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Review

Phosphotransfer networks and cellular energetics

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Summary

Precise coupling of spatially separated intracellular ATP-producing and ATP-consuming processes is fundamental to the bioenergetics of living organisms, ensuring a fail-safe operation of the energetic system over a broad range of cellular functional activities. Here, we provide an overview of the role of spatially arranged enzymatic networks, catalyzed by creatine kinase, adenylate kinase, carbonic anhydrase and glycolytic enzymes, in efficient high-energy phosphoryl transfer and signal communication in the cell. Studies of transgenic creatine kinase and adenylate kinase deficient mice, along with pharmacological targeting of individual enzymes, have revealed the importance of near-equilibrium reactions in the dissipation of metabolite gradients and communication of energetic signals to distinct intracellular compartments, including the cell nucleus and membrane metabolic sensors. Enzymatic capacities, isoform distribution and the dynamics of net phosphoryl flux through the integrated phosphotransfer systems tightly correlate with cellular functions, indicating a critical role of such networks in efficient energy transfer and distribution, thereby securing the cellular economy and energetic homeostasis under stress.

Key words: energy, metabolism, mitochondria, creatine kinase, adenylate kinase, glycolysis, carbonic anhydrase, homeostasis.

Introduction

Mechanisms responsible for communication between spatially separated intracellular ATP consumption and ATP production processes, and their precise coupling over a broad range of cellular functional activity has remained a long-standing enigma (Bessman and Carpenter, 1985; Hochachka, 1994; Saks et al., 1994; Ames, 2000). Optimal operation of the cellular bioenergetic system requires that energy-rich phosphoryls are produced and delivered to energy-consuming sites at the rate corresponding to the ATPase velocity, and that products of ATP hydrolysis, namely ADP, P_i and H^+, are removed in order to avoid kinetic and thermodynamic hindrances (van Deursen et al., 1993; Saks et al., 1994; Tian et al., 1997; Dzeja et al., 2000). Progress has been made in elucidating the cytoarchitectural, convectional and enzymatic mechanisms that facilitate coupling and coordination of energy transduction processes with the metabolic, mechanical and electrical activity of the cell (Hochachka, 1999; Janssen et al., 2000; Kaasik et al., 2001; Saks et al., 2001; Abraham et al., 2002). Cytoplasmic streaming, positioning of mitochondria and their movement in response to changes in energy utilization, along with formation of enzymatic complexes, have all been shown to contribute towards facilitating intracellular energetic communication (Harold, 1991; Hollenbeck, 1996; Hochachka, 1999; Lange et al., 2002). However, such topological arrangements apparently are insufficient on their own to fulfil all cellular energetic needs (Dzeja et al., 2000; de Groof, 2001). In this regard, a new role of spatially arranged intracellular enzymatic networks, catalyzed by creatine kinase, adenylate kinase, carbonic anhydrase and glycolytic enzymes, in supporting high-energy phosphoryl transfer and signal communication between ATP-generating and ATP-consuming/ATP-sensing processes has emerged (Wallimann et al., 1992; Saks et al., 1994; Dzeja et al., 1998; Dzeja and Terzic, 1998; Joubert et al., 2002). This ‘dynamic’ concept emphasizes that metabolic signaling through near-equilibrium enzymatic networks, along with other homeostatic mechanisms (Balaban, 2002), contributes to efficient intracellular energetic communication in maintaining the balance between cellular ATP consumption and production (van Deursen et al., 1993; Saks et al., 1994; Dzeja et al., 2000; Neumann et al., 2003).

Intracellular energy transfer

Following the discovery of adenine nucleotides, and their role in cellular energetics as a key link between spatially separated energy transducing processes, the energy transfer concept through the ‘adenylate wire’ was proposed (Lipmann,

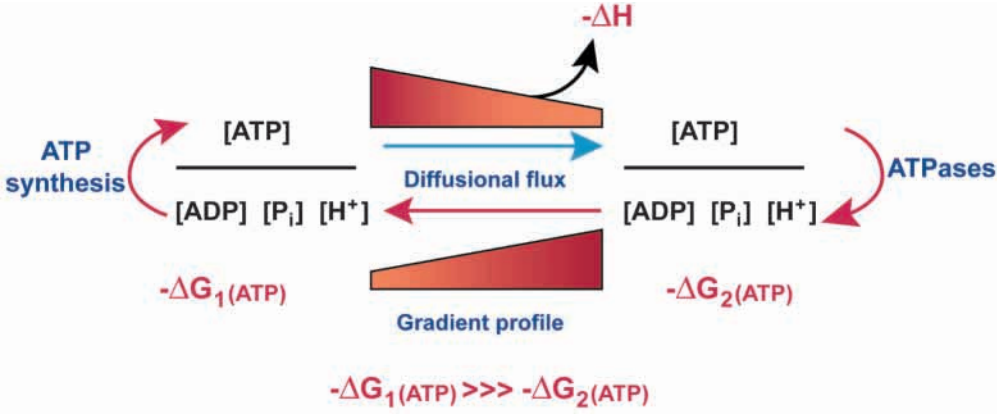

Fig. 1. Adenylate energetic cycle. Diffusional exchange of nucleotides between ATPases and ATPsynthases requires establishment of concentration gradients, which would compromise the kinetic and thermodynamic efficiency of energy transfer. –ΔH, change in enthalpy of ATP hydrolysis; $\Delta G_{1(ATP)}$ and $\Delta G_{2(ATP)}$, free energy of ATP hydrolysis at ATP synthesis and ATP consumption sites, respectively.

