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Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology

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Abstract. Creatine kinase (CK) isoenzymes, specifically located at places of energy demand and energy production, are linked by a phosphocreatine/creatine (PCr/Cr) circuit, found in cells with intermittently high energy demands. Cytosolic CKs, in close conjunction with Ca^{2+}-pumps, play a crucial role for the energetics of Ca^{2+}-homeostasis. Mitochondrial Mi-CK, a cuboidal-shaped octamer with a central channel, binds and crosslinks mitochondrial membranes and forms a functionally coupled microcompartment with porin and adenine nucleotide translocase for vectorial export of PCr into the cytosol. The CK system is regulated by AMP-activated protein kinase via PCr/Cr and ATP/AMP ratios. Mi-CK stabilizes and cross-links cristae- or inner/outer membranes to form parallel membrane stacks and, if overexpressed due to creatine depletion or cellular energy stress, forms those crystalline intramitochondrial inclusions seen in some mitochondrial cytopathy patients. Mi-CK is a prime target for free radical damage by peroxynitrite. Mi-CK octamers, together with CK substrates have a marked stabilizing and protective effect against mitochondrial permeability transition pore opening, thus providing a rationale for creatine supplementation of patients with neuromuscular and neurodegenerative diseases.

Keywords: Creatine kinase (CK), phosphocreatine shuttle, energetics of Ca^{2+}-homeostasis, CK null mutant transgenic mice, mitochondrial creatine kinase (Mi-CK), intramitochondrial inclusions, mitochondrial myopathies, AMP-activated protein kinase, mitochondrial permeability transition, peroxynitrite, porin, adenine nucleotide translocase (ANT), cell- and neuroprotective effects of creatine, creatine supplementation, neuromuscular diseases

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

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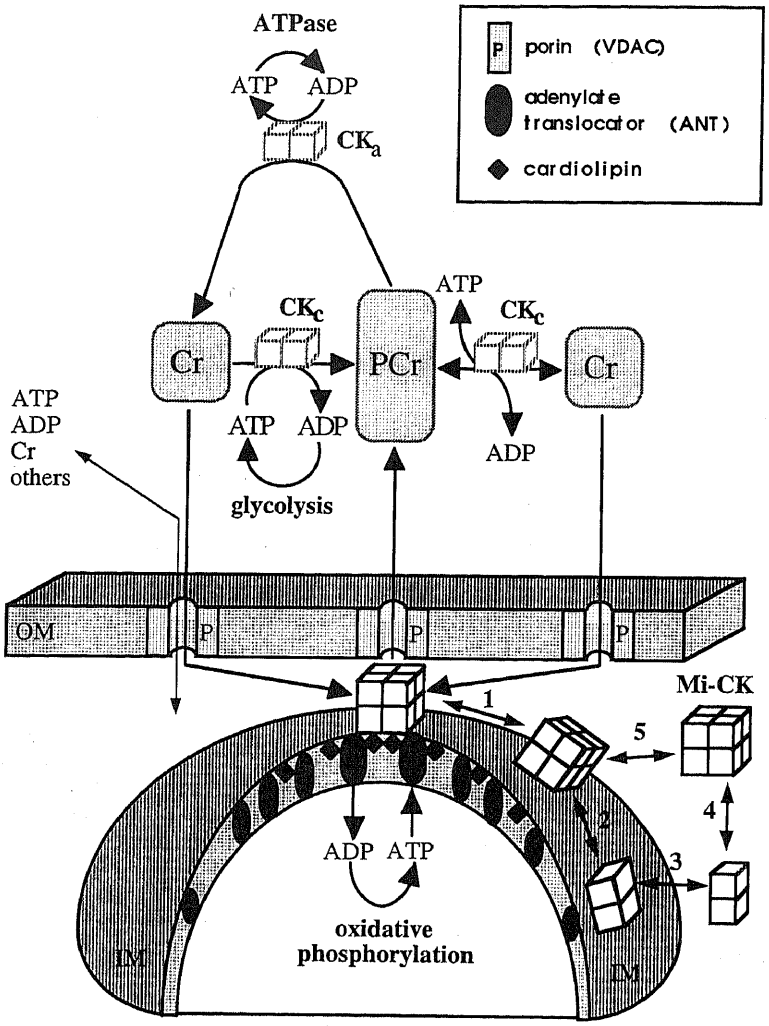

Fig. 1. The PCr-circuit: a temporal and spatial energy buffering network and regulatory system for cellular energy metabolism in cells with intermittently high energy requirements.

