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HSDJ, a Human Homolog of DnaJ, Is Farnesylated and Is Involved in Protein Import into Mitochondria[1]

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The role of HSDJ, a human homolog of bacterial DnaJ and yeast YDJ1p/MAS5, in mitochondrial protein import was examined. Recombinant HSDJ was purified and an antibody was prepared. HSDJ mRNA was heat-induced in cultured cells. In pulse-labeling and chase experiments using COS-7 cells, the endogenous HSDJ homolog was prenylated. Transiently expressed HSDJ was also prenylated, whereas its mutant C394S in which cysteine of the "CaaX box" was mutated to serine, was not. HSDJ, but not C394S, synthesized in rabbit reticulocyte lysate was farnesylated. The HSDJ antibody inhibited import of ornithine transcarbamylase precursor (pOTC) into isolated mitochondria when added prior to pOTC synthesis, but not when added prior to import assay. In transient expression of pOTC in COS-7 cells, pOTC was synthesized and processed to the mature form with an apparent half-life of 2-3 min. Coexpression of HSDJ or C394S resulted in slight retardation of the pOTC processing. These results indicate that HSDJ is involved in an early step(s) of protein import into mitochondria.

Key words: DnaJ homolog, farnesylation, HSDJ, mitochondrial protein import, molecular chaperone.

Most mitochondrial proteins are synthesized on cytosolic free ribosomes as larger precursors with NH_2-terminal presequences, released into a cytosolic pool, and then imported into mitochondria with half-lives of less than a few minutes. The presequence portions of precursor proteins are proteolytically cleaved in the mitochondrial matrix, and the mature portions are folded and assembled into their final conformations (*1-4*). The precursor proteins must be prevented from tight folding or aggregation in the cytosol prior to import. Several cytosolic factors were reported to be required for mitochondrial protein import in higher animals. They include presequence-specific cytosolic factors such as presequence binding factor (PBF) (*5, 6*), mitochondrial import stimulation factor (MSF) (*7-9*), and hsp70 molecular chaperone family members (*10-12*). We developed an *in vitro* translation-import system in which heat shock cognate 70 protein (hsc70) was completely depleted from the rabbit reticulocyte lysate, and showed that hsc70 is required during synthesis of ornithine transcarbamylase (OTC) precursor (pOTC), but not during its import into mitochondria (*13, 14*).

DnaK, the hsc70 homolog of *Escherichia coli*, is regulated by other molecular chaperones, DnaJ and GrpE (*15-17*). Recently, several eukaryotic DnaJ homologs have been found, and it seems likely that hsp70s are generally regulated by these homologs (*18*). cDNAs for three human DnaJ homologs, hsp40/HDJ-1 (*19, 20*), HSDJ/HDJ-2 (*21, 22*), and HSJ1 (*23*) have been isolated. Among the three homologs, hsp40/HDJ-1 was shown to mediate the initial folding process of the cytosolic proteins in cooperation with hsc70 and TriC complex (*24*). On the other hand, the roles of the other two homologs are totally unknown. cDNA for HSDJ/HDJ-2 was isolated in a cDNA project from human fibrosarcoma HT-1080 cells (*21*) and from human umbilical vein endothelial cells using an antibody which reacts specifically to human endothelial cells and monocytes (*22*). This cDNA encodes a protein of 397 amino acid residues whose sequence is 38% identical with DnaJ and 47% identical with YDJ1p/MAS5, a DnaJ homolog of *Saccharomyces cerevisiae* that was shown to facilitate protein translocation across mitochondrial and endoplasmic reticulum membranes (*25, 26*). HSDJ as well as YDJ1p contains a COOH-terminal "CaaX box" motif common to proteins that are modified by prenylation (*27*). We report here that HSDJ is heat-induced and farnesylated, and that it is apparently involved in mitochondrial protein import in mammals.

MATERIALS AND METHODS

Plasmids—A mammalian expression vector pCAGGS (*28*) was provided by J. Miyazaki of Osaka University and a phenylalanine hydroxylase expression plasmid pCAGGS/

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Abbreviations: MBP, maltose binding protein; pOTC and OTC, precursor and mature form of ornithine transcarbamylase.

