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Mitochondria Are Fundamental for the Emergence of Metazoans: On Metabolism, Genomic Regulation, and the Birth of Complex Organisms

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Abstract

Out of many intracellular bacteria, only the mitochondria and chloroplasts abandoned their independence billions of years ago and became endosymbionts within the host eukaryotic cell. Consequently, one cannot grow eukaryotic cells without their mitochondria, and the mitochondria cannot divide outside of the cell, thus reflecting interdependence. Here, we argue that such interdependence underlies the fundamental role of mitochondrial activities in the emergence of metazoans. Several lines of evidence support our hypothesis: (*a*) Differentiation and embryogenesis rely on mitochondrial function; (*b*) mitochondrial metabolites are primary precursors for epigenetic modifications (such as methyl and acetyl), which are critical for chromatin remodeling and gene expression, particularly during differentiation and embryogenesis; and (*c*) mitonuclear coregulation adapted to accommodate both housekeeping and tissue-dependent metabolic needs. We discuss the evolution of the unique mitochondrial genetic system, mitochondrial metabolites, mitonuclear coregulation, and their critical roles in the emergence of metazoans and in human disorders.

1. INTRODUCTION

Mitochondrial activity is essential for all tissues and the vast majority of cell types in mammals (excluding red blood cells). This is the reason why many mitochondrial dysfunction phenotypes are either multisystemic/pleiotropic or tissue-specific (20, 106), and the silencing of genetic factors that control replication and transcription of mitochondrial genes results in embryonic lethal phenotypes (40, 57). The unquestionable importance of mitochondrial function for energy metabolism, as well as for processes such as apoptosis, nucleotide biosynthesis, synthesis of metalloproteins, fatty acid biosynthesis, and synthesis of essential cellular metabolites, attests to the fundamental role of the mitochondria in the life and death of the cell.

All independent eukaryotes harbor mitochondria, and those parasites that do not have a mitochondrion or mitochondrion-like organelles (49) likely lost them during the course of evolution (112). While considering organisms that undergo programmed differentiation into multiple cell types and tissues (metazoans) and taking into account the essentiality of the mitochondria for the activity of such cells and tissues as well as for the life of the entire organism, one may ask whether it is possible that mitochondria played a pivotal role in the emergence of multitissue organisms, namely metazoans. Although the radiation of metazoans involved many functional and morphological aspects, one major aspect was the emergence of the irreversible embryonic developmental program, which occurred only in metazoans and neither in unicellular eukaryotes nor in prokaryotes and archaea. In this article, we argue that regulatory crosstalk between the mitochondria and the nucleus, the metabolic activities of the organelle, and mitochondrial-generated metabolites served as basic requirements for the establishment of complex organisms (i.e., those with different cell types and tissues) on earth.

2. THE CAMBRIAN EXPLOSION: WHAT MIGHT HAVE TRIGGERED THE EMERGENCE OF METAZOANS

The Cambrian Explosion ~530 million years ago still challenges paleobiologists, as there is no clear explanation for the molecular mechanism underlying the dramatic appearance of most metazoan animal phyla in the Early Cambrian era or the subsequent stability of these body plans ever since (52). Prior to considering the molecular mechanism that underlies the emergence of metazoans, it needs to be clarified that all metazoans are eukaryotes and no prokaryote has ever developed into a multitissue organism. The question why only eukaryotes developed into multicellular, highly differentiated organisms stirred a lively debate (91, 107). Nevertheless, unlike the differentiation of prokaryotic cells within a given bacterial community that some researchers have suggested (84), all metazoans possess irreversible programmed differentiation that gives rise to the various cell types and tissues that to date are not identified in prokaryotes. The origin of Eukarya has been heavily discussed in the past, yet most agree that it was accompanied by endosymbiosis between two formerly free-living organisms: the α-proteobacterial ancestor of today's mitochondria and the progenitor of all eukaryotic cells (23, 36, 56, 86, 112). The capability of the mitochondria to generate energy in the form of ATP in the oxygen-rich environment, along with the virtual absence of metazoans in the fossil record prior to the Cambrian era, stirred debate around the possibility that the rise in atmospheric oxygen levels played a role in the emergence of metazoans (67). However, the capability of a variety of metazoan taxa (such as certain Mollusca, Annelida, and Nematoda species) to live in low-oxygen environments raised doubts about the role of atmospheric oxygen levels in the emergence of metazoans (67). Nevertheless, all metazoans, including the species listed above that live in oxygen-poor environments, still require mitochondria to live, suggesting that other characteristics of mitochondrial activities are critical to sustain metazoan life and allowed their emergence.

