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SUBCELLULAR COMPARTMENTATION OF CREATINE KINASE ISOENZYMES, REGULATION OF CK AND OCTAMERIC STRUCTURE OF MITOCHONDRIAL CK: IMPORTANT ASPECTS OF THE **PHOSPHORYL-CREATINE CIRCUIT**

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INTRODUCTION:

In mammals and birds three types of tissue-specific cytosolic creatine kinase (CK) isoenzymes can be distinguished: ubiquitous BB-CK, expressed at high levels in brain and smooth muscle, muscle-specific MM-CK present in striated muscle, and, during muscle differentiation, a transitorily expressed MB-species which can persist in adult heart muscle (see Eppenberger et al. 1983). A fourth CK isoenzyme, mitochondrial or Mi-CK, is specifically associated with the inner mitochondrial membrane (Jacobs et al.1964, Scholte et al. 1973) and seems to be functionally coupled to the ATP/ADP-translocator (ANT) (see Jacobus 1985a), the most abundant mitochondrial transmembrane protein that is responsible for adenine nucleotide transport through the inner mitochondrial membrane (Klingenberg 1979,1985). In differentiated tissues and cells, e.g. brain, muscle, photoreceptor cells and spermatozoa, Mi-CK is usually co-expressed with either one of the cytosolic CK isoenzymes. Whereas Mi-CK is strictly compartmentalized in mitochondria, the cytosolic CK isoenzymes, BB-, MB- and MM-CK, have been considered in the past as "soluble" enzymes distributed evenly over the entire cytoplasm. However, experiments with isolated subcellular organelles and "in situ" immunolocalization studies have provided ample evidence that significant amounts of cytosolic CK isoforms are also associated with specific subcellular sites. For example, in muscle a relatively small, but physiologically significant portion (at least 3-7%) of the total MM-CK is specifically

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associated with the myofibrillar M-line (Turner et al.1973; Wallimann et al.1977a) where it forms a particular structural element (m-bridges) of this myofibrillar structure located between the two actin-myosin overlap zones of the A-band (Wallimann et al.1978, 1983). The M-line-bound CK was shown to be sufficient for intramyofibrillar regeneration of ATP hydrolysed by the actin-activated Mg^{2+}-ATPase during in vitro contraction of myofibrils (Wallimann et al.1984). The binding of CK to the M-band region is isoenzyme-specific, that is, only the structurally symmetrical homodimers of M-CK, but neither BB- nor MB-CK, although transiently present during myogenesis, are capable to integrate themselves as structural enzymes into the complex M-band architecture (Wallimann et al.1983). In chicken cardiac cells, which do not express M-CK and are devoid of an electron-dense M-band (Wallimann et al. 1977b), a skeletal muscle-like M-band architecture was generated by microinjection of "in vitro" synthesized M-CK mRNA (Schäfer and Perriard 1988), confirming that this isoprotein sorting is M-CK specific. In addition, using chimeric M-CK/B-CK mRNA it was shown that the C-terminal half of M-CK was involved in the interaction of the enzyme with the M-band (Schäfer and Perriard 1988).

CK has also been found in association with sarcolemmal membranes (SL) where it seems to be functionally coupled to the Na^+/K^+-ATPase (Sharov et al.1977). It was shown that the CK associated with the sarcoplasmic reticulum (SR) (Levitzky et al.1977) is bound specifically and tightly to purified SR vesicles where it is indeed capable to support a significant fraction of Ca^{2+}-pumping via PCr and ADP (Rossi et al. 1989). These results further support the notion that a part of the "cytosolic" CK-isoenzymes are also compartmentalized. This clearly favours earlier models on CK function (Wallimann et al.1975,1977,1985; Saks et al.1978; Bessman and Geiger 1981) suggesting that communication and transfer of "energy-rich" compounds between ATP-generating- (mitochondria and glycolysis) and ATP-utilizing sites (myofibrillar actin-activated Mg^{2+}-ATPase, Na^+/K^+-ATPase and Ca^{2+}-ATPase etc.) are facilitated via the PCr/CK system (Meyer et al. 1984, Wallimann and Eppenberger 1985). Here, (i) the in situ compartmentation of CK isozymes in frozen sections of muscle is described, (ii) evidence for the existence of octameric Mi-CK with a structure resembling a channeling protein is provided and (iii), together with the finding that cytosolic CK is subject to enzymatic regulation, a modified and extended view of the PC-shuttle, the **PCr-circuit model**, is proposed.

