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Identification of the Mitochondrial ATP-Mg/P_i Transporter

BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION*

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The mitochondrial carriers are a family of transport proteins that, with a few exceptions, are found in the inner membranes of mitochondria. They shuttle metabolites, nucleotides, and cofactors through this membrane and thereby connect and/or regulate cytoplasm and matrix functions. ATP-Mg is transported in exchange for phosphate, but no protein has ever been associated with this activity. We have isolated three human cDNAs that encode proteins of 458, 468, and 489 amino acids with 66–75% similarity and with the characteristic features of the mitochondrial carrier family in their C-terminal domains and three EF-hand Ca^{2+}-binding motifs in their N-terminal domains. These proteins have been overexpressed in *Escherichia coli* and reconstituted into phospholipid vesicles. Their transport properties and their targeting to mitochondria demonstrate that they are isoforms of the ATP-Mg/P_i carrier described in the past in whole mitochondria. The tissue specificity of the three isoforms shows that at least one isoform was present in all of the tissues investigated. Because phosphate recycles via the phosphate carrier in mitochondria, the three isoforms of the ATP-Mg/P_i carrier are most likely responsible for the net uptake or efflux of adenine nucleotides into or from the mitochondria and hence for the variation in the matrix adenine nucleotide content, which has been found to change in many physiopathological situations.

Functional studies in intact mitochondria have indicated that the inner membranes of mitochondria contain a variety of proteins that transport important metabolites, nucleotides, and cofactors into and out of the matrix. Family members have three tandem repeated sequences, each of ~100 amino acids, made of two hydrophobic transmembrane α-helices joined by a hydrophilic loop. The tandem repeats contain conserved features. So far, 18 members of the family have been identified. They are the uncoupling protein; the carriers for ADP/ATP, phosphate, 2-oxoglutarate/malate, citrate, carnitine/acylcarnitine, dicarboxylates, ornithine, succinate-fumarate, oxalacetate-sulfate, oxodicarboxylates, deoxynucleotides, aspartate-glutamate, glutamate, thiamine pyrophosphate, and *S*-adenosylmethionine; the adenine nucleotide transporter in peroxisomes; and the dicarboxylate-tricarboxylate carrier in plants (see Refs. 1–3 for reviews and Refs. 4 and 5).

The functions of other family members found in genome sequences are unknown. At the same time, there are other transport activities observed in intact mitochondria that have yet to be associated with specific proteins. An example is the net transport of adenine nucleotides across the inner mitochondrial membrane (see Ref. 6 for review), which is essential for modulation of the matrix adenine nucleotide concentration that is subject to change in many physiopathological situations (see Refs. 7 and 8 for reviews). To explain this activity, in the early 1980s, a new mitochondrial carrier was postulated (9–11), the ATP-Mg/P_i carrier, whose properties have been investigated thoroughly in isolated mitochondria (9–18). This carrier catalyzes a reversible counterexchange of ATP-Mg for P_i that accounts for the net uptake or efflux of ATP-Mg as P_i recycles rapidly through the membrane via the phosphate carrier. The carrier-mediated transport consists of an electroneutral divalent exchange between ATP-Mg^{2-} and HPO_4^{2-} as shown by the equilibrium ratios of these substrates under every condition tested and by the ability of monofluorophosphate (but not difluorophosphate) to exchange for ATP-Mg. ADP can also be transported by the ATP-Mg/P_i carrier, and nonproductive exchanges (ATP-Mg^{2-} for ATP-Mg^{2-} and HPO_4^{2-} for HPO_4^{2-}) are also catalyzed by the carrier. The transport activity is insensitive to carboxyatractyloside and inhibited by mersalyl. Furthermore, the ATP-Mg/P_i carrier is saturable and activated by calcium. However, its activity is slow compared with that of the reactions for ATP synthesis and hydrolysis and with that of most other mitochondrial carriers. Consequently, the ATP transported by this carrier distributes quickly into the ATP, ADP, and (eventually) AMP pools, and the net transport of ATP-Mg results in a change in the adenine nucleotide concentrations without changing the ATP/ADP ratios in the cytosol and mitochondrial matrix compartments.

In this study, the identification of three human isoforms of

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ619961, AJ619962, and AJ619963.

This paper is dedicated to the memory of Professor Giuseppe Porcellati.

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the mitochondrial ATP-Mg/P_i carrier (APC)[1] is described. By using the ADP/ATP carrier sequence, human expressed sequence tags (ESTs) that encode fragments of the three related proteins were identified. They provided information to complete the human cDNA sequences. The encoded proteins are 458, 468, and 489 amino acids long and ~60% identical in sequence and were found to be localized to mitochondria. They were overexpressed in *Escherichia coli*, purified, reconstituted into phospholipid vesicles, and shown to be the human isoforms of the ATP-Mg/P_i carrier. The main function of these proteins is probably to catalyze the net uptake or efflux of adenine nucleotides into or from the mitochondria. This work presents the first information on the molecular properties of the mitochondrial carriers responsible for the ATP-Mg/P_i exchange and a definitive identification of their genes.

EXPERIMENTAL PROCEDURES

Sequence Search and Analysis—Human EST and genome data bases[2] were screened with the protein sequence of human AAC1 (GenBank™/EBI accession number NM_001151) and with the protein sequences of APC1, APC2, and APC3, respectively, using TBLASTN. Amino acid or nucleotide sequences were aligned with ClustalW Version 1.8.

Construction of Expression Plasmids—The coding sequences for APC1, APC2, and APC3 and their C-terminal domains (CTDs) were amplified by PCR from human testis cDNA (APC1 and its CTD) and human brain cDNA (APC2 and APC3 and their CTDs). For amplification of APC1, APC2, and APC3, forward and reverse oligonucleotide primers corresponded to the extremities of their coding sequences (GenBank™/EBI accession numbers AJ619961, AJ619962 and AJ619963, respectively). For amplification of the CTDs, the forward primers corresponded to nucleotides 619–642, 915–934, and 626–646 of the APC1, APC2, and APC3 cDNAs, respectively. Each pair of forward and reverse primers had HindIII and BamHI or BamHI and HindIII restriction sites as linkers. The reverse primers with additional BamHI sites did not contain the stop codon. The amplified products carrying HindIII and BamHI were cloned into a modified pcDNA3 expression vector (19) in-frame with the HA1-EGFP sequence (where HA1 is a nine-amino acid epitope derived from hemagglutinin, and EGFP represents the humanized version of the S65T green fluorescent protein (GFP) mutant). The amplified products carrying BamHI and HindIII were cloned into the *E. coli* pQE30 expression vector (QIAGEN Inc.). The cloning of APC3 and its CTD was preceded by a site-directed mutagenesis reaction to remove the BamHI site present in their coding sequence. The pcDNA3 and pQE30 constructs were transformed into *E. coli* DH5α cells. Transformants were selected on 2XTY (16 g/l tryptone, 10 g/l yeast extract, 5 g/l NaCl, pH 7.4) plates containing ampicillin (100 μg/ml) and screened by direct colony PCR. The sequences of inserts were verified.

Bacterial Expression and Purification—The expression of the three APC proteins and of their CTDs was carried out at 37 °C in *E. coli* M15(pREP4) (QIAGEN Inc.) according to the manufacturer's instructions. Control cultures with the empty vector were processed in parallel. Inclusion bodies were purified on a sucrose density gradient (20) and washed at 4 °C first with 10 mM Tris-HCl and 1 mM EDTA (pH 7.0); twice with 3% (w/v) Triton X-114, 1 mM EDTA, and 10 mM PIPES (pH 7.0); and finally with 10 mM PIPES (pH 7.0). The proteins were solubilized in 1.6% (w/v) Sarkosyl. Small residues were removed by centrifugation at 258,000 × *g* for 20 min at 4 °C.