3. TRANSCRIPTIONAL REGULATION AND TRANSCRIPTIONAL CIRCUITS ARE FUNDAMENTAL TO EMBRYO DEVELOPMENT: THE MITOCHONDRIAL CONNECTION

Previously, many essays have discussed the importance of transcription factors' activities during early development and in the differentiation of pluripotent cells to specific cell types (21, 44). Additionally, a role for other regulatory factors, such as small RNAs (76), in driving differentiation in general, and in the emergence of metazoans in particular, was suggested (76). Other studies of early embryogenesis suggest that transcriptional regulation is organized in circuits that are in constant crosstalk with chromatin remodeling (44). The eukaryotic chromatin has a direct restrictive impact on gene expression, i.e., opened chromatin correlates with active gene expression whereas compact chromatin generally associates with gene silencing (94). Although the genome is largely identical in sequence between cells of the same individual, chromatin accessibility patterns differ between tissues and cell types (78). The germline chromatin accessibility pattern is largely erased upon fertilization and is remodeled during the course of embryogenesis, especially during differentiation. Chromatin remodeling during embryogenesis and differentiation is especially important for the regulation of differential gene expression among tissues, which is exemplified by the sequence conservation and activity of most classes of bilaterian transcription factors in the most ancient living metazoans: sponges (93), cnidarians, and placozoans. Moreover, comparisons of the transcriptomes, fates, and behaviors of primary sponge cell types with unicellular holozoans demonstrate similarity between sponge cell-type conversions and the temporal cell-state changes that occur in unicellular holozoans (90). This suggests that the first animal cell was most probably pluripotent and was capable of regulating differentiation into multiple cell states in a manner similar to modern stem cells.

Regulation of differentiation requires chromatin remodeling. The latter requires specific modifications of the DNA molecules themselves (mainly methylation) and the DNA-coating proteins (both methylation and acetylation), especially the histones. Strikingly, the building blocks that are required for these chromatin modifications (i.e., the methyl and acetyl residues) largely originate from mitochondrial metabolites (see Section 4).

4. METABOLITES GENERATED IN THE MITOCHONDRIA ARE ESSENTIAL COMPONENTS OF CHROMATIN REMODELING

Multiple covalent modifications are identified on chromatin and directly on nucleic acids (DNA and RNA) and proteins, of which methylation, phosphorylation, SUMOylation (110), ubiquitination, and acetylation are largely understood, but less-studied modifications, including glycosylation, crotonylation, and succinylation, are also known to carry functional consequences (reviewed in 81). Introduction and removal of these modifications are, with some exceptions, catalyzed by enzymes whose cofactors and regulators are part of metabolic pathways. As an example, several chromatin-modifying enzymes, such as the Sirtuin protein family, require mitochondrial-generated nicotinamide adenine dinucleotide (NAD+) for their activities (1); this lends the first clues for the connection between mitochondrial metabolism and chromatin remodeling. Interestingly, the building blocks of chromatin modifications, such as acetyl and methyl residues, stem from the intermediate mitochondrial metabolites S-adenosylmethionine (SAM), which has to be transported into the mitochondria where it transfers its methyl group and is converted into S-adenosylhomocysteine, and acetyl-coenzyme A (acetyl-CoA) (61), which is generated by pyruvate dehydrogenase in the mitochondrial matrix for the sake of the tricarboxylic acid (TCA) cycle. As an example, manipulation of the transcription factor c-Myc in RAT1A fibroblasts revealed the capacity of c-Myc to increase mitochondrial production of acetyl-CoA, which provided

~50% of the acetyl groups on histone H4-K16 in these cells (72). Notably, although methyl groups could be obtained from other donors, such as methyltetrahydrofolate, the methyl group from SAM, whose biosynthesis critically involves the mitochondria, is three orders of magnitude more reactive than the methyl group of N^5-methyltetrahydrofolate, thus making it much more accessible for chromatin remodelers. SAM generation is regulated by the SIRT1/c-Myc axis and is essential for mouse embryogenesis (98). Finally, an increase in mitochondrial acetyl-CoA levels has recently been reported to relieve histone acetylation patterns in cells from hypoacetylation (61).

