Dispatches

Organelle Evolution: A Mosaic of 'Mitochondrial' Functions

An ancient endosymbiosis of an α-proteobacterium produced a diverse range of organelles including mitochondria. Reconstruction of the *Pygsuia biforma* proteome adds to the mosaic of functional systems present in mitochondrial-related organelles and demonstrates the role of horizontal gene transfer.

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The endosymbiosis that gave rise to mitochondria is one of the key evolutionary innovations that marks the eukaryotic cell [1]. This organelle evolved from the endosymbiosis of an α-proteobacterium prior to the divergence of all known eukaryotes and canonically acts as the main site of aerobic ATP generation in many organisms [2]. Mitochondria also play fundamental roles in several other aspects of cellular metabolism including apoptosis, amino acid metabolism, pyruvate decarboxylation, and the biosynthesis of folate, phospholipids, heme, and iron-sulphur clusters [3–5]. A diverse range of organelles known as mitochondria-related organelles (MROs), originating from the same endosymbiotic event, have been identified in disparate anaerobic and microaerophilic lineages across nearly every major phylogenetic subdivision of the eukaryotes (Figure 1A) [5–7]. As reported in this issue of *Current Biology*, Stairs and colleagues [3] have added to this complexity by characterising the putative proteome of an MRO from *Pygsuia biforma,* a recently discovered breviate species which branches below the radiation of the fungi and animals [8]. *Pygsuia* further complicates the categorisation of MROs as it encodes a mosaic tapestry of organellar functions and demonstrates that this large family of organelles has no core conserved proteome.

We now have a multitude of terms to describe these sibling organelles, e.g. MROs, mitochondria-like organelles, mitochondrial-derived organelles, hydrogenosomes, mitosomes, cryptons, and hydrogen-producing mitochondria. Attempts to classify these diverse organelles phylogenetically have been abandoned as they have a broad and punctate distribution across the eukaryotes (Figure 1A). This suggests that MROs are likely the product of multiple independent evolutionary modifications of the same ancestral organelle. That said, Müller and colleagues have proposed a functional classification in which MROs are split into five classes on the basis of energy metabolism [6]: aerobic mitochondria (Class 1), canonical or 'text-book' mitochondria which use oxidative phosphorylation to generate ATP with oxygen as the terminal electron acceptor (e.g. *Saccharomyces cerevisiae*, *Arabidopsis thaliana*); anaerobically functioning mitochondria (Class 2), which generate ATP but use alternative electron acceptors such as fumarate or nitrate (e.g. *Ascaris lumbricoides, Trypanosoma brucei*); hydrogen-producing mitochondria (Class 3), which possess an electron transport chain and generate hydrogen while producing ATP via substrate-level phosphorylation (e.g. *Nycotherus ovalis*, *Blastocystis* sp.); hydrogenosomes (Class 4), which produce hydrogen but do not possess an electron transport chain and can produce ATP via substrate-level phosphorylation (e.g. *Trichomonas vaginalis*, *Piromyces* sp.); and mitosomes (Class 5), organelles which do not produce ATP and lack any components of the electron transport chain (e.g. *Giardia instestinalis*, *Entamoeba histolytica*, *Encephalitozoon cuniculi*) [6].

The predicted proteome of the *P. biforma* MRO demonstrates that this organelle is difficult to place in the above schema as it contains both cardiolipin and phospholipid biosynthesis pathway enzymes [3] which have only been detected previously in aerobic mitochondria (Class 1). Furthermore, this proteome indicates that the organelle has features of Class 2 anaerobic mitochondria, Class 3 hydrogen-producing mitochondria and Class 4 hydrogenosomes. Specifically, it has the protein repertoire to perform hydrogenosomal-like hydrogen production and pyruvate oxidation as well as partial components of an electron transport chain (such as quinol-reduction) [3]. This complicates functional classification, as this organelle appears to span Classes 2, 3 and 4 while containing elements common to Class 1. However, *P. biforma* is not the only MRO which defies easy categorisation; for instance, the *Mastigamoeba balamuthi* MRO has a partial electron transport chain while also generating hydrogen [9], placing it in a grey area between hydrogen-producing mitochondria and hydrogenosomes (Class 3 and 4) [5]. Another potential issue with this form of functional classification is where to classify MROs capable of facultatively modifying their energy metabolism, e.g. *Chlamydomonas reinhardtii* (which can act anaerobically [10]) or the *Trypanosoma brucei* mitochondrion (which utilises an alternative oxidase with a highly truncated electron transport chain and does not generate ATP when in the bloodstream of its host [11]). Collectively these data demonstrate, perhaps unsurprisingly, how difficult it is to apply a discrete classification to such a mosaic of organelles.

