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Microcompartmentation of transported carnitine, acetylcarnitine and ADP occurs in the mitochondrial matrix

Implications for transport measurements and metabolism

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Monitoring of the exchange-diffusion of carnitine, acetylcarnitine and ADP by measuring the influx of radioactive substrates into mitochondria or their efflux, as commonly employed, underestimated their true transport. Higher transport rates were realized when the imports were monitored by analysing, in the entire incubation medium, formation of metabolites that could proceed only after the substrate import. A recycling of substrate present in an inner microenvironment near the translocase and in the external medium appeared to be responsible for these results. Microcompartmentation of carnitine was observable also at 30°C. These findings strengthen the concept that a sharing of a microcompartment between transporters and enzymes metabolizing the entered substrates occurs and appears to offer a kinetic advantage for the reactions involved. The possibility that different segments of metabolism involving the same substrate may proceed at different loci within the matrix and thus be amenable to independent controls is also indicated by these findings.

Transmitochondrial transport of (−)-carnitine (referred to below simply as 'carnitine') and its esters is facilitated by the presence of a carnitine/acylcarnitine translocase in the inner membrane that generally catalyses a mole-to-mole exchange diffusion between carnitines of matrix and the external medium. The transmitochondrial exchange diffusion rates of metabolites are commonly determined by measuring either the uptake of radioactive metabolites or their efflux (Palmieri & Klingenberg, 1979). This is how we and others assayed carnitine/acylcarnitine translocase (Pande, 1975; Parvin & Pande, 1979; Ramsay & Tubbs, 1975; Tubbs & Ramsay, 1979). Continuing studies revealed that these methods, which monitored the turnover of the bulk of the matrix carnitine, were grossly underestimating the true translocase-catalysed transport rates because of the existence of an inner microcompartment encompassing carnitine/acylcarnitine translocase. Carnitine of this microcompartment was found to equilibrate faster with the external carnitine than the diffusion mixing with the bulk of the internal carnitine present in the matrix (Murthy & Pande, 1984). Whether the hitherto measured transport rates of other metabolites that had not taken into account the existence of such microenvironments and of diffusion barriers had given underestimates of their true transport rates then became an important question. Additional results confirming the above findings for the carnitine/acylcarnitine translocase system and showing that the conventional uptake or efflux of radioactive adenine nucleotides into/from mitochondria underestimates the true rates of adenine nucleotide translocase-catalysed exchange diffusions are described below; our data on the adenine nucleotide translocase extend the findings reported by Out & Kemp (1974) and Vignais *et al.* (1975). The possibility of the existence of microenvironments on the matrix side of the inner membrane, in close proximity to the inner-membrane transporters, frequently in association with the metabolizing enzymes, being a general phenomenon that offers a kinetic advantage for the metabolism of the substrates involved is thus strengthened by these findings.

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Experimental

Materials

[*Me*-^{3}H]Carnitine was synthesized as described previously (Daveluy *et al.*, 1982). [1-^{14}C]Acetylcarnitine was synthesized as described by Murthy & Pande (1984), and acetyl[^{3}H]carnitine was synthesized similarly except that acetyl-CoA was unlabelled and carnitine was [*Me*-^{3}H]-carnitine. [1-^{14}C]Acetyl-CoA and [2-^{14}C]pyruvate were obtained from Amersham Corp. [8-^{14}C]-ADP was from New England Nuclear, sulphobetaine (Zwittergent 3-08, *NN*-dimethyl-*N*-octyl-3-ammoniopropane-1-sulphonate) from Calbiochem, and diadenosine pentaphosphate [adenosine(5′)pentaphospho(5′)adenosine] and carboxyatractyloside were from Boehringer. Sources of other chemicals were as described previously (Parvin & Pande, 1979; Pande & Parvin, 1980).

Rat heart mitochondria were prepared as described previously (Pande & Blanchaer, 1971) and were suspended in 210mM-mannitol/70mM-sucrose/0.1mM-EDTA/10mM-Tris/HCl buffer, pH7.4 (medium A).