RESULTS:

In Situ Compartmentation of CK Isoenzymes in Muscle

Fresh chicken pectoralis muscle was prefixed by 2% paraformaldehyde and 0.2% glutaraldehyde for 2hrs on ice in relaxing solution and 0.2-0.5um cryosections, made according to Tokuyasu (1980), were stained with anti-Mi-CK and anti-M-CK antibodies. Indirect immunofluorescence staining revealed that Mi-CK is restricted to mitochondria. The fluorescence is seen clearly in areas along the myofibrils where mitochondria are clustered (Fig.1d). This was confirmed recently by immuno-EM methods (Schlegel et al.1988a) showing in addition that Mi-CK is specifically localized along the inner mitochondrial membrane and, most interestingly, seems to be clustered at the contact sites between inner and outer mitochondrial membranes. On the other hand, in situ staining of muscle by anti-M-CK revealed a regular striation pattern with strong fluorescence not only at the M-band (Fig.1b,M), where the enzyme has been localized earlier in myofibrils (Wallimann et al. 1977a), but also at the I-band, often sparing the Z-line (Fig.1b,I). Permeabilization of muscle fibers by glycerol or saponin followed by washing at physiological ionic strength prior to fixation and cryosectioning resulted in a complete loss of I-band fluorescence while a strong M-band signal remained (not shown), indicating that the M-line CK is rather firmly bound to the M-band whereas most of the sarcoplasmic CK is unevenly distributed along the myofibrils and concentrated at the I-band where it is only loosely interacting with the thin filament lattice region. Surprisingly, CK is almost completely absent from the actin-myosin overlap regions (Fig.1b). The uneven distribution of CK cannot be explained by differences in the relative void volumes between the I-band region and the actin-myosin overlap zones, for the difference in available empty space between the respective filament lattice systems differs only by a factor of 2-3, whereas the difference in the relative fluorescence intensities at the I-band versus the overlap zones differs much more. Also, there was no problem with penetration of antibodies into the overlap zone, for the latter was heavily stained in the same sections by anti-C-protein antibodies (not shown). Interestingly, staining of similar cryosections for aldolase and adenylate kinase, both considered as strictly cytosolic enzymes, also revealed strong signals at the I-band (as shown earlier also by Arnold and Pette 1970 with histochemical staining) and somewhat broader staining, com-

