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A perspective on Peter Mitchell and the chemiosmotic theory

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Abstract In 1991 Peter Mitchell wrote a last article that summarised his views on the origin, development and current status of his chemiosmotic ideas. I here review some of his views of that time on structures and mechanisms of several key bioenergetic components in relation to the subsequent advances that have been made.

Keywords Chemiosmotic theory · Peter Mitchell · Mitochondria · Energy coupling · Electron transfer

Historical perspective

Peter Mitchell wrote his final scientific article in 1991 (Mitchell 1991), 30 years after his seminal Nature paper (Mitchell 1961) that launched the chemiosmotic hypothesis (Fig. 1). The Nature publication was in its turn followed in 1966 and 1968 by two privately published 'Grey Books' (Mitchell 1966; Mitchell 1968) that established and amplified the details of the chemiosmotic theory. In his 1991 review he summarised his views on the origins, development, impact and ramifications of his chemiosmotic ideas. It is particularly interesting from several points of view. Firstly, he attempted to document the origins of the early development of his thoughts in terms of fundamental physical chemistry and thermodynamics, the nature of osmotic and diffusional forces and emerging ideas on lipid membranes and biological catalysis. He emphasized that, of all of these, it was the fundamental physical and chemical principles that the most important factors in the development of his ideas. These principles were to remain the foundation of his work and thinking.

In this final paper he also reiterated that the theory behind the chemiosmotic hypothesis is much more broadly relevant than is commonly acknowledged, being applicable not only to the membrane processes of electron/proton coupling, transport and ATP synthesis but also to ligand reactions more widely. Nevertheless, these bioenergetic systems, particularly the mitochondrial and photosynthetic electron transfer chains, were particularly conducive to experiments to test the tenets and predictions of the hypothesis and it was on the mitochondrial system that Peter Mitchell and Jennifer Moyle, his close colleague of many years, concentrated most of their experimental efforts. In the period between 1965 and 1992, his experimental and conceptual work proved extraordinarily successful in providing proof of the general hypothesis and prompting a number of explicit models of possible structures and mechanisms of key components. By the time of his Nobel award in 1978 (Mitchell 1979), his own work and a vast body of extraordinarily critical studies from around the world had provided confirmation of the basic proposals of the coupling of transmembrane electrochemical gradients of protons to energy-requiring processes of ATP synthesis and metabolite transport.

The 1991 article stands as typically prescient. In it were some of his predictions of likely structures and mechanisms of several of the key components of the respiratory and photosynthetic systems. In the years since then tremendous progress has been made, in particular on the elucidation of atomic models of many key bioenergetic proteins and the chemistry and dynamics of their catalytic processes. It is sad that he missed these developments; it would have been

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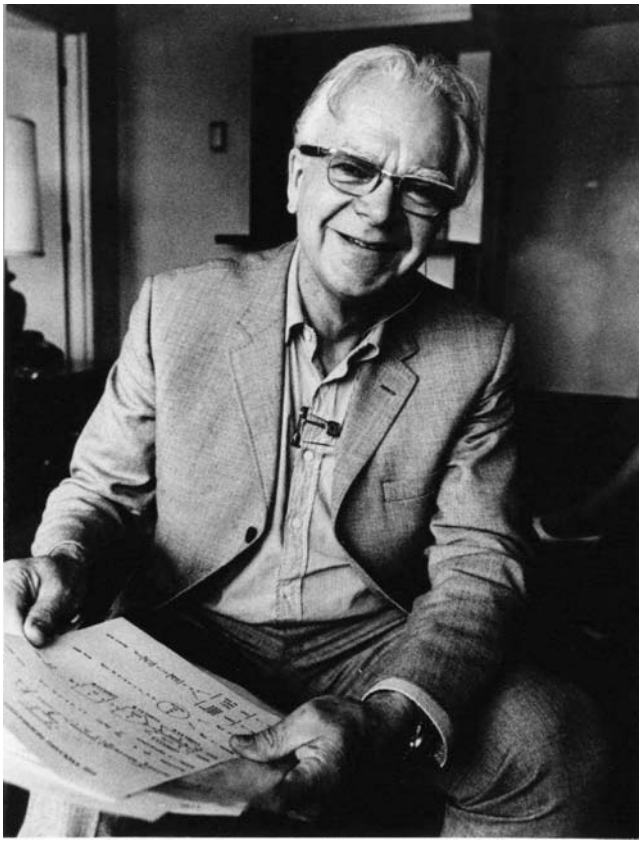

Fig. 1 Peter Mitchell in the 1980s. From the archive of Glynn photographs held at the Glynn Laboratory of Bioenergetics at UCL

fascinating to see how he would have inevitably used them to develop further his unique perspective. Peter's views and predictions of 1991, made without the knowledge that has come from these advances, were very much shaped by his abstract formalism and with emphasis on the direct coupling between the driving vectorial reaction and the chemical or spatial transformation that was being catalysed. Below, I have summarised his 1991 views of four key bioenergetic protein complexes in relation to current understanding. Some of these have been remarkably prescient, others less so, though in all cases they clearly illustrate how his inventions of specific models were governed by the underlying formalism of his chemiosmotic theory.

