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

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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 protoeukaryote. Variation among protoeukaryotes 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. In animals, this pathway regulates the maturation of sperm. While the particular features of this pathway may be expected between these seemingly distinct major transitions. Such parallels may be particularly likely, because each transition requires mechanisms that mediate conflict among the lower-level units. Conflicts arise because selection on lower-level units can favor competition, rather than cooperation. Competition for substrate is particularly likely; the lower-level unit that obtains more substrate can achieve an advantage in replication rate and outcompete other lower-level units. As described by Michod (38), conflicts can be mediated by mechanisms that decrease heritable variation of the lower-level units (making selfish behavior less likely to evolve) or increase heritable variation of higher-level units (making cooperative groups more likely to be favored).

In the many independent origins of multicellular eukaryotes, cell signaling can be regarded as a mechanism of conflict mediation (see epigraph; 14, 37, 40). For instance, signaling pathways that govern germ line formation and sequestration limit the extent to which selfish lower-level units can form by mutation and gain access to the next generation. More broadly, any pathway that downregulates cell division also diminishes the likelihood that selfish lower-level units will form by mutation. Policing (e.g., programmed cell death pathways) provides an alternative mechanism to hold selfish lower-level units in check.

Were such signaling pathways created de novo? An evolutionary view might deem this unlikely, since similar conflicts had to be mediated in the origin of eukaryotes. Perhaps eukaryotic multicellularity could be achieved so easily relative to the origin of eukaryotes precisely because the latter preceded the former (8). By this view, the roots of cell signaling in all multicellular eukaryotes may be found in the mechanisms that mediated conflict in early eukaryotes. Hence, to understand the roots of cell signaling, the mechanisms of conflict mediation in eukaryotes must be understood. To this end, the endosymbiotic hypothesis will be briefly reviewed, with attention to its beginnings as an alternative to “Darwinian” conflict. Then the nature of the conflicts, as well as likely mechanisms of conflict mediation, will be examined. Finally, the relevance to cell signaling in multicellular eukaryotes will be revisited.

The Endosymbiont Hypothesis

Early and late 20th century discussion of the endosymbiotic 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, Kellogg, 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 endosymbiotic hypothesis (31), Margulis and Sagan (32) wrote, “Next, the view of evolution as chronic bloody competition among individuals and species, a
popular distortion of Darwin’s notion of ‘survival of the fittest,’ dissolves before a new view of continual cooperation, strong interaction, and mutual dependence among life forms. Life did not take over the globe by combat, but by networking. Life forms multiplied and complexified by co-opting others, not by killing them.” Interestingly, despite the extensive development of level-of-selection theory between 1986 and 2001 (14, 23, 35–37, 44), Margulis did not seem to change her views and later published an identically worded statement (33).

Margulis’ endorsement of the endosymbiont theory met with extensive scientific criticism (11, 45, 50). Perhaps, surprisingly, none of these authors criticized the endosymbiont hypothesis on general evolutionary grounds, i.e., because stable symbiosis was unlikely due to conflicts between levels of selection. This is particularly surprising, because, in the 1960s, and 1970s, there was near-absolute condemnation of any kind of group-selection thinking in evolutionary theory (34, 53). As nucleotide sequence data built overwhelming support for the endosymbiont hypothesis (20, 48, 55), the issue of conflict began to emerge. As pointed out by Williams (54), “The subsequent stability of these eukaryotic cell lineages through geologic time, despite potential disruption from selection among cellular components, presents an evolutionary problem that deserves detailed attention.” Earlier (16) and later (7) comments in the same vein had equally little impact. Rather, the empirical discovery of the role of mitochondria and cytochrome c release in programmed cell death (25, 56) firmly established the notion of conflicting relationships in the formation of the eukaryotic cell (18, 26, 39). A careful reexamination of the evolutionary conflicts inherent in the origin of eukaryotes constitutes the first step toward consideration of the mediation of these conflicts.

