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Life of mice – development of cardiac energetics

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Production and transfer of metabolites like ATP and phosphocreatine within cardiomyocytes is crucial for the robust availability of mechanical work. In mammalian cardiomyocytes, mitochondria, the main suppliers of usable chemical energy in the form of ATP, are situated adjacent to both the ATPases near the mechanical apparatus, and the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) calcium pumps. Operation of these ATPases requires a high ATP/ADP ratio, which is maintained by two parallel energy transfer systems – creatine kinase (CK) and direct adenine nucleotide channelling (DANC). Compartmentation of energy metabolites works to lessen the impact of dynamic changes in the availability of usable energy on the operation of these ATPases, and allows for a higher phosphorylation potential where it is required most.

The operational mechanism, structure and development of the barriers responsible for energetic compartmentation within cardiomyocytes have yet to be elucidated despite intensive research in this area. A recent article in *The Journal of Physiology* by Piquereau *et al.* (2010) is an extensive investigation into how the structural and energetic properties of mouse heart muscle change during postnatal development. It includes observations on structural changes and cellular morphology using electron microscopy, quantification of mitochondrial, myofibrillar and SR proteins, assessment of organelle functionality, and the quantification of the energy flux in both the CK and DANC transfer systems. SERCA function was measured via calcium mediated tension generation, while myosin ATPase function was quantified by measuring rigor tension development. Total activity of CK and mitochondrial CK (mi-CK) were estimated.

The article by Piquereau *et al.* builds upon a strong research tradition at Inserm U769, Univ. Paris-Sud, which focuses on studying how cardiac mechanisms function in response to both pathological and physiological stimuli. This includes work on contractile, sarcoplasmic reticulum (SR), and mitochondrial proteins, membrane receptors, ion channels and signalling. Their work has inspired new areas of inquiry into the function of energy compartmentation in the heart with various implications for therapeutic targets to improve both function and clinical outcomes.

As main results of their recent publication, Piquereau *et al.* concluded that the formation of energetic microdomains occurs very early in postnatal development, and that the maturation of cellular architecture plays an important role in achieving maximal flexibility in regulation of ATP production by mitochondria. They found that the development of regulatory energetic pathways does not happen simultaneously. Throughput of energy transfer between mitochondria and myosin ATPases is correlated with the changes in the cytoarchitecture in contrast to the CK supported energy transfer which seems to depend on specific localization and expression of CK. Development between days 3 and 7 is crucial in increasing the capacity of energy transfer and involves major remodelling of the contacts between organelles. The density of intracellular organelles increases at the expense of free cytosolic space. Contacts between mitochondria and longitudinally oriented myofibrils and between SR and mitochondria are established to form an effective intracellular energetic unit. After the first week (*post natum*), a different phase of hypertrophy occurs without major structural changes to the contacts between organelles. After 3 weeks, the respiratory capacity of mitochondria increases, whereas heart weight to body weight ratio decreases. The main results of the article are summarized in Fig. 1.

Considerable effort has been invested by Piquereau *et al.* in determining various changes during cardiomyocyte maturation. Several questions arise, however, when comparing the publication with previous studies. Firstly, in 3-day-old cells, based on results from electron microscopy and SR protein expression experiments, the authors deduce SR not to be present in quantities high enough to enable SR Ca^{2+} content measurement. However, volume measurements from electron microscopy are known to be very sensitive to sample preparation procedures, especially as dimensions of different organelles can change in different ratios as a result of fixation. The low level of SR protein expression in 3-day-old cells could be explained by results obtained in embryonic mouse cardiomyocytes (Takeshima *et al.* 1998), where SR Ca^{2+} release channels do not play a major role in excitation–contraction (EC) coupling but, instead, are required for cellular Ca^{2+} homoestasis. Full SR function develops rapidly in neonates, possibly explaining both the dramatic increase in SR Ca^{2+} content between day 3 and day 7 fibres, and the difficulty the authors had in conducting the experiment with fibres from 3-day-old mice.