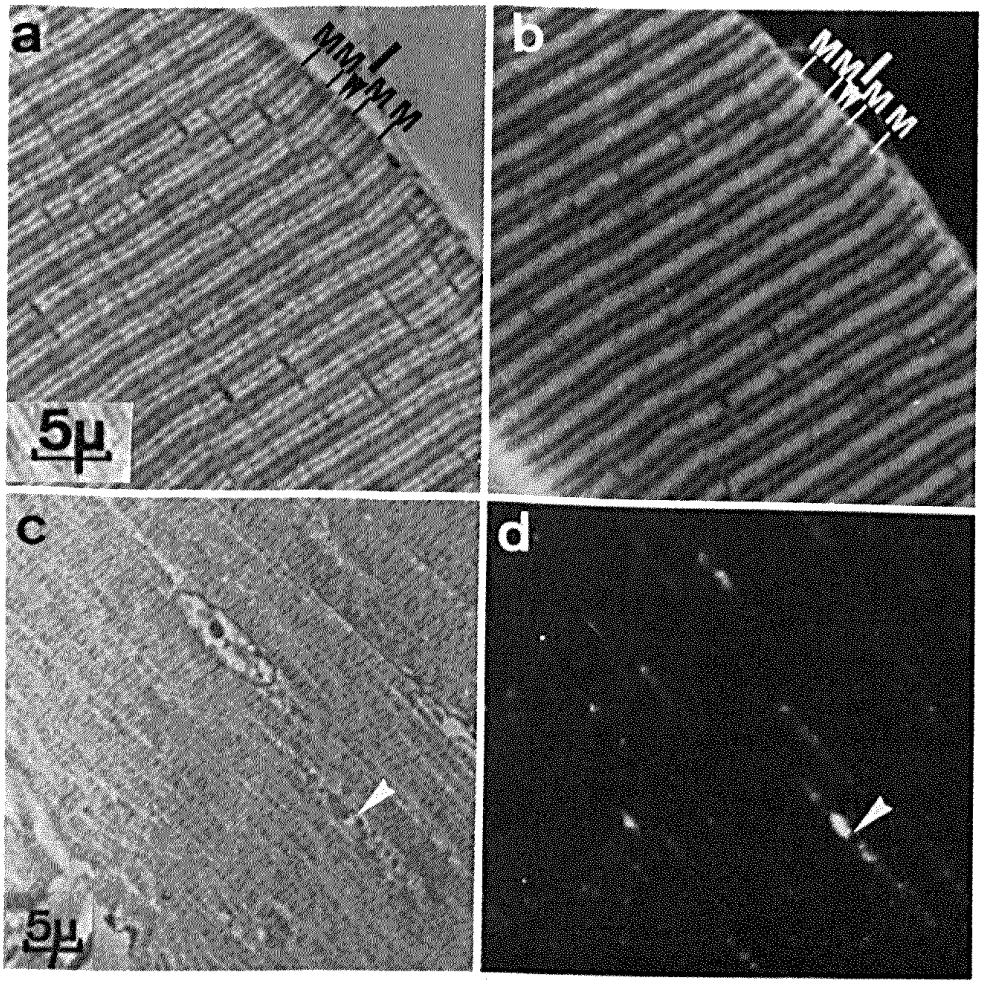


Figure 1: Compartmented localization of CK isoenzymes in muscle
Indirect immunofluorescence localization of M-CK (a,b) and Mi-CK (c,d) on 0.5 μm cryosections (Tokuyasu 1980) of chicken pectoralis muscle using subunit-specific anti-M and anti-Mi-CK antibodies followed by FITC-conjugated second antibody. The corresponding phase contrast pictures are shown in (a) and (c), respectively. Panel b: broad fluorescence staining by anti-M-CK antibody of the I-band (I, with double arrowheads) often sparing the Z-line, and sharp staining signal at the M-band (M). Panel d: patchy staining of clusters of mitochondria (arrowhead). If muscle fibers were permeabilized by glycerol or detergents and washed under physiological conditions prior to chemical fixation and freezing, the I-band CK was completely washed out, but the fluorescence signal at the M-line (Wallimann et al.1983) remained unaltered indicating that M-line CK is bound strongly to this myofibrillar structure whereas the I-band CK is mostly soluble or only loosely interacting with myofibrillar structures of the thin-filament lattice region.

pared to that with anti-M-CK antibodies, at the H-zone. Again, fluorescence staining was absent from the actin-myosin overlap zone (not shown). However, unlike M-CK, both of the latter enzymes were readily washed out from both the I-bands and the H-zones of unfixed muscle. Our interpretation of these results is that the actin-myosin overlap zone is not readily accessible to sarcoplasmic proteins which seem to be excluded from this region possibly by molecular sieving and electrostatic repulsion. Indeed, Donnan potential measurements have shown that the electric charges are quite different in the A-band compared to the I-band region (Bartels and Elliott 1985).

Furthermore, soluble CK is co-localized together with glycolytic enzymes at the I-band where it is thought to be coupled to glycolysis (see Wallimann and Eppenberger 1985) by forming loosely interacting, but functionally coupled multienzyme complexes in this sarcomeric region as shown recently (Maughan and Lord 1988; and Dr. Maughan this volume). Considering that there is no CK in the actin-myosin overlap zone where the actual ATP-hydrolysing events of muscle contraction take place, the localization of the enzyme between two of these zones, at the M-band, makes physiological sense, for cytoplasmic steaming due to cross-bridge cycling and piston-like interdigitation of the two myofilament lattices during contraction would tend to push the products of ATP-hydrolysis from both sides towards the M-band as postulated by Wallimann and Eppenberger (1985) and actually demonstrated experimentally by Dr. Winegard (this volume). Thus the M-line-bound CK shown to be an efficient intramyofibrillar in situ ATP-regenerator (Wallimann et al.1984) seems to be ideally positioned in this respect.

Mitochondrial Mi-CK Forms Octameric Channeling Molecules

From recent publications (Schlegel et al.1988a,b; and Schnyder et al.1988) describing a new procedure for the isolation of Mi-CK and the subsequent biochemical and biophysical characterization of the enzyme it has become clear (i) that Mi-CK can form highly ordered octameric structures with a M_r of 328-364'000 (Table 1) and with a central cavity or channel (Fig. 2), (ii) that in vitro octameric Mi-CK is in a dynamic equilibrium with dimeric Mi-CK which is affected by protein concentration, ionic strength, pH, and nucleotides, indicating that these parameters may also be regulatory factors in vivo, (iii) that under certain conditions Mi-CK octamers rebind preferentially and with higher affinity than Mi-CK