1941). Fritz Lipman, the author of this concept, was among the first to notice the analogy between the energy-carrying adenine nucleotide system and the electrical circuit. Indeed, basic principles of energy transfer, in terms of the rate and efficiency, apply equally to both industrial and metabolic networks (Peusner, 1974; Jeong et al., 2000).

The localization of mitochondria in close proximity to cellular energy-utilizing processes, and their movement in response to activation of ATP-utilizing reactions (Hollenbeck, 1996), suggest that the distance of energy transfer is critical for adequate energy supply. However, energy transfer by diffusional exchange of adenine nucleotides is kinetically and thermodynamically inefficient since it requires a significant concentration gradient (Meyer et al., 1984; Jacobus, 1985), and would result in ATPase inhibition by end products (P_i, ADP, H^+), inability to sustain the high free energy of ATP hydrolysis (ΔG_{ATP}) at sites of ATP utilization (Fig. 1), and ultimately energy dissipation (–ΔH) during transmission (Kammermeier, 1997; Dzeja et al., 2000). The difference between $\Delta G_{1(ATP)}$ and $\Delta G_{2(ATP)}$, signifying energy loss (–ΔH), would increase at higher rates of ATP turnover, and the drop of $\Delta G_{2(ATP)}$ below a threshold would impair cellular functions (Kammermeier, 1997; Taegtmeyer, 2000).

Part of intracellular energy transfer proceeds in the narrow mitochondrial inner membrane infoldings, known as cristae (Fig. 2). The cristae arrangement increases, by several folds, the capacity of mitochondrial ATP production without occupying additional intracellular space. However, it creates difficulties in ATP export from the mitochondrial intracristal space, as diffusional flux requires a significant concentration gradient. Accordingly, ATP accumulation in the mitochondrial intracristal space would inhibit export of ATP from the mitochondrial matrix by locking the adenine nucleotide translocator (Mannella et al., 2001). In principle, this limitation can be overcome by either placing in the intracristal space near-equilibrium phosphotransfer systems, capable of accelerating ATP export/ADP import, and/or by establishing high-throughput contact sites between inner and outer membranes, thereby providing direct access to ATP in the mitochondrial matrix (Fig. 2). Available data suggest that in mitochondrial physiology both possibilities are employed, and their functional significance may vary depending on the physiological conditions or functional load (Gerbitz et al., 1996; Ziegelhoffer, 2002). This view is supported by the observation that the presence of creatine kinase, adenylate kinase and nucleoside diphosphate kinase in the intermembrane space facilitates ATP/ADP exchange between mitochondria and cytosol (Saks et al., 1994; Laterveer et al., 1997; Roberts et al., 1997; Dzeja et al., 1999b). Conversely, disruption of the adenylate kinase gene impedes ATP export from mitochondria (Bandlow et al., 1988). Taken together, this would indicate that in the absence of facilitating mechanisms, cell architecture and diffusional hindrances would obstruct free movement of molecules, impeding efficient intracellular communication.

Near-equilibrium enzymatic flux transfer networks

In searching how cells overcome diffusional limitations for substrate movement in the highly structured intracellular milieu, Nagle (1970) and Goldbeter and Nicolis (1976) suggested that displacement of equilibrium in creatine kinase or glycolytic reactions in one cellular locale could be rapidly transmitted through a near-equilibrium network in the form of a sharp concentration wavefront over macroscopic distances. This view was supported by Reich and Sel'kov (1981) introducing the concept of flux transfer chains along which an incoming flux wave could be instantaneously transmitted in either direction. The principle of vectorial ligand conduction, as a basic mechanism for operation of metabolic and transport processes within the cell, was developed by Peter Mitchell (Mitchell, 1979). Taken together, these principles were applied to the chains of sequential rapid equilibrating reactions catalyzed by creatine kinase and adenylate kinase ('phosphoryl wires') as a mechanism for facilitated high-energy phosphoryl transfer between ATP-consuming and ATP-generating sites in the cell (Zeleznikar et al., 1995; Dzeja et al., 1998). In these chains, a series of rapidly equilibrating reactions provide the driving force for high-energy phosphoryl flux (Wallimann et al., 1992; Saks et al., 1994; Dzeja et al., 1998). According to this mechanism, incoming ligands at one end of the system 'push' adjacent ligands, triggering a propagation of a 'flux