Secondly, the authors concluded that the functional coupling of adenine nucleotide translocase (ANT) and mi-CK ('functional activity' in Piquereau *et al.* 2010) was considerably higher in adult myocytes. This conclusion, however, seems to be based on misinterpreting the K_{mADP}/K_{Cr} ratio graph (article Fig. 5*F*). As is evident from the K_m plots in the article (article Fig. 5E), K_{mCr} is constant throughout the ageing process, whereas K_{mADP} increases notably in older fibres. The increase in K_{mADP}/K_{Cr} ratio stems from the increase of K_{mADP} and is not, in this case, indicating increases in mi-CK–ANT coupling nor mi-CK activity. Rather, it can be interpreted as indication of an increase in diffusion restrictions to adenine nucleotides in the cytosol caused by changes in either mitochondrial outer membrane or myofibrillar and other cytosolic structures, or both (Vendelin & Birkedal, 2008; Sepp *et al.* 2010). In order to measure the coupling between mi-CK and ANT, different experimental techniques need to be employed, such as measuring changes in respiration in response to ATP titration.

Two observations can be made from further analysing SR calcium uptake and rigor tension sensitivity results from the article (article Figs 2 and 4). By looking at ratios of values obtained during different

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conditions, it is possible to eliminate auxiliary effects and focus on how the role of energy supply pathways change in relation to one another as the cell matures. Two examples are given in Fig. 1 (bottom row). Firstly, from the difference in rigor tension levels ($\Delta pMgATP_{50}$) supported by CK and ATP energy supply systems (Fig. 1, line b), it is evident that myosin ATPase activity supported by CK is consistently higher than exogenous ATP throughout the growing process. On the other hand, the capacity of the CK system to load the SR increases ~2 times by day 61 (Fig. 1, line d). We suggest that this is further evidence of the role of SR transitioning from maintaining Ca^{2+} homoestasis (Takeshima *et al.* 1998) to playing an essential role in EC coupling. A possible explanation for this could be activation of SR-bound CK by day 21, whereas myofibril bound CK is already active from day three. Secondly, $pMgATP_{50}$(DANC) – $pMgATP_{50}$(ATP) (Fig. 1, line a) indicates that after an initial increase caused by changes in mitochondrial positioning, myofibrils stay constantly more sensitive to stimulation via direct channelling compared to exogenous ATP. At the same time, however, direct channelling is able to maintain an increasingly higher SR load than exogenous ATP (Fig. 1, line c). This can be explained by structural changes in the cell, whereby SR becomes more closely situated with respect to mitochondria (article Fig. 8*D*). Clearly, these interpretations should be verified through further experiments and modelling.

Building on results obtained by Piquereau *et al.* some directions could be explored in the future. One matter of interest would be how the role of glycolysis changes during maturation. It has been shown that embryonic mouse heart responds in a similar manner to inhibition of either glycolysis or oxidative phosphorylation and that in early stages of postnatal development, ATP consumed by ion pumps is preferentially supplied through glycolysis (Chen *et al.* 2007). Additionally, in 1-day-old rabbit, 44% of consumed ATP comes from glycolysis, whereas by day 7 this goes down to 7% (Lopaschuk *et al.* 1992). In the paper under discussion, the possible contribution of glycolysis to ATP supply was not directly addressed. Especially in young mouse cells, the effect from this could be considerable and might impact some of the conclusions of the article.

Another possible area to explore in the future could be to analyse these results with the aid of a computational model.