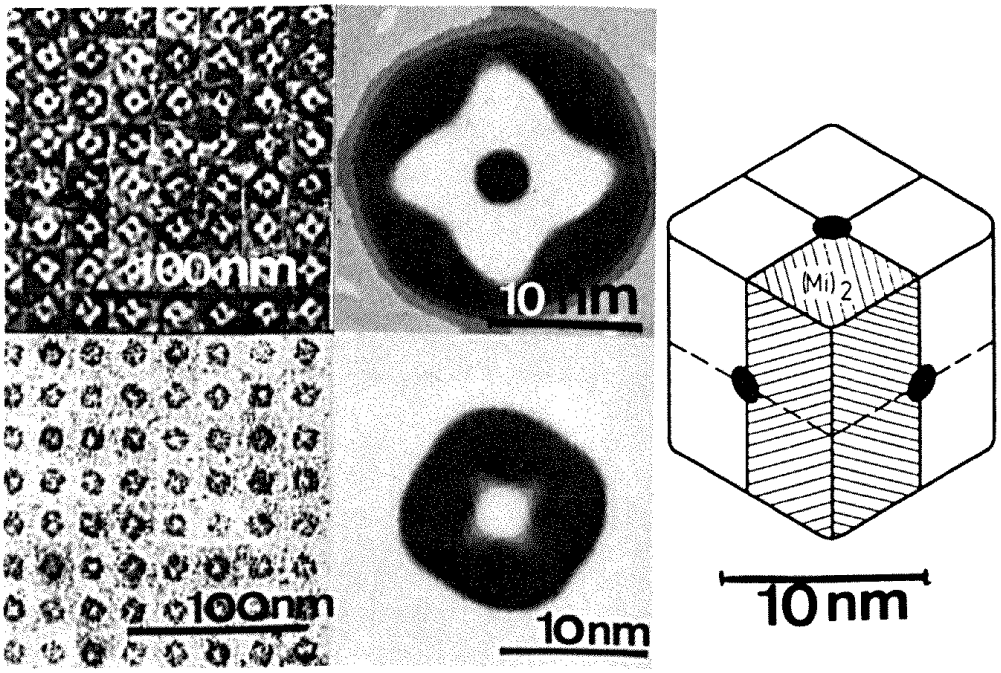


Figure 2: Mitochondrial CK forms octameric molecules
Selected, aligned Mi-CK octamers after negative staining by uranyl acetate (upper left, protein mass is white) and rotary shadowing by heavy metal (lower left, protein mass is dark) and the corresponding averaged structures obtained by image processing. Note the four-fold symmetry of the molecules and the negative stain-filled central cavity as well as the indentation in the heavy metal relief of the molecules (lower middle). An idealized model drawing of the Mi-CK octamer stressing the arrangement of four Mi-CK dimers arranged around a central cavity to form the cube-like molecule which is reminiscent of a channeling molecule is shown on the right. This structure-function relationship, a very intriguing feature of the Mi-CK molecule, is most likely to be of physiological importance as will be discussed later.

Table 1: Biophysical characterization of purified Mi-CK (for details see Schnyder et al. 1988)

measured by:	M_r of Mi-CK octamer	M_r of Mi-CK dimer
gel permeation chromatography.....	364'000 ± 30'000	86'000 ± 8'000
analytical ultra-centrifugation.....	328'000 ± 25'000	83'000 ± 8'000
mass measurement by STEM	340'000 ± 55'000	89'000 ± 27'000

dimers to the inner mitochondrial membrane, and (iv) that Mi-CK is not evenly distributed over the inner mitochondrial membrane, but seems to be concentrated at contact sites between inner and outer mitochondrial membranes (Kottke et al. 1988; Adams et al.1989) where it is thought to form functionally coupled multienzyme energy channeling complexes together with ATP/ADP translocators and voltage-gated ion-selective pores of the outer membrane as suggested by the model in Fig.5. The existence of octameric Mi-CK within mitochondria has recently been confirmed by radiation inactivation experiments (Quemeneur et al.1988), and a report on the redistribution of Mi-CK towards the periphery of the mitochondrion as a function of stimulation of cardiac muscle has also been published very recently (Biermans et al.1988).

Cytosolic CK is Subject to Regulation

Cytosolic CK isoenzymes from chicken separated on 2D-gels display a heterogeneous pattern (shown for chicken B-CK in Fig. 4a) consisting of the two major B-CK subunits, acidic B_a-CK and basic B_b-CK (Rosenberg et al.1981), plus one or more rather prominent additional spots (Fig. 4a-d, indicated by stars). The actual appearance of these spots depends on the animal species (Fig.4). It has been recently shown that the formation of dimeric BB-CK from the two B-CK subunit species mentioned above is tissue-specific (Quest et al.1989) and that fractions of chicken BB-CK enriched in the main satellite species (Fig.4a, B_{b1}) display altered enzyme kinetics which is likely due to post-translational modification by phosphorylation (Quest et al. 1988;1989b; and Soldati et al.1989). Similar single or multiple satellite spots have been noted in the past with CK's from other species, like Torpedo, rat and cow (see Fig.4) that all can be explained by phosphorylation (Saito and Changeux 1980; Mahadevan et al. 1984; Barrantes et al.1985; Quest 1988). Thus the possibility that cytosolic CK itself may be regulated by post-translational modification opens up a multidude of regulatory mechanisms by which local intracellular ATP-levels may be fine-tuned precisely. This would require only relatively small amounts of cytosolic CK associated at those intracellular sites where ATP-dependent molecules, e.g. ATP-gated K^+-channels are located (Weiss and Lamp 1987). The top portion of the PCr-circuit model in Fig.5 tries to depict the cytosolic part of the complex regulatory network possibly coupled to cellular signaling pathways, whereas the bottom part of the model shows the regulatory possibilities involving Mi-CK on the producing side of the PCr-circuit.