(cellular ATPases, such as the acto-myosin ATPase and SR-Ca^{2+}-ATPase). Thus they form an intricate, highly regulated energy distribution network, the PCr-circuit or PCr-shuttle (Fig. 1 [26]).

Upper, cytosolic side: the bulk of soluble, cytosolic CK (CK_c) equilibrates global ATP/ADP and PCr/Cr ratios by its equilibrium reaction (depicted in the right middle of the figure). In skeletal muscle at rest, these metabolite levels are approximately 3–5 mM/10–20 μM and 20–40 mM/10–15 mM, respectively (see [26]). One of the main functions of CK_c is to keep the concentration of free global ADP very low and thus to maintaing global [ATP] remarkably stable also during cell activation. This part of the PCr-circuit model represents the classical textbook function of CK as a temporal energy buffer, being backed up by adenylate kinase as a second safeguard against declining ATP and rising ADP levels. Some of the cytosolic CK_c is functionally coupled to glycolysis and, during periods of anaerobic work-output and recovery, preferentially accepts glycolytic ATP to replenish the very large PCr pool (ATP from glycolysis, depicted in the left middle of the figure). Additionally, however, some fractions of cytosolic

CK, are very specifically associated (CK_a) with ATP requiring processes at sites of energy consumption. For example, CK_a is associated with the contractile apparatus and the sarcoplasmic reticulum, where it forms functionally coupled microcompartments with the acto-myosin ATPase and the SR-Ca^{2+}-ATPase, respectively, or with other ATP requiring processes (see top of figure). There, ATP is directly regenerated *in situ* by CK_a via PCr, thus keeping local ATP/ADP ratios very high in the immediate vicinity of these ATPases.

Lower mitochondrial side: mitochondrial Mi-CK is bound to the outer side of the inner mitochondrial membrane (IM) and localized along the cristae membranes, as well as at mitochondrial contact sites, where IM and OM are in close vicinity (Brdickzka, 1991). At these sites, Mi-CK octamers are forming microcompartments with porin and adenine nucleotide translocase (ANT) for energy transfer from ATP to Cr, followed by vectorial transport of PCr into the cytosol. ATP generated by oxidative phosphorylation is preferentially accepted by Mi-CK octamers, transphosphorylated onto Cr, which is entering through the pore, to give PCr which then is exported into the cytosol. Thus, under high work-load, high-energy phosphate would be shuttled from mitochondria to sites of energy consumption (ATPases, top of figure), where it is then used by CK_a to regenerate ATP locally *in situ* to fuel these ATP-requiring processes and to keep local ATP/ADP ratios very high. Cr would diffuse back to the mitochondria to be recharged again. This part of the model represents the spatial buffering function of the PCr-circuit. In this model, the specifically localized CK isoenzymes at sites of energy consumption and energy production are connected via PCr and Cr as mediators, generating metabolic waves and dampening oscillations of metabolites [29].

The dynamic recruitment of either free or membrane-bound Mi-CK octamers, possibly depending on the metabolic state of the mitochondria, the dynamic octamer/dimer equilibrium of Mi-CK, as well as octamerization of Mi-CK dimers bound on the IM, all observed *in vitro*, are schematically visualized as potential modulatory events for long-term metabolic regulation. The interaction of Mi-CK with porin and complex formation with ANT, most likely facilitated by cardiolipin associated with ANT, are also illustrated. Under the conditions expected to prevail in the mitochondrial intermembrane space, however, the equilibria of these reactions, as observed *in vitro*, would clearly favour the membrane-bound octamer as depicted in Fig. 2 [17,19]. Since the formation of contact sites and the establishment of the protein complexes are thought to be rather dynamic, a on/off recruitment of Mi-CK octamer into contact sites is easily to be envisaged. After all, these events may be relevant also for the control of the permeability transition pore ([1,14], see also [2a]).

