



## Creatine kinase: An enzyme with a central role in cellular energy metabolism

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The enzyme creatine kinase (CK), catalyzing the reversible transfer of the N-phosphoryl group from phosphocreatine (PCr) to ADP to regenerate ATP, plays a key role in the energy homeostasis of cells with intermittently high, fluctuating energy requirements, e.g. in skeletal and cardiac muscle, neurons, photoreceptors, spermatozoa and electrocytes. Cytosolic CK isoenzyme(s) (MM-, MB- and BB-CK) are always co-expressed in a tissue-specific fashion together with a mitochondrial isoform. Using biochemical fractionation and *in situ* localization, one was able to show that the CK isoenzymes, earlier considered to be strictly soluble, are in fact compartmentalized subcellularly and coupled functionally and/or structurally either to sites of energy production (glycolysis and mitochondria) or energy consumption (cellular ATPases, such as the actomyosin ATPase and SR-Ca<sup>2+</sup>-ATPase), thus forming an intricate, highly regulated energy distribution network, the PCr-circuit or PCr-shuttle (Fig. 1, [1]).

This non-equilibrium energy transport model has been challenged, based upon global <sup>31</sup>P-NMR experiments, measuring CK-mediated flux in muscles at different work-loads [2,3]. The conclusions reached were that the CK system is in equilibrium with the substrates and behaves like a solution of well-mixed enzymes, that effects of compartmentation were negligible with respect to total cellular bioenergetics and that thermodynamic characteristics of the cytosol could be predicted as if the CK metabolites were freely mixing in solution.

However, based on the organizational principles of striated muscle, as well as on our findings concerning the highly structured subcellular CK-compartments, this interpretation seemed rather unlikely and thus has been questioned subsequently by a CAVEAT! [4]. In support of this, <sup>31</sup>P-NMR CK-flux measurements with transgenic mice with graded reductions of MM-CK expression in their muscles, show a strikingly unexpected, 'anomalous' CK-flux behaviour [5], indicating that some flux through CK, presumably bound CK, and possibly also some PCr and/or ATP, is NMR-invisible or otherwise not amenable to this analysis [4,6]. In the meantime, more evidence from NMR-measurements [7–10], as well as from recent *in vivo* <sup>14</sup>C-Cr-tracer studies [11], is accumulating in favour of compartmentation of the CK system and for the existence of different pools of CK substrates. As a matter of fact, it has now become clear that in muscle, Cr and PCr molecules do not tumble freely, but display partial orientational ordering, which is in contrast to what is expected for small molecules dissolved in water [7]. Furthermore, <sup>31</sup>P-NMR saturation transfer experiments with sea-urchin spermatozoa show that the CK-flux increases by a factor of 10–20 upon sperm activation [12]. These specialized sperm cells derive their energy for motility entirely from fatty oxidation within the single large mitochondrion located just behind the sperm head, from where PCr is diffusing along the 50 μm long sperm tail to fuel the dynein/tubulin ATPase. It is obvious that in these polar, elongated cells, the diffusional limitation of ADP is the key limiting factor

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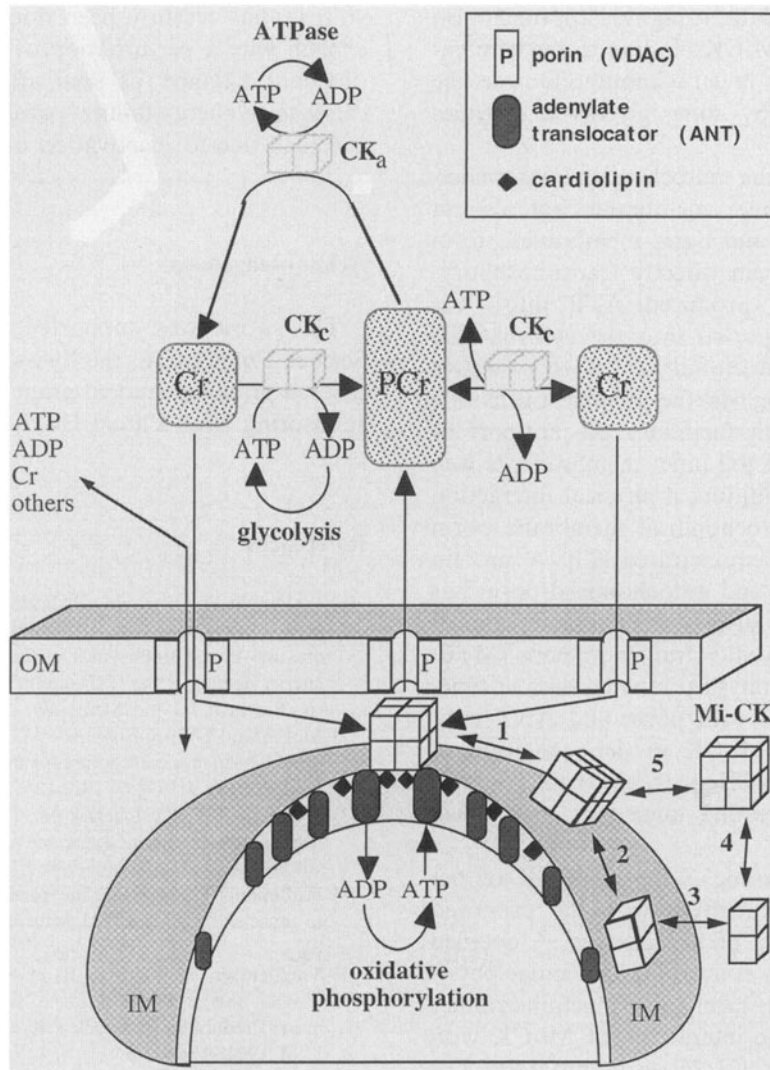