Specific examples

The cytochrome bc complexes

The cytochrome bc_1 complex is a central component of the mitochondrial electron transfer chain and part of a superfamily of homologous enzymes that occur widely in respiratory and photosynthetic electron transfer chains. All share a common catalytic core of three subunits that contain a 2Fe2S iron sulphur center, heme C and two hemes B prosthetic groups. These catalyse its ubiquinol-cytochrome *c* oxidoreductase and associated proton transfer activities. A crowning achievement of Mitchell was his proposal of the 'Q-cycle' mechanism of proton/electron coupling (Mitchell 1976) in which two ubiquinone-reactive sites are in protonic contact with opposite sides of the membrane and are connected electronically via the two haems B. The two-electron oxidation of ubiquinol at the ubiquinol-binding 'Q_o' site results in a bifurcation of electron transfer in which one electron is transferred to the iron sulphur center and the other to the lower potential heme B, termed b_L. The electron on b_L moves across the membrane to reduce the higher potential heme b_H which in turn reduces ubiquinone to ubiquinol.

This Q cycle mechanism was formulated and largely proven long before the first atomic structural models of *bc* complexes were available, and also before the identity of the electron and proton transfer cofactors had been identified with any certainty. These atomic models, together with physical and chemical characterisation of cofactors, confirmed the basic structural features that the Q cycle mechanism demands and have provided a wealth of specific structural details. Even so, key aspects of the basic electron/proton transfer mechanism still remain unclear. For example, the chemical and physical factors that cause the strict bifurcation of electron transfer at the Q_o site remain controversial (Osyczka et al. 2004) and possible long-range concerted actions within and between monomeric units of the dimeric complexes are still debatable (Crofts et al. 2008; Covian and Trumpower 2008). Nevertheless, the Q cycle mechanism in its 'modified Q cycle' form (Crofts et al. 2008) remains clearly the mechanism by which this enzyme maximises the conversion of redox and protonmotive energy. It stands out as an example of a chemiosmotically coupled device that precisely fits the abstract formalism that Mitchell envisaged.

Cytochrome c oxidase

Mitochondrial cytochrome *c* oxidase catalyses the transfer of electrons from cytochrome *c* to molecular oxygen and is again a member of a large and diverse superfamily. The enzyme is arranged so that cytochrome *c* binds and donates electrons from the intermembrane space but the protons are taken up from the opposite, matrix phase. It was later discovered that the each full catalytic cycle also results in the translocation of four additional protons across the inner mitochondrial membrane (Wikström 1977), greatly increasing the efficiency of storage of released energy.

Peter Mitchell was very reluctant to accept the fact that cytochrome *c* oxidase has a protonmotive function that is

additional to its protonmotive action caused simply by the fact that oxygen reduction is achieved with electrons donated from the intermembrane 'Positive' phase and protons from the matrix 'Negative' phase. He subsequently acknowledged that this was a mistake that had held the field back (Mitchell 1991) and it is probably fair to say that he only fully embraced the additional protonmotive function when he had also formulated an explicit possible model, the 'O' cycle (Mitchell 1987), that was again firmly based on his chemiosmotic principles. Figure 2 shows his first formulation of this type of model, where mobile oxygen species act as proton carriers in the same manner that ubiquinone acts with *bc* complexes; a more detailed model was given in (Mitchell 1991). In these cases, he envisaged the P/N barrier to be located at Cu_B, with rotations of protonated oxygen-based ligands providing the protonmotive action.

Although the actual protonmotive coupling mechanism of C*c*O remains unresolved and controversial, it now seems highly improbable that a mechanism of this type could be