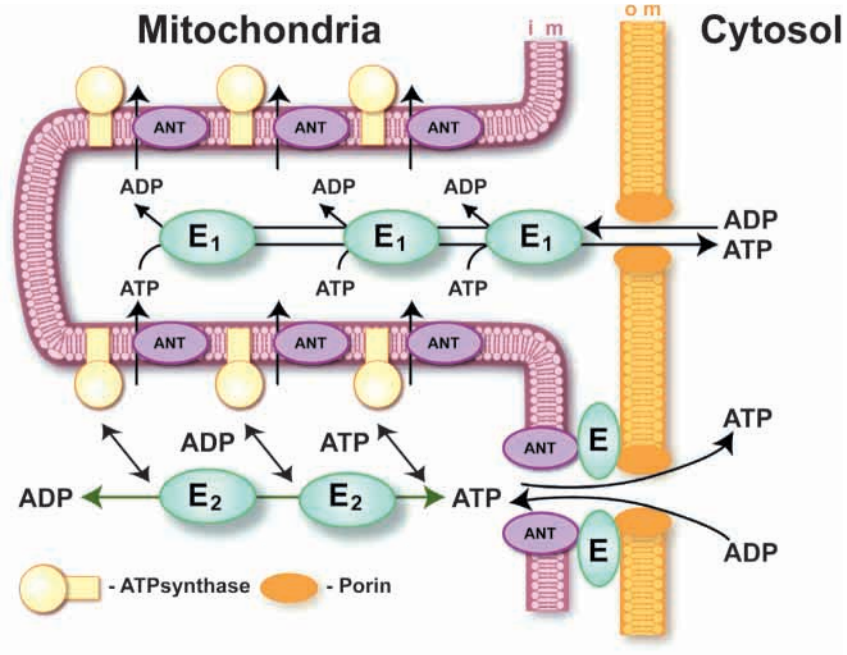

Fig. 2. Energy export from mitochondria matrix and intracristal space. Restricted diffusion of nucleotides from the narrow intracristal space to the external compartment could be overcome by an enzymatic system and/or by high-throughput contact sites traversing the inner (im) and outer (om) membranes. In some circumstances, contact sites could provide direct access to ATP in the matrix compartment, while intracristal space could be used for proton confinement leading to efficient ATP synthesis. E, E_1, E_2, near-equilibrium phosphotransfer enzymes; ANT, adenine nucleotide translocator.

wave' through a network of enzymes catalyzing rapid equilibrium among substrates. Thereby, ligands do not move the entire length of the pathway, as molecules arriving at the distal sites of this sequence represent the equivalent rather than the specific molecule generated at the origination site. This phenomenon, which was recently refined using modern computer simulations (Tuckerman et al., 2002), is referred to as 'walking without moving'. It is known that flux wave propagation along rapid equilibrating chemical and biological reactions can proceed much faster than diffusion of reactants (Goldbeter and Nicolis, 1976; Mair and Muller, 1996). The rate of wave front movement in these systems is equal to the square root of the reaction velocity constant and diffusion coefficient (Mair and Muller, 1996). These calculations provide an important indication as to why the total cellular activity of enzymes catalyzing near-equilibrium reactions surpasses apparent physiological needs. Moreover, the enzymatic ligand conduction system is capable of operating with minimal or no concentration gradients, underscoring its thermodynamic efficiency (Dzeja et al., 1998). This could explain why changes in cellular adenine nucleotide concentrations are most often not observed even with marked increases in metabolic flux (Zeleznikar et al., 1995; Balaban, 2002).

Produced by the ATPase reactions, ADP apparently cannot diffuse freely and serve as a feedback signal to ATP-regenerating processes, as abundant and catalytically active creatine kinase, adenylate kinase and glycolytic enzymes residing throughout a cell would process a large portion of the ADP produced by ATPase reactions (Saks et al., 1994; Dzeja et al., 2000). The high rate of unidirectional phosphoryl exchange in these phosphotransfer systems would promote metabolic flux wave propagation and ligand conduction at cellular distances.

Creatine kinase phosphotransfer system: a conduit for high-energy phosphoryls

Creatine kinase is a major phosphotransfer system in cells with high-energy demand, and it acts in concert with other enzymatic systems to facilitate intracellular energetic communication (Bessman and Carpenter, 1985; Jacobus, 1985; Saks et al., 1994; Joubert et al., 2002; Neumann et al., 2003; Dzeja et al., 2003). The metabolic significance of the creatine kinase-catalyzed reaction depends on total enzyme catalytic capacity, intracellular localization of creatine kinase isoforms, and the ability of creatine kinase to propagate displacement of the local ATP/ADP equilibrium to other cellular sites to maintain energetic homeostasis (Ingwall, 1991; Wallimann et al., 1992; Saks et al., 1994; Zeleznikar et al., 1995; Dzeja et al., 2003). ^{31}P-NMR saturation transfer (Kingsley-Hickman et al., 1987; Ingwall, 1991; Neeman et al., 1987) and ^{18}O-phosphoryl labeling data (Zeleznikar et al., 1995; Dzeja et al., 1998) indicate that rapidly equilibrating enzymatic systems can operate by the ligand conduction mechanism, providing a conduit for high-energy phosphoryls (Saks et al., 1994; Dzeja et al., 1998). This would increase the rate and efficiency of phosphotransfer in the highly structured intracellular environment (Carrasco et al., 2001; Abraham et al., 2002; Dzeja et al., 2002).