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hPAH by Y. Matsubara and K. Narisawa of Tohoku University. pCAGGS/HSDJ was constructed by inserting the *Eco*RI (partial)-*Not*I 1.5 kb fragment of HSDJ cDNA (*21*) into the *Xho*I site of pCAGGS. pCAGGS/pOTC was constructed by inserting the *Eco*RI fragment of human OTC cDNA (*29*) into the *Eco*RI site of pCAGGS. The C394S mutant of HSDJ was generated by PCR to mutate codon 394 TGT (cysteine) to AGT (serine). The PCR oligonucleotides for C394S were 5′-CGGTGAGAGGCGG-AGGAGCGGTAACTA-3′ (sense) and 5′-CACCGGATCC-ATTAAGAGGTATGACTCTGAACA-3′ (antisense), with a fragment size of 1,306 bp. An expression plasmid for HSDJ antisense RNA was constructed by inserting the full-length HSDJ cDNA (*21*) in the antisense orientation into pCAGGS.

Expression and Purification of Histidine-Tagged HSDJ—A DNA fragment encoding N-His-HSDJ, generated by polymerase chain reaction, was inserted into pMAL-c2 (New England Biolabs) in-frame with maltose binding protein (MBP). The histidine-tag was induced for two reasons. Firstly, MBP-HSDJ fusion protein without the His-tag could not be cleaved by Factor Xa. Secondly, the His-tag could be used for purification of N-His-HSDJ after Factor Xa digestion. N-His-HSDJ was expressed as an MBP fusion protein in a protease-deficient *E. coli* strain BL21 (Novagen). The fusion protein was purified from the cell lysate using an amylose resin (New England Biolabs), and cleaved with Factor Xa (Haematologic Technologies Inc., 2.5 μg/mg of the fusion protein) for 2 h at 25°C. N-His-HSDJ was purified by nickel chelate affinity chromatography (Pharmacia).

Antibodies—Anti-HSDJ antibody was raised in a rabbit by injecting 1.0 mg of the purified N-His-HSDJ mixed with Freunds' complete adjuvant intracutaneously two times biweekly and 0.5 mg of HSDJ mixed with Freunds' incomplete adjuvant three times biweekly. Anti-OTC antibody was raised in a rabbit by injecting the *E. coli*-expressed and purified human mature OTC, as described for anti-HSDJ. Anti-phenylalanine hydroxylase was provided by S. Kaufman of National Institutes of Health, Bethesda, Maryland, USA.

Cell Culture and DNA Transfection—COS-7 cells were cultured in 10-cm culture dishes in Dulbecco's modified Eagle's medium (DMEM) plus fetal calf serum. Subconfluent cells were transfected with eukaryotic expression vectors by a calcium phosphate precipitation procedure.

Pulse and Pulse-Chase Experiments—COS-7 cells were transfected with 10 or 20 μg of expression vectors. After 24 h, the cells were harvested with trypsinization, washed twice with phosphate-buffered saline and suspended in 1 ml of methionine-free DMEM. The cell suspensions were preincubated at 37°C for 1 h in a 50-ml conical tube and radiolabeled with 8 MBq of Pro-mix™ containing L-[^{35}S]-methionine and L-[^{35}S]cysteine (Amersham) for the indicated periods. Aliquots (0.6 ml) were removed at the indicated times and mixed with 0.4 ml of ice-cold 25 mM Tris-HCl (pH 7.4) containing 5 mM EDTA, 0.25% SDS, 0.25% Triton X-100, 125 μM chymostatin, 125 μM pepstatin, 125 μM leupeptin, and 125 μM antipain. The cell lysates were clarified at 10,000 × *g* for 10 min. Radiolabeled proteins were immunoprecipitated, subjected to 10% SDS-PAGE and fluorography and quantified by imaging plate analysis using a FUJIX BAS2000 analyzer (Fuji Film). In pulse-chase experiments, the cells were suspended in 1 ml of methionine-free medium, preincubated for 1 h, radiolabeled for 5 min, and then chased by adding 2 ml of medium containing 20 mM methionine.