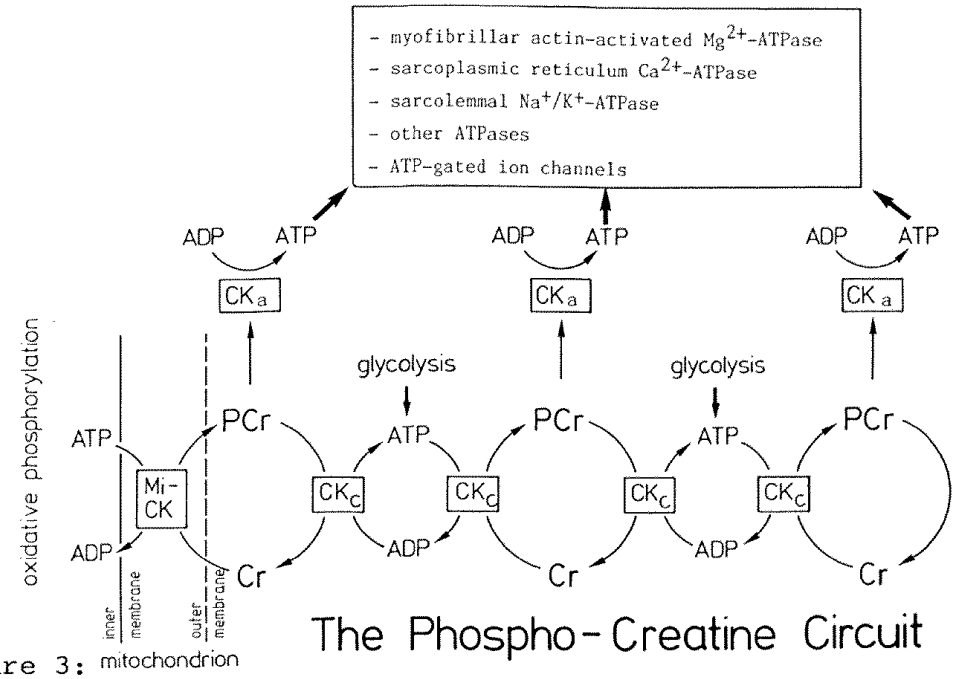
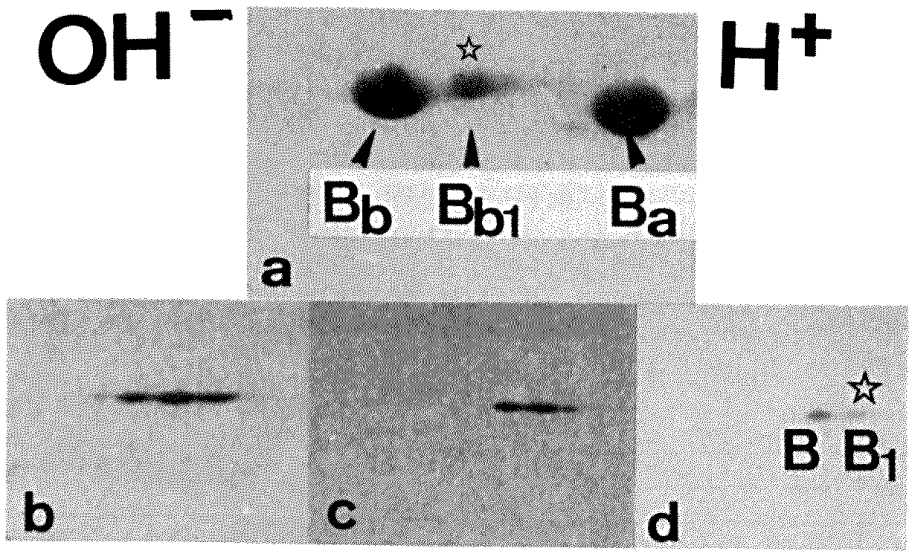


Figure 3: A modified version of the CP-shuttle model by Wallimann and Eppenberger (1985) incorporates the facts of subcellular compartmentation of mitochondrial CK (Mi-CK) and cytosolic CK (CK_c) as well as the subcellular association of some fractions of CK (CK_a) at strategically important sites. There, functionally and/or structurally coupled microcompartments are formed involving CK at the producing side of the PCr-circuit, e.g. Mi-CK coupled to ATP/ADP-translocators at the contact sites between inner and outer mitochondrial membranes (Fig.5), and at the consuming side of the PCr-circuit, e.g. CK_a at the sarcoplasmic reticulum coupled to the Ca^{2+}-ATPase, CK_a at the myofibrillar M-line coupled to the acto-myosin ATPase, CK_a at the sarcolemma coupled to the Na^+/K^+-ATPase and possibly to ATP-gated ion-channels. The model suggests that PCr produced by oxidative phosphorylation via mitochondrial matrix-generated ATP and via Mi-CK enters the cytosolic PCr/Cr - ATP/ADP equilibrium governed by the large amount of cytosolic CK_c which in muscle is localized together with glycolytic enzymes at the I-band (Fig.1) where it is thought to be functionally coupled to the glycolysis, the second producing side of the PCr-circuit. This model visualizes the aspects of facilitated diffusion (Meyer et al.1984), but in addition incorporates the aspects of the compartmented subcellular localization of CK isoforms. The PCr-circuit serves i) as an energy buffer, ii) as an energy transport and channeling system and iii) as a regulator of local ATP levels.