This non-equilibrium energy transport model has been challenged, based upon gobal ^{31}P-NMR experiments, measuring CK-mediated flux in muscles at different work-loads [10,28]. The conclusions reached were (i) that the CK system is in equilibrium with the substrates and behaves like a solution of well-mixed enzymes, (ii) that effects of compartmentation were negligible with respect to total cellular bioenergetics and (iii) 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 questionned subsequently by a caveat [24]! In support of this, ^{31}P-NMR CK-flux measurements with transgenic mice showing graded reductions of MM-CK expression in their muscles, revealed a strikingly unexpected, "anomalous" CK-flux behaviour [23], 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 [24,25]. In the meantime, more evidence from NMR-measurements [8,9,12,27], as well as from recent *in vivo* ^{14}[C]Cr-tracer studies [4], is accumulting 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 [8]. Furthermore, ^{31}P-NMR saturation transfer experiments with sea-urchin spermatozoa show that the CK flux increases by a factor of 10–20 upon sperm activation [22]. 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 with respect to high-energy phosphate provision [5]. In support of the PCr-shuttle model [25,26] the calculated diffusional flux of ADP in these sperm cells is by 2 and 3 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 Ca^{2+}-transients in myotubes after stimulation, as well as remarkable remodelling of the contractile apparatus with increased numbers of mitochondria and grossly over-produced tubular SR membranes [20]. The obvious difficulties of these mice with muscle 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 [16], where it is crucial for fueling the energetically highly demanding Ca^{2+}-ATPase [6,11]. Therefore, the most crucial function of the CK system in muscle is related to the energetics of Ca^{2+}-hoemostasis.

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 [15]. 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 [26]. 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 [21]. This M-band interaction domain could be narrowed down to two lysine charge-clamps, symmetrically organized on a exposed face of each M-CK monomer (unpubl.). 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 [7].

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 [17,29]. In addition, a physical interaction of Mi-CK with outer mitochondrial membrane porin (VDAC) has also been demonstrated [2]. 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 [1]. 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 [1], plus creatine or creatine analogues, can delay MTP [14].

The recently solved atomic X-ray structure of octameric Mi-CK [3] 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 [17,19] 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 [13]. Furthermore, a pronounced sensitivity of Mi-CK towards reactive oxygen species (ROS), especially peroxynitrite, has recently been documented [18]. 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 [18].

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References

[1] G. Beutner, A. Rück, B. Riede, W. Welte and D. Brdiczka, Complexes between kinases, mitochondrial porin and adenylate translocator in rat brain resemble the permeability transition pore, *FEBS Lett.* **396** (1997), 189–195.
[2] D. Brdiczka, P. Kaldis and T. Wallimann, *In vitro* complex formation between the octamer of mitochondrial creatine kinase and porin, *J. Biol. Chem.* **269** (1994), 27 640–27 644.
[2a] D. Brdiczka, G. Beutner, A. Rück, M. Dolder and T. Wallimann, The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition, *Biofactors* **8** (1998), 235–242.
[3] K. Fritz-Wolf, T. Schnyder, T. Wallimann and W. Kabsch, Structure of mitochondrial creatine kinase, *Nature* **381** (1996), 341–345.
[4] P.W. Hochachka and M.K. Mossey, Does muscle creatine phosphokinase have access to the total pool of phosphocreatine plus creatine?, *Am. J. Physiol.* **274** (1998), R868–R872.
[5] P. Kaldis, G. Kamp, T. Piendl and T. Wallimann, Functions of creatine kinase isoenzymes in spermatozoa, *Adv. Develop. Biol.* **5** (1997), 275–312.
[6] P. Korge and K.B. Campbell, Local ATP regeneration is important for sarcoplasmic reticulum Ca^{2+}-pump function, *Am. J. Physiol.* **267** (1994), C357–C366.
[7] Th. Kraft, V. Nier, B. Brenner and T. Wallimann, Binding of creatine kinase to the I-band of skinned skeletal muscle fibers is mediated by glycolytic enzymes: an *in situ* biochemical approach, *Biophys. J.* **70** (1996), A292.
[8] R. Kreis, M. Koster, M. Kamber, H. Hoppeler and C. Boesch, Peak assignment in localized 1H MR spectra of human muscle based on oral creatine supplementation, *Magn. Reson. Med.* **37** (1997), 159–163.
[9] E. LeRumeur, N. LeTallec, F. Kernec and J.D. de Certaines, Kinetics of ATP to ADP β-phosphoryl conversion in contracting skeltal muscle by *in vivo* 31P-NMR magnetization transfer, *NMR Biomed.* **10** (1997), 67–72.
[10] E.W. McFarland, M.J. Kushmerick and T. Moerland, Activity of creatine kinase in a contracting mammalian muscle of uniform fiber type, *Biophys. J.* **67** (1994), 1912–1924.
[11] A. Minajeva, R. Ventura-Calapier and V. Veksler, Ca^{2+}-uptake by cardiac sarcoplasmic reticulum ATPase *in situ* strongly depends on bound creatine kinase, *Pflügers Arch.* **432** (1996), 904–912.