RNA Blot Analysis—Total RNAs (2 μg) were isolated and electrophoresed in a formaldehyde-containing 1.0% agarose gel and transferred to a nylon membrane. The membrane was hybridized with digoxigenin-labeled RNA according to the protocol supplied by Boehringer. Detection was performed using the DIG luminescence detection kit (Boehringer).

In Vitro Translation of HSDJ in Rabbit Reticulocyte Lysate—Prior to *in vitro* transcription, pTZ18R plasmids (Pharmacia) containing HSDJ or C394S inserts were linearized by digestion with *Bam*HI. The linearized plasmids (6 μg) were transcribed with T7 RNA polymerase. *In vitro* translation of the RNA transcripts was carried out in a rabbit reticulocyte system [Promega, 40% (v/v) lysate]. A standard 50 μl reaction containing 2 μg of mRNA, 2.7 MBq [^{35}S]Promix™, 50 μM antipain, and 50 μM leupeptin was incubated at 25°C. Two microliter aliquots were withdrawn from the reaction at appropriate times, and subjected to 10% SDS-PAGE and fluorography.

In Vitro Incorporation of [1-^{3}H]Farnesyl Pyrophosphate into HSDJ—The ability of *in vitro*-translated HSDJ protein to serve as an acceptor for farnesylation was checked by adding 9.3 kBq of [1-^{3}H]farnesyl pyrophosphate, triammonium salt (2.1 TBq/mmol, American Radiolabeled Chemicals) instead of [^{35}S]Promix™ to the standard *in vitro* translation system. Eight microliter aliquots were withdrawn from the reaction mixture at appropriate times, and subjected to 10% SDS-PAGE and fluorography.

In Vitro Import of pOTC into Mitochondria—Translation was performed *in vitro* in a rabbit reticulocyte lysate system [Promega, 40% (v/v) lysate] as described previously (*13*). The import mixture (50 μl) containing 4.0 μl of the lysate and ^{35}S-labeled pOTC (2.0–40 kBq) was incubated with isolated rat liver mitochondria (100 μg) at 25°C for 12 min. The reaction was stopped by diluting the import mixture into the ice-cold mitochondria isolation buffer containing 0.1 mM dinitrophenol (*13*). The mitochondria were reisolated by centrifugation and subjected to 10% SDS-PAGE. The radioactive polypeptides were visualized by fluorography and quantitated by imaging plate analysis using a FUJIX BAS2000 analyzer.

RESULTS AND DISCUSSION

Expression and Purification of HSDJ—MBP-N-His-HSDJ fusion protein was expressed in *E. coli* cells, purified by amylose affinity chromatography and cleaved to MBP and N-His-HSDJ with factor Xa (Fig. 1). N-His-HSDJ was purified to apparent homogeneity by nickel chelate column chromatography. Anti-HSDJ antibody was raised in a rabbit.

Farnesylation of HSDJ—In immunoblot analysis of COS-7 cell extracts using the anti-HSDJ antibody, a polypeptide with an estimated molecular mass of 46 kDa that corresponds to the mature form of the COS-7 HSDJ homolog was detected (Fig. 2A). When HSDJ cDNA in a potent mammalian expression vector pCAGGS (*28*) was transfected into COS-7 cells, the amount of the 46 kDa form increased, indicating expression of the mature form of

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HSDJ. In addition, a polypeptide with an estimated molecular mass of 48 kDa appeared. When a cDNA for C394S, in which cysteine of the "CaaX box" was mutated to serine, was transfected, the expressed mutant HSDJ remained in the 48 kDa form. These results indicate that the 48 kDa form is the unprenylated precursor of HSDJ and the 46 kDa form is the prenylated mature form. An increase of mobility in SDS-PAGE after farnesylation was reported for YDJ1p (*27*) and *ras*-related GTP-binding proteins (*30, 31*).