Mitochondrial transport of carnitine and acetylcarnitine

Import of carnitine into mitochondria by [^{14}C]-acetylcarnitine formation was measured by monitoring the [^{14}C]acetylation of the entering carnitine in mitochondria whose CoA was converted into [^{14}C]acetyl-CoA in the presence of [2-^{14}C]pyruvate and malonate. Correction for [^{14}C]acetylcarnitine formed from endogenous carnitine was applied by including controls to which sulphobetaine, an inhibitor of carnitine/acylcarnitine translocase, was added before unlabelled carnitine (Murthy & Pande, 1984). Similarly, the transport of [1-^{14}C]-acetylcarnitine into mitochondria was measured by monitoring the formation of ^{14}C-labelled anions (Murthy & Pande, 1984). The direct mitochondrial uptake of [^{3}H]-carnitine and acetyl[^{3}H]carnitine was assayed by using a silicone-oil centrifugation procedure (Pande & Parvin, 1980).

Matrix carnitine content was varied by incubating about 20mg of mitochondria at 37°C in 250mM-mannitol/25mM-Hepes buffer (pH7.4)/0.05mM-EDTA/3mM-ADP/1mM-malate/5mM-potassium phosphate buffer (pH7.4)/bovine serum albumin (5mg/ml) containing various concentrations of carnitine (0–50mM); the final volume was 2ml. After 10min, 20ml of chilled medium A was added to each mixture, the tubes were centrifuged at 27000*g* for 5min at 0°C and the pellets were suspended in 20ml of medium A and re-centrifuged. Finally, mitochondria were suspended in medium A. Mitochondrial carnitine was determined as described previously (Parvin & Pande, 1977; Pande & Parvin, 1980).

Adenine nucleotide transport into mitochondria

Transport of [8-^{14}C]ADP into respiring (State-3) heart mitochondria was measured either by direct uptake or by monitoring carboxyatractyloside-sensitive formation of [^{14}C]ATP. State-3 conditions were employed purposely to allow phosphorylation of entering [^{14}C]ADP to [^{14}C]ATP so that any recycling of newly entered substrate between the microenvironment and the external medium may become detectable as [^{14}C]ATP formation.

Assay of direct uptake

The constantly stirred incubation mixture, in a final volume of 100μl at 0°C, contained 250mM-mannitol, 25mM-Hepes buffer, pH7.4, 0.5mM-EDTA, 5mM-potassium phosphate buffer, pH7.4, 5mM-malate, 5mM-glutamate and 0.5mM-diadenosine pentaphosphate (to inhibit adenylate kinase; Melnick *et al.*, 1979). After 3min incubation at 0°C of mitochondria (about 1mg of protein) in the above mixture, [8-^{14}C]ADP was added to a final concentration of 0.1mM and then, 10s later, 50μl of 0.3mM-carboxyatractyloside/200mM-mannitol/50mM-Tris/HCl buffer, pH7.4, was added to arrest further ADP transport. In controls, [^{14}C]-ADP and carboxyatractyloside were added simultaneously. After mixing, 125μl portions were processed for silicone-oil centrifugation (Pande & Parvin, 1980).

Assay of [^{14}C]ATP formation

Incubation conditions were as for the assay of direct [^{14}C]ADP uptake, except that 75μl of 7.5% (w/v) $HClO_4$ was added 30s after the addition of carboxyatractyloside. After neutralization with KOH and centrifugation, the supernatants were processed through Dowex AG-1 X8 resin (400 mesh; Cl^- form) as described by Davis & Lumeng (1975), and the [^{14}C]ATP fraction was monitored for radioactivity. Separate recovery experiments showed that the separation of [^{14}C]ATP from [^{14}C]ADP was at least 98%.

Manipulation of mitochondrial adenine nucleotide content

Heart mitochondria (10–20mg) were incubated at 37°C for 15min in a medium containing 200mM-mannitol, 25mM-Hepes buffer, pH7.4, 5mM-potassium phosphate buffer, pH7.4, 5mM-malate, 5mM-glutamate, 0.05mM-EDTA, 5mM-$MgCl_2$ and bovine serum albumin (5mg/ml) without (for ATP+ADP depletion) or with 10mM-ATP (to load adenine nucleotides), in a final volume of 10ml. Then 15ml of ice-cold medium A was added

and the tubes were centrifuged at 27000*g* for 5min. The pellets were suspended in medium A and re-centrifuged. Finally, all the pellets were suspended in medium A.

Labelling of matrix adenine nucleotide pool with [^{14}C]ADP

Normal or adenine nucleotide-depleted mitochondria (5–7mg) were suspended in 100μl of medium A containing 4.5nmol of [^{14}C]ADP (40Ci/mol) and incubated at 20°C for 2min. Then 20ml of ice-cold medium A was added and the tubes were centrifuged at 27000*g* for 5min. The pellets were washed once by suspension in medium A and re-centrifuging, and the mitochondria were resuspended in medium A.