All of this evidence suggests that the building blocks of chromatin remodeling (for both DNA and histone modifications) and the machinery that modulates chromatin changes rely on the metabolite synthesis that occurs in the mitochondria. Interestingly, the chromatin modifications that have been mentioned likely preceded the emergence of metazoans, as they already exist in baker's yeast, a unicellular eukaryote (47). Secondly, the synthesis of SAM and acetyl-CoA already occurs in bacteria, thus further attesting to their antiquity and suggesting that their adaptation for modification of histones and chromatin remodeling occurred after the radiation of eukaryotes. Considered together, chromatin remodeling most likely preceded the radiation of metazoans and preferentially used mitochondrial-generated metabolites, which are essential components of the modulation of gene expression patterns and their programming. This is of particular interest since, during early embryogenesis (four/eight-cell stage in humans, one- to two-cell stages of mouse preimplantation embryos), certain TCA cycle enzymes are found not only in the mitochondria but also transiently localized to the nucleus, which may give rise to the required acetyl residues for histone modifications on site (i.e., in the nucleus) prior to embryo implantation (74). This finding raises the question about the importance of mitochondrial activities for embryogenesis and subsequent differentiation.

5. MITOCHONDRIAL ACTIVITY IS REQUIRED FOR DIFFERENTIATION

So far, we have presented evidence suggesting that the activity of transcription factors and chromatin remodelers largely relies on mitochondrial-generated metabolites. Because transcription factors control embryogenesis and differentiation, and these are fundamental processes in metazoans, we now assess the importance of mitochondrial activities during development (**Figure 1**).

The central role of the mitochondria in the differentiation of multiple tissues and cell types, such as myocytes (42) and cardiomyocytes (18), hematopoietic stem cells (27), spermatogonia (103), and neurons (51), has been thoroughly discussed (30, 31) (**Figure 1**). First and foremost, the requirement of mitochondrial activities for differentiation is best exemplified by the impaired capability of human embryonic stem cells to differentiate when mitochondrial energy production is compromised (64). Nevertheless, not only does mitochondrial activity play a major role in differentiation, but changes in mitochondrial activity and morphology occur during, and are required for, major embryogenesis stages. First, mitochondrial morphology and cristae formation, which correlate with mitochondrial ATP production, change from the absence of cristae in the ova and in preimplanted embryos to the well-developed cristae-containing mitochondria in the blastocyst stage (reviewed in 101). This is important, as the blastocyst stage marks the final transition from maternal to zygote gene expression in many studied metazoans (96), which occurs just before gastrulation and emergence of the three germ layers (i.e., endoderm, mesoderm, and ectoderm)—the first mark for differentiation. Notably, the remaining maternal mitochondrial transcripts and proteins, as well as mitochondrial proteins that are generated in the first major zygotic gene activation prior to the blastocyst stage (during the two-cell embryo in mice) (99), drive