Reconstitution into Liposomes and Transport Measurements—The recombinant proteins in Sarkosyl were reconstituted into liposomes in the presence of cardiolipin (0.7 mg/ml for APC1 and its CTD and 0.3 mg/ml for APC2 CTD) and 20 mM PIPES-NaOH (pH 7.0) with or without substrate as described (21). External substrate was removed from proteoliposomes on Sephadex G-75 columns pre-equilibrated with 100 mM sucrose and 20 mM PIPES-NaOH (pH 7.0) (buffer A) or with 1 mM PIPES-NaOH (pH 7.0) in the experiments reported in Table III. Transport at 25 °C was started by addition of the indicated labeled substrate to substrate-loaded proteoliposomes (exchange) or to unloaded proteoliposomes (uniport). In both cases, transport was terminated by addition of 0.2% tannic acid (the "inhibitor-stop" method) (21). In controls, the inhibitor was added at the beginning together with the radioactive substrate. All transport measurements were carried out in the presence of 20 mM PIPES (pH 7.0) in the internal and external compartments, except in the experiments reported in Table III, where 1 mM PIPES (pH 7.0) was used. The external substrate was removed, and the radioactivity in the liposomes was measured (21). The experimental values were corrected by subtracting control values. The initial transport rate was calculated from the radioactivity taken up by proteoliposomes in the initial linear range of substrate uptake. For efflux measurements, proteoliposomes containing 2 mM substrate were labeled with 5 μM radioactive substrate by carrier-mediated exchange equilibration (21). After 60 min, the external radioactivity was removed by passing the proteoliposomes through Sephadex G-75. Efflux was started by adding unlabeled external substrate or buffer A alone and terminated by adding the inhibitor indicated above.

Expression Analysis by Real-time PCR—Total RNAs from human tissues (Invitrogen) were reverse-transcribed with the GeneAmp RNA PCR Core kit (Applied Biosystems) with random hexamers as primers. For real-time PCRs, primers and probes based on the cDNA sequences of APC1, APC2, and APC3 were designed with Primer Express (Applied Biosystems). The forward and reverse primers corresponded to nucleotides 143–169 and 226–252 (APC1), nucleotides 247–269 and 306–326 (APC2), and nucleotides 490–510 and 550–575 (APC3), respectively. The carboxyfluorescein-Dark Quencher-labeled probes corresponded to nucleotides 171–197, 272–293, and 513–537 of the APC1, APC2, and APC3 cDNAs, respectively. Real-time PCRs were performed in a MicroAmp optical 96-well plate using the automated ABI Prism 7000 sequence detector system (Applied Biosystems). 50 μl of reaction volume contained 5 μl of template (reverse-transcribed first-strand cDNA), 1× TaqMan universal master mixture (Applied Biosystems), 200 nM probe (for APC1, APC2, or APC3), and 900 nM each primer. To correct for differences in the amount of starting first-strand cDNAs, the human β-actin gene was amplified in parallel as a reference gene. The relative quantification of APC1, APC2, or APC3 in various tissues was performed according to the comparative method ($2^{-\Delta\Delta C_t}$) (22, 23; User Bulletin 2 P/N 4303859, Applied Biosystems), with the pancreas ΔC_t for APC1, the spleen ΔC_t for APC2, and the liver ΔC_t for APC3 as internal calibrators. $2^{-\Delta\Delta C_t} = 2^{-(\Delta C_t \text{ sample} - \Delta C_t \text{ calibrator})}$, where ΔC_t sample is C_t sample $-$ C_t reference gene and C_t is the threshold cycle, *i.e.* the PCR cycle number at which emitted fluorescence exceeds 10 times the S.D. of base-line emissions.

Subcellular Localization—For the subcellular localization of the three APC proteins and their CTDs in Chinese hamster ovary cells, the cells were grown on 24-mm round coverslips to 50–70% confluence and cotransfected according to a standard calcium phosphate procedure with 3 μg of pcDNA1/mtEBFP (24); 3 μg of modified pcDNA3 plasmid containing the coding sequence of APC1, APC2, or APC3 or each of their CTDs fused with the EGFP sequence; and (in some experiments) 3 μg of DsRed2-peroxi plasmid (Clontech). About 30–40% of the cells were transfected. EGFP, enhanced blue fluorescent protein (BFP), and peroxisome-targeted DsRed2 fluorescence was detected by an inverted Zeiss Axiovert 200 microscope equipped with epifluorescence. Cells were imaged with a CoolSNAP HQ CCD camera (Roper Scientific, Trenton, NJ) using Metamorph software (Universal Imaging Corp., Downingtown, PA).

Other Methods—A K^+ diffusion potential was generated by adding valinomycin (1.5 μg/mg of phospholipid) to proteoliposomes in the presence of a potassium gradient. For the formation of ΔpH (acidic outside), nigericin (50 ng/mg of phospholipid) was added to proteoliposomes in the presence of an inwardly directed K^+ gradient. Proteins were separated by SDS-PAGE and stained with Coomassie Blue. The N termini were sequenced, and the amount of purified proteins was estimated by laser densitometry of stained samples using carbonic anhydrase as a protein standard (25). The amount of protein incorporated into liposomes was measured as described (25). In all cases, it was ~30% of the protein added to the reconstitution mixture. All of the specific activities reported in this study were calculated using the amount of inserted protein.

RESULTS

Identification and Characterization of the APC1, APC2, and APC3 cDNAs—The sequence of human AAC1 (encoded by the *SLC25A4* gene) was used to try to find candidates for human APC. Several related EST sequences were found in human EST data bases; we considered only the clones that encode proteins

[1] The abbreviations used are: APC, ATP-Mg/P_i carrier; EST, expressed sequence tag; AAC, ADP/ATP carrier; CTD, C-terminal domain; GFP, green fluorescent protein; EGFP, enhanced green fluorescent protein; PIPES, 1,4-piperazinediethanesulfonic acid; BFP, blue fluorescent protein.

[2] Available at www.ebi.ac.uk, ensembl.ebi.ac.uk, and www.ncbi.nlm.nih.gov.

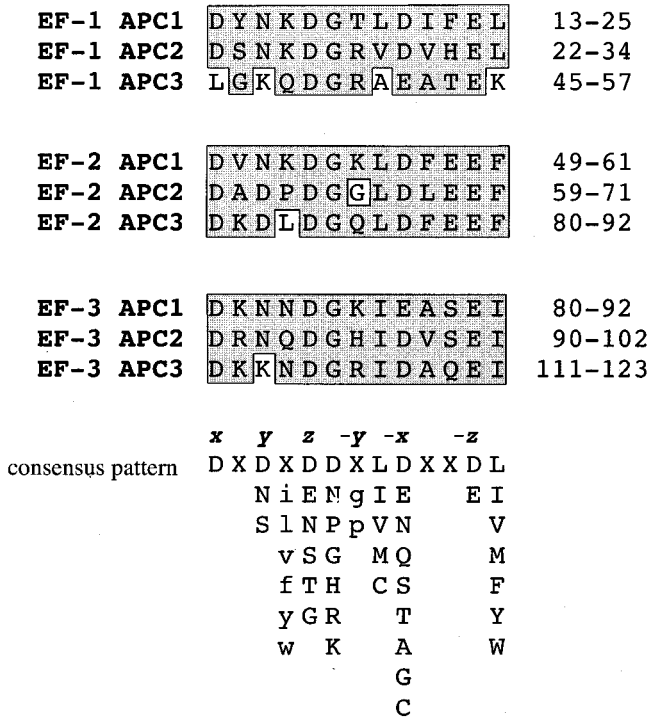

FIG. 1. **Alignment of the EF-hand calcium-binding motifs in APCs.** The consensus sequence for EF-hands is from the PROSITE Database (available at www.expasy.org/cgi-bin/nicedoc.pl?PDOC00018). The six residues involved in calcium binding are denoted by x, y, z, $-y$, $-x$, and $-z$, and the *lowercase letters* below an X indicate residues that usually do not appear at that position. The *shaded boxes* represent the residues conserved in the three APC EF-hand motifs compared with the consensus sequence.

