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Abstracts

S3 Membrane Transporters

Lectures

3L.1 The ups and downs of mitochondrial calcium signalling in the heart☆

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Regulation of intramitochondrial free calcium ($[Ca^{2+}]_m$) is critical in both physiological and pathological functioning of the heart. The full extent and importance of the role of $[Ca^{2+}]_m$ is becoming apparent as evidenced by the increasing interest and work in this area of the last two decades. However, controversies remain, such as the existence of beat-to-beat mitochondrial Ca^{2+} transients; the role of $[Ca^{2+}]_m$ in modulating whole-cell Ca^{2+} signalling; whether or not an increase in $[Ca^{2+}]_m$ is essential to couple ATP supply and demand; and the role of $[Ca^{2+}]_m$ in cell death by both necrosis and apoptosis, especially in formation of the mitochondrial permeability transition pore. The role of $[Ca^{2+}]_m$ in heart failure is an area that has also recently been highlighted. $[Ca^{2+}]_m$ can now be measured reasonably specifically in intact cells and hearts thanks to developments in fluorescent indicators and targeted proteins and more sensitive imaging technology. This has revealed interactions of the mitochondrial Ca^{2+} transporters with those of the sarcolemma and sarcoplasmic reticulum, and has gone a long way to bringing the mitochondrial Ca^{2+} transporters to the forefront of cardiac research. Mitochondrial Ca^{2+} uptake occurs via the ruthenium red sensitive Ca^{2+} uniporter (mCU), and efflux via an Na^+/Ca^{2+} exchanger (mNCX). The purification and cloning of the transporters, and development of more specific inhibitors, would produce a step-change in our understanding of the role of these apparently critical but still elusive proteins. In this article I will summarise the key physiological roles of $[Ca^{2+}]_m$ in ATP production and cell Ca^{2+} signalling in both adult and neonatal hearts, as well as highlighting some of the controversies in these areas. I will also briefly discuss recent ideas on interactions of nitric oxide with $[Ca^{2+}]_m$.

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3L.2 Mitochondrial ion transport in metabolic disease

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Mitochondria are the central coordinators of energy metabolism and alterations in their function and number have long been associated with metabolic disorders such as obesity, diabetes and hyperlipidemias. Since oxidative phosphorylation requires an electrochemical gradient across the inner mitochondrial membrane, ion channels in this membrane certainly must play an important role in the regulation of energy metabolism. However, in many experimental settings, the relationship between the activity of mitochondrial ion transport and metabolic disorders is still poorly understood. We will cover aspects of mitochondrial H^+ and K^+ transport which may be determinants in metabolic disorders, including the impact of mitochondrial uncoupling on energy metabolism and aging and the role of K^+ channels in metabolic disorders.

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3L.3 The transport mechanism of mitochondrial carriers based on the analysis of pseudo-symmetry

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Mitochondrial carriers link the biochemical pathways of the cytosol and mitochondrial matrix by transporting substrates across the mitochondrial inner membrane [1]. The structures of mitochondrial carriers are three-fold pseudo-symmetrical [2,3], but their substrates and coupling ions are not. Thus, deviations from symmetry are to be expected in the substrate and ion binding sites in the central aqueous cavity [4]. By analyzing the three-fold pseudo-symmetrical repeats from which their sequences are made, conserved asymmetric residues were found to cluster in a region of the central cavity identified previously as the common substrate binding site [5,6]. Conserved symmetrical residues required for the transport mechanism were found at the water–membrane interfaces, flanking the substrate binding sites [4]. Three PX[DE]XX[RK] motifs form a salt bridge network on the matrix side of the cavity, when the carrier is in the cytoplasmic state with the substrate binding site open to the mitochondrial intermembrane space [3,7]. Three [FY][DE]XX[RK] motifs are present on the cytoplasmic side of the cavity and they could form a salt bridge network when the carrier is in the matrix state with the substrate binding site accessible from the mitochondrial matrix [4]. It is proposed that the opening and closing of the carrier could be coupled to the disruption and formation of the two salt bridge networks induced by substrate binding. The interaction energy of the cytoplasmic network allows members of the transporter family to be classified as strict exchangers or importers.

