Evolution and cell physiology. 2. The evolution of cell signaling: from mitochondria to Metazoa

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Submitted 15 July 2013; accepted in final form 17 July 2013

Blackstone NW. Evolution and cell physiology. 2. The evolution of cell signaling: from mitochondria to Metazoa. Am J Physiol Cell Physiol 305: C909–C915, 2013. First published July 24, 2013; doi:10.1152/ajpcell.00216.2013.—The history of life is a history of levels-of-selection transitions. Each transition requires mechanisms that mediate conflict among the lower-level units. In the origins of multicellular eukaryotes, cell signaling is one such mechanism. The roots of cell signaling, however, may extend to the previous major transition, the origin of eukaryotes. Energy-converting protomitochondria within a larger cell allowed eukaryotes to transcend the surface-to-volume constraints inherent in the design of prokaryotes. At the same time, however, protomitochondria can selfishly allocate energy to their own replication. Metabolic signaling may have mediated this principal conflict in several ways. Variation of the protomitochondria was constrained by stoichiometry and strong metabolic demand (state 3) exerted by the proteokaryote. Variation among proteokaryotes was increased by the sexual stage of the life cycle, triggered by weak metabolic demand (state 4), resulting in stochastic allocation of protomitochondria to daughter cells. Coupled with selection, many selfish protomitochondria could thus be removed from the population. Hence, regulation of states 3 and 4, as, for instance, provided by the CO2/soluble adenylyl cyclase/cAMP pathway in mitochondria, was critical for conflict mediation. Subsequently, as multicellular eukaryotes evolved, metabolic signaling pathways employed by eukaryotes to mediate conflict within cells could now be co-opted into conflict mediation between cells. For example, in some fungi, the CO2/soluble adenylyl cyclase/cAMP pathway regulates the transition from yeast to forms with hyphae. In animals, this pathway regulates the maturation of sperm. While the particular features (sperm and hyphae) are distinct, both may involve between-cell conflicts that required mediation.

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PERSPECTIVES IN ANIMAL PHYLOGENY AND EVOLUTION

The Endosymbiont Hypothesis

Early and late 20th century discussion of the endosymbiont theory seems to share a disdain of Darwin’s theory, in general, and the notion of conflict, in particular. For instance, Wallin (52) wrote, “Modern writers have recognized the insufficiency of Darwin’s hypothesis to explain the origin of species. The ‘unknown factor’ in organic evolution has been especially emphasized by Osborne, Bateson, Kellog, and other recent writers. This ‘unknown factor’ is especially concerned with the origin of species.” Over a half-century later, and subsequent to Margulis’ resurrection of the endosymbiont hypothesis (31), Margulis and Sagan (32) wrote, “Next, the view of evolution as chronic bloody competition among individuals and species, a
that allocates energy to its own selfish replication. If such variant protomitochondria are not held in check by mechanisms of conflict mediation, the symbiosis will fail.

Discussion of conflict mediation has largely focused on the transfer of the mitochondrial genome to the nucleus (27). Doubtless, this transfer and the consequent reduction in the mitochondrial genome and mutational space did diminish the heritable variation of the lower-level units. Close examination, however, reveals difficulties with the hypothesis that genome loss was the primary mechanism of conflict mediation (8). First, all modern mitochondria that function in energy conversion retain a small genome (2, 3), so they retain some heritable variation. Second, before a functional protomitochondrion can lose any heritable variation, the mitochondrial protein-import apparatus has to evolve. This is a nontrivial system. Furthermore, it is difficult to conceive of selection driving its formation, except in a protoeukaryote that had already mediated conflicts and begun to emerge as a higher-level unit. Generally, the role of selection presents the biggest obstacle for genome loss evolving to mediate conflicts: if there is a selfish advantage for a protomitochondrion to retain a gene, how did selection then lead to gene loss? In this context, various arguments can be contrived, but as long as strong selection acted on promitochondria, loss of heritable variation remained unlikely (8).