Prenylation of HSDJ was analyzed by pulse-chase experiments (Fig. 2B). When untransfected COS-7 cells were labeled with [^{35}S]methionine for 5 min, the unprenylated form of endogenous HSDJ homolog and a lesser amount of the prenylated form were detected. In chase experiments, the unprenylated form was converted into the prenylated

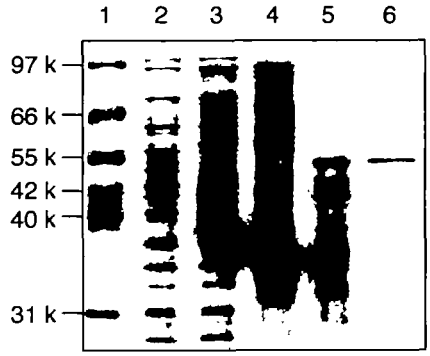

Fig. 1. **Expression and purification of histidine-tagged HSDJ.** Fractions at each purification step were analyzed by 10% SDS-PAGE. Proteins were stained with Coomassie Brilliant Blue. Lane 1, molecular mass markers; lane 2, uninduced cells (10 μg of protein); lane 3, induced cells (10 μg); lane 4, amylose column fraction (4 μg); lane 5, fraction after factor Xa cleavage (4 μg); lane 6, the purified protein (2 μg). Molecular mass markers were phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), glutamate dehydrogenase (55 kDa), ovalbumin (42 kDa), aldolase (40 kDa), and carbonic anhydrase (31 kDa). An attempt to express HSDJ using a T7 polymerase expression vector pET-3a (Novagen) failed.

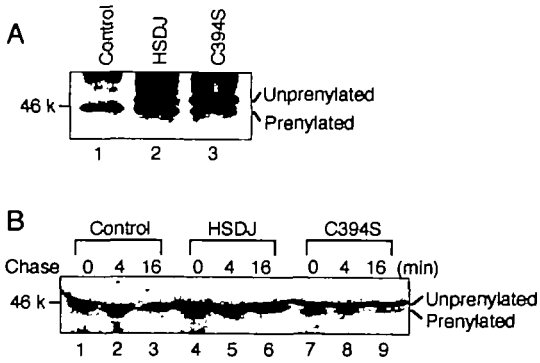

Fig. 2. **Immunoblot and pulse-chase analyses of endogenous HSDJ homolog and overexpressed HSDJ and C394S.** (A) COS-7 cells were cotransfected with 10 μg each of a eukaryotic expression vector for pOTC (pCAGGS/pOTC) plus 10 μg of pCAGGS (lane 1), pCAGGS/HSDJ (lane 2), or pCAGGS/C394S (lane 3). pCAGGS/pOTC was added so that conditions were the same as those for Fig. 6. After 24 h, the cells were harvested with trypsinization and washed twice with phosphate-buffered saline. Cell extracts (10 μg of protein) were subjected to 10% SDS-PAGE and proteins were electrotransferred to a nitrocellulose membrane. Immunodetection was performed using rabbit anti-HSDJ serum (×5,000 diluted) and an ECL kit (Amersham) according to the protocol supplied by the manufacturer. (B) COS-7 cells were transfected with 10 μg each of pCAGGS (lanes 1-3), pCAGGS/HSDJ (lanes 4-6), or pCAGGS/C394S (lanes 7-9). After 24 h, pulse-chase experiments were performed as described in "MATERIALS AND METHODS." Radiolabeled wild-type and mutant HSDJs were immunoprecipitated with 20 μl of anti-HSDJ serum and 200 μl of 10% suspension of protein A-Sepharose as described (*35*), and subjected to 10% SDS-PAGE and fluorography. Radioactive polypeptides were visualized by imaging plate analysis.