Understanding of creatine kinase function was limited when the cell was considered as a homogenous system where enzymes are in equilibrium, and metabolites have uniform distributions and concentrations (Meyer et al., 1984; Kushmerick, 1995). Recently, a new experimental approach that allows quantification of unidirectional fluxes of creatine kinase localized in different subcellular compartments provided strong evidence for the involvement of creatine kinase in intracellular energy transfer (Joubert et al., 2002). Moreover,

transgenic animal studies demonstrate that creatine kinase deficiency compromises energy delivery for muscle contraction and intracellular calcium handling, as well as signal communication to membrane metabolic sensors such as the K_{ATP} channel (van Deursen et al., 1993; Steeghs et al., 1997; Saupe et al., 1998; Kaasik et al., 2001; Abraham et al., 2002).

In creatine kinase-deficient muscles, phosphotransfers catalyzed by adenylate kinase as well as by glycolytic enzymes provide the major route for intracellular high energy phosphoryl transfer (Dzeja et al., 1998, 2003; de Groof et al., 2001). Such alternative high-energy phosphoryl routes may rescue cellular bioenergetics in cells with compromised creatine kinase (CK)-catalyzed phosphotransfer (Boehm et al., 2000; Dzeja et al., 2000). In this regard, observations following deletion of brain B-CK indicate that this isoform is fundamental to processes that involve habituation, spatial learning and seizure susceptibility (Jost et al., 2002). Mitochondrial isoforms ScCKmit and UbMi-CK are critically necessary to maintain normal high-energy phosphate metabolite levels in heart and brain during stress (Kekelidze et al., 2001; Spindler et al., 2002). In addition, reduction in cellular B-CK activity by dominant negative gene expression abrogates thrombin-mediated, energy-dependent signal transduction during cytoskeletal reorganization (Mahajan et al., 2000). These findings emphasize the importance of creatine kinase in providing energetic efficiency in support of various cellular functions.

Adenylate kinase phosphotransfer system: managing β- and γ-ATP phosphoryls and cellular energetics economy

Adenylate kinase-catalyzed reversible phosphotransfer between ADP, ATP and AMP molecules has been implicated in processing metabolic signals associated with cellular energy utilization (Noda, 1973; Bessman and Carpenter, 1985; Dzeja et al., 1998). To date, five isoforms of adenylate kinase have been identified (Van Rompay et al., 1999). Adenylate kinase isoforms have been found in mitochondria and cytosol, and also membrane-bound (Tanabe et al., 1993; Carrasco et al., 2001). Recently, the existence of another *AK1* gene product, p53-inducible membrane-bound AK1β, has been reported and implicated in p53-dependent cell-cycle arrest (Collavin et al., 1999). Inside myofibrils, adenylate kinase molecules are clustered into linear arrays (Wegmann et al., 1992) and can form polymeric rods in other cell types (Wild et al., 1997). Coordinated action of mitochondrial and cytosolic isoforms of adenylate kinase (AK_2 and AK_1, respectively) are thought to provide a mechanism for transfer of two high-energy phosphoryls (i.e. β and γ) in one molecule of ATP from its generation to utilization sites (Zeleznikar et al., 1995; Dzeja et al., 1998). This exclusive property of adenylate kinase catalysis doubles the energetic potential of the ATP molecule and cuts by half the cytosolic diffusional resistance (Dzeja et al., 1999b).

Recent evidence indicates that the adenylate kinase-catalyzed relay indeed facilitates intracellular energetic communication, and that loss of adenylate kinase function can be complemented by activation of creatine kinase phosphotransfer (Carrasco et al., 2001; Dzeja et al., 2002). Moreover, interaction between adenylate kinase and creatine kinase phosphorelays determines metabolic signal transmission to the prototypic membrane metabolic sensor, the K_{ATP} channel (Dzeja and Terzic, 1998; Carrasco et al., 2001; Abraham et al., 2002), and mediates energetic remodeling in preconditioned (Pucar et al., 2001) and failing hearts (Dzeja et al., 1999b, 2000). AK1 knockout muscles display lower energetic efficiency and increased vulnerability to metabolic stress, associated with a compromised ability to maintain nucleotide pools and intracellular metabolic signal communication (Janssen et al., 2000; Pucar et al., 2002). Also, muscle exercise performance correlates with adenylate kinase activity, suggesting that this enzyme is an integral part of cellular energetic homeostasis (Linossier et al., 1996).