In contrast, metabolic regulation seems considerably more plausible (8). Energy metabolism can be regarded as a series of living redox couples that are linked to environmental sources and sinks of electrons (2). To the extent that replication requires energy (5, 29, 47), replication is regulated by the environment. In the case of the protomitochondria, the environment is the cytosol. A symbiosis based on metabolic complementation (17) provides a predictable framework for the interaction of the mutualistic partners (Fig. 2). Substrate is taken up into the cytosol. Energy-converting processes then oxidize the substrate, resulting in the reduction of an electron carrier. The reduced electron carrier is subsequently taken up by a protomitochondrion and oxidized by its energy-converting processes. Finally, the oxidized electron carrier is excreted by

Fig. 2. Metabolic complementation between a protomitochondrion and the cytosol of the protoeukaryote. Substrate is taken up from the environment (1) and oxidized and excreted as waste (2a). An electron carrier, X, is reduced (3) and then taken up by the protomitochondrion and oxidized (4). The cytosol and the protomitochondrion carry out energy conversion in this fashion. Stoichiometry prevents defection of the lower-level units because of the necessary complementation of redox reactions. As the protoeukaryote evolves, waste may be taken up by the protomitochondrion (2b), rather than excreted.
the protomitochondrion, and the cycle can be repeated. Both partners reap benefits from this sort of metabolic complementation. At the same time, neither partner can easily defect from the cooperative aspects of the relationship without losing the benefits as well. Such metabolic complementation constrains the variation of the protomitochondria through simple stoichiometry. Evolution of selfish protomitochondria in these circumstances thus seems unlikely.

Given that the central limitation of prokaryotes is the presence of energy-converting membranes on the outside of the cell, a key innovation of eukaryotes was the loss of the energy-converting function of the plasma membrane. Once this occurred, the cytosol could not be supplied with ATP derived from chemiosmosis. The cytosol could still carry out substrate-level phosphorylation. If the environment is very rich in electron donors, substrate-level phosphorylation may provide an efficient mechanism to exploit it (e.g., yeast and cancer cells). Indeed, since size increase likely drove the evolution of eukaryotes (8, 12), the protoeukaryote was likely larger than all other contemporaneous cells. Consuming these smaller cells may have provided the protoeukaryote with abundant substrate. Nevertheless, at some point, a protoeukaryote population would likely deplete these abundant resources. In a somewhat depleted environment, substrate-level phosphorylation in the cytosol would be out-of-balance with oxidative phosphorylation in the protomitochondria. Energetic accounts could be rectified if, periodically, some of the protomitochondria were consumed by the cytosol. This would translate into a lower mortality rate. If ANT-containing protomitochondria replicated at the same rate as those that did not contain ANT, the ANT-containing protomitochondria would come to predominate because of lower mortality rates (Fig. 3). Ion exchangers that diminish the transmembrane proton gradient (e.g., the mitochondrial Ca$$^{2+}$$ unipporter) also simulate metabolic demand and may have evolved under selective dynamics similar to ANT.

Likely more than any other factor, the evolution of ANT-containing protomitochondria transformed the protoeukaryote. Energy conversion in the cytosol became less and less important. Increasingly, the cytosol became devoted to two tasks: 1) providing protomitochondria with substrate and 2) converting the ATP exported from protomitochondria back to ADP. As discussed above, given its position at the top of the food chain, the protoeukaryote was likely well equipped for task 1. Equally crucial was task 2; if the cytosol could not utilize the available ATP, ANT-containing protomitochondria would lose their advantage over those that did not contain ANT. Successful protoeukaryotes likely consumed this ATP by maintaining a high replication rate and by developing an energetically expensive store of information (29, 30).

Nevertheless, under some environmental conditions, the metabolic demand of the cytosol may have slackened. Without ATP to import, even ANT-containing protomitochondria would enter state 4. ANT-less protomitochondria would no longer be at a disadvantage. The extensive production of reactive oxygen by the community of protomitochondria, possibly enhanced by cytochrome c release (9), may have caused sufficient damage to the protoeukaryote, such that a sexual phase of the life cycle would have evolved (10). As commonly found in unicellular eukaryotes, sex likely involved fusion of two protoeukaryotes and recombination. Sex also likely randomly apportioned protomitochondria to the two daughter cells. By chance, the variance between the higher-level units may be increased, so that the ANT-less protomitochondria would be disproportionately found in one of the daughter cells.

![Diagram of protomitochondria](http://ajp-cell.physiology.org/)
Subsequent selection would, of course, favor the more cooperative group, i.e., the protoeukaryote with a greater proportion of ANT-containing protomitochondria.

To summarize, once ANT evolved, conflict in the protoeukaryote was mediated by metabolic state. High metabolic demand led to state 3 and constrained the variation of the lower-level units, favoring cooperation. Low metabolic demand led to state 4 and increased the variation of the higher-level units via sex. Selection subsequently favored the more cooperative protoeukaryotes. Given the critical nature of the state 3-to-state 4 transition, selection for additional mechanisms that regulate this metabolic transition would occur.

