d). However, no major changes in the ultrastructure of the cells were detected. As indicated before, control sections incubated without the specific antibody displayed negligible labeling (under 1 gp in 20 μm^2 of area, not shown).

3.2. Immunolabeling densities in granulosa-lutein cells at different stages of the luteal phase and after administration of GnRH antagonist

Table 1 summarizes the densities of StAR immunolabeling in the cytoplasmic and mitochondrial compart-

Table 1
Immunogold labeling density for StAR in LR-Gold sections of human granulosa-lutein cells

Luteal stage/treatment	No. of CL	gp/5 μm^2 cyt[a]	gp/5 μm^2 mit[*]
Early	3	4.0 ± 0.5	8.0 ± 0.4
Mid	3	6.0 ± 0.3	13.0 ± 1.1
Late	3	0.9 ± 0.1[b]	1.1 ± 0.2[b]
Cetrotide®	2	1.2 ± 0.3[b]	1.2 ± 0.2[b]

[a] Values are expressed as mean ± SEM of gold particles counted in a 5 μm^2 of cytoplasm or mitochondria area; $n = 60$.
[b] $P < 0.05$ significantly different vs. early and mid-CL.

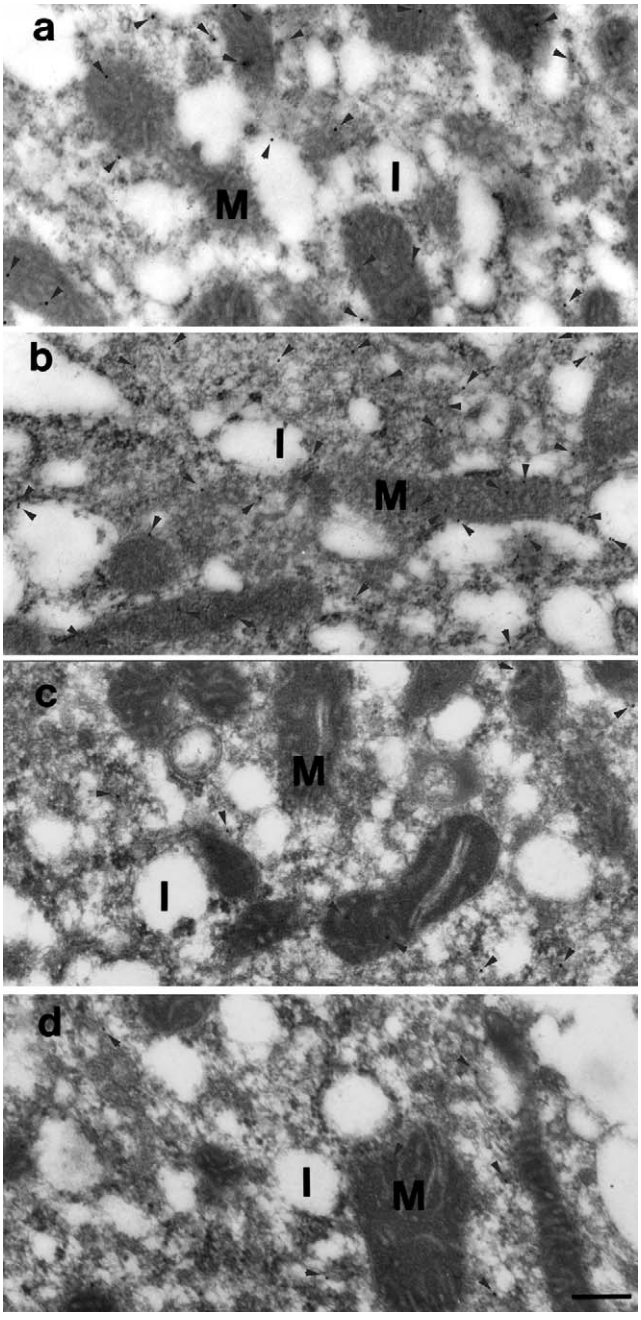

Fig. 1. Immunogold labeling of StAR in LR-Gold sections of granulosa-lutein cells from corpora lutea at different times of the luteal phase and after administration of a GnRH antagonist; representative images of: (a) early, (b) mid, (c) late, (d) Cetrotide®-treated woman. Arrows indicate gold particles. M: mitochondria, l: lipid droplet. Bar = 350 nm.