A sensitive radioenzymic procedure was employed for the determination of ATP+ADP contents (Goswami & Pande, 1984). All values shown are means ± S.E.M. for four or more observations.

Results

We have previously reported (Murthy & Pande, 1984) (*a*) that the carnitine/acylcarnitine translocase-catalysed import rates of carnitine and acyl esters of carnitine, judged from the uptake of the radioactive substrates, markedly decreased with any decrease in the matrix carnitine content to below normal values, (*b*) that such was not the case when carnitine import rates were calculated from the [^{14}C]acetylation of the entering carnitine in matrix observed in the presence of [2-^{14}C]pyruvate and malonate, (*c*) that the latter technique revealed higher transport rates than those calculated from the observed uptake (i.e. association with mitochondrial pellet) of carnitine radioactivity from the medium, and (*d*) that, in carnitine-depleted mitochondria having less than 10% of the normal carnitine content, an appreciable exchange diffusion of the substrate was proceeding as revealed by the acetylation technique, but no concurrent carnitine uptake could be observed because in this situation the entering radioactive carnitine soon exited in exchange with another molecule of radioactive carnitine moving in from the medium. Accordingly, we had inferred that there is a substrate pool in a microenvironment near carnitine/acylcarnitine translocase, carnitine of which equilibrated faster with the carnitine of the medium than with the bulk of the mitochondrial carnitine present in the matrix. The repeatedly noted (for references see Murthy & Pande, 1984) increase in the uptake of radioactive carnitine with an increase in the carnitine content of mitochondria employed arose from the enhanced ability of the larger matrix carnitine pool to trap the incoming radioactive carnitine so that the chances of the newly entering radioactive carnitine molecule itself exiting out in exchange for another entering molecule of radioactive carnitine were lessened (Murthy & Pande, 1984). If the preceding interpretations were correct, then one would expect that on elevation of the carnitine content of mitochondria to high values, a point would be reached at which most of the entering carnitine would become 'trapped' and the observed uptake rate then would not increase with a further increase in matrix carnitine. Fig. 1 shows that such indeed was the case; the direct-influx rate increased with an increase in matrix carnitine content with the activity levelling off at $\geqslant$10nmol of matrix carnitine/mg of mitochondrial protein. The inward-transport rate of carnitine, monitored by the acetylation procedure, depended on matrix carnitine content only when this was $\ll$0.7nmol/mg; the rate was not affected by a further increase in the matrix carnitine content of up to 8nmol/mg of mitochondrial protein. [The import rates of palmitoylcarnitine show dependence on matrix carnitine only when the latter falls below 0.13nmol/mg of mitochondrial protein (Murthy & Pande, 1984).] Inasmuch as the maximally observed carnitine-influx rates by these two procedures approached similar values, it is likely that under these conditions both procedures were picking up the true translocase-catalysed rates of exchange diffusions and that none of the transport

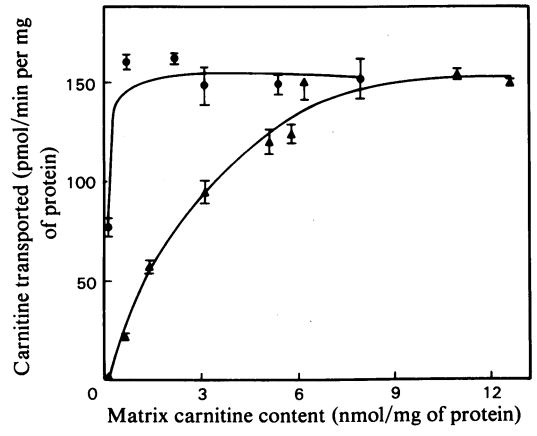

Fig. 1. *Effect of matrix carnitine content on the apparent import rates of carnitine from the medium as determined by the direct-uptake method* (▲) *and the acetylcarnitine-formation method* (●)

Details of the assay procedures were as described previously (Murthy & Pande, 1984) except that the final concentration of carnitine was 0.5mM and that heart mitochondria having various amounts of matrix carnitine were used.

events were now escaping detection in the uptake assay.

Table 1 shows that a similar picture prevailed also during the import of acetylcarnitine in (*a*) that the rates of direct uptake rose with increase in matrix carnitine of from 0.5 to 8.9nmol/mg of protein whereas those of the indirect import assay based on formation of ^{14}C-labelled anions were not so affected, (*b*) that at the lower, near-physiological, matrix carnitine contents the anion-formation assay picked up higher transport rates than did the direct-uptake assay, and (*c*) that at the high matrix carnitine content of 8.9nmol/mg of mitochondrial protein the acetylcarnitine-import rates determined by the direct-uptake assay were comparable with those obtained by the indirect anion-formation assay.