Glycolytic phosphotransfer system: delivering mitochondrial high-energy phosphoryls in exchange for P_i, NADH and ADP

That glycolytic enzymes contribute to intracellular high-energy phosphoryl transfer and spatial distribution is being increasingly recognized (Dzeja et al., 1998, 2003; de Groof et al., 2001). Energy-rich phosphoryls from ATP, used to phosphorylate glucose and fructose-6-phosphate at the mitochondrial site, traverse the glycolytic pathway and can phosphorylate ADP through the pyruvate kinase-catalyzed reaction at remote ATP utilization sites (Fig. 3). Additional phosphoryls can be transferred through the near-equilibrium reaction system catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase (GAPDH/PGK) (Dzeja et al., 2003). The significance of this pathway of energy transfer is underscored by the finding that the unidirectional phosphoryl exchange rate through the GAPDH/PGK glycolytic enzyme couple approaches that of mitochondrial oxidative phosphorylation and creatine kinase (Kingsley-Hickman et al., 1987; Portman, 1994), and that tight regulation of hexokinase binding to the mitochondrial outer membrane depends on cellular energetic needs (Parra et al., 1997; Penso and Beitner, 2003). As the GAPDH/PGK couple catalyzes a rapidly equilibrating reaction between P_i and γ-ATP, it has been implicated in transferring P_i, NADH and ADP from myofibrils to mitochondria (Dzeja et al., 1999a). In this regard, disequilibrium created at one specific intracellular locale of the near-equilibrium glycolytic network would be translated to other cellular compartments (Goldbeter and Nicolis, 1976; Mair and Muller, 1996). Indeed, ‘metabolic waves’ have been observed to propagate rapidly throughout the entire cell, and oscillations in energy metabolism appear to govern cellular electrical activity, biological information processing and functional response (O’Rourke et al., 1994; Welch, 1996). High energy phosphoryls generated by glycolysis can be preferentially delivered and used to support specific cellular functions, such as maintenance of membrane ionic gradients, cell motility, muscle contraction and nuclear