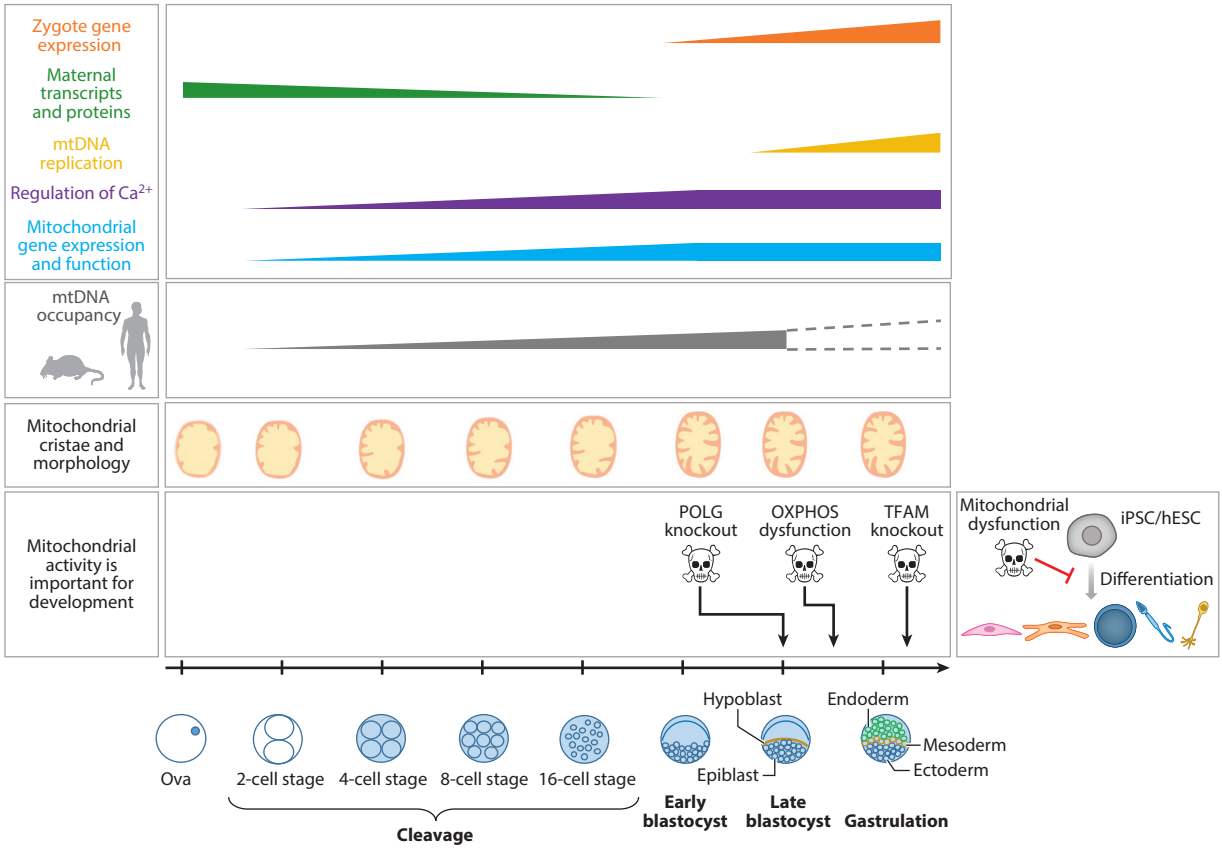

Figure 1

Mitochondrial changes during embryogenesis and the impact of mitochondrial dysfunction on development and differentiation. Embryo developmental stages (until gastrulation) are indicated on the *x*-axis. The processes varying during embryogenesis stages are indicated in the left column. Dashed lines indicate missing information. Abbreviations: hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; POLG, DNA polymerase gamma; TFAM, mitochondrial transcription factor A.

mitochondrial function and oxidative phosphorylation (OXPHOS) activity during the course of preimplanted embryonic cleavage stages. In line with these findings, large-scale RNA-seq analysis of mouse embryos during embryonic days 3.5 (blastocyst), 7.5 (epiblast), and 10.5 postfertilization revealed consistently elevated gene expression [mostly selected from the MitoCarta (12)] belonging to lipid metabolism, antioxidation, and transmembrane transport Gene Ontology terms (82). The authors of this study also observed reduced expression of master mitochondrial regulators, such as PPARGc1b and Dmc1, and glucose/amino acid metabolism and mitochondrial membrane organization genes, primarily after the transition from the embryonic day 3.5 to the embryonic day 7.5 epiblast stage. These changes were accompanied by elevated reactive oxygen species (ROS) production and epigenetic changes, particularly reflected by increased de novo promoter methylation in nuclear DNA-encoded mitochondrial genes with reduced expression levels. Furthermore, Lei An and coworkers (82) compared the above-mentioned changes between in vitro and naturally fertilized mouse embryos and found a notably higher consistency in the values obtained from healthy embryos, which correlates with the importance of mitochondrial activity for healthy

embryogenesis. Finally, during preimplantation, the mitochondria serve as hubs for regulation of intracellular free Ca^{2+}, which is critical for cell division, via their interaction with the surfaced endoplasmic reticulum (85). The pivotal role of mitochondria in modulating Ca^{2+} flux in preimplanted embryos is especially interesting in light of the importance of Ca^{2+} for the activation of embryonic deadenylation element-binding protein (EDEN-BP) in *Xenopus laevis* embryos (75), where it is a major player in degradation of maternal transcripts—the first phase prior to maternal-to-zygote transition of gene activation. Taken together, the transition from less-active to highly active mitochondria correlates with major developmental transitions during embryogenesis. However, is this just a correlation, or does it mark a much more profound involvement of mitochondrial activity in embryogenesis?