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3L.4 Structure based study of the functionality of NhaA in pH and Na^+ homeostasis

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Na^+/H^+ antiporters are essential for homeostasis of Na^+, H^+ and volume and are critical to cell viability. The crystal structure of *Escherichia coli* NhaA determined at pH 4 has provided insights into the mechanism of activity of a pH-regulated Na^+/H^+ antiporter [1,2]. The structural fold of NhaA is novel; six of the twelve transmembranes (TM) form an inverted repeat of which two TMs are interrupted in the middle of the membrane forming a unique electrostatic organization, important for activity. Two funnels, a deep cytoplasm-facing and a shallow periplasm-facing are separated by a barrier. The functional unit of NhaA is a monomer [3] with a "pH sensor" separated from the active site. Novel structure-based experimental and computational approaches demonstrate that amino acid residues in TM II contribute to the cation pathway of NhaA and its unique pH activation between pH 6 and 8.5 [4,5]: 1) the highly conserved residues of TM II are located on one side of the helix facing either the cytoplasmic or periplasmic funnels of NhaA. 2) Cys replacements of the conserved residues and measuring their antiporter activity in everted membrane vesicles identified new functional important residues. 3) Several of the Cys replacements were significantly alkylated by a membrane permeant probe implying the presence of water-filled cavities in NhaA. 4) Several Cys replacements were modified by MTSES and/or MTSET, membrane impermeant, negatively and positively charged reagents, respectively, that could reach the Cys replacements only via water filled funnel(s). Remarkably, MTSES but not MTSET repaired the mutant D65C implying the importance of Asp65 negative charge for pH activation of NhaA. The crystal structure of NhaA allowed to model the eukaryotic NHE1 [6] and NHA2 [7].

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3L.5 Novel secretory pathway pumps

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The super family of P-type ATPases constitutes a large class of membrane proteins involved in the active transport of ions and lipids across biological membranes. Among the prominent members are the Na^+/K^+-ATPase, the Ca^{2+}-ATPase of sarcoplasmic reticulum and the plasma membrane H^+-ATPases of plants and fungi. The family can be divided phylogenetically into five distinct subfamilies (P1–P5), each divided into additional subgroups (A, B etc.). The importance of these biological pumps is underlined by the fact that its members are found in all forms of life, from bacteria to man and are involved in fundamental physiological processes, ranging from ion homeostasis and signal transduction to heavy metal and lipid transport across membranes. P4 ATPases constitute the largest P-type ATPase subfamily in eukaryotes but are absent from prokaryotes. They are found in all membranes of the secretory pathway, except the endoplasmic reticulum. P4 ATPases have been associated with flipping of lipids across membranes, a process likely to be the initial event in vesicle budding in the secretory pathway. However, our understanding of lipid translocation, vesiculation and the involvement of P4-type ATPases in these processes is just beginning to emerge and further biochemical characterization of P4-ATPases is required in order to clarify whether these transporters indeed are capable of directly catalyzing transmembrane phospholipid flipping. The β-subunit of P4-ATPases shows unexpected similarities between the β- and γ-subunits of the Na^+/K^+-ATPase. It is likely that these proteins provide a similar solution to similar problems, and might have adopted similar structures to accomplish these tasks. P5 ATPases remains the least characterized group of P-type ATPases. They evolved at the branching point between eukaryotic and prokaryotic organisms and thus are associated with the event of compartmentalization in eukaryotes. Localization studies indicate that they reside in internal membrane systems, a hallmark of eukaryotic cells. As no P4-ATPases have been identified in the endoplasmic reticulum, where P5-ATPases are present, it remains an intriguing possibility that in this compartment P5A-ATPases are functional homologues of P4-ATPases.

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3P.1 Probing the conformation of the yeast ADP/ATP carrier by fluorescent probes

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The mitochondrial ADP/ATP carrier exchanges cytosolic ADP for ATP synthesised in the mitochondrial matrix. During the transport cycle the carrier opens the central substrate binding site to the intermembrane space in the cytoplasmic state and to the mitochondrial matrix in the matrix state [1,2]. The charged residues of the PX[DE]XX[RK] motifs form a salt bridge network on the matrix side of the cavity, when the carrier is in the cytoplasmic state [3,4], whereas the charged residues of the [FY][DE]XX[RK] motifs, which are present on the cytoplasmic side of the cavity, could form a salt bridge network when the carrier is in the matrix state [2]. A structure of the