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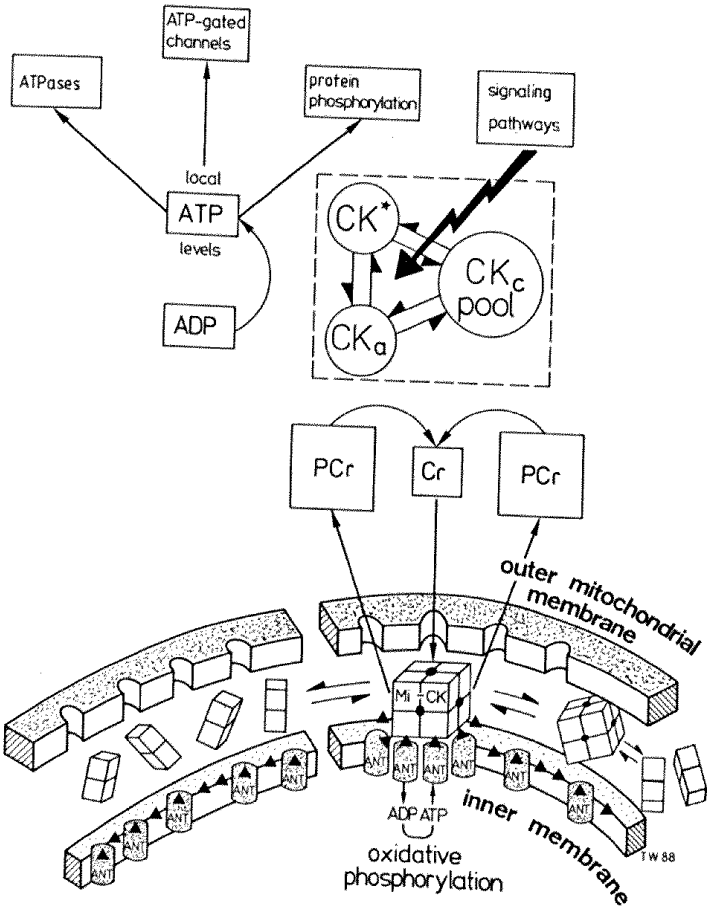

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(Legends for Fig. 4 and Fig. 5 appear on page 168.)

Figure 4: Heterogeneity of B-CK resolved by two-dimensional gel electrophoresis: (a) In chicken, B_a and B_b are the two major acidic and basic B-CK subunit species (Rosenberg et al. 1981) with B_b showing multiple satellite spots (B_{b1}) most likely due to phosphorylation of B_b (Quest et al.1989; Soldati et al. 1989). Similar satellite spots are found in soluble (b) as well as membrane-bound CK (c) of Torpedo electrocytes (Barrantes et al.1985) which may be explained by phosphorylation (Saitoh and Changeux 1980). Also in CK from mammals, e.g. in rat or bovine CK (d) (Mahadevan et al.1984), a single satellite next to the main CK spot indicated by a star is seen regularly.

Figure 5: The PCr-circuit as a complex regulatory network controlled at several levels for directing intracellular energy flux and for controlling local subcellular ATP levels
In activated mitochondria, Mi-CK octamers, concentrated at the contact sites between inner and outer mitochondrial membranes (Adams et al.1989), form functionally coupled microcompartments with ATP/ADP-translocators(ANT), possibly via cardiolipin (▲) linkages (Müller et al.1985) and with voltage-gated ion-selective pores of the mitochondrial outer membrane (Kottke et al.1988) to constitute an efficient trans-membrane energy channeling complex (Schnyder et al.1988). Dimerization or dissociation of Mi-CK octamers from the inner membrane (affected by pH, P_i and nucleotides) as well as the preferential reattachment of Mi-CK octamers to this membrane can all be envisaged as regulatory parameters on the mitochondrial side of the PCr-circuit (Schlegel et al.1988). The PCr produced by mitochondria is the net product of oxidative phosphorylation in cells with sudden high energy turnover. After its equilibration with cytosolic PCr/Cr and ATP/ADP pools via cytosolic CK_c which itself is coupled to glycolysis (Fig.3), PCr will be available not only as a simple energy buffer, but also as an inert transport form of energy at those places in a cell where ATP-supply and proper ATP-levels are critical for cell function. There, PCr is utilized by CK_a associated at these locations and forming functionally coupled microcompartments for in situ ATP-regeneration (Wallimann et al.1984; Rossi et al.1989) and for the regulation of local ATP-levels near ATPases and ATP-gated processes, respectively. There is evidence that CK itself is enzymatically regulated, e.g. by phosphorylation (Mahadevan et al.1984; see Fig.4) affecting the kinetic parameters(Quest et al. 1989) and possibly also the subcellular distribution of the enzyme. Therefore, it is very tempting to speculate that a signalling pathway-dependent stimulation of excitable cells is likely to regulate also the receiving end of the PCr-circuit and thus facilitates the regulation of energy supply and of subcellular ATP-levels.