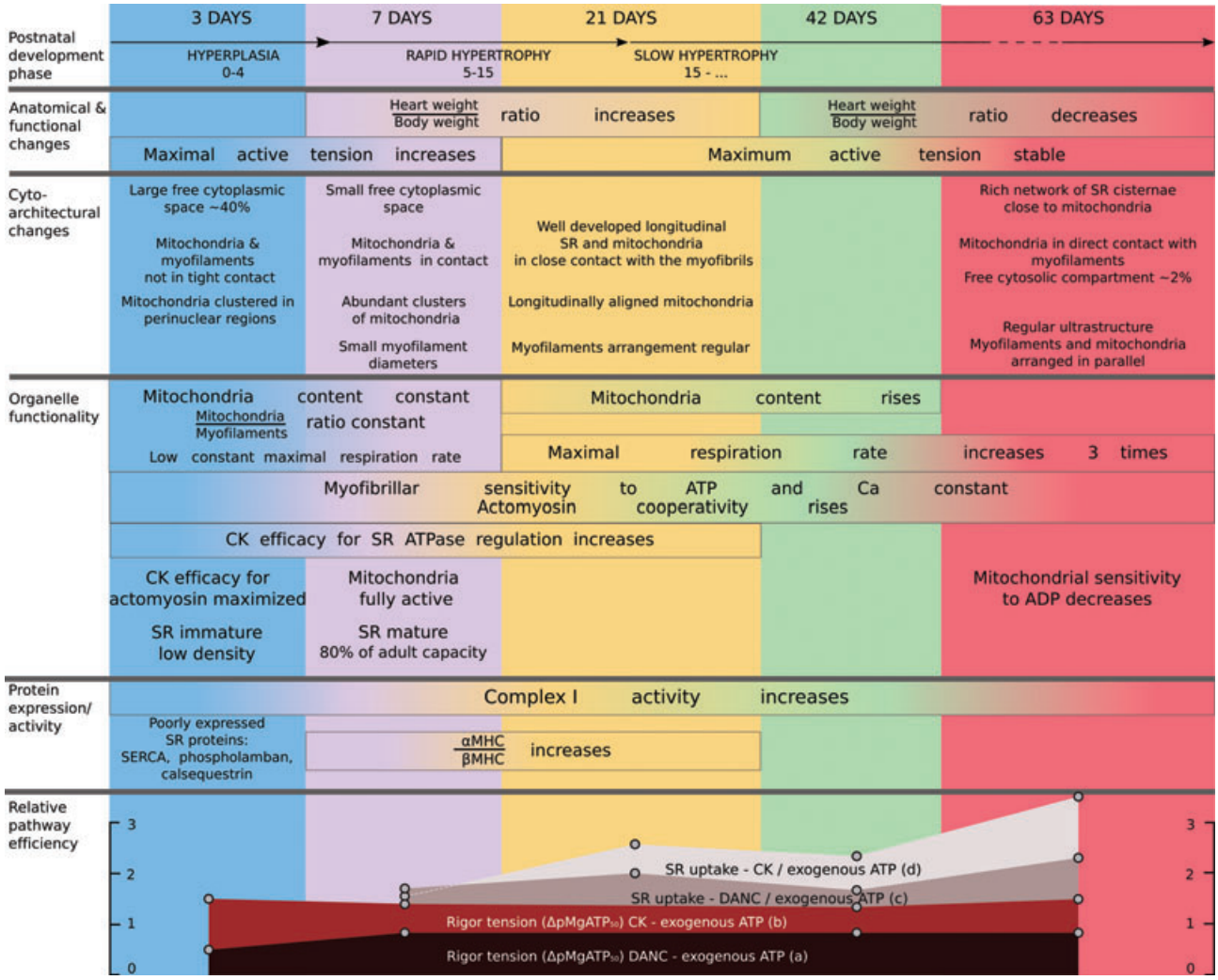

Figure 1. Summary of results from the article by Piquereau *et al*.

This would help in further unravelling the interplay between different factors during cell maturation, especially in questions where experimental methods fail to yield clear results. Different mathematical models could be compared with statistical methods in order to determine the role of various pathways and the existence of metabolite pools or spatial compartmentation in the developing cell (Sepp *et al.* 2010).

In summary, the extensive experimental work performed in the work by Piquereau *et al.* covers various aspects of energy metabolism and morphological changes in the cell during maturation. The work provides new information on postnatal development of heart energetics in mice – a popular animal model used for studying the effects of genetic manipulation.

References

Chen F, De Diego C, Xie L, Yang J, Klitzner T & Weiss J (2007). Effects of metabolic inhibition on conduction, Ca transients, and arrhythmia vulnerability in embryonic mouse hearts. *Am J Physiol Heart Circ Physiol* **293**, H2472–2478.

Lopaschuk G, Collins-Nakai R & Itoi T (1992). Developmental changes in energy substrate use by the heart. *Cardiovasc Res* **26**, 1172–1180.

Piquereau J, Novotova M, Fortin D, Garnier A, Ventura-Clapier R, Veksler V & Joubert F (2010). Postnatal development of mouse heart: formation of energetic microdomains. *J Physiol* **588**, 2443–2454.

Sepp M, Vendelin M, Vija H & Birkedal R (2010). ADP Compartmentation analysis reveals coupling between pyruvate kinase and ATPases in heart muscle. *Biophys J* **98**, 2785–2793.

Vendelin M & Birkedal R (2008). Anisotropic diffusion of fluorescently labeled ATP in rat cardiomyocytes determined by raster image correlation spectroscopy. *Am J Physiol Cell Physiol* **295**, C1302–C1315.

Takeshima H, Komazaki S, Hirose K, Nishi M, Noda T & Iino M (1998). Embryonic lethality and abnormal cardiac myocytes in mice lacking ryanodine receptor type 2. *EMBO J* **17**, 3309–3316.

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