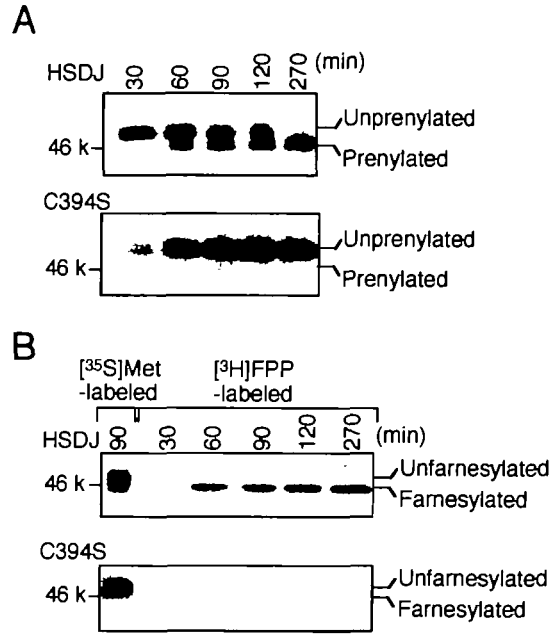

Fig. 3. **[^{35}S]Methionine (A) and [^{3}H]farnesyl pyrophosphate (B) labeling of HSDJ and C394S mRNA in a rabbit reticulocyte lysate system.** mRNAs coding for HSDJ and the C394S mutant were translated *in vitro* in standard reticulocyte lysate reactions (50 μl) as described in "MATERIALS AND METHODS." Samples were removed from each translation reaction (2 μl from reactions containing [^{35}S]methionine and 8 μl from reactions containing [^{3}H]farnesyl pyrophosphate) at the indicated times and subjected to 10% SDS-PAGE followed by fluorography. The fluorographs in A were exposed for 3 h, whereas those in B were exposed for 72 days. The reason for the presence of two adjacent bands for both prenylated and unprenylated forms in A is not known. 46 k shows the position of ovalbumin (Rainbow colored protein markers, Amersham).

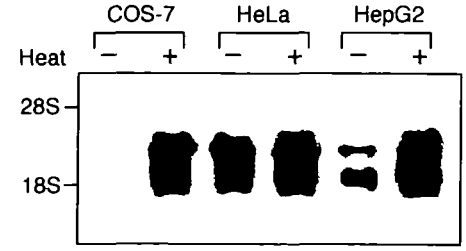

Fig. 4. **Induction of HSDJ mRNA in COS-7, HeLa, and HepG2 cells by heat treatment.** Cells were untreated (−) or heated at 45°C for 15 min (+) and allowed to recover at 37°C for 3 h. Total RNAs (2 μg) were subjected to RNA blot analysis using as a probe digoxigenin-labeled HSDJ RNA. The positions of 28S and 18S rRNAs are shown on the left. Integrity of the RNAs was verified by the apparently identical intensities of 28S and 18S rRNA bands following the ethidium bromide staining.

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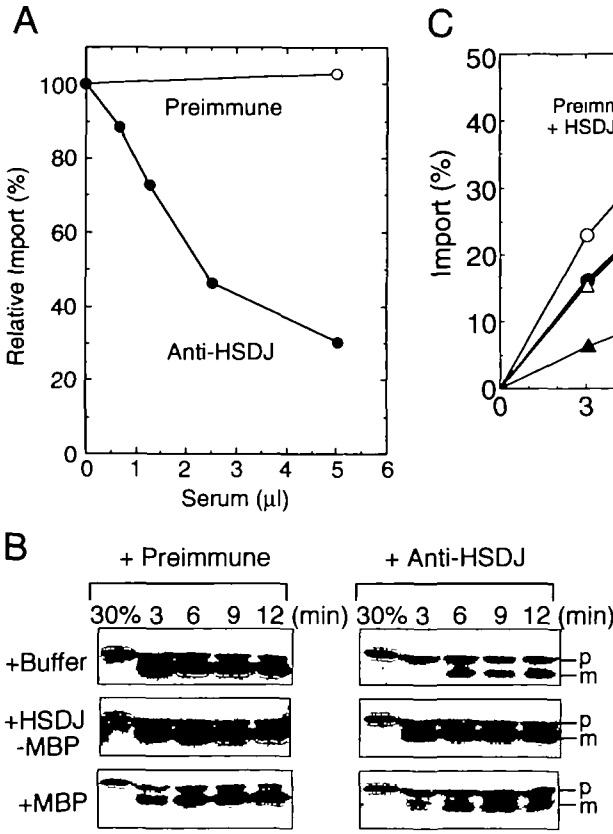