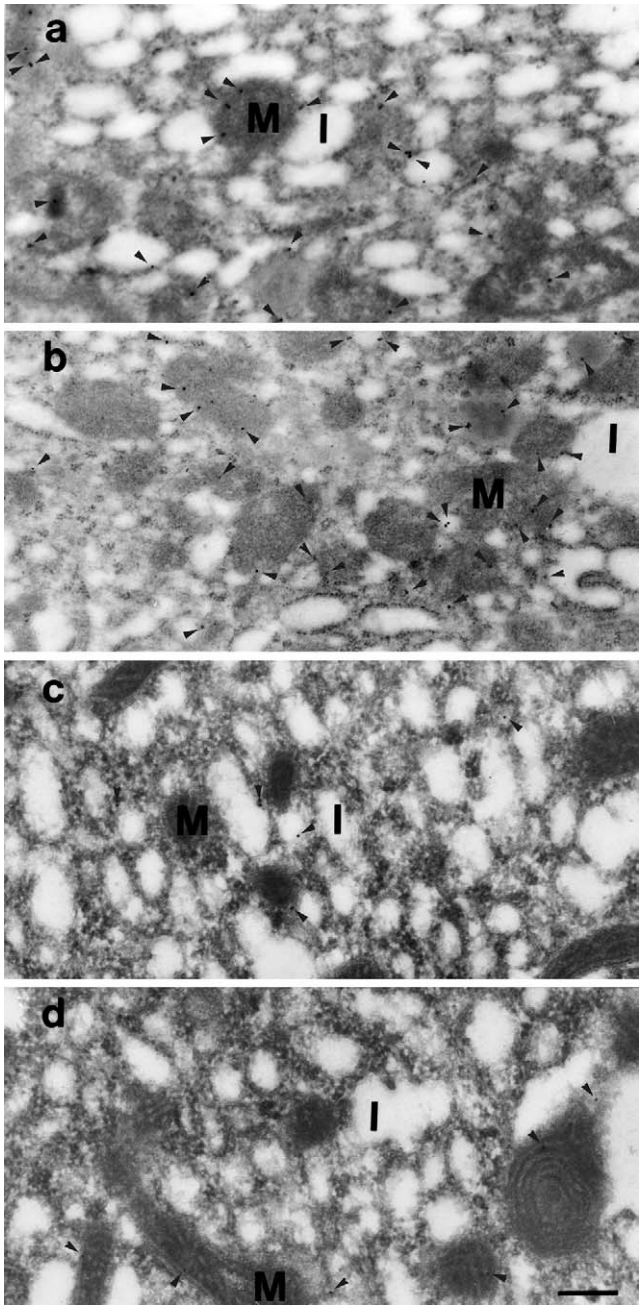

Fig. 2. Immunogold labeling of StAR in LR-Gold sections of thecal-lutein cells from corpora lutea at different times of the luteal phase and after administration of a GnRH antagonist; representative images of: (a) early, (b) mid, (c) late, (d) Cetrotide®-treated woman. Arrows indicate gold particles. M: mitochondria, l: lipid droplet. Bar = 350 nm.

ments in granulosa-lutein cells. The focus on this cell type was based on the finding of greater number of granulosa-lutein cells in our samples, allowing for statistical analysis. The cells from mid- and early-luteal CL displayed higher levels of labeling in the cytoplasm and mitochondria than those of late CL or of CL from Cetrotide®-treated subjects.

3.3. Immunoblot analyses of StAR in mitochondria and post-mitochondrial supernatants from CL of different ages and after administration of GnRH antagonist

A representative Western blot for StAR proteins in the mitochondria and cytoplasm of CL homogenates at different stages of the luteal phase and after Cetrotide® administration is shown in panel A of Fig. 3. Immunoreactive bands