of unknown function and that contain Ca^{2+}-binding EF-hand motifs in their sequences because it had been shown that ATP-Mg/P_i carrier activity is stimulated by calcium (13, 14, 17). Three related human EST clones (GenBank™/EBI accession numbers AF123303, AK054901, and AB067483) matched these criteria; they were extended to the 3′- and 5′-ends, and their full-length cDNAs were obtained and sequenced. The final cDNAs (GenBank™/EBI accession numbers AJ619961 (for APC1), AJ619962 (for APC2), and AJ619963 (for APC3)) consisted of 3345 (APC1), 3430 (APC2), and 3660 (APC3) nucleotides and encoded protein sequences of 458, 468, and 489 amino acids, respectively. These proteins belong to a subfamily of Ca^{2+}-binding mitochondrial carriers with a characteristic bipartite structure. Their N-terminal domains (the first 166, 176, and 196 amino acids of APC1, APC2, and APC3, respectively) contain three EF-hand Ca^{2+}-binding motifs, as shown in Fig. 1, and have 43–46% identical amino acids. The three C-terminal domains of the APCs contain the common sequence features of the mitochondrial carrier family, and their amino acids are 66–75% identical to each other and 27–30% identical to those of human AAC1. They also contain a sequence at residues 319–323 (APC1), 329–333 (APC2), and 350–354 (APC3) in the second matrix loop that conforms to the sequence motif EG*XX*A, the P-box of the DNA-binding domain of nuclear receptors (26). The same motif is present in the deoxynucleotide carrier, the ADP/ATP carrier, and the uncoupling protein UCP1 (27–29) and may be involved in the binding of nucleotide substrates because it was shown to bind purine nucleotides in rat UCP1 (29).

By screening the human genome data bases with the three APC cDNAs, the corresponding genes were found. The APC1 gene (gene name *SLC25A24*) is spread over 58 kb on chromosome 1p13.3; the APC2 gene (gene name *SLC25A23*) is spread

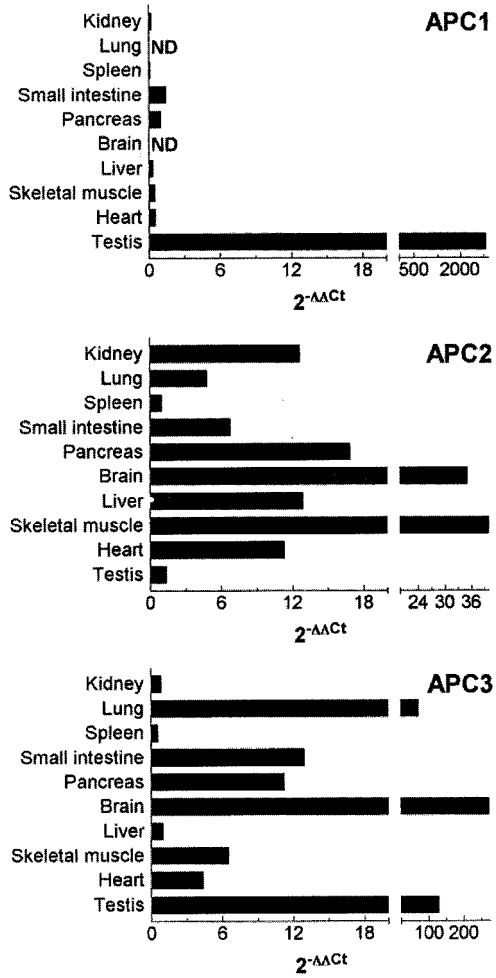

FIG. 2. **Expression of human APC1, APC2, and APC3 in various tissues.** Real-time PCR experiments were conducted on cDNAs prepared by reverse transcription of total RNAs from various human tissues. The $2^{-\Delta\Delta C_t}$ values on the *abscissa* were calculated as described under "Experimental Procedures." For the internal calibrator (pancreas, spleen, and liver for APC1, APC2, and APC3, respectively), $\Delta\Delta C_t = 0$, and $2^0 = 1$. For the remaining tissues, the value of $2^{-\Delta\Delta C_t}$ indicates the -fold change in gene expression relative to the calibrator. *ND*, not detectable.

over 20 kb on chromosome 19p13.3; and the APC3 gene (gene name *SLC25A25*) is spread over 18 kb on chromosome 9q34.13. All three genes contain 10 exons separated by nine introns. In the three genes, all of the splicing junctions occur in the same nucleotide regions, indicating a triplication of a common ancestral gene.

Expression of Human APC1, APC2, and APC3 in Various Tissues—The tissue distribution of mRNAs for the human APC proteins determined by real-time PCR is summarized in Fig. 2. The amount of APC1 mRNA in the pancreas was the same as the amount of APC3 mRNA in the liver (as they have the same ΔC_t in these tissues), and so this value served as an internal calibration in the relative quantification of these two proteins in various tissues. In all of the tissues investigated except testis, the APC3 mRNA was expressed at higher levels than the APC1 mRNA. It was expressed most strongly in the brain, testis, and lung; in reasonable abundance in the small intestine, pancreas, skeletal muscle, and heart; and at lower levels in the kidney, spleen, and liver. The APC1 mRNA was expressed in very abundant amounts in the testis; at low levels in the small intestine and pancreas; and poorly in the kidney, spleen, liver, skeletal muscle, and heart. It was not detectable in the brain and lung. For APC2, the ΔC_t in the spleen was used

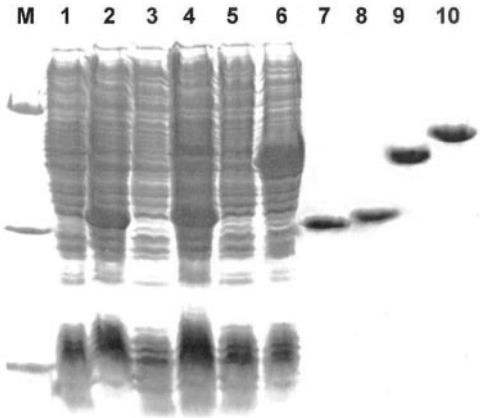

FIG. 3. **Overexpression in *E. coli* and purification of APC1, APC3, and the C-terminal domains of APC1 and APC2.** Proteins were separated by SDS-PAGE and stained with Coomassie Blue. *Lane M*, markers (bovine serum albumin, carbonic anhydrase, and cytochrome *c*). *Lanes 1–6*, *E. coli* M15(pREP4) containing the expression vector with the coding sequence of APC1 CTD (*lanes 1* and *2*), APC2 CTD (*lanes 3* and *4*), or APC1 (*lanes 5* and *6*). Samples were taken at the time of induction (*lanes 1*, *3*, and *5*) and 5 h later (*lanes 2*, *4*, and *6*). The same number of bacteria were analyzed in each sample. *Lanes 7–9*, purified APC1 CTD, APC2 CTD, and APC1 (~2 μg each) originating from bacteria shown in *lanes 2*, *4*, and *6*, respectively. *Lane 10*, purified APC3 (~3 μg) originating from bacteria containing the expression vector with the coding sequence of APC3 sampled 5 h after induction.