The Phosphoryl-Creatine Circuit Model

Based on our results and the work of many authors in the field we would like to propose the model described in Fig.3 and Fig.5 depicting the energy flux in muscle and its regulation, respectively, emphasizing the central role of Mi-CK in the energy channeling process and the importance of the subcellular compartmentation of cytosolic CK's as in situ regenerators and regulators of local ATP-levels. By implementing three main functions for the creatine kinase system, namely (i) energy buffering, (ii) energy transport and channeling and (iii) regulation of local intracellular ATP-levels, and by extending the concept of the model from a simple "CP-shuttle" (Bessman and Geiger 1981) to a more complex, regulated **PCr-circuit**, a hopefully more realistic picture of the highly sophisticated network may be suggested. With this concept in mind the long- standing debates over "CP-shuttle only" (Bessman and Geiger 1981), a name that has led to much confusion anyway, or over "facilitated diffusion only" (Meyer et al.1984) should have an end here by saying that both aspects, energy buffering and channeling, are important together with the third aspect of regulation of local ATP-levels. Depending on the energy need, an excitable cell may have to put emphasis either on one, two or all three aspects at any one time. Such a PCr-circuit model is supported by immunolocalization studies on CK isoenzymes, by recent structural work on Mi-CK as well as by many physiological and ^{31}P-NMR studies with intact muscle (see legends of Fig.3 and 5, and Discussion).

DISCUSSION

We would like to stress the point that the PCr-circuit is not an absolute prerequisite for muscle function per se, for we have provided evidence ourselves that (i) certain muscle types, although predominantly slow tonic ones which are lacking M-line-bound CK and at the same time are missing an electron dense M-band structure (Wallimann et al. 1977; 1985), or that (ii) hearts of chicken (Wallimann et al.1977b) or of neonatal mammals (Perry et al. 1988) which are lacking M-line-bound CK or both M-line CK plus mitochondrial CK, respectively, seem yet to function perfectly well within their appropriate physiological range. In addition, non-excitable cells and organs with a still relatively high, but continuous energy flux, e.g. liver which contain only small amounts of

CK or none at all, function without a PCr-circuit. However, it is our contention that the addition of the PCr-circuit system on top of the basic energy ground-state of a cell is likely to have a profound physiological impact for cellular energetics and performance that will be most advantageous at high work loads. This idea is supported by the fact that in fully differentiated and highly specialized cells with high sudden energy turnover, e.g. skeletal and cardiac muscle (Wallimann and Eppenberger 1985), brain and retina photoreceptor cells (Wallimann et al.1986a), spermatozoa (Tombes and Shapiro 1985; Wallimann et al.1986b) and Torpedo electrocytes (Wallimann et al.1985) mitochondrial CK is generally found in conjunction with cytosolic CK's with a significant fraction of the latter being associated subcellularly in a compartmented fashion at intracellular sites of high energy turnover. It is also becoming apparent that some of the cytosolic CK is specifically associated with membranes, possibly via membrane anchors, e.g., with the SR-membrane where CK was shown to be functional by supporting a significant portion of the maximal Ca^{2+}-pumping rate (Rossi et al.1988,1989). Similar membrane associations of CK have been shown with the post-synaptic acetylcholin-receptor-rich membrane, the invaginated, and non-innervated face membrane of electrocytes rich in Na^+ / K^+-ATPase as well as with synaptic vesicles (Wallimann et al.1985), with the sperm-tail plasma membrane (Walliman et al. 1986a) or recently also with rod outer segment plasma membranes of bovine photoreceptor cells (Quest et al.1987; Hemmer et al.1989). Thus, for all the above cells the PCr-circuit seems to represent an efficient, flexible and highly responsive accessory crucial not only as an energy back-up system, but also as a regulator of energy flux (channeling) and as a fine-tuning device of local ATP-levels. The strength of such a regulated channeling circuit operating at relatively low adenine nucleotide levels compared to the high total PCr and Cr pools, which are metabolically inert, is its high sensitivity towards ADP (μM) (Wallimann et al. 1984) that is preventing in excitable cells the accumulation of ADP and AMP unless severe stress, like hypoxia or ischaemia is imposed. Additional details concerning the PCr-circuit model in muscle and our current ideas about the structure-function relationships of mitochondrial CK have been described elsewhere (Wallimann and Eppenberger 1985; Schlegel et al.1988; and Schnyder et al.1988).

While it is accepted that in living muscle the CK reaction velocity could be severalfold faster than the rate of ATP-hydrolysis by the acto-myosin ATPase and that of ATP-