Fig. 5. Effect of anti-HSDJ serum on pOTC import into rat mitochondria. (A) Effect of varying amounts of anti-HSDJ serum on pOTC import. The indicated volume of anti-HSDJ serum or preimmune serum was added to the translation mixture prior to translation. Translation and mitochondrial import of pOTC were performed as described in "MATERIALS AND METHODS." Import without added serum was set at 100%. (B) Effect of anti-HSDJ serum and HSDJ-MBP on pOTC import. Translation (50 μl) was performed in the presence of 2.5 μl of buffer A [20 mM Hepes-KOH (pH 7.6), 120 mM potassium acetate, 20% (v/v) glycerol] alone, or 0.54 μM (50 μg/ml) HSDJ-MBP in buffer A or 0.94 μM (38 μg/ml) MBP in buffer A with 5 μl of the indicated serum. Import reaction and subsequent analysis were performed as described in A. p, pOTC; m, mature form of OTC; 30%, 30% of input pOTC. (C) The results in B were quantified by imaging plate analysis.

form almost completely within 16 min. When HSDJ was overexpressed, prenylation was greatly retarded. On the other hand, prenylation of C394S was not observed.

Prenylation of HSDJ was further analyzed in an *in vitro* translation system (Fig. 3). Studies on a set of GTP-binding proteins showed that the rabbit reticulocyte lysate system contains all the components necessary for prenylation of proteins, including the enzymes which supply activated isoprenoids and prenyltransferases (*32, 33*). When HSDJ mRNA was translated *in vitro*, the unprenylated precursor form of 48 kDa appeared rapidly, reached a maximum in 60 min, and then decreased (Fig. 3A). The precursor form of 48 kDa was converted to the prenylated mature form of 46 kDa. The amount of the mature form reached a plateau in 120 min. No mature form of 46 kDa was observed when C394S mRNA was translated. These results accord well with the results obtained from cultured cells (Fig. 2).

Prenylation of HSDJ seems to be farnesylation, because C-terminal serine in the CaaX box of HSDJ favors farnesylation (*31, 34*). This was confirmed by [^{3}H]farnesyl pyrophosphate incorporation into HSDJ synthesized *in vitro* (Fig. 3B). Incorporation was not observed when C394S mutant was translated. *In vitro* synthesis of ^{35}S-labeled HSDJ in the presence of α-hydroxyfarnesylphosphonic acid, a potent inhibitor of protein farnesyltransferase, reduced the formation of the 46 kDa mature form (data not shown). Thus, HSDJ is farnesylated *in vitro*, and most probably *in vivo*, as well.

Heat Induction of HSDJ mRNA—The effect of heat shock on HSDJ gene expression in cultured cells was examined (Fig. 4). COS-7 cells, HeLa cells, and HepG2 cells were heated at 45°C for 15 min, then allowed to recover at 37°C for 3 h, and RNA blot analysis of HSDJ mRNA was performed. Two HSDJ mRNA species of about 3.5 and 1.8 kb were detected in all cell lines, and both species were induced several-fold in these cell lines by the heat-treatment.

Inhibition of pOTC Import by Anti-HSDJ Serum—We asked whether HSDJ is involved in protein import into mammalian mitochondria. We examined the effect of an HSDJ antibody on pOTC import into isolated rat liver mitochondria. pOTC was synthesized in a rabbit reticulocyte lysate system and subjected to import assay. When the HSDJ antiserum was added after translation (prior to import), pOTC import was not inhibited (data not shown). On the other hand, when the antiserum was added prior to synthesis of pOTC, its import was markedly inhibited (Fig. 5A). This inhibition was mostly recovered by the addition of purified HSDJ-MBP fusion protein, but not by the addition of MBP (Fig. 5, B and C). Immunodepletion of HSDJ from the reticulocyte lysate markedly decreased pOTC import (data not shown). However, this reduction could not be restored by the readdition of recombinant MBP-N-His-HSDJ. Attempts to prepare functionally active HSDJ are under way.