as an internal calibrator in its relative quantification in various tissues. APC2 was expressed in the kidney, lung, small intestine, pancreas, liver, and heart and especially in the brain and skeletal muscle at levels severalfold higher than the amount present in the spleen. Only in the testis was the APC2 mRNA expressed at a level comparable with that in the spleen. Although the levels of expression of APC2 (Fig. 2) were not directly comparable with those of APC1 and APC3, it is noteworthy that the ΔC_t of APC2 mRNA in the spleen was lower than that used as an internal calibrator for APC1 and APC3; and consequently, the amount of APC2 mRNA in the spleen was higher than that of APC1 and APC3 in the tissues used as internal calibrators (pancreas and liver, respectively). It should also be borne in mind that, as post-transcriptional mechanisms may be in operation, the levels of mRNAs reported in Fig. 2 do not necessarily reflect the ratios of transport activities.

Bacterial Expression of APC Proteins—APC1, APC3, and the CTDs of APC1 and APC2 (corresponding to the last 292 amino acids of APC1 and APC2) were overexpressed in *E. coli* M15(pREP4) (Fig. 3, see *lanes 2*, *4*, and *6* for APC1 CTD, APC2 CTD, and APC1, respectively). They accumulated as inclusion bodies and were purified by centrifugation and washing. The apparent molecular masses of the purified proteins (yield of 60–80 mg/liter) (Fig. 3, *lanes 7–10*) were ~51 and 32 kDa for APC1 and its CTD, respectively; 32.5 kDa for APC2 CTD; and 55 kDa for APC3, in good agreement with the calculated values. Their identities were confirmed by N-terminal sequencing. The proteins were not detected in bacteria harvested immediately before the induction of expression (Fig. 3, *lanes 1*, *3*, and *5*) or in cells harvested after induction, but lacking the coding sequence in the expression vector (data not shown). APC2 and APC3 CTD were not expressed at appreciable levels in *E. coli* M15(pREP4) or in *E. coli* BL21(DE3) or CO214(DE3) using the pET expression system.

Functional Characterization of Recombinant APC Proteins—All four recombinant proteins were reconstituted into liposomes, and their transport activities for a large variety of potential substrates were tested in homo-exchange experiments (*i.e.* with the same substrate inside and outside). Using external and internal substrate concentrations of 1 and 20 mM, respectively, reconstituted APC1, APC1 CTD, and APC2 CTD catalyzed active [^{14}C]ADP/ADP and [^{33}P]P_i/P_i exchanges, which were totally inhibited by 0.2% tannic acid. They did not catalyze homo-exchanges for pyruvate, succinate, malate, oxoglutarate, ketoisocaproate, citrate, carnitine, ornithine, lysine, arginine, glutamate, aspartate, histidine, glutathione, choline, spermine, proline, and threonine. Of importance, no [^{14}C]ADP/ADP or [^{33}P]P_i/P_i exchange activity was detected if APC1, APC1 CTD, and APC2 CTD had been boiled before incorporation into liposomes or if proteoliposomes were reconstituted with Sarkosyl-solubilized material from bacterial cells lacking the expression vector for APC1, APC1 CTD, or APC2 CTD or harvested immediately before induction of expression. At variance with APC1, APC1 CTD, and APC2 CTD, recombinant and reconstituted APC3 showed no activity under any of the experimental conditions tested, which included variation of the parameters that influence solubilization of the inclusion bodies and reconstitution of the protein into liposomes.

The substrate specificities of reconstituted APC1, APC1 CTD, and APC2 CTD were examined in detail by measuring the uptake of [^{14}C]ADP or [^{33}P]P_i into proteoliposomes that had been preloaded with various potential substrates (Table I). With APC1 and its CTD, the highest activities were observed in the presence of internal ATP-Mg, ADP, P_i, AMP, and ATP. Interestingly, with ATP (but not ADP), the uptake of labeled substrate was greater in the presence of internal Mg^{2+} than in its absence. [^{14}C]ADP and [^{33}P]P_i also exchanged with internal deoxynucleotides of adenine, 3′-AMP, 3′,5′-ADP, and pyrophosphate, although to a lesser extent. A low activity was observed with G, C, T, and U nucleotides. In contrast, the uptake of

TABLE I
Dependence on internal substrate of the transport properties of proteoliposomes reconstituted with recombinant APC1, APC1 CTD, and APC2 CTD

Proteoliposomes were preloaded internally with various substrates (20 mM). Transport was started by the addition of 0.3 mM [^{14}C]ADP or 1.5 mM [^{33}P]P_i to proteoliposomes reconstituted with APC1 and APC1 CTD or by the addition of 0.5 mM [^{14}C]ADP or 1.5 mM [^{33}P]P_i to proteoliposomes reconstituted with APC2 CTD. The reaction time was 30 s (APC1 and APC1 CTD) or 2 min (APC2 CTD). Similar results were obtained in at least three independent experiments for each carrier investigated.

Internal substrate	[^{14}C]ADP uptake			[^{33}P]P_i uptake	
	APC1	APC1 CTD	APC2 CTD	APC1	APC2 CTD
	μmol/min/g protein			*μmol/min/g protein*	
None (NaCl)	1.9	2.1	0.5	1.8	0.6
AMP	98.1	153.4	26.6	76.8	23.9
ADP	181.1	295.3	33.9	126.6	36.1
ADP-Mg	182.2	283.3	34.1	120.6	35.9
ATP	74.9	115.3	19.6	76.7	18.1
ATP-Mg	186.7	305.3	40.5	172.8	39.8
P_i	137.3	230.6	35.4	170.0	38.9
P_iP_i	36.1	53.8	18.1	32.8	21.6
dAMP	46.4	70.3	13.5	56.2	10.7
dADP	96.6	150.8	19.4	57.5	18.5
dATP	39.0	65.4	11.5	43.7	12.3
3′-AMP	52.2	77.8	7.7	31.2	15.6
3′,5′-ADP	43.8	65.9	6.3	37.5	4.1
GMP	23.5	36.7	3.1	25.6	6.4
GDP	30.3	54.2	4.4	30.7	9.7
GTP	16.5	28.3	3.0	16.2	5.9
CMP	7.4	9.7	2.9	6.2	5.3
CDP	12.2	20.1	3.5	13.8	4.5
CTP	6.9	11.8	4.5	15.2	6.5
TMP	7.9	14.2	4.4	23.7	8.2
TDP	24.3	37.4	6.3	25.0	8.5
TTP	9.8	18.7	2.4	15.2	8.5
UMP	4.7	6.9	1.9	10.3	6.0
UDP	7.8	12.8	6.0	6.2	1.3
UTP	10.5	14.8	6.4	21.1	2.3