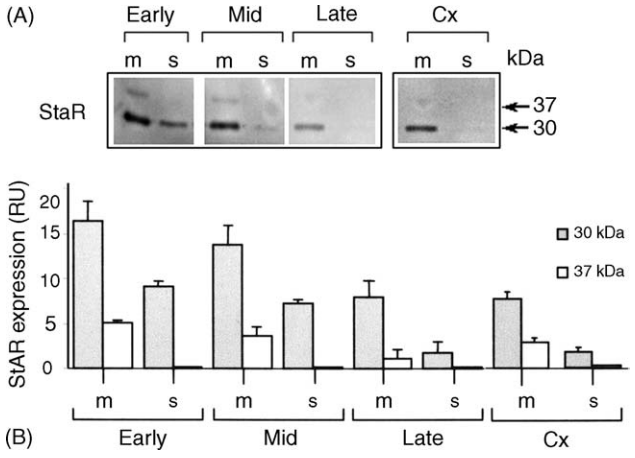

Fig. 3. Immunoblot analyses of StAR in the mitochondria and cytosol of CL homogenates.Panel A shows a representative immunoblot for StAR proteins in the mitochondria and the post-mitochondrial supernatant of CL homogenates at different stages of the luteal phase and after Cetrotide®. The blot illustrates immunoreactive bands at 37 and 30 kDa that represent the StAR pre-protein and the mature StAR protein, respectively. Panel B shows the histogram of Western blot data with the relative content of 37 and 30 kDa StAR in 10 ug protein aliquots of these subcellular fractions. Values represents the mean ± SEM of early- (3), mid- (4), late- (3) and Cx-treated (2) CL. Cx: Cetrotide®.

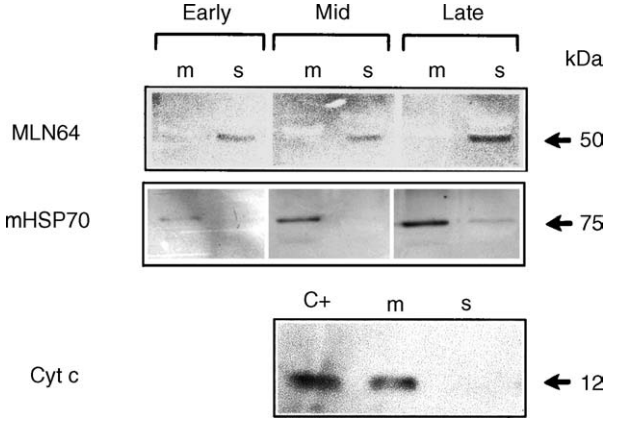

Fig. 4. The upper panel depicts a representative Western blot for MLN64 protein in the mitochondria and the post-mitochondrial supernatants of CL homogenates at different stages of the luteal phase. The blot depicts the immunoreactive band of MLN64 at 50 kDa.The middle panel displays a representative Western blot for mHSP70 in the mitochondria and the post-mitochondrial supernatants of CL homogenates at different stages of the luteal phase. The blot depicts the immunoreactive band of mHSP70 at 75 kDa. The lower panel shows a representative Western blot for denaturated cytochrome *c* in aliquots of post-mitochondrial supernatant and mitochondria from homogenates of mid-luteal phase CL. Cyt *c*: cytochrome *c*; s: post-mitochondrial supernatant; m: mitochondrial pellet; C+: sonicated mitochondria.

at 37 and 30 kDa, representing the StAR pre-protein and the mature StAR protein respectively, were detected in all the mitochondrial extracts, while in the cytosol mainly the 30 kDa protein was found. The association of the 37 kDa StAR protein with the mitochondrial pellet illustrates the efficient nature of the targeting of the nascent StAR pre-protein. Because the antibody recognizes the StAR C-terminus and the molecular weights of the cytosolic and mitochondrial proteins are similar, it seems that processed StAR exists outside of the mitochondria. The densitometric analysis is illustrated in panel B of Fig. 3. On a per unit protein basis, the relative abundance of mature StAR in mitochondria is higher than in the cytosol. However, the total protein content of the mitochondrial fractions was almost one order of magnitude lower than that of the cytosols.

3.4. Immunoblot analysis of MLN64, mHSP70 and cytochrome c in mitochondria and post-mitochondrial supernatants of CL

A representative Western blot for MLN64 in CL is shown in the upper panel of Fig. 4. A single band at 50 kDa was detected in the post-mitochondrial supernatants but not in the mitochondrial sediment throughout the luteal phase. This finding confirms that the StAR antiserum does not cross react with other proteins containing a START domain, like MLN64. MLN64 has been localized to endosomes, which is consistent with the presence of the protein in the post-mitochondrial supernatant.

The middle panel of Fig. 4 displays a representative immunoblot for mitochondrial heat shock protein 70 (mHSP70) in fractions from CL homogenates. In CL from different ages, this 75 kDa protein was detected in the organelles and not in the post-mitochondrial supernatants. These results demonstrate that during the homogenization of CL, the integrity of both, the outer and inner membranes of the organelles was preserved. Thus, the presence of mature StAR in the cytoplasm is not likely to be the result of a disruption of mitochondria.