To examine whether microenvironments on the matrix side of the inner membrane would be discernible during the exchange diffusion of other metabolites as well, mitochondrial transport of ADP was assayed by monitoring the uptake of [^{14}C]ADP from the medium or the efflux of matrix [^{14}C]ADP. Simultaneously the [^{14}C]ADP import was monitored also by analysing for the carboxyatractyloside-sensitive formation of [^{14}C]ATP in the whole incubation system. The results showed (Table 2) that higher transport rates were revealed by the ATP-formation assay as compared with the ADP-influx or ADP-efflux assays and that a 90% depletion of mitochondrial adenine nucleotides decreased the latter assay values to a greater extent than that of the [^{14}C]ATP formation. Thus, whereas the influx or the efflux assay picked up

Table 1. *Rate of transport of acetylcarnitine into mitochondria as affected by variations in matrix carnitine content*

Acetylcarnitine influx was followed by uptake measurements made with acetyl[^{3}H]carnitine and also by assay of formation of ^{14}C-labelled anions with the use of [*Ac*-^{14}C]acetylcarnitine. The constantly stirred assay system, in a final volume of 50 μl at 0°C, contained 250mM-mannitol, 25mM-Hepes buffer, pH 7.4, 0.05mM-EDTA, 3mM-ADP, 5mM-potassium phosphate buffer, pH 7.4, 1mM-malate and 0.5mM-acetylcarnitine, and the reactions were started by the addition of 0.5–1 mg of mitochondria having various amounts of matrix carnitine. The reactions were terminated by 100 μl of a mixture containing 3mM-mersalyl, 200mM-mannitol and 50mM-Tris/HCl buffer, pH 7.4 (direct uptake), or by 200 μl of a mixture containing 2.5mM-mersalyl, 200mM-mannitol and 50mM-Tris/HCl buffer, pH 7.4 (formation of ^{14}C-labelled anions). All tubes were further incubated for 10min at 0°C and then processed as described in Murthy & Pande (1984).

Matrix carnitine (nmol/mg)	Acetylcarnitine transport (pmol/min per mg of protein)	
	Measured as direct uptake	Measured as formation of ^{14}C-labelled anions
0.5±0.1	42.5±3*	110±9
1.4±0.1	54.1±1*	132±23
3.3±0.2	84.6±4*	106±7
8.9±1.1	99.5±1	119±15

* $P<0.05$ as compared with values from formation of ^{14}C-labelled anions.

Table 2. *Effect of matrix ADP+ATP content on [^{14}C]ADP transport in heart mitochondria as measured by different procedures*

Details of these procedures were as indicated in the Experimental section, except that, where the efflux of matrix [^{14}C]adenine nucleotides was to be measured, after carboxyatractyloside addition the tubes were immediately centrifuged at 10000*g* for 90s and the supernatants were monitored for radioactivity.

	Mitochondrial ATP+ADP content (nmol/mg)	[^{14}C]Adenine nucleotide transport (nmol/min per mg of protein)		
		Measured as efflux of matrix [^{14}C]adenine nucleotide	Measured as uptake of medium [^{14}C]ADP	Measured as conversion of medium [^{14}C]ADP into [^{14}C]ATP
Adenine nucleotide-loaded mitochondria	7.3±0.10	—	4.9±0.05†	8.7±0.82*
Normal mitochondria	5.2±0.01	3.0±0.10	3.3±0.20	7.9±0.40*
Adenine nucleotide-depleted mitochondria	0.5±0.01	0.3±0.05	0.3±0.03	3.0±0.01*

* $P \ll 0.05$ compared with the uptake of [^{14}C]ADP with the corresponding mitochondria.
† $P \ll 0.05$ compared with uptake in normal mitochondria.

Table 3. *Not all the entering [^{14}C]ADP is phosphorylated to [^{14}C]ATP in the matrix of actively respiring mitochondria*

[^{14}C]ADP uptake by mitochondria was measured by using the silicone-oil centrifugation procedure except for the use of 50 μl of 2.5% $HClO_4$ in 0.8 M-mannitol as the bottom layer. After mitochondria had been sedimented the acid layer was neutralized with KOH and processed for the separation of adenine nucleotides. Other details were as indicated in the Experimental section. Abbreviation: N.D., not detectable.