As energetics metabolism is likely the defining reason behind the acquisition and, in most cases, the maintenance of this endosymbiotic organelle, Müller and colleagues' classification has great utility when considering MROs purely from the perspective of energetics [1,12]. However, problems emerge when the scheme is generalised beyond its initial formulation, principally because energy generation is not a conserved unifying characteristic of MROs. Therefore, these sibling organelles

show complex patterns of gain and loss (Figure 1B), for example, gain of fumarate and nitrate based respiration [5]. The paper by Stairs *et al.* also provides another interesting example of function acquisition. Namely, the *Pygsuia* MRO proteome has acquired a rhodoquinone biosynthesis protein. This potentially allows the completion of the *Pygsuia* TCA cycle by producing a low electron potential quinone that would allow complex II to function as a fumarate reductase [3], like a Class 2 mitochondrion.

So if energy generation isn't a common feature of all MROs, are there any other alternative possibilities? Prime candidates would include the localisation and function of endosymbiotically derived chaperonin proteins (e.g. mtHsp70, mtCpn60), protein import machinery (e.g. Tim/Tom/Sam/Pam), and the biosynthesis of iron-sulphur clusters via the ISC system. However, in various MRO lineages there are clear examples of the loss of these features (Figure 1B) suggesting in the right circumstances these systems are also dispensable [9,13,14]. Even the ISC system, widely believed to be one of the essential conserved features of MROs, has been lost in *Entamoeba histolytica* [15] and *M. balamuthi* [16]. These amoeba have acquired an alternative means of iron-sulphur cluster biosynthesis in the form of an ε-proteobacterial-derived nitrogen fixation system [16,17]. Stairs and colleagues have also demonstrated the loss of the ISC system in the *P. biforma* MRO as well as the acquisition of an analogous archael sulphur-mobilisation protein[s] (SUFCB) via horizontal gene transfer (HGT) from *Blastocystis* sp. and/or Methanomicrobiales archaea. The SUFCB MRO protein is encoded by a gene fusion and possesses an amino-terminal MRO-targeting sequence. Localisation of this protein to the MRO was confirmed using fluorescence-microscopy methods, and this contrasts with *Blastocystis* sp. where the protein is localised in the cytosol [3]. This means ISC has been functionally replaced by this non-homologous HGT-acquired sulphur mobilisation system [3]. These data demonstrate that the conserved ISC MRO system, the only known function of some MROs (e.g. *Encephalitozoon cuniculi* [18]) and the only essential function of the yeast mitochondrion [19], is liable to loss.

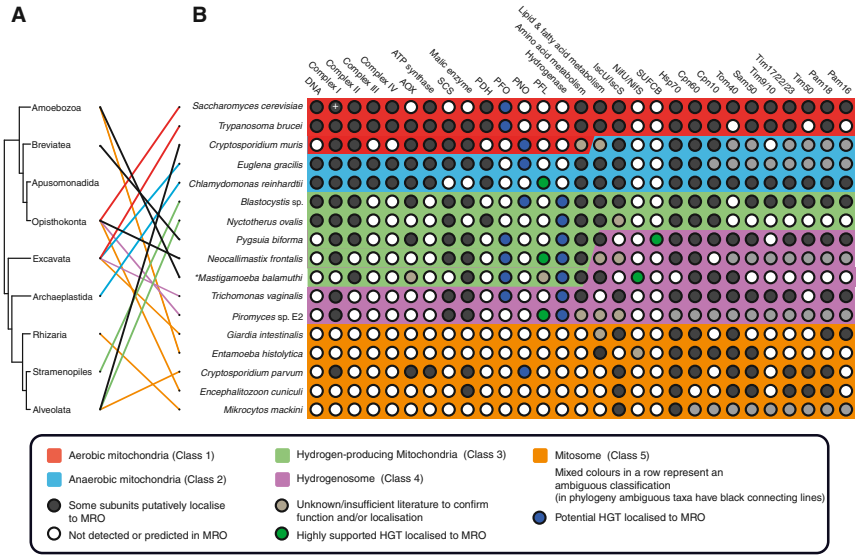

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Figure 1. Mosaic of functions in mitochondria-related organelles.