Fig. 1.

with respect to high-energy phosphate provision [13]. In support of the PCr-shuttle model [6,1] the calculated diffusional flux of ADP in these sperm cells are by two and three orders of magnitude smaller than those of ATP and PCr, respectively.

Transgenic CK(-/-) double knock-mice show significantly increased relaxation times of their limb muscles, altered  $\text{Ca}^{2+}$ -transients in myotubes after stimulation, as well as remarkable remodelling of the contractile apparatus with increased numbers of mitochondria and a grossly over-produced tubular SR membranes [14]. The obvious difficulties of these mice with muscle  $\text{Ca}^{2+}$ -handling, as the main phenotype, is in line with biochemical and functional data showing that some MM-CK is specifically associated with SR membranes [15], where it is crucial for fueling the energetically highly demanding  $\text{Ca}^{2+}$ -ATPase [16,17]. Therefore, the most crucial function of the CK-system in muscle is related to the energetics of  $\text{Ca}^{2+}$ -homeostasis.

According to recent findings, AMP-activated protein kinase is able to bind rather tightly to muscle-type MM-CK and phosphorylate the latter to inhibit its activity. AMPK itself is regulated not only by the ATP/AMP ratio, but also by the PCr/Cr ratio [18]. This invalidates the long-held dogma that PCr and Cr are metabolically completely inert compounds. Thus, AMPK, as an energy sensor system, could represent the missing link for regulation of adaptive metabolic regulation.

Some CK is additionally associated with the myofibril [1]. The isoenzyme-specific association domain of MM-CK with the sarcomeric M-band has been localized by an in situ biochemical approach, using heterologously expressed, fluorescently labelled site-directed mutants, as well as M/B-CK chimaeras for diffusion into chemically skinned muscle fibers [19]. This M-band interaction domain could be narrowed down to two lysine charge-clamps, symmetrically organized on an exposed face of each M-CK monomer

(unpublished data). New data, using this approach also indicate that the weak MM-CK binding to the sarcomeric *I*-band, observed by in situ immunofluorescence localization is mediated by some glycolytic enzymes [20].

Mi-CK is located in the mitochondrial intermembrane space along the inner membrane, but also at contact sites where inner and outer membranes are in close proximity. Mi-CK can directly transphosphorylate intramitochondrially produced ATP into PCr, which subsequently is exported into the cytosol. The functional coupling of mitochondrial CK to oxidative phosphorylation, occurring via the adenine nucleotide translocator (ANT), which facilitates the antiport of ATP versus ADP through the inner membrane, is well documented [21,22]. In addition, a physical interaction of Mi-CK with outer mitochondrial membrane porin (VDAC) has also been demonstrated [23]. A protein complex containing ANT and mitochondrial porin has recently been described to display the characteristics of the mitochondrial permeability transition pore (MTP) or megachannel [24]. The physical interaction and functional coupling of Mi-CK with porin and ANT indicates an involvement of Mi-CK in the regulation of MTP, since octameric Mi-CK in this protein complex [24], plus creatine or creatine analogues, can delay MTP [25].

The recently solved atomic X-ray structure of octameric Mi-CK [26] is consistent with the proposed energy channeling function of this enzyme. A detailed structure/function analysis concerning molecular physiology, catalytic site and mechanism, octamer/dimer equilibrium, as well as the interaction of Mi-CK with mitochondrial membranes [21,27] is in progress. The identical top and bottom faces of the octamer contain putative membrane binding motifs likely to be involved in binding of Mi-CK to mitochondrial membranes. The central 20 Å wide channel of the Mi-CK octamer may be of functional significance for the exchange of energy metabolites between mitochondria and cytosol. Furthermore, Mi-CK may follow a 'back door' mechanism by which PCr is expelled into the central channel of the Mi-CK octamer to facilitate a vectorial transport of PCr from the mitochondrial matrix into the cytosol. Finally, the CK/PCr system is now recognized as an important metabolic regulator during health and disease. Creatine supplementation seems helpful not only for athletes to improve physical performance, but is also emerging as a therapeutic aid for neuromuscular and neurodegenerative diseases. In some of these diseases, especially in mitochondrial myopathies, a compensatory over-expression of Mi-CK, due to cellular energy deficit, can lead to the formation of pathological intramitochondrial, crystalline Mi-CK inclusions [28]. Furthermore, a pronounced sensitivity of Mi-CK towards reactive oxygen species (ROS), especially perox-

ynitrite, has recently been documented [29]. This may explain why a perturbation of cellular pro-oxidant/antioxidant balance, as seen after ischemia/reperfusion, can lead to energy failure, paralleled by chronic calcium overload due to inactivation of CK [29].

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