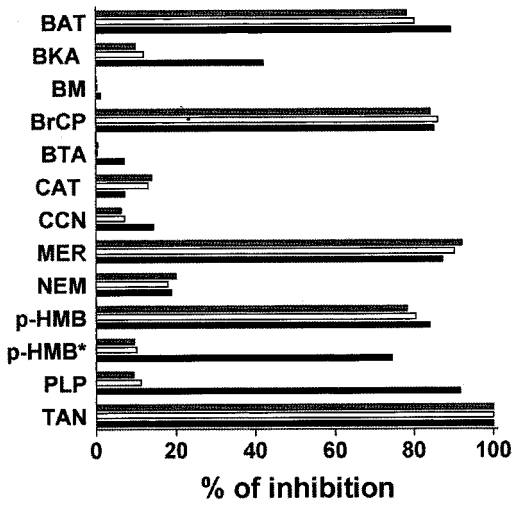

FIG. 4. **Effect of inhibitors on the [^{14}C]ADP/ADP exchange by APC1, APC1 CTD, and APC2 CTD.** Proteoliposomes were preloaded internally with 20 mM ADP. Transport was initiated by adding 0.3 mM [^{14}C]ADP to proteoliposomes reconstituted with APC1 (*gray bars*) or APC1 CTD (*white bars*) or by adding 0.5 mM [^{14}C]ADP to proteoliposomes reconstituted with APC2 CTD (*black bars*). The incubation time was 30 s for APC1 and its CTD and 2 min for APC2 CTD. Thiol reagents were added 2 min before the labeled substrate; the other inhibitors were added together with the labeled substrate. The final concentrations of the inhibitors were 20 mM bathophenanthroline (*BAT*) and pyridoxal 5′-phosphate (*PLP*); 10 μM bongkrekic acid (*BKA*), carboxyatractyloside (*CAT*), and *p*-hydroxymercuribenzoate (*p-HMB**); 2 mM butylmalonate (*BM*) and 1,2,3-benzenetricarboxylate (*BTA*); 0.3 mM bromcresol purple (*BrCP*); 1 mM α-cyano-4-hydroxycinnamate (*CCN*) and *N*-ethylmaleimide (*NEM*); 0.1 mM mersalyl (*MER*) and *p*-hydroxymercuribenzoate (*p-HMB*); and 0.2% tannic acid (*TAN*). The extent of inhibition (percent) for each carrier from a representative experiment is reported.

labeled substrates was negligible in the presence of internal NaCl and cAMP, adenosine, thiamine, thiamine mono- and diphosphates, FAD, NAD, NMN, *S*-adenosylmethionine, 5-phospho-α-D-ribosyl-1-pyrophosphate, succinate, fumarate, aspartate, glutamate, malate, malonate, citrate, oxoglutarate, oxalacetate, pyruvate, phosphoenolpyruvate, L-carnitine, L-ornithine, and L-citrulline (data not shown). Reconstituted APC2 CTD behaved similarly to APC1 and its CTD (Table I). However, the rates of the APC2 CTD-mediated [^{14}C]ADP and [^{33}P]P_i exchanges were lower than the corresponding rates catalyzed by APC1 and its CTD.

The [^{14}C]ADP/ADP exchange reactions catalyzed by reconstituted APC1, APC1 CTD, and APC2 CTD were inhibited strongly by bathophenanthroline, mersalyl, and *p*-hydroxymercuribenzoate (inhibitors of several mitochondrial carriers) (30) as well as by bromcresol purple and tannic acid (inhibitors of the glutamate carrier) (23) (Fig. 4). In contrast, little inhibition was observed with *N*-ethylmaleimide, α-cyano-4-hydroxycinnamate, butylmalonate, and 1,2,3-benzenetricarboxylate (inhibitors of other mitochondrial carriers) (30). The behavior of APC1 and APC2 toward carboxyatractyloside and bongkrekic acid (powerful inhibitors of the ADP/ATP carrier) (31, 32) was interesting. At a concentration (10 μM) that completely inhibits the ADP/ATP carrier, carboxyatractyloside had little effect on the activities of APC1, APC1 CTD, and APC2 CTD, whereas bongkrekic acid considerably inhibited APC2 CTD (42% inhibition) and poorly inhibited APC1 and its CTD (10 and 12% inhibition, respectively). It is also notable that 20 mM pyridoxal 5′-phosphate and *p*-hydroxymercuribenzoate at a low concentration (10 μM) strongly inhibited the activity of APC2 CTD, whereas they had little effect on the activities of APC1 and its CTD. In studies with intact mitochondria, ATP-Mg/P_i carrier activity was found to be inhibited by pyridoxal 5′-phosphate, by sulfhydryl reagents in a concentration-dependent manner, and partially by *N*-ethylmaleimide (33).

Kinetic Characteristics of Recombinant APC Proteins—In Fig. 5 (*A* and *B*), the kinetics are compared for the uptake by proteoliposomes of 1 mM [^{14}C]ADP measured either as uniport (in the absence of internal ADP) or as exchange (in the presence of 20 mM ADP). The exchange reactions catalyzed by both APC1 and APC2 CTD followed first-order kinetics, with isotopic equilibrium being approached exponentially. The rate constants and the initial rates of ADP exchange deduced from the time courses (21) were 0.76 and 0.11 min^{-1} and 280.4 and 44.0 μmol/min/g of protein for APC1 and APC2 CTD, respectively. In contrast, the uniport uptake of ADP by both APC1 and APC2 CTD was negligible, suggesting that APC1 and APC2 CTD do not catalyze a unidirectional transport (uniport) of ADP, but only the exchange reaction. This issue was further investigated by measuring the efflux of [^{33}P]P_i and of other substrates from prelabeled active proteoliposomes, as this provides a more sensitive assay for unidirectional transport (21). With both reconstituted APC1 and APC2 CTD, in the absence of external substrate, no efflux of [^{33}P]P_i or [^{14}C]ADP was observed upon 4–10 min of incubation (Fig. 5, *C–F*) and even after 30 min of incubation (data not shown). There was also no efflux of [^{33}P]P_i or [^{14}C]ADP upon addition of external 20 mM pyruvate, oxoglutarate, citrate, carnitine, ornithine, or glutamate (data not shown), which are substrates of other mitochondrial carriers. However, upon addition of external P_i, ADP, ATP-Mg, or ATP, an extensive efflux of radioactivity occurred, and this efflux was prevented completely by the presence of the inhibitor tannic acid (Fig. 5, *C–F*). A substantial efflux of [^{14}C]ATP and [^{14}C]AMP from prelabeled proteoliposomes reconstituted with APC1 and APC2 CTD was also observed in the presence of external P_i, ADP, ATP-Mg, or ATP, whereas the efflux was not observed without added substrate (data not shown). These results demonstrate that APC1 and APC2 CTD catalyze an obligatory and reversible exchange reaction of substrates.

The kinetic constants of APC1, APC1 CTD, and APC2 CTD were determined from the initial transport rate of homo-exchanges at various external labeled substrate concentrations in the presence of a constant saturating internal substrate concentration of 20 mM (Table II). For all three recombinant proteins, the half-saturation constants (K_m) for external ATP-Mg, ATP, and ADP (between 0.2 and 0.5 mM) were lower than those for external AMP and P_i (between 0.9 and 1.6 mM). The K_m value for each substrate was not significantly different for APC1, APC1 CTD, and APC2 CTD, except that for ADP, which was 1.8-fold higher for APC2 CTD than for APC1 and its CTD. The maximal activities (V_{max}) of all five homo-exchanges were virtually the same for each carrier. It is notable that the V_{max} value for the C-terminal domain of APC1 was higher than that for the corresponding intact protein, whereas the turnover numbers were very similar. Therefore, the C-terminal domain of APC1 accounts for the entire transport activity of APC1, as previously found for the two aspartate/glutamate carrier isoforms, which also have an extensive N-terminal domain with Ca^{2+}-binding sites (34). It is also noteworthy that both the activity and the turnover number of APC1 CTD were ~7.5-fold greater than those of APC2 CTD. Furthermore, the K_m values of the ATP-Mg/P_i carrier for ATP-Mg and ADP determined using intact mitochondria (11, 14, 18, 35) are somewhat variable and usually higher than those reported here with recombinant purified proteins.