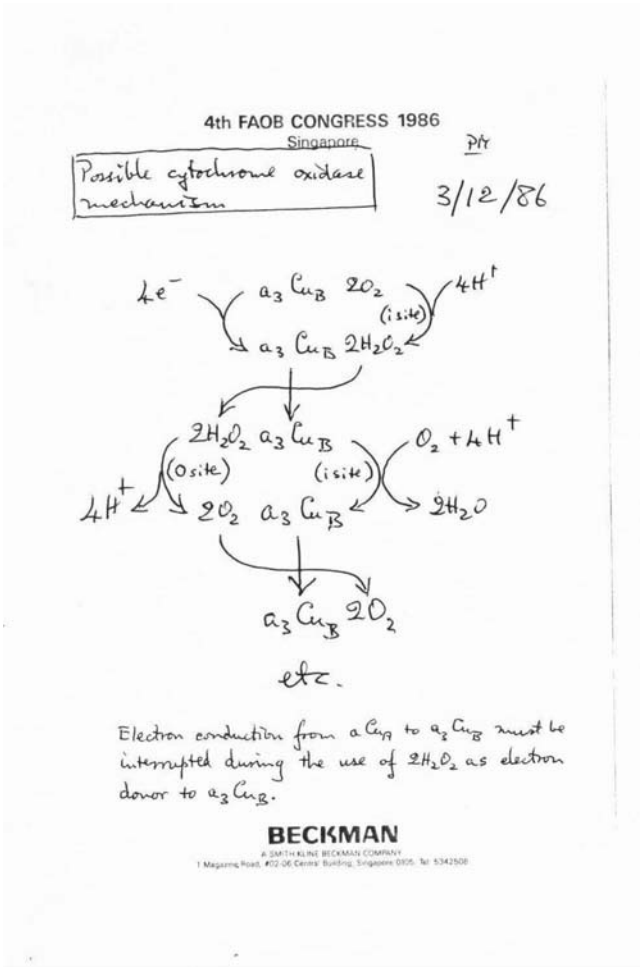

Fig. 2 Peter Mitchell's first formulation of a coupling mechanism for cytochrome oxidase involving mobile oxygen species

operative. During the late 1980s, we began investigations to directly test such mechanisms. Experiments to detect two simultaneous oxygen-reactive sites, analogous to two Q sites in the *bc* complexes, were unsuccessful. Also unsuccessful were attempts to show that some catalytic intermediates in the 'O loop' became 'supercharged' with protons so that the then supposed $2H^+/e^-$ stoichiometry of the last two steps (Wikström 1989) could be achieved. Notably, it was this latter empirical direction that led us to the eventual recognition that stable charges changes in the catalytic centre tend to be electroneutral through associated protonation changes (Mitchell et al. 1992). This recognition played a critical part in the subsequent formulation of a general coupling model involving such charge neutralisation, associated with vectorially positioned protonation sites (Rich 1995). This electrostatic model remains a viable basis for coupling mechanism that is firmly rooted in the formalism of Peter's chemiosmotic theory.

Solute porters

Peter's work on bacterial transport and group transfer preceded his formulation of the chemiosmotic theory and is a major stream that contributed to it. As early as 1957 (Mitchell 1957) he proposed a mechanism for phosphoryl transfer that involved a gated binding site that could, through protein conformational changes, face either side of the membrane. He later coined the term 'mobile barrier' to distinguish it from the 'mobile carrier' mechanisms above. In the context of the chemiosmotic theory, the notion was expanded to explain a range of active transport symport and antiport processes. Even in the light of present day advances in structures and mechanisms of active transport systems, such as the lactose transporter (Guan and Kaback 2008), these ideas provide the foundation for understanding of their actions.

ATP synthases

In considering possible mechanisms for ATP synthesis, Peter remained firmly with the central notion of direct linkage between the driving vectorial process and the coupled chemical or vectorial outcome. Even in his 1991 article, he favoured a relatively direct coupling between the translocated protons and the site of ATP synthesis, with a proton-driven antiport action between (ADP + P_i) and ATP and protonation of the oxide product of phosphorylation. He extended this same type of model to the sodiummotive ATPase whose existence had been taken as evidence that such a directly coupled mechanism was not possible. However, subsequent work ATP synthase structure and the direct demonstration of rotary motion of the central stalk have clearly pointed to a mechanism in which the

ionmotive force acts to drive a membrane-embedded rotor formed by a ring of *c* protein subunits, which in turn transmits forces via a rigid rotating rod structure to the catalytic sites of the α/β subunits at a considerable distance from the protonic events (Walker 1998).

In conclusion

The advances in understanding of bioenergetic membrane proteins since 1992 have been tremendous, particularly the crystallographic data that have transformed the understanding of membrane protein structure at the atomic level and the range of advanced spectroscopic and imaging methods that have provided spatial and mechanistic details of the redox centres. For the most part these advances have provided further support for Peter Mitchell's sometimes revolutionary ideas. Always firmly based on his chemiosmotic principles, many predictions have been borne out and extended by modern advances—the mechanism of the *bc* complexes and the structures of porter proteins are particularly elegant examples. In other cases, and inevitably so, Peter's predictions have been less successful. In the case of cytochrome *c* oxidase, the last experimental work in which he was involved already argued against the 'O loop' type of mechanism but subsequently led to a still viable electrostatic model of coupling. In contrast, the weight of modern data on ATP synthase appear to firmly rule out the type of direct coupling between driving ions and chemical actions that he envisaged. Complex I, with all known cofactors located in the peripheral matrix arm of the complex (Sazanov and Hinchliffe 2006), also continues to present a formidable challenge for any direct type of chemiosmotic coupling model. Nevertheless, the chemiosmotic theory provides a universal, readily understood, description that explains structures and mechanisms of these intricately complicated molecular machines that lie at the energetic heart of biology.

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