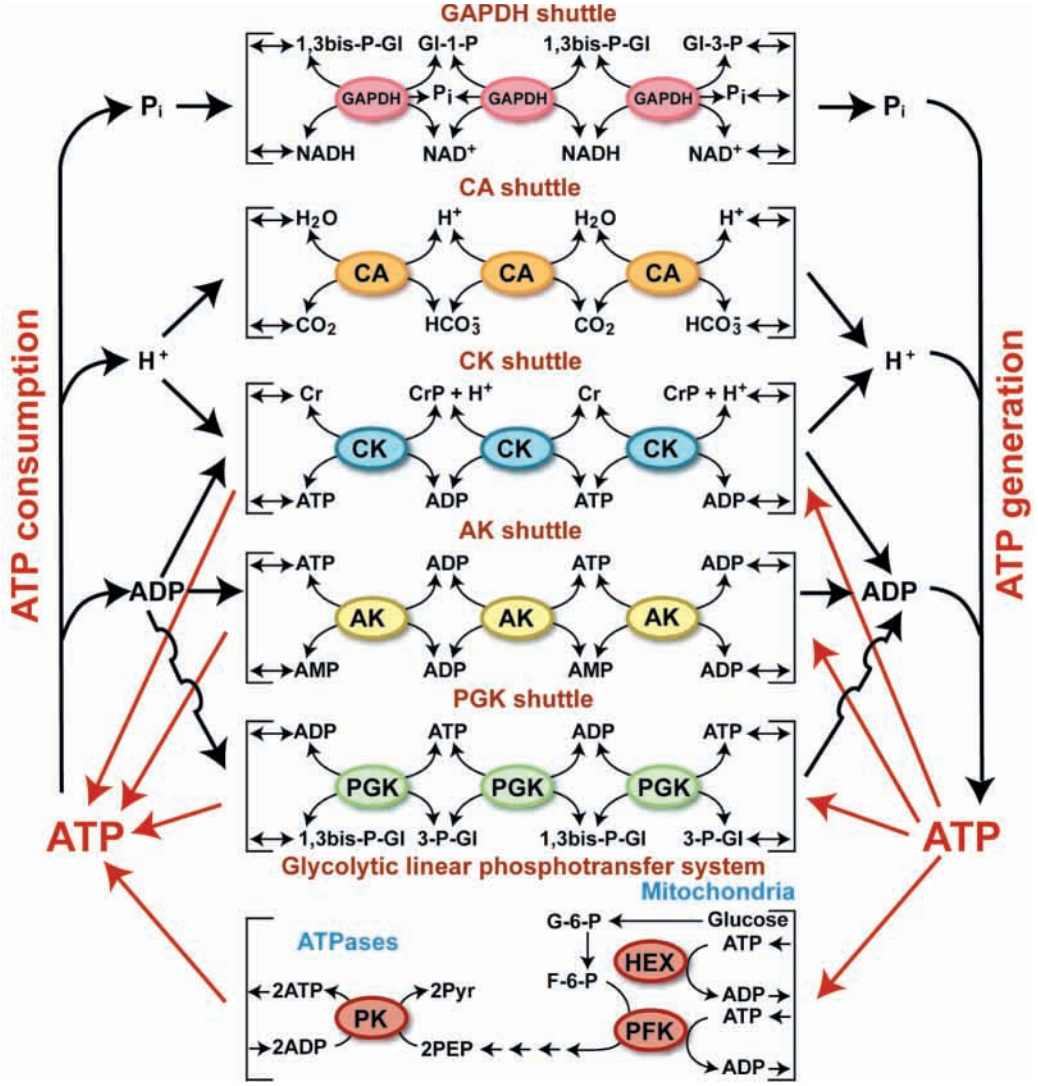

Fig. 3. Integrated communication between cellular sites of ATP-utilization and ATP-generation. Cells utilize enzymatic shuttles to promote ATP delivery and removal of ATPase byproducts, ADP, P_i and H^+, to sustain efficient energy utilization. Shuttles comprise near-equilibrium enzymes capable of facilitating ligand transfer between cellular compartments by rapidly relaying the displacement of equilibrium. ATP delivery is facilitated through creatine kinase (CK), adenylate kinase (AK), and the glycolytic system, which includes hexokinase (Hex), pyruvate kinase (PK) and 3-phosphoglycerate kinase (PGK). ADP is removed by CK, AK and PGK shuttles. P_i transfer is catalyzed by the near-equilibrium glyceraldehyde 3-phosphate dehydrogenase (GAPDH) shuttle. H^+ removal is facilitated by CK and carbonic anhydrase (CA) shuttles. As these shuttle systems operate in parallel, a diminished activity of a single enzyme is rather well tolerated. However, a decrease in the activity of several enzymes could lead to a cumulative impairment in the communication between ATP-generating and ATP-consuming sites (Dzeja et al., 2000). Gl, glucose; PEP, phospho-enol pyruvate; Pyr, pyruvate; Cr, creatine.

processes (Ottaway and Mowbray, 1977; Masters et al., 1987). Glycolytic enzymes have also been recognized as an important component in the regulation of ATP/ADP-sensitive cellular components such as the K_{ATP} channel (Weiss and Lamp, 1987; Dzeja and Terzic, 1998), providing energetic signaling between mitochondria and plasmalemma. Recently, an adaptor protein involved in anchoring metabolic enzymes, such as creatine kinase, adenylate kinase and phosphofructokinase, to sites of high-energy consumption in the cardiac sarcomere has been discovered (Lange et al., 2002). Such scaffolding proteins can support spatial organization of phosphotransfer networks, thus increasing the efficiency and specificity of high-energy phosphoryl distribution.

Carbonic anhydrase ligand conduction system: speeding up protons and disposing of CO_2

The commonly held view is that carbonic anhydrase acts as a single near-equilibrium enzyme to provide HCO_3^- or H^+ for biosynthetic reactions and H^+-ATPases, respectively (Dodgson et al., 1980; Geers and Gros, 1991). Immunocytochemical data show that carbonic anhydrase molecules are arranged in clusters along membranes, protruding myofibrils along I-bands and localized in the narrow extracellular space (Swenson, 1997; Sender et al., 1998). Such spatial heterogeneity and directionality of enzyme-catalyzed process (Harold, 1991) may contribute to the ability of carbonic anhydrase to facilitate the intracellular diffusion of CO_2 and H^+ (Geers and Gros, 1991). Furthermore, by increasing proton movement carbonic anhydrase-catalysis dissipates intracellular pH gradients, maintaining the spatiotemporal uniformity of cellular pH (Stewart et al., 1999).

As most ATPases, especially actomyosin ATPase, are inhibited by the buildup of protons in their vicinity, the necessity for H^+ removal system is warranted (Dzeja et al., 1999a). In this regard, inhibition of carbonic anhydrase reduces

