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LETTER TO THE EDITOR

# Metabolite Channelling in Aerobic Energy Metabolism

The question of whether "metabolite channelling" is only applicable to stable enzyme complexes or if it is also valid for dynamic complexes dominates current discussions (Batke, 1991; Cárdenas, 1991; Gutfreund & Chock, 1991; Ovádi, 1991*b*; Petterson, 1991; Welch & Marmillot, 1991). Several authors argued that metabolite channelling by dynamic complexes is irrelevant, since by the high degree of structural organization in the cytosol (Goodsell, 1991) and in mitochondria, the diffusion of proteins is reduced to such an extent that "accidental" complex formation by consecutive enzymes of a reaction pathway seems unlikely. However, the likeliness of metabolite channelling by "dynamic" complexes can be increased by inferring the existence of "metastable" complexes, in which the catalytic turnover is much faster than the rates of association and dissociation (Batke, 1991; Ovádi, 1991*a*). The purpose of this commentary is first, to show that metabolite channelling by creatine kinase (CK) isoenzymes is of prime importance for aerobic energy metabolism, and second, to argue that mitochondrial creatine kinase (Mi-CK) is an attractive candidate to prove that metabolite channelling by "dynamic" enzyme complexes occurs.

CK isoenzymes are primarily found in tissues with high and fluctuating energy demands, like skeletal and cardiac muscle, brain, photoreceptor cells and spermatozoa, where they are thought to be involved in "buffering" of [ATP] and [ADP] as well as in the "transport" of high-energy phosphates from sites of ATP production (mitochondria, glycolysis) to sites of ATP consumption (Wallimann *et al.*, 1992; Wyss *et al.*, in press). As far as the "transport" function of the CK system is concerned, Mi-CK in the mitochondrial intermembrane space catalyses the transphosphorylation of ATP (generated in the mitochondrial matrix) and creatine to ADP and phosphocreatine. The latter compound leaves the mitochondria as the net product of oxidative phosphorylation and diffuses through the cytosol to sites of ATP consumption. There, cytosolic CK isoenzymes continuously regenerate ATP in order to maintain a high ATP/ADP ratio, which seems to be crucial for many ATPases. Diffusion of creatine back to the mitochondria closes the cycle. One of the cornerstones of the "transport" function is the isoenzyme-specific subcellular compartmentation of CK isoenzymes, whereby the different members of the CK isoenzyme family are involved in distinct metabolic pathways, as suggested by Ureta (1991) for hexokinase. Mi-CK is the only CK isoenzyme coupled to oxidative phosphorylation (see below). In contrast, the cytosolic CK isoenzymes are either free in the cytosol, or are bound to subcellular structures like the sarcolemma, sarcoplasmic reticulum or myofibrils where they were shown to be functionally coupled to $Na^+/K^+$-ATPase, $Ca^{2+}$-ATPase, and myosin ATPase, respectively (for review see Wallimann *et al.*, 1992). To us, it seems rather amazing that the beauty of the CK isoenzyme system has gone



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completely unrecognized so far by the "metabolic channelling" community (see *J. theor. Biol.* **152,** 1991).

Since highest CK activities are found in tissues with the most abrupt changes in workload, it was recently hypothesized that a main function of the CK system is to *reduce transient times* to reach a new steady state of high-energy phosphate concentrations (Wyss *et al.*, in press). Because ATP and ADP are key regulators of many fundamental metabolic pathways, large changes in their concentrations upon changes in workload might heavily disturb the whole cellular metabolism. Therefore, by balancing the rates of ATP consumption and ATP production more rapidly, the CK system helps to avoid energy dissipation and ensures proper functioning of cellular ATPases. According to Ovádi (1991*a*) and Heinrich & Schuster (1991), transient times can be reduced in two different ways: (1) by making the reactions very fast so that they approach quasi-equilibrium. In fact, the enzymatic activity of *soluble* cytosolic CK is much higher than the maximal rates of ATP production or ATP consumption, thus pointing to near-equilibrium conditions of the CK reaction in the cytosol; (2) by metabolite channelling. Metabolite channelling is likely to occur between *myofibril-* or *membrane-bound* cytosolic CK isoenzymes and various ATPases of these subcellular structures; discussion of this topic is beyond the scope of this commentary. Additionally, as summarized below, a large number of recent studies strongly suggest that a multienzyme complex, comprising the adenine nucleotide translocator of the mitochondrial inner membrane, Mi-CK of the intermembrane space, and porin of the outer membrane, is involved in channelling of high-energy phosphates from the mitochondrial matrix to the cytosol (for reviews see Brdiczka, 1991; Wallimann *et al.*, 1992; Wyss *et al.*, in press; see also Fig. 1).

(a) In mitochondrial subfractionation experiments, as much as 50% of Mi-CK was found in a fraction enriched for contact sites between the inner and outer mitochondrial membranes. Contact sites are thought to be involved in mitochondrial import of precursor proteins as well as in lipid transport between both mitochondrial membranes. Furthermore, contact sites increase in extent upon stimulation of mitochondrial respiration, thus suggesting that they are also involved either in regulation of oxidative phosphorylation or, more likely, in export of high-energy phosphates out of the mitochondria. Within the contact sites, Mi-CK is rather inaccessible to externally added (extramitochondrial) substrates and negatively charged inhibitors, which argues strongly for a distinct microcompartment for CK substrates within the contact sites.

(b) The fact that a synthetic polyanion known to block porin also inhibited Mi-CK activity within the contact sites, even when the outer membrane was selectively broken by digitonin, strongly favours functional coupling between Mi-CK and porin.