Effect of Overexpression of HSDJ, C394S, and HSDJ Antisense RNA on pOTC Import in Cultured Cells—We next tested the effects of overexpression of HSDJ, C394S, and HSDJ antisense RNA on mitochondrial import of pOTC in cultured cells. An assay system to analyze the import in cultured cells was developed. COS-7 cells were transfected with expression plasmids and short-term pulse and pulse-chase experiments were performed by using suspended cells. Mitochondrial import of pOTC was assessed in terms of its processing to the mature form (*35*). When the cells transfected with the pOTC expression plasmid were labeled with [^{35}S]methionine, newly synthesized pOTC of 40 kDa appeared in 1 min, increased up to about 5 min and reached a plateau. On the other hand, mature OTC of 36 kDa appeared with a lag time of 2 min and increased with time up to 10 min (Fig. 6A).

Then pulse-chase experiments were performed. In a pulse of 5 min, 65% of the newly synthesized OTC was processed to the mature form (Fig. 6, B-a and C). When the

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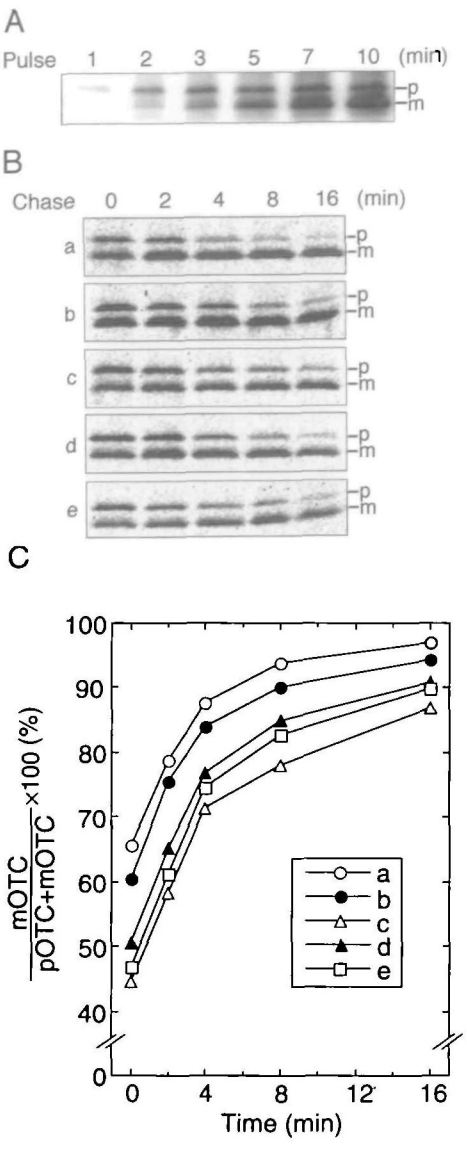