(A) A sketch of the major groupings of the eukaryotic tree of life demonstrating the taxonomic membership (via lines) of various MRO-bearing lineages linked to (B). Phylogeny is a simplified version of the tree shown in [8]. The connecting lines are coloured to match the respective 'Class' of MRO sensu Müller [6] (see the key) with black lines linking to those lineages of ambiguous 'Class'. (B) A dot plot derived from similar tables in [5] and [6] showing the presence of a subset of proteins suggested/demonstrated to localise/function in MROs (some homologous proteins are not indicated as they putatively function in the cytosol of the taxa listed). Organisms are grouped by coloured boxes corresponding to their 'Class' as shown in the key. Strongly supported HGT events are highlighted in green with hypothesised HGTs in blue. Reference detail for data summarised in Figure 1 can be found in the form of a table at http://richardslab.exeter.ac.uk/?p=212. **M. balamuthi* data are derived from a Sanger sequencing based EST project and thus proteins with relatively low expression such as the membrane translocases may not be detected. $^{+}$*S. cerevisiae* complex 1 is potentially a non-canonical NADH oxidoreductase. (AOX, alternative oxidase; SCS, succinyl-coA synthetase; PDH, pyruvate dehydrogenase; PFO, pyruvate:ferredoxin oxidoreductase; PNO, pyruvate:NADP oxidoreductase; PFL, pyruvate formate lyase; ISC, iron-sulphur cluster biosynthetic system; NIF, nitrogen fixation biosynthetic system; SUF, sulphur mobilisation biosynthetic system; HSP, heat-shock protein; CPN, chaperonin; TOM, translocase of the outer membrane; TIM, translocase of the inner membrane; SAM, sorting and assembly machinery; PAM, presequence translocase associated motor.)

However, even in the rare cases of loss, the iron-sulphur cluster appears to be replaced with a system of analogous function. This implies that an ability to make proteins containing iron-sulphur clusters is a cellular necessity and that this process requires compartmentalisation in most eukaryotes [3,19] in contrast to prokaryotes.

The work of Stairs and co-authors [3] emphasises the prevalence of lineage-specific re-modelling of MROs as well as the role HGT plays in this process. *Pygsuia* demonstrates that MROs do not display sets of discrete functional traits but instead are a product of a complex mosaic of diverse functions and evolutionary origins. Taking these data together (Figure 1), what emerges is a glimpse of an ancestral organelle functioning in cellular energetics, facultatively anaerobic, aerobic and which may [12] or may not have [20] had hydrogenosomal functions.

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Neuroethology: Self-Recognition Helps Octopuses Avoid Entanglement

How an octopus performs complex movements of its eight sucker-studded arms without entanglement has been a mystery. A new study has found that self-recognition of the octopus's skin by its suckers inhibits reflexive grasping of its own arms, simplifying the mechanisms needed to generate intricate arm behavior.

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A couple learning to dance soon realizes how easy it is for eight active limbs to become entangled. The challenge to an octopus is far greater because each of its eight supple arms can bend in almost any direction from any point along its boneless length. Worse, the arms have numerous suckers that reflexively grasp whatever they touch. But with a few hundred million years more time than that available to human dancers to solve the limb entanglement problem, the octopus has evolved a solution based on a mechanism more familiar to immunologists than to neurobiologists: chemical self-recognition. In a study reported in this issue of *Current Biology*, Nesher *et al.* [1] demonstrate that a cue in the skin of octopus inhibits sucker attachment, helping to avoid inadvertent grasping of its own arms as each arm performs its graceful routines.

Unlike human couples who struggle to synchronize movements commanded by just two brains, an octopus effectively has nine brains that have their own agendas: each of its eight arms has a large and relatively complete nervous system, which seems barely to communicate with the other arms [2,3]. The central brain sends general executive commands to all the arms at once, but these messages lack detailed instructions, leaving the individual arms remarkable autonomy to control their own movements [4,5]. Central encoding of arm position appears to be lacking; for example, somatotopically arranged sensory and motor representations of the octopus body within its brain are absent [6]. And while octopuses can learn to use visual feedback to guide an arm to a specific location [7], visual control of more than one arm at a time is not apparent. So, without the brain or eyes telling each arm where it is and where the seven others are, some sort of local sensing and control are needed.

In a series of systematic experiments using the common octopus (*Octopus vulgaris)*, Nesher *et al.* [1] first showed that the suckers of amputated octopus arms recognize skin from the same species. Suckers attached avidly to abiotic surfaces and to potential food items, but the suckers of amputated arms neither grasped skin of their own arm nor of other arms from the same octopus or other octopuses. Strong evidence for species recognition by individual suckers came from offering to amputated arms a petri dish containing a semi-circular slice of isolated skin covering half the glass: the suckers attached firmly to the glass, but adjacent suckers touching the skin refused to attach. What is the cue that tells an octopus sucker to avoid skin from its own species? Avoidance did not occur when the researchers presented amputated arms with skinned pieces of octopus arm, indicating that cues for species recognition are in the skin. Presentation of various skin extracts suggested that the cue molecules are hydrophobic, but their identity remains a mystery.

The new findings of Nesher *et al.* [1] are the first evidence for the use of a