Influence of Membrane Potential and pH Gradient on the APC-mediated Exchange Reactions—Because the ATP-Mg/P_i carrier has been shown to catalyze the neutral exchange between ATP-Mg and P_i in mitochondria (15), we investigated the

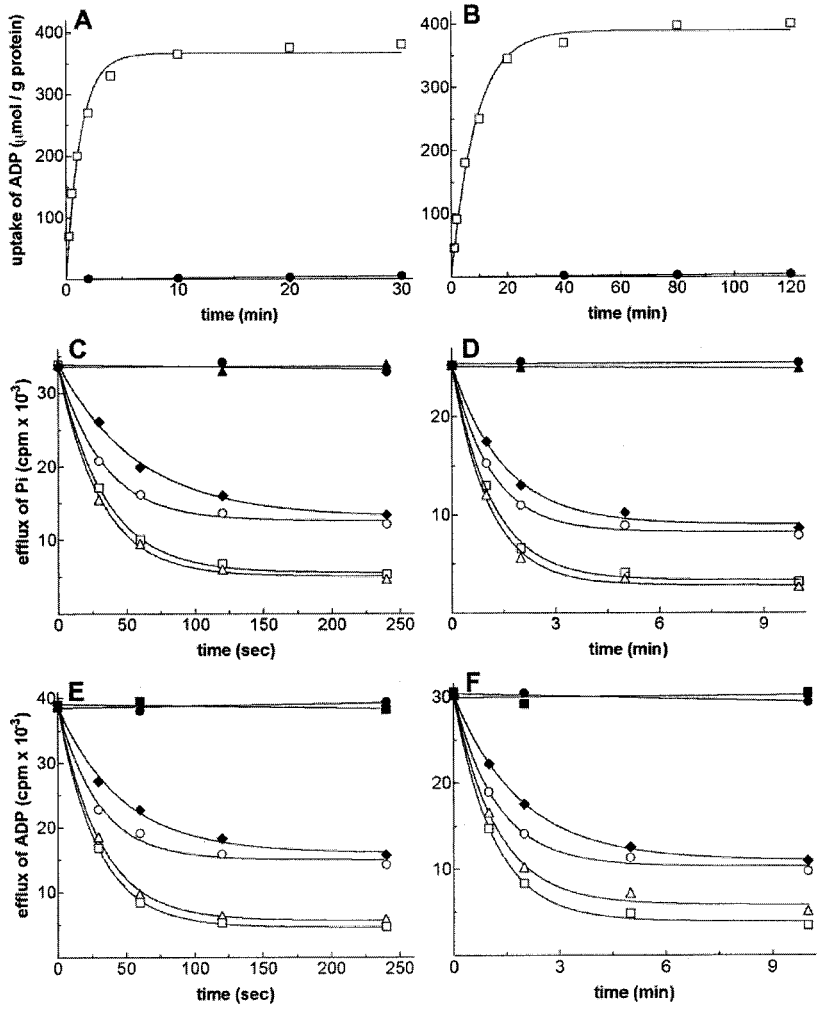

FIG. 5. **Kinetics of exchange reactions catalyzed by APC1 and APC2 CTD.** Proteoliposomes were reconstituted with recombinant APC1 (*A*, *C*, and *E*) or APC2 CTD (*B*, *D*, and *F*). *A* and *B*, 1 mM [^{14}C]ADP was added to proteoliposomes containing 20 mM ADP (□) or 20 mM NaCl (●); *C–F*, the internal substrate of proteoliposomes (2 mM P_i (*C* and *D*) or 2 mM ADP (*E* and *F*)) was labeled with [^{33}P]P_i (*C* and *D*) or [^{14}C]ADP (*E* and *F*) by carrier-mediated exchange equilibration. After removal of the external substrate by Sephadex G-75, the efflux of [^{33}P]P_i (*C* and *D*) or [^{14}C]ADP (*E* and *F*) was started by adding buffer A alone (●), 2 mM ATP (♦), 2 mM ATP-Mg (○), 20 mM P_i (△), 20 mM ADP (□), 20 mM P_i and 0.2% tannic acid (▲), or 20 mM ADP and 0.2% tannic acid (■).

TABLE II

Kinetic constants of recombinant APC1, APC1 CTD, and APC2 CTD

The values were calculated from double-reciprocal plots of the rates of homo-exchanges (the same substrate inside and outside) under variation of the external substrate concentration. The exchange was started by the addition of appropriate concentrations of labeled substrate to proteoliposomes preloaded internally with the same substrate at 20 mM. The reaction time was 30 s (APC1 and APC1 CTD) or 2 min (APC2 CTD).

Carrier and substrate	K_m	V_{max}	Turnover number
	mM	*µmol/min/g protein*	*per min*
APC1			
AMP	0.97 ± 0.12	337 ± 48	17.3
ADP	0.30 ± 0.04	345 ± 38	17.7
ATP	0.33 ± 0.03	320 ± 55	16.4
ATP-Mg	0.20 ± 0.03	365 ± 33	18.4
P_i	1.64 ± 0.13	380 ± 42	19.5
APC1 CTD			
AMP	1.11 ± 0.16	505 ± 70	16.2
ADP	0.29 ± 0.03	542 ± 64	17.4
ATP	0.36 ± 0.04	523 ± 58	16.8
ATP-Mg	0.22 ± 0.02	580 ± 72	18.6
P_i	1.54 ± 0.19	535 ± 55	17.2
APC2 CTD			
AMP	1.30 ± 0.12	68 ± 9	2.2
ADP	0.54 ± 0.06	73 ± 12	2.3
ATP	0.31 ± 0.04	65 ± 10	2.1
ATP-Mg	0.22 ± 0.03	79 ± 12	2.5
P_i	1.40 ± 0.15	70 ± 8	2.3

influence of the membrane potential on ATP-Mg/P_i and other hetero-exchanges catalyzed by APC1 and APC2 CTD. A K^+ diffusion potential was generated across the proteoliposomal membranes with valinomycin in the presence of a K^+ gradient of 1 mM/50 mM (in/out), corresponding to a calculated value of ~100 mV positive inside (Table III). The rate of the ATP-Mg/P_i exchange and the rates of all of the other exchanges tested were unaffected by valinomycin in the presence of the K^+ gradient. In contrast, the aspartate$_{out}$/glutamate$_{in}$ exchange, mediated by the aspartate/glutamate carrier, which is known to catalyze an electrophoretic exchange between aspartate$^-$ and glutamate$^-$ + H^+ (34, 36), was stimulated under the same experimental conditions (data not shown). These results indicate that APC1 and APC2 CTD catalyze an electroneutral exchange of substrates. In view of the different charges carried by ATP, ATP-Mg, and P_i at physiological pH values, we became interested in the question as to whether the charge imbalance of the ATP/ATP-Mg and ATP/P_i hetero-exchanges catalyzed by APCs is compensated by proton movement in the same direction as ATP. A pH difference across the liposomal membranes (basic inside the vesicles) was created by the addition of the K^+/H^+ exchanger nigericin to proteoliposomes in the presence of a potassium gradient of 1 mM/50 mM (in/out) (Table III). Under these conditions, the uptake of [^{14}C]ATP in exchange for internal ATP-Mg or P_i increased, whereas the uptake of [^{14}C]ATP-Mg in exchange for internal ATP decreased. By contrast, the ATP/ATP, ATP-Mg/ATP-Mg, and ATP-Mg/P_i exchanges were not affected. Therefore, the ATP/ATP-Mg and ATP/P_i hetero-exchanges are driven by the ΔpH, indicating that the charge imbalance of the exchanged substrates is compensated by the movement of protons. It should be noted that none of the exchanges tested was affected by either valinomycin or nigeri-

TABLE III
Influence of membrane potential and ΔpH on the activity of reconstituted APCs

The exchanges were started by the addition of 0.3 mM [^{14}C]ATP or 0.2 mM [^{14}C]ATP-Mg to proteoliposomes containing various internal substrates (20 mM). 1 mM K^+_{in} was included as KCl in the reconstitution mixture, whereas 50 mM K^+_{out} was added as KCl together with the labeled substrates. 1 mM PIPES-NaOH (pH 7.0) was present in the internal and external compartments. Valinomycin or nigericin was added in 10 μl of ethanol/ml of proteoliposomes. In the control samples, the solvent alone was added. The exchange reactions were stopped after 30 s. Similar results were obtained in three independent experiments.