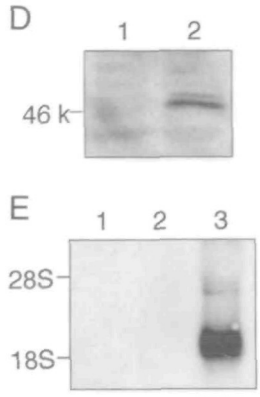

Fig. 6. **Synthesis and processing of pOTC in COS-7 cells and effect of overexpression of HSDJ, C394S, and antisense HSDJ RNA on pOTC processing.** (A) COS-7 cells were transfected with 10 μg of pCAGGS/pOTC and 10 μg of pCAGGS. After 24 h, the cells (3 ml) were labeled with 8 MBq of Pro-mix™. Aliquots (0.6 ml) were removed at the indicated times and pOTC (p) and mature OTC (m) were immunoprecipitated with 20 μl of anti-human OTC serum as described for Fig. 2B. (B) COS-7 cells were cotransfected with 10 μg of pCAGGS/pOTC plus 10 μg of pCAGGS (a), pCAGGS/hPAH (b), pCAGGS/HSDJ (c), pCAGGS/C394S (d), or pCAGGS containing HSDJ antisense cDNA (e). After 24 h, the cells (1 ml) were labeled for 5 min with 8 MBq of Pro-mix™ and chased for the indicated times as described for Fig. 2B. pOTC and mature OTC were immunoprecipitated as described in A. (C) The radioactive pOTC and mature OTC on the SDS-polyacrylamide gel were quantified by imaging plate analysis and percent values of mature OTC (mOTC) in pOTC plus mOTC are shown. (D) Expression of phenylalanine hydroxylase. COS-7 cells were cotransfected with pCAGGS/pOTC plus pCAGGS (lane 1) or pCAGGS/hPAH (lane 2) under the same conditions as for Fig. 6B-b. Cell extracts (10 μg of protein) were subjected to immunoblot analysis using anti-rat phenylalanine hydroxylase IgG and the ECL kit as described for Fig. 2A. (E) Expression of HSDJ antisense RNA. COS-7 cells were transfected with pCAGGS (lane 1), pCAGGS/HSDJ (lane 2), or pCAGGS/HSDJ cDNA in opposite orientation (lane 3). Total RNAs (2 μg) were subjected to RNA blot analysis using as a probe digoxigenin-labeled HSDJ sense RNA. The positions of 28S and 18S rRNAs are shown on the left.

cells were chased with non-labeled methionine after the 5-min pulse, pOTC decreased with an apparent half-life of 2–3 min and mature OTC increased concomitantly. These results accord with previous data obtained using isolated rat hepatocytes (*35*) and HeLa cells transfected with pOTC cDNA (*36*). Coexpression of a cytosolic protein, phenylalanine hydroxylase, had little effect on pOTC import (Fig. 6, B-b, C, and D). On the other hand, when HSDJ was coexpressed, only 30% of the newly synthesized OTC was processed in a 5-min pulse, and pOTC decreased more slowly in the chase (Fig. 6, B-c and C). The retardation of pOTC import by HSDJ overexpression was marginal, but was repeatedly observed in several independent experiments. Assuming that HSDJ participates in mitochondrial protein import in cooperation with hsc70 and other factors, the retardation of pOTC import by the HSDJ overexpression is thought to be due to an imbalance of these factors. In fact, *in vitro* import of yeast pre-F_1 ATPase β subunit into yeast mitochondria and that of yeast prepro-α-factor into canine pancreatic and yeast microsomes were inhibited by DnaJ and the inhibition of prepro-α-factor translocation was restored by the addition of DnaK, a bacterial hsp70 member, plus GrpE (*37*). Synthesis of pOTC was not affected by the HSDJ overexpression. Cotransfection of decreasing amounts of the HSDJ plasmid resulted in less marked inhibitions of pOTC import and not in its stimulation (data not shown). pOTC import was also inhibited by overexpression of the HSDJ mutant C394S, but the inhibition was somewhat weaker than that by wild-type HSDJ (Fig. 6, B-d and C). Under these conditions, expressed wild-type HSDJ was partly prenylated and partly unprenylated, whereas C394S was totally unprenylated (Fig. 2A). Therefore, unprenylated HSDJ may be less inhibitory than the prenylated form. pOTC import was also inhibited by overexpression of HSDJ antisense RNA (Fig. 6, B-e, C, and E). This inhibition may be due to a decrease in intracellular HSDJ homolog concentration, but this could not be confirmed because of the low efficiency of transient transfection.

These results suggest strongly that HSDJ is involved in pOTC import into mammalian mitochondria. HSDJ is apparently required during pOTC synthesis and not during import into the mitochondria, the results being similar to those for hsc70 (*13*). HSDJ presumably binds to the growing pOTC polypeptide in association with hsc70 and maintains it in an import-competent unfolded or loosely folded conformation. This is in sharp contrast to the recent finding that hsc70 and hsp40, another mammalian DnaJ homolog, mediate the initial folding process of the cytosolic proteins (*24, 38*). It remains to be tested whether hsp40 can replace HSDJ in mitochondrial protein import, and conversely whether HSDJ can replace hsp40 in protein folding. The molecular mechanisms of sorting between the folding (cytosolic proteins) and anti-folding (mitochondrial proteins) of the newly synthesized proteins by the hsc70-DnaJ molecular chaperone family members also require further study.

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