	Normal mitochondria	Adenine nucleotide-depleted mitochondria
ATP+ADP content (nmol/mg)	5.21±0.01	0.53±0.01*
Appearance of [^{14}C]ATP in the matrix (nmol/min per mg)	0.51±0.03	0.11±0.01*
Appearance of [^{14}C]ADP in the matrix (nmol/min per mg)	2.56±0.23	0.17±0.01*
Appearance of [^{14}C]AMP in the matrix (nmol/min per mg)	0.25±0.01	N.D.

* $P \ll 0.05$ as compared with normal mitochondria.

Table 4. *Microcompartmentation of the imported carnitine is discernible even at ambient temperature and in uncoupled mitochondria*

Details of the assay system were described previously (Murthy & Pande, 1984) except that the final concentration of carnitine was 0.5 mM. In Expt. I, the reactions were started by the addition of 0.5–1 mg of mitochondria (matrix carnitine was 1.9±0.1 nmol/mg of protein) and the incubations were for 3 s at 30°C. In Expt. II, the assay system contained 10 μM-carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and the reactions were started by carnitine after prior incubation of the mitochondria (matrix carnitine was 2.5±0.2 nmol/mg of protein) for 1 min at 0°C. Incubations were for 2 min at 0°C. All the tubes were processed as described in Murthy & Pande (1984).

	Method used for measurement of carnitine transport	Rate of carnitine transport (nmol/min per mg of protein)
Expt. I	Direct influx	5.6±0.3*
	[^{14}C]Acetylcarnitine formation	22.9±4.8
Expt. II	Direct influx	0.093±0.01*
	[^{14}C]Acetylcarnitine formation	0.187±0.01

* $P \ll 0.05$ as compared with [^{14}C]acetylcarnitine-formation value.

40% of the transport rates revealed by the [^{14}C]-ATP-formation method, after adenine nucleotide depletion this value fell to 10%.

It should be added that, although the [^{14}C]ATP-formation assay measured the [^{14}C]ATP formed in the entire assay system (i.e. mitochondria plus medium), it still underestimated the true [^{14}C]ADP transport; appreciable [^{14}C]ADP that escaped phosphorylation by F_1 ATP synthase was detected in the matrix (Table 3). During the assay of carnitine import, based on carnitine's acetylation in matrix, or of palmitoyl[^{3}H]carnitine import, based on free [^{3}H]carnitine release in matrix, higher rates are realized when after arrest of the transport by a stop inhibitor, a further incubation is allowed to complete the acylation/deacylation of the already entered substrate (Murthy & Pande, 1984). The possibility that higher transport rate of [^{14}C]ADP, based on [^{14}C]ATP formation, might be picked up on the inclusion of a post-carboxyatractyloside incubation step (up to 10 min at 0°C was tested) to complete phosphorylation of any [^{14}C]ADP that had reached matrix but not yet become phosphorylated to [^{14}C]ATP was explored, but given up when the results showed that for some reason the total [^{14}C]ATP of the incubation system gradually declined when such a post-carboxyatractyloside incubation was allowed.

Since in the preceding experiments evidence for the microcompartmentation was obtained in incubations performed at 0°C, the question whether the same was likely at near-physiological temperatures arose, particularly because the possibility of microcompartments has previously been alluded to as being an artefact of low-temperature incubations (Hartung *et al.*, 1983). Table 4 shows that at 30°C also higher import rates of carnitine were found when monitored as acetylcarnitine formation than as the association of medium carnitine's radioactivity with mitochondria; we should stress that this was found under the conditions where the uptake assays showed that $\leqslant 15\%$ of the matrix's carnitine had been replaced. Thus consideration of the possibility that microcompartments exist under physiological conditions and might have some metabolic role appears justified.

Discussion

Our results show that, under conditions permitting further metabolism, a large part of the entering substrates became simultaneously metabolized

(acetylation of free carnitine, deacylation of acylcarnitines, oxidative phosphorylation of ADP) and that portions of the metabolites thus formed were re-transported back to the medium unless matrix had high enough concentration of endogenous substrate to trap effectively the newly entering molecules and/or the transportable metabolites derived therefrom. When mitochondria have little endogenous substrate to participate in exchange diffusion, the newly entering substrate and its transportable metabolites themselves exchange out, with substrate molecules entering subsequently, and consequently many of these transport events fail to register as uptake, i.e. as association of radioactive substrate with mitochondria. Even with mitochondria having the normal contents of carnitine and adenine nucleotides, their usual influx/efflux assays register less than half of the proceeding exchange-diffusion events. Clearly the conventional influx and efflux assays are inadequate for assessing true transport rates of carnitines and ADP, and there is nothing to indicate that this phenomenon would not be a general one applicable to the transport of other substrates in mitochondria and perhaps also in cells. It is probable, therefore, that misleading conclusions have at times been drawn with regard to the kinetic and regulatory significance of various transports based on rate measurements made by employing solely the usual influx or efflux assays.