The lower panel of Fig. 4 illustrates a representative immunoblot for cytochrome *c* in fractions from CL homogenates using an antibody that reacts with the denatured protein. Under these conditions, we detected cytochrome *c* in the mitochondrial sediments but not in the supernatants. An immunoblot assessment of cytochrome *c* distribution under native conditions, using an antibody raised against the native protein confirmed the exclusive presence of the protein inside the mitochondria (not shown). Therefore, during the homogenization of CL, the integrity of the outer membrane of the organelles was preserved. Altogether, these biochemical findings also argue against a leakage of StAR from the mitochondria into the cytosol during the processing and fixation of CL for immunoelectron microscopy.

4. Discussion

StAR, a cAMP-regulated phosphoprotein expressed primarily in non-placental steroidogenic cells, is responsible for the translocation of cholesterol from the outer to the inner mitochondrial membrane and represents the rate-limiting step in the biosynthesis of steroid hormones. The present

study, using immunogold labeling, detected StAR protein in thecal and granulosa-lutein cells of luteal tissue collected at different times throughout the luteal phase. There was a change in overall labeling density with higher values in early and mid-luteal phase CL and lowest in late luteal phase CL, confirming our previous immunohistochemical data (Devoto et al., 2001, 2002; Kohen et al., 2003).

In this study, the immunoblot analysis of StAR distribution in subcellular fractions of luteal cells confirmed the finding of Arakane et al. (1998) demonstrating the presence of mature (30 kDa) protein in the cytosol and mitochondria of COS-1 cells transfected with wild-type StAR cDNA. In contrast to Arakane et al. (1998), who detected StAR by immunoelectron microscopy only in the mitochondria of transfected COS-1 cells, we visualized it by the same technique and noted the presence of this protein in the cytoplasm of CL steroidogenic cells.

Beside methodological differences, one possible explanation for the apparent disparity in findings is that the "in vivo" system operating in CL relies on different mechanisms for processing StAR than the transfected cells in culture, resulting in higher levels of 30 kDa protein in the cytoplasm of CL steroidogenic cells.

Our analyses revealed that "mature" StAR is the most abundant form present in the mitochondria and the post-mitochondrial supernatant of homogenates from CL at different ages. Collectively, these findings argue for the presence "in vivo" of mature StAR protein in the cytoplasm. Indeed, based on total protein content, the 30 kDa StAR form in CL cytosol is greater than in the mitochondrial compartment. When the same cell fractions were analyzed for mHSP70, we detected this protein in the mitochondrial sediments from early, mid- and late-CL homogenates. In homogenates from late CL we saw, in addition to the strong mitochondrial band, a faint band of HSP70 in the supernatant. These results demonstrate that the integrity of mitochondria membranes was preserved in the gentle homogenization followed and support the view that the presence of 30 kDa StAR outside the mitochondria is not a product of membrane disruption nor the leakage of proteins from the matrix.

We found that cytochrome *c* was not released into the cytoplasm during homogenization and centrifugation. Since cytochrome *c* is located in the intermembrane space of the mitochondrion, its absence in the post-mitochondrial supernatants of gently-disrupted luteal tissues demonstrate again the preservation of (outer) mitochondrial membrane's integrity. It is thought that in steroidogenic cells the ultimate destination of StAR is the mitochondrial matrix, the space limited by the inner mitochondrial membrane. Thus, the discharge of StAR from the mitochondrial matrix would require its passage through both, the outer- and the inner-mitochondrial membranes. Disruption of only the outer membrane would allow the release of cytochrome *c* but not that of soluble macromolecules from the mitochondrial matrix. Consequently, the presence of StAR and simultaneous absence of cytochrome *c* in the cytosol supports the idea that mature StAR protein is not likely to have been released from mitochondria during tissue processing.

In normal tissues, the release of cytochrome *c* into the cytoplasm is considered an initial event in cell apoptosis. In hundreds of fields of early and mid-CL inspected under the electron microscope for this work, we did not observe cells exhibiting the morphological characteristics of this type of cell death. Therefore, the presence of mature StAR in the cytoplasm is unlikely to be a consequence of the initiation of programmed cell death due to cytochrome *c* release.