Supportive evidence for the requirement of both mitochondrial function and regulation for embryogenesis comes from knockout experiments of several core mitochondrial regulatory proteins, such as the mitochondrial DNA polymerase gamma (POLG) (40), leading to developmental arrest between mouse embryonic days 7.5 and 8.5; mitochondrial transcription factor A (TFAM), causing mouse embryonic lethality on day 10.5 (57); and mitochondrial RNA polymerase, which led to reduced brood size and impaired ova development in *Caenorhabditis elegans* (16). Secondly, TEAD4, a known regulator of embryo development, was recently found to be a regulator not only of nuclear gene expression, but also of mitochondrial DNA (mtDNA) gene expression, a phenomenon which was essential for the development of extraembryonic tissues (54). Finally, regulated mtDNA replication during oocyte maturation was essential for successful porcine embryonic development (92). All of this evidence strongly supports the requirement of mitochondrial activities and regulation for various stages of embryogenesis.

As differentiation of cell types and tissues is a fundamental metazoan trait, and since mitochondrial function is essential for differentiation (as discussed above), one may envision that better understanding of the regulation of mitochondrial activities and their interaction with the rest of the cell during the life of the organism is crucial to disentangle the role of mitochondria in the emergence of metazoans.

6. MITOCHONDRIAL-NUCLEAR CROSSTALK REQUIRES COREGULATION: ADAPTATION TO TISSUE-SPECIFIC BIOENERGETIC NEEDS

As already alluded to, mitochondrial activities are driven by genes that are encoded by both the mitochondrial and nuclear genomes. Specifically, during endosymbiosis, the α-proteobacterial ancestor of the mitochondrion lost most of its genes, which either were transferred to the host genome or were selected against and were lost during the course of evolution. As a result, most factors required for mitochondrial function (~1,500) are currently encoded by the nuclear genome (nDNA), while as few as 39 genes remained in the vertebrate mtDNA, including 13 protein-coding subunits of the OXPHOS system, 22 transfer RNAs (tRNAs), 2 ribosomal RNAs, and two long noncoding RNAs (66, 77). The protein-coding OXPHOS subunits and RNA members of the mitochondrial translation system have been shown to physically interact and coevolve with their nDNA-encoded partners (reviewed in 58). In addition, several reports argue that microRNA genes are also encoded by mammalian mtDNAs, as well as the putative peptide humanin (97), both with yet-undetermined roles. As factors encoded by the mitochondrial and nuclear genomes interact to maintain mitochondrial activities in all studied eukaryotic cell types, it is not surprising that such interactions directly impact female reproduction and embryo development in *Drosophila* (114) and have been shown to play a central role in the formation of reproductive barriers, which is a pivotal step toward the emergence of new species (34).

Interactions between factors encoded by the mtDNA and the nDNA required reciprocal adaptive processes of the host and its organelle. Such adaptation has occurred at many levels: first, the invention of a mitochondrial protein import machinery; second, the adaptation of the newly immigrating genes to the nuclear transcriptional regulatory system; third, the recoding of the new mitochondrial inhabitants in the nucleus to replace mitochondrial codons by cytoplasmic codons; and, finally, the invention of a signaling system, including signals traveling from the mitochondria to the nucleus and vice versa (i.e., retrograde and anterograde signaling, respectively—see below). All of these adaptive processes should have been accompanied by the emergence of regulatory systems that coordinate the activities of the mitochondrial and nuclear genomes. Such cooperation likely preceded the emergence of metazoans, since mitochondrial and nuclear genes are coregulated in unicellular eukaryotes (e.g., baker's yeast) (19) and across 48 different tissues in humans, a representative complex organism (5). Nevertheless, there are profound differences between the mitochondrial and nuclear genomes in terms of higher-order organization [as well as their epigenetic control (9, 69)], the multiple mtDNA copies per cell as compared to the two cellular copies of each nuclear locus, and the polycistronic transcription of mtDNA as compared to the separate gene-by-gene regulation of the nDNA. Given such (and other) differences, how does mitonuclear coregulation work, at least at the level of gene expression?