Carrier and uptake	Internal substrate	Transport activity		
		Control	+Valinomycin	+Nigericin
		μmol/min/g protein		
APC1				
[^{14}C]ATP-Mg	ATP-Mg	193	178	184
[^{14}C]ATP-Mg	ATP	145	149	95
[^{14}C]ATP-Mg	P_i	120	107	113
[^{14}C]ATP-Mg	None	1.3	1.2	1.0
[^{14}C]ATP	ATP-Mg	200	208	297
[^{14}C]ATP	ATP	154	150	147
[^{14}C]ATP	P_i	107	103	176
[^{14}C]ATP	None	1.7	1.5	1.8
APC2 CTD				
[^{14}C]ATP-Mg	ATP-Mg	37	39	41
[^{14}C]ATP-Mg	ATP	34	37	23
[^{14}C]ATP-Mg	P_i	32	30	33
[^{14}C]ATP-Mg	None	0.5	0.8	0.5
[^{14}C]ATP	ATP-Mg	42	42	59
[^{14}C]ATP	ATP	35	29	34
[^{14}C]ATP	P_i	28	25	40
[^{14}C]ATP	None	0.7	0.4	0.8

cin in the absence of the K^+ gradient, *i.e.* by adding 1 mM KCl and 49 mM NaCl together with the labeled substrate (data not shown). Furthermore, no uptake of [^{14}C]ATP-Mg or [^{14}C]ATP by unloaded liposomes was observed even in the presence of an energy input (either membrane potential or pH gradient).

Subcellular Localization of APC Proteins—Because some members of the mitochondrial carrier family are localized in non-mitochondrial membranes (37–39) and because a rabbit protein (87% identical to APC1, but of unknown function) has been reported to be localized in peroxisomes (40), the intracellular localization of ectopically expressed APC1, APC2, and APC3 was investigated using GFP-tagged proteins (Fig. 6). After 36–48 h of transfection, the green fluorescence revealed a typical mitochondrial localization of APC1, APC2, and APC3 (Fig. 6, *A–C*). The fluorescence of the GFP-tagged proteins completely overlapped with the blue fluorescence of a mitochondrially targeted BFP (24) coexpressed in the same cells (Fig. 6, *A–C*). In contrast, the green fluorescence of GFP-APC1 (Fig. 6, *D–G*) and GFP-APC2 and GFP-APC3 (data not shown) did not overlap with the red fluorescence of peroxisome-targeted DsRed2. The same intracellular localization was observed for the C-terminal domains of APC1, APC2, and APC3 fused with GFP and individually expressed in Chinese hamster ovary cells (data not shown). These results demonstrate that both the entire APC proteins and their CTDs localize to mitochondria and that the truncated forms of APCs, which retain the transport properties of the entire proteins, contain in their amino acid sequence the structural information for import into mitochondria, in accordance with data available for other mitochondrial carriers (41–43).

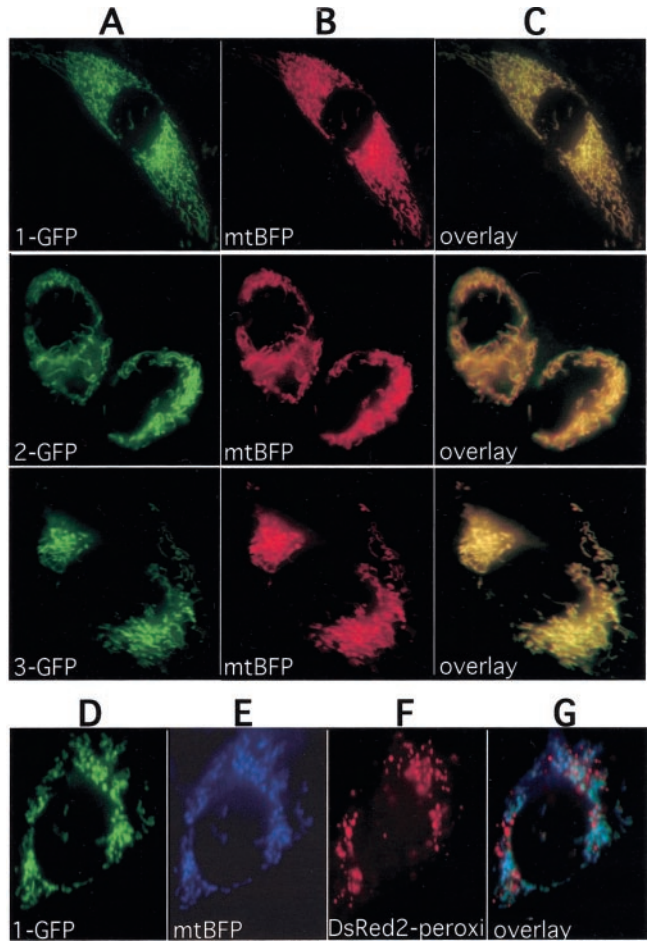

FIG. 6. **Subcellular localization of ATP-Mg/P_i carrier isoforms.** *A–C*, Chinese hamster ovary cells were transiently cotransfected with pcDNA3 vectors carrying the cDNA sequence coding APC1, APC2, or APC3 in-frame with GFP DNA sequence and pcDNA1 plasmid carrying the DNA sequence of mitochondrially targeted BFP (see "Experimental Procedures"). *A*, fluorescence of GFP fused with APC1 (*1-GFP*), APC2 (*2-GFP*), or APC3 (*3-GFP*); *B*, fluorescence of mitochondrially targeted BFP (*mtBFP*; shown here in red); *C*, overlaid images of mitochondrially targeted BFP fluorescence with GFP fluorescence of the three APC proteins. *D–G*, Chinese hamster ovary cells were transiently cotransfected with the DsRed2-peroxi plasmid carrying the DNA sequence of peroxisome-targeted DsRed2 and the pcDNA1 plasmid and the pcDNA3 vector as described for *A* (see "Experimental Procedures"). *D*, fluorescence of GFP fused with APC1 (*1-GFP*); *E*, fluorescence of mitochondrially targeted BFP; *F*, fluorescence of peroxisome-targeted DsRed2; *G*, overlaid images of mitochondrially targeted BFP and peroxisome-targeted DsRed2 fluorescence with GFP fluorescence of APC1. Images were acquired by a fluorescence microscope equipped with appropriate filters, and identical fields are presented.