The Western blot analyses revealed higher levels of StAR pre-protein (37 kDa) in the mitochondrial fraction as compared with the post-mitochondrial supernatant of CL throughout the luteal phase. This presumably reflects an efficient targeting of 37 kDa StAR to the mitochondria, together with a rapid processing of the pre-protein that does not reach the organelle in time (Tajima et al., 2001; Granot et al., 2003). The degradation of StAR pre-protein by proteasomes of rat granulosa cells has been described, but there is no evidence that this process could lead to a release of mature protein into the cytosol (Tajima et al., 2001; Granot et al., 2002, 2003). Our observations may also suggest that in CL cells, mature StAR protein is selectively released into the cytoplasm after mitochondrial processing. It might be that StAR is exposed to physicochemical forces operating during importation and resulting, like for fumarase, in a dual distribution between cytosol and the mitochondrial matrix (Sass et al., 2003). An additional explanation for the presence of cytoplasmic 30 kDa StAR could be the association with other proteins in this cellular compartment; it is noteworthy that StAR has been shown to interact with cytoplasmic proteins involved in cholesterol metabolism, like hormone sensitive lipase (Shen et al., 2003)

The presence of mature StAR in the cytoplasm challenges the thinking about the itinerary of StAR (Christenson and Strauss, 2000). Recent evidence shows that the C-terminal of StAR associates with phospholipid head groups of artificial membranes resembling the outer-mitochondrial coating (Yaworsky et al., 2004). It appears that the last 28 amino acids of StAR C-terminus are essential in the transference of cholesterol through these membranes; the mitochondrial targeting peptide of 37 kDa StAR would play a role in the duration of cholesterol passage (Yaworsky et al., 2004). If this model is correct, cytoplasmic mature StAR, incapable of importation into the mitochondria but carrying an intact C-terminal, could sustain cholesterol supply for the steroidogenic process, a desirable attribute in mid-luteal phase CL cells which must generate large quantities of progesterone. Indeed, it is possible that the presence of mature StAR in the cytoplasm is restricted to cells with a large capacity to produce steroid hormones, like those of CL. In this context, our results are consistent with the data presented by LeHoux et al. (1999) demonstrating the presence of 29–30 kDa StAR proteins in the cytosol of rat adrenal homogenates. According to these authors, both the cytosolic and the mitochondrial mature forms of StAR are related to the

steroidogenic response observed after stimulation with ACTH- and/or sodium restriction (LeHoux et al., 1999).

MLN64 contains a C-terminal StAR-related lipid transfer (START) domain and that it may participates in the intracellular trafficking of cholesterol in animal cells, including follicular thecal and granulosa cells (Watari et al., 1997). The presence of MLN64 in the supernatant of CL extract throughout the luteal phase, supports the idea that in cells with high steroidogenic activities, protein(s) with a START domain, other than StAR, might participate in the intracellular trafficking of cholesterol or of other lipids (Kishida et al., 2004).

The drop in LH output, resulting from the administration of the GnRH antagonist during mid-luteal phase, causes a dramatic reduction in StAR immunolabeling in both the cytoplasm and mitochondria of thecal and granulosa-lutein cells of mid-luteal CL, suggesting that the levels of StAR protein in both cell compartments are LH dependent. In contrast to the results reported by others (Fraser et al., 1999), we did not observe deleterious actions of the GnRH antagonist on the ultrastructural characteristics of luteal steroidogenic cells; this disparity may be related to the differences in chemical structures and activities between the GnRH antagonist used: Atarelix® versus Cetrotide®. In addition, we must consider that the data of Fraser et al. (1999) were obtained in the marmoset using doses of GnRH antagonist much greater than those administered here. Thus, the decline of luteal StAR protein levels in GnRH antagonist treated women is presumably the result of the decline in plasma LH and not a deleterious action of the GnRH antagonist on the luteal steroidogenic cells.

The underlying mechanisms controlling the simultaneous reduction of StAR in the mitochondria and the cytosol of late luteal phase CL presumably include changes in abundance of StAR mRNA, but post-translational mechanisms may also play a role. The cell death processes initiated during the natural regression of late luteal phase CL may also contribute to the alterations in StAR. The simultaneous morphological and molecular assessment of CL StAR reported herein contribute to the understanding of the complex cellular changes that govern the endocrine function of the Cl during its life cycle.

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