In this context, consider the CO2/soluble adenylyl cyclase (sAC)/cAMP pathway (Fig. 5A). In eukaryotic mitochondria (1), CO2 produced by the oxidation of substrate in the tricarboxylic acid (Krebs) cycle is converted by carbonic anhydrases to bicarbonate. Bicarbonate, in turn, stimulates sAC to form cAMP. cAMP then activates protein kinase A, which phosphorylates various mitochondrial proteins that are integral to or associated with the electron transport chain. As a result, the oxidation of substrate and reduction of coenzymes closely correspond to the activity of the electron transport chain. If a large quantity of substrate is oxidized and the NADH pool becomes relatively reduced, high levels of activity of the mitochondrial electron carriers quickly oxidize the NADH. Metabolic state 3 is thus maintained and state 4 is avoided even as the substrate supply rapidly changes.

The CO2/sAC/cAMP pathway is found in modern cyanobacteria (13) and may have been present in the ancestors of the protomitochondria prior to the endosymbiosis. No doubt, this pathway provides efficient utilization of substrate and regulation of reactive oxygen species production in free-living bacteria. As the endosymbiosis progressed, however, these functions became even more critical. As the cytosol became more specialized in providing substrate for protomitochondria, at times the supply of substrate may have strained the capacity of protomitochondria to process it. At the same time, the association between metabolic state 4 and consumption by the cytosol required efficient metabolic regulation. The CO2/sAC/cAMP pathway...
pathway thus became a key component of metabolic conflict mediation in the emerging protoeukaryote.

**Cell Signaling in Multicellular Eukaryotes**

The evolution of multicellularity has much in common with the evolution of eukaryotes (35, 36). Both transitions involved selection for increased size and concomitant ecological advantages (27). As in eukaryotes, lower-level units in multicellular organisms can be selfish in terms of energy and replication. Nevertheless, there is one striking difference between these major transitions: eukaryotes only evolved once and, hence, are monophyletic, while multicellular eukaryotes evolved many times in diverse eukaryotic lineages. Mediation of conflicts in the origin of eukaryotes appears to have been extremely challenging but, by contrast, was relatively easy in the origin of multicellular eukaryotes (8). This difference seems even more remarkable, in that a structure analogous to the eukaryotic nucleus never evolved in any multicellular lineage. In a multicellular organism, levels-of-selection conflicts thus can never be mediated by genome transfer. On the other hand, while metabolism of a multicellular organism is vastly more complex than metabolism of a single cell, metabolic signaling can still be employed to mediate conflicts. In this case, pathways of metabolic regulation in eukaryotes can be coopted again and again as multicellularity evolves repeatedly.

Consider again the CO$_2$/sAC/cAMP pathway (Fig. 5). CO$_2$/sAC/cAMP signaling is found in animals, where, among many other functions, it influences the development of sperm (49). Features of sperm and spermatogenesis are derived for animals (4), so involvement of CO$_2$/sAC/cAMP in sperm development is likely also derived. At the same time, CO$_2$/sAC/cAMP signaling is also found in fungi and mediates the transition from unicellular to multinucleate, filamentous forms (24). While fungi and animals are opisthokonts, the hyphae that characterize filamentous forms do not resemble any kind of animal multicellularity. We can thus expect that hyphal structures and the role of the CO$_2$/sAC/cAMP pathway in their differentiation were independently derived from animals. Nevertheless, in both cases, germ line formation in animals and multinucleate hyphae formation in fungi, potential levels-of-selection conflicts require mediation. In a multicellular organism, constituent cells inevitably compete to form the next generation. By setting aside the cells that will do this and by limiting the mutational variation of cells, the germ line mediates conflicts in many animals (14, 37). Similarly, multinucleate hyphae invite competition among constituent nuclei. Mechanisms [e.g., septa (14)] are required to mediate this competition. Possibly such mediation evolved by co-opting a protomitochondrial pathway of metabolic signaling. These sorts of data support the larger hypothesis.