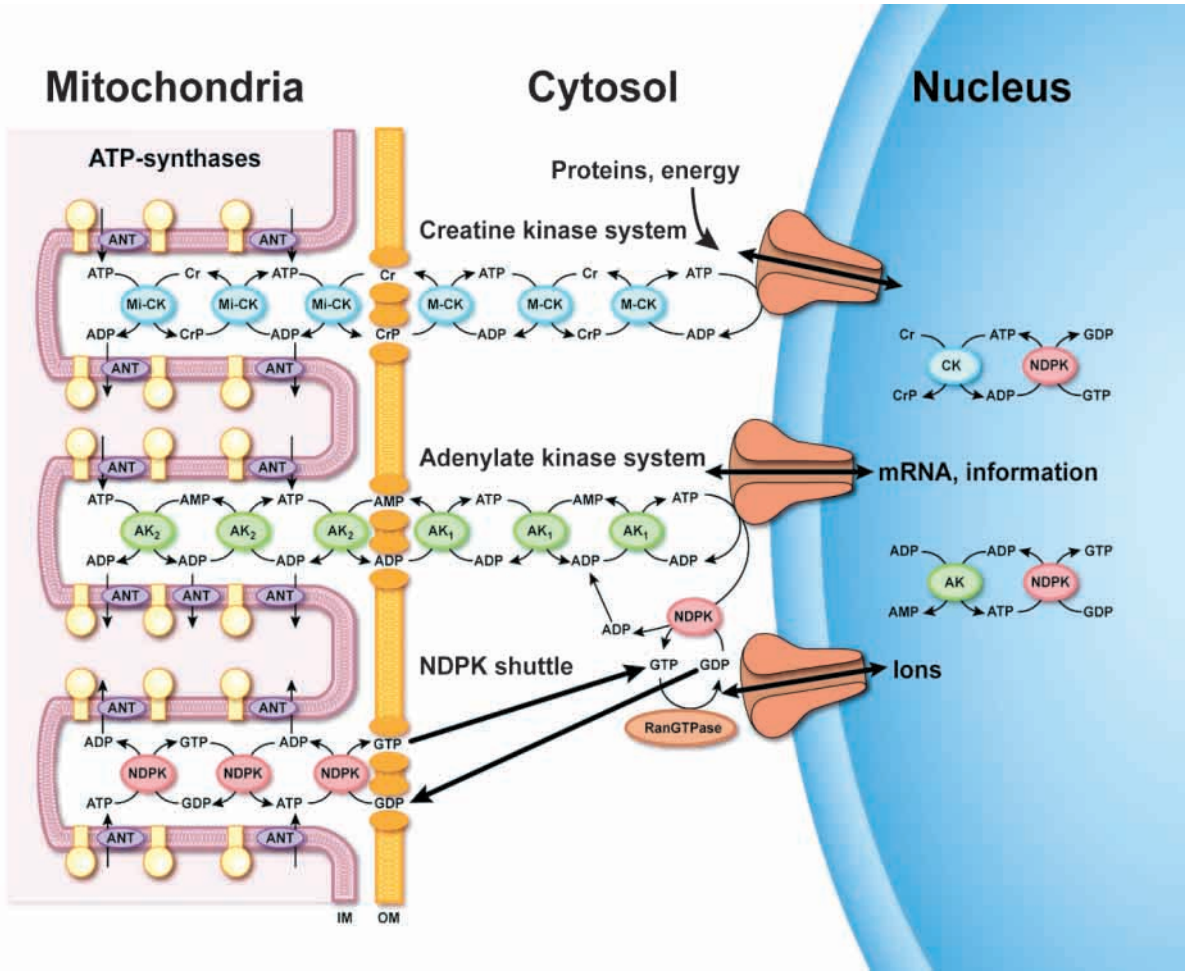

Fig. 4. Energy support relays for nucleocytoplasmic communication. Mitochondria clustered around the nucleus generate the majority of ATP required for nuclear processes. Export of ATP from the mitochondrial intracristal space is facilitated by near-equilibrium reactions catalyzed by mitochondrial isoforms of adenylate kinase (AK_2), creatine kinase (Mi-CK) and nucleoside diphosphate kinase (NDPK). Subsequently, high-energy phosphoryls are navigated through the diffusionally restricted perinuclear space to ATP consumption sites at the nuclear envelope and inside the nucleus by cytosolic and nuclear isoforms of AK, CK and NDPK. Interaction and complementation between these systems secure proper nucleotide ratios at and across the nuclear envelope, sustaining the high energy of ATP and GTP hydrolysis. For other abbreviations, see Fig. 3.

muscle contractility and calcium handling (Geers and Gros, 1991), and could contribute to the development of heart failure (Dzeja et al., 1999a). It was proposed that sequentially arranged carbonic anhydrase molecules catalyzing rapid equilibrium among reactants could provide ligand conduction pathways for transferring protons from ATPases to ATP-generating sites inside the cell, as well as for facilitated transfer of CO_2 to the cell membrane and consequently out of the cell to the capillaries (Dzeja et al., 1999a). In fact, 'proton waves' have been observed to spread throughout the entire cell and also from one cell to another (Grandin and Charbonneau, 1992; Mair and Muller, 1996).

The creatine kinase phosphotransfer system can also participate in proton transfer from ATPases (Fig. 3), and its function may be interrelated with that of carbonic anhydrase (Wallimann et al., 1998; Dzeja et al., 2000). In this regard, creatine kinase deficient muscles have a reduced capability to regulate intracellular pH (in't Zandt et al., 1999). Thus, carbonic anhydrase is emerging as a dynamic player in intracellular and paracellular H^+ and CO_2 trafficking, and as an integral part of the cell energetic infrastructure.

Nucleoside diphosphate kinase system: energy currency exchange, delivery and feedback signaling