DISCUSSION

Knowledge about the mitochondrial ATP-Mg/P_i carrier has existed for a long time, and its main properties have been studied in intact mitochondria (see Ref. 33 for review). However, the protein(s) responsible for the ATP-Mg/P_i exchange had not been identified hitherto. The transport properties and kinetic characteristics of recombinant and reconstituted APC1 and APC2 and their targeting to mitochondria, reported here, show that they are distinct isoforms of the ATP-Mg/P_i carrier. For the closely related sequence APC3, which was also cloned and expressed in *E. coli* in this study, no biochemical data are available, as we did not manage to renature and/or reconstitute it functionally. However, the high degree of similarity of APC3 to APC1 and APC2 (57–62% comparing the entire proteins and 66–75% comparing the C-terminal domains), which is similar to that found for other mitochondrial carrier isoforms (23, 34), makes it very likely that APC3 is another isoform of the ATP-Mg/P_i carrier in human.

In a phylogenetic tree of the human mitochondrial carriers of known function, APCs cluster together with the three isoforms of the ADP/ATP carrier (44–47) and with the carrier for coenzyme A (48). Apart from the relatively low homology that the

three APC C-terminal domains have to these carriers (25–30% similarity), APCs do not show significant sequence homology to any other mitochondrial carrier functionally identified until now in mammals, yeast, and plants (see Refs. 1–3 for reviews and Refs. 4 and 5) above the basic homology existing between the different members of the mitochondrial carrier family. However, several protein sequences available in data bases are likely orthologs of this transporter in other organisms. These sequences include GenBank™/EBI accession number AF004161 from *Oryctolagus cuniculus*, accession number NM_145677 from *Rattus norvegicus*, accession number AK032806 from *Mus musculus*, BC043993 from *Xenopus laevis*, accession number AE003541 from *Drosophila melanogaster*, accession number EAA11419 from *Anopheles gambiae*, accession number F55A11.4 from *Caenorhabditis elegans*, accession number AP002483 from *Oriza sativa*, accession number AL163912 from *Arabidopsis thaliana*, and accession number YNL083W from *Saccharomyces cerevisiae*. The wide distribution of APCs in the eukaryotic kingdom indicates that this transporter plays an important role that has been conserved throughout evolution. The rabbit protein named Efinal (accession number AF004161), which is an ortholog of APC1 with 87% similarity, has been reported to have a peroxisomal location (40), which contrasts with the mitochondrial localization of the human APCs shown here. This discrepancy may be due to a low specificity of the anti-Efinal antibody used, which indeed displays immunoreactivity both at peroxisomal and mitochondrial levels (40). Consistently with our results, the rat protein (accession number NM_145677) that is an ortholog of APC3 with 85% similarity has recently been found to be localized to mitochondria (49).

Besides transporting ATP, ADP, AMP, and P_i efficiently, reconstituted APC1 and APC2 also transport structurally related nucleotides and pyrophosphate to a much lesser extent, but none of the many other compounds tested. Their substrate specificity is distinct from that of any other previously characterized member of the mitochondrial carrier family. Compared with the substrate specificity of the well known ADP/ATP carrier (31, 32), that of reconstituted APCs is much broader. The name ATP-Mg/P_i carrier was originally assigned to this transporter because mitochondria were loaded primarily with ATP-Mg. Despite the rather broad specificity exhibited by the recombinant proteins in the reconstituted system, this name still appears to be justified for APCs, as *in vivo* ATP-Mg is their main substrate because of its abundance within the cells. The biochemical properties of the recombinant APCs indicate that the main function of these transporters is probably to catalyze the electroneutral exchange of adenine nucleotides, in particular ATP-Mg, for P_i. Because, in mitochondria, P_i transported by APCs can recycle via the phosphate carrier, our results are consistent with and further support the previous proposal, derived from studies with isolated mitochondria, that the ATP-Mg/P_i carrier is responsible for the net uptake or efflux of adenine nucleotides into or from the mitochondria and hence for the modulation of the matrix adenine nucleotide concentration (33). By changing the matrix adenine nucleotide content, the APC isoforms play an important role in the regulation of the metabolic pathways that have adenine nucleotide-dependent enzymes compartmentalized in the mitochondrial matrix. These include gluconeogenesis from lactate; urea synthesis; mitochondrial DNA replication, transcription, and protein synthesis; and import of nuclear encoded proteins into mitochondria. The physiological significance of APC isoforms is highlighted by the observation that changes in the adenine nucleotide concentration in the mitochondrial matrix, without variation in the whole cell content, occur in many adaptive states. For example, the mitochondrial adenine nucleotide pool has been found to be increased in newborn liver mitochondria 3–4 times within 3–4 h after birth (35) and in adult liver mitochondria upon fasting (50, 51), after glucagon treatment (50–52), and in altered thyroid states (53). On the other hand, it is decreased by hypoxia (54) and ischemia (55), in fast growing hepatomas (56), during hibernation (57), and in copper deficiency (58). Furthermore, recent data have shown that P_i-induced opening of the permeability transition pore of rat liver mitochondria is secondary to depletion of the intramitochondrial adenine nucleotide content via the ATP-Mg/P_i carrier (59). This has important implications for hepatocytes in hypoxia or ischemia or during reoxygenation/reperfusion as well as in cellular dysfunction under conditions of hypoxia and ischemia that can ultimately lead to apoptotic or necrotic cell death.

ATP-Mg/P_i carrier activity is known to be regulated by Ca^{2+} in isolated mitochondria and by Ca^{2+}-mobilizing hormones in hepatocytes (13, 14, 17). The presence of three EF-hand Ca^{2+}-binding motifs in the N-terminal domains of the identified APCs suggests that the mechanism of Ca^{2+} regulation of the ATP-Mg/P_i carrier requires interaction of Ca^{2+} with the N-terminal domains of the APC molecules. In fact, both the rabbit and rat APC orthologs have been shown to bind Ca^{2+} (40, 49), although their functions remained unknown. In the case of the rabbit ortholog, it was further demonstrated that Ca^{2+} binds to the N-terminal domain of the protein, which contains the EF-hand Ca^{2+}-binding sites (40). Unfortunately, the Ca^{2+} dependence of the APC activity could not be detected in our reconstituted system. This may be due to a number of reasons, among which the following can be quoted: the presence of unknown amounts of Ca^{2+} and other divalent ions (especially in the lipids used for the reconstitution), the unknown orientation of the protein in the liposomes, and the fact that added Ca^{2+} may interact primarily with the phospholipids of the liposomal membrane. In the case of the two isoforms of the aspartate/glutamate carrier, which are the only known members of the mitochondrial carrier family that share with APC the presence of EF-hand Ca^{2+}-binding motifs in their N-terminal domains, it has been shown that the carrier is stimulated by Ca^{2+} using a different system, *i.e.* cultured cells (43). Clearly, further work is needed to investigate the Ca^{2+} regulation of APCs.

The tissue specificity of APCs differs markedly from that of the isoforms of the phosphate carrier (25), the ADP/ATP carrier (44–47), and other mitochondrial proteins involved in oxidative phosphorylation. The common feature of all of these proteins is the presence of at least one heart-type isoform, which is expressed abundantly only in muscles, and one liver-type isoform, which is expressed ubiquitously in all tissues. In agreement with the importance of the ATP-Mg/P_i carrier in intermediary metabolism and mitochondrial biogenesis (7), at least one APC isoform is present in all of the tissues investigated. In light of the observed difference in V_{max} for APC1 and APC2, it may be that APC2, expressed at higher levels and with a lower specific activity, matches the basic requirement of all tissues, whereas APC1 and APC3 satisfy the higher demands associated with special tissue-specific metabolic functions.

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Identification of the Mitochondrial ATP-Mg/P_i Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION

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