[12] V. Ntziachristos, R. Kreis, C. Boesch and B. Quistorff, Dipolar resonance frequency shifts in 1H MR spectra of skeletal muscle: confirmation in rats at 4,7 T *in vivo* and observation of changes postmortem, *Magn. Reson. Med.* **38** (1997), 33–39.
[13] E. O'Gorman, K.-H. Fuchs, P. Tittmann, H. Gross and T. Wallimann, Crystalline mitochondrial inclusion bodies isolated from creatine-depleted rat soleus muscle, *J. Cell Sci.* **110** (1997), 1403–1411.
[14] E. O'Gorman, G. Beutner, M. Dolder, A.P. Koretsky, D. Brdiczka and T. Wallimann, The role of creatine kinase in inhibition of mitochondrial permeability transition, *FEBS Lett.* **414** (1997), 253–257.
[15] M. Ponticos, Q.L. Lu, J.E. Morgan, D.G. Hardie, T.A. Partridge and D. Carling, Dual regulation of AMP-activated protein kinase provides a novel mechanism for the control of creatine kinase in skeletal muscle, *EMBO J.* **17** (1998), 1688–1699.
[16] A.M. Rossi, H.M. Eppenberger, P. Volpe, R. Cotrufo and T. Wallimann, Muscle type MM-creatine kinase is specifically bound to sarcoplasmic reticulum and can support Ca^{2+}-uptake and regulate local ATP/ADP ratios, *J. Biol. Chem.* **265** (1990), 5258–5266.
[17] U. Schlattner, N. Forstner, M. Eder, O. Stachowiak, K. Fritz-Wolf and T. Wallimann, Functional aspects of the X-ray structure of mitochondrial creatine kinase: a molecular physiology approach, *Mol. Cell Biochem.* **184** (1998), 125–140.
[18] O. Stachowiak, M. Dolder, T. Wallimann and Ch. Richter, Mitochondrial creatine kinase is a prime target of peroxynitrite-induced modification and inactivation, *J. Biol. Chem.* **273** (1998), 16 694–16 699.
[19] O. Stachowiak, U. Schlattner, M. Dolder and T. Wallimann, Oligomeric state and membrane binding behaviour of creatine kinase isoenzymes: implications for cellular function and mitochondrial structure, *Mol. Cell Biochem.* **184** (1998), 141–151.
[20] K. Steeghs et al., Altered Ca^{2+}-response in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies, *Cell* **89** (1997), 93–103.
[21] M. Stolz and T. Wallimann, Myofibrillar interaction of cytosolic creatine kinase (CK) isoenzymes: allocation of N-terminal binding epitope in MM-CK and BB-CK, *J. Cell Sci.* **111** (1998), 1207–1216.
[22] F. VanDorsten, M. Wyss, T. Wallimann and K. Nicolay, Activation of sea urchin sperm motility is accompanied by an increase in the creatine kinase exchange flux, *Biochem. J.* **325** (1997), 411–416.
[23] J. VanDeursen et al., Creatine kinase in skeletal muscle energy metabolism: a study of mouse mutants with graded reduction in muscle CK expression, *Proc. Natl. Acad. Sci. USA* **91** (1994), 9091–9095.
[24] T. Wallimann, ^{31}P-NMR-measured creatine kinase reaction flux in muscle: a CAVEAT!, *J. Muscle Res. Cell Motil.* **17** (1994), 177–181.
[25] T. Wallimann, Dissecting the role of creatine kinase, *Curr. Biol.* **1** (1994), 42–46.
[26] T. Wallimann, M. Wyss, D. Brdiczka, K. Nicolay and H.M. Eppenberger, Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the "PCr-circuit" for cellular energy homeostasis, *Biochem. J.* **281** (1992), 21–40.
[27] J.P. Williams and J.P. Headrick, Differences in nucleotide compartmentation and energy state in isolated and in sit rat heart: assessment by ^{31}P-NMR spectroscopy, *Biochim. Biophys. Acta* **1276** (1996), 71–79.
[28] R.W. Wiseman and M. Kushmerick, Creatine kinase equilibrium follows solution thermodynamics in skeletal muscle: ^{31}P-NMR studies using creatine analogs, *J. Biol. Chem.* **270** (1995), 12 428–12 438.
[29] M. Wyss, J. Smeitink, R. Wevers and T. Wallimann, Mitochondrial creatine kinase: a key enzyme of aerobic energy metabolism, *Biochim. Biophys. Acta* **1102** (1992), 119–166.