Several recent review papers, including our own, discuss this in detail (6, 39, 46, 58). In brief, it has been argued that such coordination is maintained mainly by signals traveling either from the nucleus to the mitochondria (i.e., anterograde signaling) or from the mitochondria to the nucleus (i.e., retrograde signaling) (14). Interestingly, retrograde signaling, traveling as ROS, was offered as an explanation for altered chromatin modification patterns (53, 104) and nuclear gene expression in response to elevated levels of mtDNA pathological mutations within an individual organism. However, accumulating evidence suggests an additional mechanism for mitonuclear coregulation that relies on transcription factors that localize in both the nucleus and mitochondria, bind both genomes, and regulate transcription (recently reviewed in 6). This mechanism challenges the prevailing view of mtDNA transcriptional regulation: It is currently accepted that mammalian mtDNA transcription is regulated solely by a set of mitochondrial-dedicated factors. These factors initiate mtDNA transcription at strand-specific promoters [one light-strand promoter (LSP) and two heavy-strand promoters (HSP1 and HSP2)] (10, 15, 71) via the transcription initiation complex, including the mitochondrial RNA polymerase POLRMT (32, 80, 83), transcription factors TFB2M and TFAM (28, 59), and elongation factor TEFM (39, 68). mtDNA transcription termination is currently thought to rely on the activity of the mitochondrial termination factor family MTERF (4, 25). Although all these factors are encoded by the nuclear genome, they are transported to the mitochondria and are dedicated to regulate only mtDNA.

As mentioned above, accumulating evidence suggests that apart from this small set of core mitochondrial-dedicated factors that regulate mtDNA transcription, certain known regulators of nuclear gene expression are also imported into the mitochondria, bind the mtDNA, and regulate its transcription (6). The initial discovery of such factors, including the p43 thyroid hormone receptor (24, 73), MEF2D (89), MOF (17), NFATC1 (55), and TEAD4 (54), was part of the analysis of particular cellular phenotypes caused by these specific factors. Only relatively recently a hypothesis-free screen was performed, which led to the identification of additional transcription factors (c-Jun, JunD, and CEBPB) with in vivo mtDNA binding within negatively selected sites in the coding mtDNA sequence (11). Therefore, we argue that the mechanism underlying mitonuclear coregulation involves both signaling and actual dual (mitonuclear) localization of transcription factors, which allows direct regulation of both the nuclear and mitochondrial genomes. Such an argument has evolutionary implications: Although some mitochondrial regulatory factors have bacterial orthologs, such as the structural similarity of the mitochondrial RNA polymerase

POLRMT to the bacteriophage T7 RNA polymerase (43, 83), some mitochondrial regulatory factors likely gained their mitochondrial function after endosymbiosis and were not originally in the prokaryote genome. We speculate that the regulation of mitochondrial gene expression evolved in several steps, with the core elements essential for mtDNA regulation likely being the most ancient, and the emergence of mitochondrial roles in factors that are tissue-dependent or dual-localized in the cell (i.e., mitonuclear) belongs to the later phases of adaptation. This hypothesis is supported by the finding that mtDNA binding of some of the dual-localized transcription factors occurred in a tissue-dependent manner, suggesting that they might participate in modulating differences in mitochondrial gene expression (and mitochondrial biogenesis) between tissues (5, 26, 41). Obviously, tissues became apparent only in metazoans as programmed differentiation emerged, and hence it is yet to be studied when during evolution regulators of nuclear gene expression, such as c-Jun and others, acquired their mitochondrial function and became dual-localized (i.e., mitonuclear). Tissue-dependent regulation of mitochondrial gene expression adds to the discovery of tissue-specific OXPHOS gene paralogs (3, 5, 102) and splice variants (5, 22, 38), which reflect posttranscriptional tissue differentiation in mitochondrial activities. Taken together, these findings further support the essentiality of coordinated mitonuclear regulation, not only for general cell activities, but for the emergence of tissue-specific bioenergetics needs—a major outcome of the transition from unicellular organisms to metazoans.

It is well known that chromatin accessibility and gene regulation evolved during the course of embryogenesis and regulate cell-type differentiation. Specifically, genome-wide analysis of chromatin accessibility using DNase-seq and ATAC-seq reveals changes in chromatin accessibility patterns during embryogenesis, which associate with altered gene expression (62, 111). Our analysis of both DNase-seq and ATAC-seq experimental datasets revealed a gradual increase in mtDNA occupancy during the course of human and mouse embryo development (65). This is interesting, as conserved patterns of mtDNA occupancy were discovered in multiple human samples that associate mainly with known regulatory elements of mtDNA transcription (9). These findings suggest that regulatory crosstalk between the nuclear and mitochondrial genomes is not limited to gene expression but might extend to chromatin remodeling in adult tissues and during the course of embryogenesis. Notably, although mitonuclear coexpression and coregulation have recently been demonstrated by RNA-seq analysis of multiple adult human tissues (5), it will be interesting to assess mitonuclear coregulation of gene expression during the course of embryogenesis, especially during the various differentiation steps.