Insofar as under conditions permitting metabolism a good part of the entering substrate becomes metabolized, an appreciable portion of which is transported out before mixing with the major part of the substrate present in the matrix, it is evident that the involved metabolizing enzymes, of the inner membrane and of matrix, reside close to the translocases to share the same microcompartment(s). Evidence of such a sharing between heart mitochondrial carnitine/acylcarnitine translocase and carnitine acyltransferases (Murthy & Pande, 1984; the present study), adenine nucleotide translocase and F_1-ATPase (Out & Kemp, 1974; Vignais *et al.*, 1975; Out *et al.*, 1976; the present study), pyruvate transporter and the pyruvate dehydrogenase system (Pande & Parvin, 1978), malate transporter and malic enzyme in tumour mitochondria (Moreadith & Lehninger, 1984), glutamine transporter and P_i-dependent glutaminase in kidney mitochondria (Simpson, 1983) and glutamate/aspartate transporter and glutamate:oxaloacetate transaminase in liver mitochondria (Duszynski *et al.*, 1978) is now available; in many of these cases the entering substrate is concurrently metabolized despite evidence that transport and the subsequent metabolism are not obligatorily linked events. Similarly during [^{14}C]ADP's import into normal mitochondria (Table 3) the major part of the [^{14}C]ATP formed was present in the medium and the major part of the ^{14}C associated with the mitochondria was in the form of ADP. Out & Kemp (1974) and Vignais *et al.*, (1975) have previously considered the possibility that adenine nucleotide translocase and F_1-ATPase either interact directly or share the same microcompartment on the matrix side of the inner membrane. In a subsequent study Out *et al.* (1976) interpreted their results in favour of the former possibility, and postulated further that the functional interaction between the adenine nucleotide translocase and the F_1-ATPase was lost in the non-energized state. Inasmuch as with carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone-uncoupled mitochondria also the [^{14}C]acetylation assay of carnitine import (Expt. II in Table 4) gave higher transport rates than did the carnitine-uptake assay, it is evident that the existence of microcompartments was not obliterated by the non-energization of mitochondria. A similar comparison for the adenine nucleotide translocase is not expected to yield unequivocal information in light of the knowledge (LaNoue & Schoolwerth, 1979) that the substrate preferences and affinity of the adenine nucleotide translocase are markedly influenced by the energy status of mitochondria. Data reported by Hamman & Haynes (1983) and our present results support the existence of a microcompartment near the translocase.

The existence of more than one substrate pool inside mitochondria, at least one near the inner membrane and the other away from it, substrates of which turn over at different rates, could be advantageous for metabolic regulation. Since the make-up of the enzymes in the above two compartments will be different, at least for substrates that require the functionally linked participation of transporters and enzyme(s) of the inner membrane for one segment of their metabolism but not for the other, it follows that potential for the independent control of the different steps of the metabolism of the same substrate in different mitochondrial subcompartments exists. Demonstration of microcompartment near the inner membrane was facilitated in the present study most probably owing to the close proximity of the relevant membrane-associated proteins, the translocases and the enzymes that metabolized the entered substrate; it should be stressed that evidence for the existence of microcompartments would be difficult to obtain with metabolic systems that do not meet the requirement of organizational proximity. The sharing of the microcompartment(s) by the different enzymes and translocases involved in the successive steps of a segment of a metabolic pathway could offer a kinetic advantage similar to that derived from the existence of multienzyme com-

plexes. Indirect pieces of evidence have indicated that the mitochondrial enzymes of the citric acid cycle and the β-oxidation spiral behave as organized systems (Srere, 1984). The possibility that such a functional organization involves, in part, sharing of the same microcompartment by the related enzymes is raised by the present findings. Finally, it is plausible that similar microcompartmentation occurs around cell-membrane transporters and hormonal receptors, resulting in the segregation of discrete metabolic steps for higher metabolic efficiency and rapid signal amplification. Besides, it should also be recognized that relevant changes in metabolite concentrations that occur only within the microcompartment may go unrevealed by their measurement in whole cells or tissue portions as is commonly practiced currently.

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