(c) A large number of kinetic, thermodynamic as well as labelling experiments with $\gamma$-[$^{32}$P]-ATP or [$^{33}$P]-inorganic phosphate demonstrated functional coupling between Mi-CK and the adenine nucleotide translocator. Most importantly, an apparent violation of the second law of thermodynamics (Westerhoff, 1991) was observed, in as far as mitochondrial phosphocreatine production by respiring mitochondria still proceeded even when, as calculated from the extramitochondrial

substrate concentrations, the Mi-CK reaction should have proceeded in the opposite direction.

(d) Finally, in contrast to the dimeric cytosolic CK isoenymes, Mi-CK forms octamers. The highly symmetrical octameric structure of Mi-CK (Schnyder *et al.*, 1991; see Fig. 1), with a four-fold symmetry, two identical top and bottom faces and possibly a channel through the molecule, is also indicative for metabolite channelling. Recently, octameric Mi-CK was found to induce close contacts between a monolayer of outer mitochondrial membrane lipids or spread outer membranes on one hand and large unilamellar vesicles of inner membrane lipids or inner membranes, respectively, on the other hand, thus corroborating that octameric Mi-CK is ideally suited for a contact site localization (Rojo *et al.*, 1991). On the basis of these experiments, one can hypothesize that tetrameric adenine nucleotide translocator of the inner membrane, octameric Mi-CK, and oligomeric porin of the outer membrane form a highly organized multienzyme complex in the contact sites, allowing phosphocreatine synthesis even at high cytosolic ATP/ADP ratios (Fig. 1).

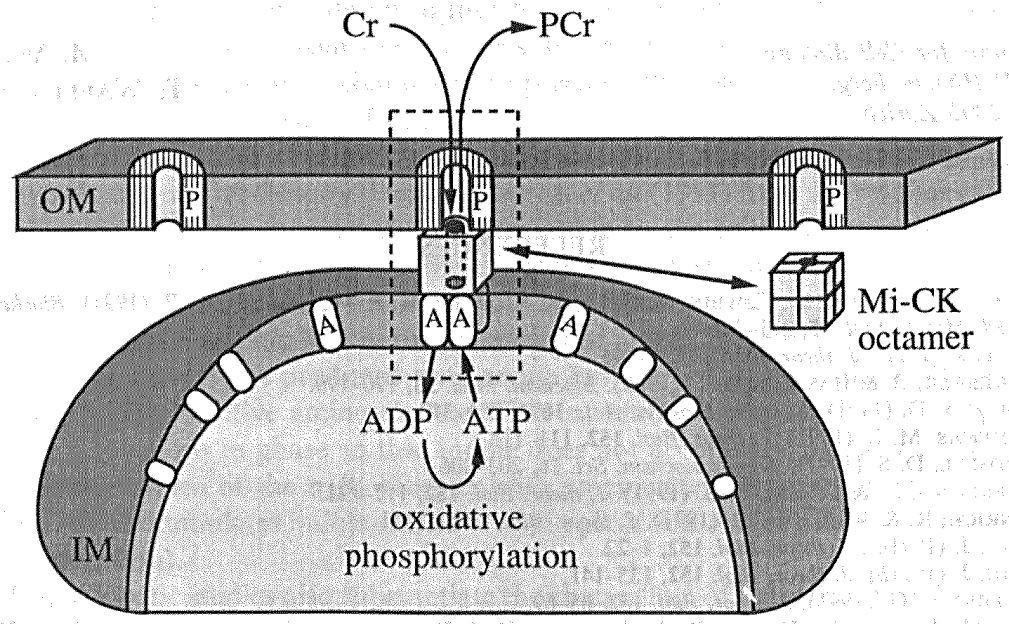


FIG. 1. Involvement of octameric mitochondrial creatine kinase (Mi-CK) in channelling of high-energy phosphates at the mitochondrial contact sites. Within contact sites (surrounded by a dashed line), exclusively octameric Mi-CK interacts with both the inner (IM) and the outer mitochondrial membrane (OM) and seems to be functionally coupled to the adenine nucleotide translocator (A) of the inner and porin (P) of the outer membrane. Tetrameric adenine nucleotide translocator, octameric Mi-CK and oligomeric porin may form a highly organized multienzyme complex, thereby creating a "microcompartment" allowing efficient substrate channelling between the three enzymes. The central channel of the Mi-CK octamer suggested by electron microscopical investigations is probably of prime importance for this substrate channelling and thus for export of high-energy phosphates out of the mitochondria. By the arrow, it is schematically indicated that formation of contact sites and, most likely, also of the multienzyme complex between Mi-CK, adenine nucleotide translocator and porin are dynamic processes, thereby allowing for efficient regulation of high-energy phosphate channelling and thus of whole cellular energy metabolism. For simplicity, the subunit "boundaries" were omitted in the Mi-CK octamer localized within the contact sites. Cr, creatine; PCr, phosphocreatine.

In this same context, it seems worth mentioning that functional coupling was also demonstrated between oxidative phosphorylation and hexokinase or glycerol kinase, whereby the latter two enzymes are bound to porin at the contact sites on the outer side of the outer mitochondrial membrane (Adams *et al.*, 1991). Interestingly, in intact respiring rabbit heart mitochondria, creatine depressed the formation of glucose-6-phosphate by mitochondrially bound hexokinase, whereas glucose had no effect on phosphocreatine production, indicating that even within contact sites, rapid equilibration of substrates does not occur (Borrebaek & Haviken, 1985).

To conclude, Mi-CK may become a valuable candidate to corroborate metabolite channelling by "dynamic" enzyme complexes. First, much evidence for dynamic microcompartmentation of Mi-CK in contact sites is available; second, the extent of contact sites and therefore, indirectly, also the proportion of Mi-CK in contact sites can be measured by freeze-fracture electron microscopy; and third, factors influencing the extent of contact sites are already known.

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