7. MITOCHONDRIAL RESPONSE TO ENVIRONMENTAL CUES

One key factor that underscores the importance of mitochondrial function for the emergence of metazoans is the capability of the organelle to display programmable flexibility to environmental and physiological cues. This property is mainly due to the need to respond to differential bioenergetic requirements of tissues and cell types. Mitochondrial biogenesis and function are affected by a variety of environmental conditions. For example, mitochondrial activity responds to conditions such as a high-fat diet (60), changes in carbon source (109), ultraviolet light (50), climate (13, 70), and changes in atmospheric oxygen levels (87). Recent analysis of the regulatory response of the mitochondria to carbon source shift in yeast revealed that mitochondrial translation was the first to respond to such change, followed by a later transcriptional response (19). Although these results are interesting, it is yet to be determined whether a similar response pattern will be observed in different cells and tissues from complex organisms, i.e., metazoans. Moreover, as mentioned above, tissue differences in patterns of mitochondrial gene expression, the repertoire of mitochondrial gene splice variants, and the expression of mitochondrial gene paralogs add

another level of complexity that interferes with the direct application of deductions from findings in unicellular model organisms to metazoans.

As mitochondrial activity is primarily oxidative, mitochondrial response to changes in oxygen levels requires special attention. How do the mitochondria cope with reduced oxygen concentrations (i.e., hypoxia)? The oxidative response of organisms to differences in atmospheric oxygen has been studied in the bar-headed geese (*Anser indicus*) that tend to migrate at high altitudes (88), in mitochondrial gene expression patterns of highland versus lowland populations of deer mice (*Peromyscus maniculatus*) (87), in the apparent association of certain mtDNA genetic backgrounds with adaptation to high elevation in Tibet in humans (37, 48, 100), and in glyptosternoid fishes (Sisoridae, Siluriformes) (63). How is such adaptation reflected at the cellular level? The impact of hypoxia was measured in normal human pulmonary artery endothelial cells, with special focus on TFAM (113). Zarrabi et al. (113) showed that hypoxia led to decreased expression of TFAM as well as of the transcriptional coactivator PGC-1β, an upstream regulator of TFAM. Interestingly, TFAM and another member of the PGC-1 family (PGC-1α) were among the genes with expression pattern differences between high- and low-altitude deer mice (87), thus suggesting evolutionary conservation of the phenomenon. In addition, knockdown of TFAM significantly decreased mitochondrial respiration in colon cancer cells, which correlated with upregulated glycolysis genes independent of hypoxia-inducible factor 1 (HIF1) (108).

How is such flexibility in mitochondrial function allowed in changing environmental conditions? We interpret the above-mentioned results as reflecting a suboptimal adaptation of the mitochondria to the host cell. This interpretation stems from the following logic: A suboptimal state of mitochondrial activity resembles the flexibility of organisms to cope with changing environments, compared to organisms that are fully adapted to a certain niche (8). Suboptimal activity of the mitochondria is best exemplified by the mitochondrial membrane potential: It has a very narrow range that responds to changes in ATP and ROS production, accommodating the needs of different cell types and environments encountered by the cells (33). This may explain why mitochondrial function is important for the life of the organism as a whole yet differs in activity and morphology between tissues and between organisms that encounter different environmental conditions. These characteristics of the mitochondria explain why mitochondrial dysfunction phenotypes could be both tissue-specific and systemic.