Nucleoside diphosphate kinase (NDPK) catalyzes transfer of γ-phosphate from nucleoside 5′-triphosphates to nucleoside 5′-diphosphates, ATP+NDP↔ADP+NTP, and links ATP-based energetics with the cellular nucleoside triphosphate pool (Lacombe et al., 2000). NDPK, which is localized in mitochondria, cytosol and nucleus, facilitates channeling of nucleoside triphosphates into protein synthesis and DNA replication complexes (Ray and Mathews, 1992; Gerbitz et al., 1996). Simultaneously, sequential NDPK reactions could

communicate nucleoside diphosphate feedback signals to mitochondria (Gerbitz et al., 1996). NDPK-deficient cells have highly biased nucleoside triphosphate pools, including marked elevations of CTP and dCTP, and a strong mutator phenotype (Bernard et al., 2000). Other reports indicate that imbalance in cellular nucleotide ratios results in increased genetic error frequency (Bebenek et al., 1992), and that NDPK is a product of the tumor supressor gene *Nm23* (Lacombe et al., 2000). In addition to regulating nucleic acid synthesis, NDPK controls GTP/GDP exchange on G-proteins and receptor-mediated signal transduction (Hippe et al., 2003). Also, NDPK could facilitate GTP/GDP exchange on Ran GTPase, an essential factor in nuclear transport (Kraeft et al., 1996; Dzeja et al., 2002). Moreover, NDPK catalysis is linked to Krebs cycle activity and high-energy phosphoryl export from mitochondria (Roberts et al., 1997; Janssen et al., 2000). In AK1-deficient muscles, phosphoryl flux through enzymes catalyzing GTP production, including NDPK, is increased and could contribute to energetic compensation (Janssen et al., 2000). The essential role of NDPK in cell growth is indicated by coupling between reduced NDPK levels and suppression of proliferative activity (Kimura et al., 2000). In addition, NDPK levels are enhanced with cell development and differentiation (Kimura et al., 2000). Thus, NDPK isoforms provide an important link between ATP generation and energy distribution to synthetic processes through other nucleoside triphosphates, as well as through regulation of signal transduction and gene expression.

Phosphotransfer systems and nuclear processes: trading metabolic energy for information

Intense nuclear functions, including DNA replication, chromatin remodeling, gene transcription and transport of macromolecules across the nuclear envelope require efficient energy supply, yet principles governing nuclear energetics and energy support for nucleocytoplasmic communication are still poorly understood (Mattaj and Englmeier, 1998; Dzeja et al., 2002). Recently, it was demonstrated that mitochondrial ATP production is required to support energy-consuming processes at the nuclear envelope, while glycolysis alone was insufficient to perform such a function (Dzeja et al., 2002). Although mitochondrial clustering around the nucleus reduced the distance of energy transfer, oxidative phosphorylation and simple nucleotide diffusion were inefficient to meet energy requirements for nucleocytoplasmic communication. Adenylate kinase phosphotransfer was identified to direct transmission of high-energy phosphoryls from mitochondria to the nucleus, maintaining the optimal nucleotide ratios required for active nuclear transport. Moreover, adenylate kinase coupled with NDPK secured phosphoryl transfer between ATP and GTP, as both nucleoside triphosphates are necessary for active nuclear transport (Mattaj and Englmeier, 1998; Perez-Terzic et al., 2001). Inhibition of nuclear transport by disruption of the adenylate kinase relay could be rescued through upregulation of alternative phosphotransfer pathways, such as the creatine kinase system, underscoring the plasticity of the cellular energetic network (Wallimann et al., 1992; Dzeja et al., 2003; Neumann et al., 2003).

These data implicate phosphotransfer enzymes in the energy-linked regulation of matter and information exchange between the cytosol and nucleus (Fig. 4). In this way, sequential phosphotransfers are responsible for transmission of ATP and GTP from mitochondria and maintenance of ATP/ADP and GTP/GDP ratios at ATP/GTP-utilization sites. Variations of phosphotransfer enzyme activity in the cytosol and nucleus correlate with the intensity of nuclear processes in normal and diseased conditions, underscoring the significance of maintained phosphotransfer in directing cellular energy flow (Manos and Bryan, 1993; Dzeja et al., 2000; Perez-Terzic et al., 2001). In this regard, glycolytic enzymes have also been identified in nuclei of several cell types, including regenerating hepatocytes where they furnish a considerable portion of increased nuclear energy requirements (Ottaway and Mowbray, 1977). Thus, integration of the nuclear compartment with mitochondrial energetics is accomplished through specialized enzymatic networks, securing the metabolic demands of nuclear processes.

Concluding remarks

In summary, coordination between ATP consumption and ATP production processes can be achieved through a coupled near-equilibrium enzymatic network, which has the ability to provide precise control similar to a ‘digital’ type of regulation, such that each ATP conversion to ADP, P_i and H^+ will be signaled to an equivalent stoichiometry of ADP, P_i and H^+ transformation to ATP (Saks et al., 1994; Dzeja et al., 2000; Neumann et al., 2003). Facilitated communication between cellular energy transforming and consuming processes minimizes metabolite gradients, reducing energy dissipation and providing a capability to direct high-energy phosphoryls into specific pathways according to physiological needs. Distribution of cellular energy could be accomplished by altering phosphotransfer enzyme isoform composition and their intracellular localization (Wallimann et al., 1992; Lange et al., 2002; Dzeja et al., 2003). In this regard, derangement in cellular energy flow and distribution has been implicated in cardiovascular (Dzeja et al., 2000; Perez-Terzic et al., 2001) and neurodegenerative (Ames, 2000) diseases, as well as in determining uncontrolled growth and metastatic potential of tumor cells (Kimura et al., 2000; Penso and Beitner, 2003).

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