Many other potential examples can be found in signaling pathways that involve mitochondria and are also used in between-cell signaling in animals (8). For the most part, it is not known how widely distributed these pathways are in other multicellular eukaryotes. For pathways that are known to be widely distributed, unraveling the complex evolutionary histories remains challenging. Programmed cell death provides a case in point. While best known in animals, similar forms of cell death are found in plants as well (46). Possibly, these pathways evolved independently in plants and animals from primordial interactions of protomitochondria and proteo- eukaryotes involving reactive oxygen species. This seems unlikely, however, since similar processes are found fully developed in unicellular eukaryotes (41). More likely, a pathway serving a different function (or no function at all) in unicellular eukaryotes (41) was independently co-opted into a policing function in plants and animals. By this view, support is again found for the hypothesis that pathways once mediating conflicts in proteo-eukaryotes can be recruited to a similar role in multicellular organisms.

Ca$^{2+}$ signaling pathways are also known to be widely distributed in eukaryotes. In the absence of available ADP, protomitochondria may have released Ca$^{2+}$ to diminish the transmembrane proton gradient and avoid metabolic state 4. Arguably, the complex interplay between Ca$^{2+}$ signaling and metabolic state in modern eukaryotes arose from this interaction. In animals, at least, the link between Ca$^{2+}$ signaling and cell growth and proliferation (6) suggests a connection to mediation of levels-of-selection conflicts. Beyond animals, the specifics of Ca$^{2+}$ signaling are poorly known in many groups. For the many protein components of this signaling system, pumps and exchangers, plasma membrane channels, organelle channels, and buffers, among others, Collins and Meyer (15) carried out a phylogenetic analysis of the eukaryotic orthologs of the human genes for these proteins. Their analysis strongly supports the idea that the first eukaryotes utilized Ca$^{2+}$ signals and points to some interesting evolutionary patterns. For instance, Ca$^{2+}$-regulated PLC$_6$, which can produce inositol triphosphate (IP$_3$), is more broadly distributed and possibly arose earlier than the IP$_3$ receptor. The hydrolysis of phosphoinositide lipids by PLC may thus have had more ancient roles than the current role of producing IP$_3$ to bind to the IP$_3$ receptor and trigger Ca$^{2+}$ release. As eukaryotes diversified, signaling pathways themselves underwent substantial evolutionary change.

In any event, additional clarification of the role of Ca$^{2+}$ signaling, as well as other mitochondrial pathways in levels-of-selection conflicts, awaits further work in diverse multicellular eukaryotes. Some added insight into the role of metabolic signaling, however, can be obtained by pathophysiological evidence in animals. For instance, in diseases that involve levels-of-selection conflicts, is metabolic regulation also perturbed? Extensive discussion of the “Warburg effect” in recent years suggests that such a connection may indeed exist. The increasing recognition that the metabolic features of cancer cells may be generally found in proliferating mammalian cells does not diminish the significance of this connection. For instance, evidence suggests that the mitochondria of highly proliferative macrophages are inhibited by nitric oxide (43). In contrast to the above discussion of proteo-eukaryotes, proliferation thus seems to correspond to state 4, while differentiation corresponds to state 3. Such a correspondence of metabolic state and proliferation makes sense, given the much greater access of animals than single cells to large quantities of substrate. Highly proliferative glycolytic cells in state 4 are heavily dependent on a rich stream of nutrients. They require these nutrients from the organism and, thus, may be more easily regulated by the higher-level unit. It follows that cancer cells are characterized by mutations that allow them to trigger vascularization and circumvent the mechanisms that would usually limit their access to the necessary nutrient resources (51).
CONCLUSIONS

The origins of eukaryotes and of multicellularity show a number of parallels, yet they diverge sharply in one respect: the latter evolved a number of times, while the former evolved only once. Evolution is a historical process: could it be that multicellularity is a relatively simple transition precisely because it was preceded by the origin of eukaryotes? Since conflict mediation is a crucial aspect of every major transition, multicellular organisms may have repeatedly co-opted mechanisms of conflict mediation that evolved during the origination of eukaryotes. By this view, signaling pathways that mediate conflicts in multicellular organisms may trace their origin to the dynamics of conflict and mediation in protoeukaryotes. In particular, the metabolic regulation that characterized the emergence of eukaryotes may also pervade cell-cell signaling in multicellular eukaryotes. A greater emphasis on comparative studies of cell signaling among the diverse lineages of multicellular eukaryotes is needed to further test this hypothesis.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

N.W.B. prepared the figures; N.W.B. drafted the manuscript; N.W.B. edited and revised the manuscript; N.W.B. approved the final version of the manuscript.

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