8. MITOCHONDRIAL DISEASES: SYSTEMIC AND TISSUE-SPECIFIC PHENOTYPES

The fundamental role that is attributed to mitochondria in the radiation of metazoans predicts that mitochondrial dysfunction is rarely benign. Indeed, mitochondrial dysfunction phenotypes range from early to late onset, systemic to tissue-specific manifestations, and mild to severe diseases (20, 105). Such range could be the result of the multiple genes involved in mitochondrial function but may also stem from the differential impact of a single mutation, especially if the latter occurred in the mtDNA, mainly due to differences in the percentage of mutated mitochondria (heteroplasmy) between tissues and between affected and healthy individuals. One of the best examples for the latter is the $tRNA^{Leu}$ mutation in mtDNA position 3243 that can cause the devastating Leigh syndrome (95), or the MELAS (myoclonic epilepsy with lactic acidosis and stroke-like episodes) syndrome (35), or that may associate with the tendency to develop type 2 diabetes (2). Such phenotypic variability is consistent with the fundamental role of the mitochondria in many different tissues and during development.

But are phenotypes that include mitochondrial dysfunction limited to OXPHOS and energy metabolism? Above, we discussed the direct involvement of the mitochondria in methyl synthesis via the donor of most methyl groups in the cell, SAM. When SAM synthesis is affected in

yeast, various events of genome instability emerge (45). Accordingly, a variety of cancer types, such as mixed-lineage leukemia, display affected SAM synthesis (7). Moreover, inhibition of S-adenosylmethionine decarboxylase has broad-spectrum antiapoptotic effects (79). Also, it is noteworthy that gain-of-function mutants of isocitrate dehydrogenase, the third enzyme in the TCA cycle, lead to chromatin remodeling via hypermethylation of CTCF binding sites in gliomas (29). This adds another aspect of mitochondrial involvement in chromatin and gene expression regulation via metabolism of methyl residues. As mentioned above, chromatin remodeling also involves histone acetylation/deacetylation, which largely relies on acetyl-CoA biosynthesis: a major donor of cellular acetyl residues. Notably, mtDNA depletion in cells led to changes in acetyl-CoA biosynthesis (61). Accordingly, such changes were also associated with histone hypoacetylation, reduced activity of histone acetyltransferases, and altered expression of multiple genes. These findings further support the involvement of mitochondrial function in nuclear gene regulation and shed new light on the potential role of mitochondrial dysfunction in human disorders far beyond metabolic diseases.

9. CONCLUSIONS

Much attention has been given to the essentiality of the mitochondria to the emergence of eukaryotes, and many theories have been put forward to provide explanations for the possible advantage provided by the mitochondria for the emerging eukaryotic cell as well as for intracellular complexity. However, much less consideration has been given to the possible role the mitochondria had in the radiation of metazoans, i.e., organisms with multiple cell types and tissues and programmed differentiation. We addressed this possibility by integrating data from the fields of developmental biology, bioenergetics, biochemistry, genetics, and genomics. In brief, we summarized findings that demonstrate the essentiality of mitochondrial function for early embryonic development and for tissue differentiation. Then, based on the notion that the well-known transcriptional controls of such events depend on chromatin modifications and transcriptional regulation, we assembled evidence supporting the idea that chromatin modifications rely on methyl and acetyl residues, both originating from mitochondrial activities. Finally, we argued for the role of coordinated regulation between mtDNA- and nDNA-encoded factors in the adult, in healthy embryogenesis and differentiation, and in response to environmental cues.

These pieces of evidence support our thought that mitochondrial regulation and function are fundamental for the emergence, maintenance, and differentiation of cell types and tissues, and hence they likely played a crucial role in the emergence of metazoans. This hypothesis has far-reaching implications beyond evolution and basic genomics as it provides an evolutionary logic for the systemic and tissue-specific nature of the various mitochondrial disease phenotypes. It also urges the community to consider mitochondrial involvement in a variety of disorders due to not only impaired OXPHOS and ROS production but also the inherent involvement of the mitochondria in the transcriptional and epigenetic regulation of the cell. Thus, the fundamental role of the mitochondria in the emergence of metazoans explains why mitochondrial function is critical for the life and embryonic development of most metazoans and why its dysfunction has pleiotropic characteristics.

FUTURE ISSUES

1. It remains to be investigated when the regulatory factors of today's mitochondria acquired their mitochondrial role and localization.

2. The mechanism by which the regulatory factors that are localized in both the mitochondria and the nucleus operate remains unclear.
3. Because mitochondrial dysfunction hampers differentiation, will improving mitochondrial function enhance the differentiation capabilities of stem cells?
4. It will be interesting to assess the role of the mitochondria in the emergence of holozoans, the multicellular life-form that is currently believed to have preceded, and may even have given rise to, metazoans.

DISCLOSURE STATEMENT

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