Conflict and Mediation in the Origin of Eukaryotes

Prokaryotes suffer from at least one serious design limitation: their energy-converting membranes are on the outside of the cell. Any increase in size thus results in relatively less surface for carrying out energy conversion and relatively more volume requiring such conversion (28). These constraints can be transcended, if small, energy-converting cells reside within a larger one. A clever engineering solution to surface-to-volume constraints, however, results in a levels-of-selection nightmare. Because the protomitochondria now carry out energy conversion and allocation, selection will inexorably favor the protomitochondrion 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 protomitochondria, 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 protomitochondrion, 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

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Fig. 1. Two higher-level units (large circles) containing two kinds of lower-level units (● and ○). In the emergence of a higher-level unit, conflicts among the lower-level units can be mediated in two ways (38): 1) by mechanisms that decrease the heritable variation of the lower-level units (making selfish behavior less likely to evolve) or 2) by mechanisms that increase the heritable variation of higher-level units (making cooperative groups more likely to be favored by selection).

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. Indeed, such a process occurs in modern cells in the form of mitophagy (19).

In an environment that provided sufficient, but not unlimited, substrate, the imbalance between the cytosol and the protomitochondria might have other consequences. If the cytosol were carrying out substrate-level phosphorylation while the protomitochondria were carrying out oxidative phosphorylation and if both were utilizing the same (limited) substrate, the protomitochondria were carrying out oxidative phosphorylation while the protomitochondria were consuming the cytosol, balancing the energy budget of the protoeukaryote. Under these circumstances, the evolution of the adenine nucleotide translocator (ANT) would be adaptive for the individual protomitochondrion. Because ANT exchanges ATP in protomitochondria with ADP in the cytosol, a protomitochondrion with ANT could effectively generate metabolic demand, maintain metabolic state 3, and avoid state 4. Protomitochondria with ANT would be less likely to damage themselves and be 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\(^{2+}\) uniporter) 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 proprotoeukaryote. 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 proprotoeukaryote 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 proprotoeukaryotes 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 ADP 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 proprotoeukaryote, 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.
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 CO$_2$/soluble adenylyl cyclase (sAC)/cAMP pathway (Fig. 5A). In eukaryotic mitochondria (1), CO$_2$ 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 NAD$^+$ 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 CO$_2$/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 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 CO$_2$/sAC/cAMP pathway was co-opted to mediate conflicts between cells in germ cell formation and maturation in animals (B) and between nuclei in the transition from yeast to hyphal growth in fungi (C).
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 CO2/sAC/cAMP pathway (Fig. 5). CO2/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 CO2/sAC/cAMP in sperm development is likely also derived. At the same time, CO2/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 CO2/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 promitochondria and protoeukaryotes 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 protoeukaryotes can be recruited to a similar role in multicellular organisms.

Ca2+ signaling pathways are also known to be widely distributed in eukaryotes. In the absence of available ADP, protomitochondria may have released Ca2+ to diminish the transmembrane proton gradient and avoid metabolic state 4. Arguably, the complex interplay between Ca2+ signaling and metabolic state in modern eukaryotes arose from this interaction. In animals, at least, the link between Ca2+ signaling and cell growth and proliferation (6) suggests a connection to mediation of levels-of-selection conflicts. Beyond animals, the specifics of Ca2+ 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 Ca2+ signals and points to some interesting evolutionary patterns. For instance, Ca2+-regulated PLC, which can produce inositol triphosphate (IP3), is more broadly distributed and possibly arose earlier than the IP3 receptor. The hydrolysis of phosphoinositide lipids by PLC may thus have had more ancient roles than the current role of producing IP3 to bind to the IP3 receptor and trigger Ca2+ release. As eukaryotes diversified, signaling pathways themselves underwent substantial evolutionary change.

In any event, additional clarification of the role of Ca2+ 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 protoeukaryotes, 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 the manuscript; N.W.B. approved the final version of the manuscript.

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