synthesis by oxidative phosphorylation, a positive correlation between oxygen consumption of perfused hearts and a concomitant increase of the CK reaction velocity has only been verified recently by ^{31}P-NMR-techniques (Kupriyanov et al.1984) or by direct biochemical measurements (Mahler 1985) and model calculations (Connett 1988). This correlation factor, seems to depend strongly on the relative amount of Mi-CK (Perry et al.1988) which in mammalian cardiac muscle is gradually expressed in increasing amounts only during postnatal development (see Dr. McAuliffe in this volume). Thus, the flux of energy through the CK- and PCr-system increases with progressive differentiation of cardiac muscle where the accumulation of Mi-CK becomes physiologically relevant especially at higher work loads (Perry et al.1988). During chronical stimulation of skeletal muscle (Schmitt and Pette 1986) or training for long distance running (Apple and Rogers 1986) the conversion from fast-twitch to slow-twitch fibers is accompanied by a significant decrease in total CK activity, but at the same time by a severalfold elevation of Mi-CK activity. The absolute amounts of Mi-CK at different times during this metabolic adaptation correlate with the oxidative potential of the corresponding muscles. In our opinion this is a very good example or even an experimental proof for the two main functions of the PCr-circuit, that is, for the energy buffering function more prominent in glycolytic, fast-twitch white-muscles containing high levels of cytosolic CK, and for the energy transport or channeling function more pronounced in oxidative slow-twitch red-muscles containing relatively high levels of Mi-CK. During the observed fiber-type conversion the gradual increase in the relative amounts of Mi-CK is characteristic for shifting emphasis from the energy buffering towards the energy channeling function with Mi-CK assuming an increasingly more important role as an intra-extra-mitochondrial energy transfer system. In addition, the evidence from ^{31}P-NMR studies for compartmentation of the CK/PCr-system and for the existence of distinct ATP-pools (Gudbjarnason et al.1970, Nunally and Hollis 1979, Barbour et al.1984, Zahler et al.1987; for more references see Schlegel et al.1988) some of which are NMR-invisible as shown by Murphy et al. (1988) is certainly in agreement with our results concerning the immunolocalization of compartmentalized CK isoforms in muscle. Therefore, compartmentation can no longer be neglected if ^{31}P-NMR results are to be evaluated. Furthermore, PCr-depletion and O_2-uptake measurements (Mahler 1985) as well as studies measuring the dependence of relaxation and tension development of muscle fibers on PCr-content (Ventura-

Clapier et al.1987) also indicate the physiological importance of the PCr-circuit.

In this context the results concerning the depletion of creatine by the poorly metabolized analogue beta-guanidinopropionic acid (GPA, Shoubridge et al.1985), which accumulates in muscle in preference over creatine if given for prolonged periods of time, do not represent serious arguments against the physiological importance of the PCr-circuit, for several long-term metabolic adaption and compensatory mechanisms have to be considered (Moreland and Kushmerick,1987). It seems that the PCr levels in animals, intoxicated with GPA, are still higher than the K_m range of myofibrillar CK and larger than the PCr concentration differential (uM range, Jacobus 1985b) necessary to support maximal energy flux in the form of PCr from mitochondria accross the sarcoplasm to the myofibrils. There, the M-line-bound CK was shown to sustain contractions also at relatively low PCr concentrations (Wallimann et al.1984). In a very recent ^{31}P-NMR study by Hoerter et al. (1988) a normoxic heart model perfused with 2-deoxy-D-glucose and insulin was used to reduce the PCr-level in the cardiac muscle to as low as 15% of the original value and the ATP-level such that it was no longer measurable by NMR. Surprisingly, however, the heart was still beating with 65% of its original systolic pressure. The decrease in heart function was attributed to the fact that it was mainly the turnover rate of CK in the vicinity of the myofibrils, presumably the M-line-bound CK (Wallimann et al. 1977a) that was affected by the very low PCr-levels (4.5mM) which were comparable to the K_m values of CK for PCr. Thus the turnover rate of the myofibrillar CK became the limiting factor for work output and energy transport in this animal model. From these results, however, one can also conclude that perfusion with deoxy-glucose and insulin is mainly interfering with the energy buffering function, as indicated by the relatively low level of PCr, but not so much with the energy transport or channeling function of the PCr-circuit since the latter was still operational under normoxic conditions, for Mi-CK was able to maintain a reasonably high energy flux to support 65% of normal heart function at very low levels of "high-energy" phosphates. Blockage of oxidative phosphorylation, however, with the typical fall in PCr- and rise in P_i-levels at relatively high ATP-levels which is seen also under anoxia, had a dramatic effect on heart function (Hoerter et al.1988) due to the fact that a major supply and transport function of the PCr-circuit was interrupted and the buffering function on its own was not able to support contraction for longer

periods of time. Thus, once again, the myofibrillar and mitochondrial compartmentation of CK and their in situ function as intramyofibrillar ATP-regenerators and mitochondrial energy channeling transporters, respectively, may be critical parameters for optimal muscle function, especially in fast muscles and muscles under high work load.

To conclude, the best living example so far for the physiological significance of the PCr-circuit are spermatozoa where also two compartmented CK isoforms were identified, Mi-CK being localized in the midpiece and B-CK in the tail portion (Wallimann et al.1986). Dr. Shapiro and his coworkers were able to show that in sea urchin spermatozoa oxidative phosphorylation and flagellar motility were linked together via the CK reaction (Tombes and Shapiro 1985) and that inactivation of the CK-system with increasing, but very low concentrations of dinitro-fluoro-benzene (DNFB) resulted in a progressive loss of sperm tail oscillation starting from the distal end of the sperm tails (Tombes et al.1987). Therefore, the PCr-cicuit hypothesis remains a very attractive idea to be tested by further experimentation.

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