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Review

Mitochondrial inheritance in yeast☆

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ABSTRACT

Mitochondria are the site of oxidative phosphorylation, play a key role in cellular energy metabolism, and are critical for cell survival and proliferation. The propagation of mitochondria during cell division depends on replication and partitioning of mitochondrial DNA, cytoskeleton-dependent mitochondrial transport, intracellular positioning of the organelle, and activities coordinating these processes. Budding yeast *Saccharomyces cerevisiae* has proven to be a valuable model organism to study the mechanisms that drive segregation of the mitochondrial genome and determine mitochondrial partitioning and behavior in an asymmetrically dividing cell. Here, I review past and recent advances that identified key components and cellular pathways contributing to mitochondrial inheritance in yeast. This article is part of a Special Issue entitled: 18th European Bioenergetic Conference. Guest Editors: Manuela Pereira and Miguel Teixeira.

1. Introduction

Most membrane-bounded organelles cannot be made de novo. Rather they grow and multiply from pre-existing organelles and must be inherited upon cell division [1]. Mitochondria are semi-autonomous cell organelles that contain their own genome encoding a small subset of mitochondrial proteins. Growth of mitochondria depends on replication and expression of the mitochondrial genome and import of nuclear-encoded proteins. Multiplication of mitochondria is facilitated by dynamin-related membrane fission proteins, while their appropriate intracellular distribution is ensured by cytoskeleton-dependent transport mechanisms. In sum, these processes are essential for inheritance of mitochondria, maintenance of bioenergetic capacity, and cell survival [2–7].

Nearly half a century has elapsed since the discovery of mitochondrial DNA (mtDNA) [8–10]. However, the molecular and cellular mechanisms of mtDNA inheritance and maintenance remain poorly understood [3,11,12]. Unlike nuclear DNA, the replication and partitioning of mtDNA are not strictly linked to the cell cycle. The mitochondrial genome encodes 13 mitochondrial proteins in humans, 8 in budding yeast, and 2 rRNAs and several tRNAs [3,13]. As these gene products include some of the core subunits of the respiratory chain complexes they are indispensable for the biogenesis of respiratory-competent mitochondria. An intact respiratory chain is essential for life in metazoan animals and humans. Thus, it is not surprising that several maternally inherited diseases are associated with mutations in the mitochondrial genome [14,15]. In addition, even healthy born individuals inevitably suffer from an accumulation of mitochondrial mutations during aging. The respiratory chain produces reactive oxygen species (ROS) as byproducts of ATP production during oxidative phosphorylation. As mitochondria are a major source for ROS, mtDNA is particularly vulnerable to ROS-induced mutations and lesions. As a consequence, gradual and progressive accumulation of mtDNA mutations leads to a loss of functional respiratory chain complexes, resulting in a decline of bioenergetic capacity and eventually age-associated pathologies and death [16]. Thus, inheritance of functional mitochondria requires replication and partitioning of the mitochondrial genome together with selection mechanisms that ensure that intact mtDNA molecules are passed on to the next generation.

The cytoskeleton is essential for intracellular positioning of mitochondria, for their ordered inheritance upon cytokinesis, and for maintenance of mitochondrial tubular shape. Depending on the organism and cell type, mitochondria interact with different cytoskeletal elements. In animal tissues, microtubule-dependent long-distance transport of mitochondria is of major importance, while actin filaments are required for local organellar movements [6,17,18]. Myosin-driven actin-dependent transport of mitochondria was described in several metazoans and higher plants. For example, Myo19 is expressed in multiple tissues of vertebrates, localizes to mitochondria and functions in actin-based mitochondrial motility [19], and plant class XI myosins colocalize with mitochondria in maize [20] and mediate mitochondrial trafficking in leaf cells of tobacco [21,22]. In fungi, the use of cytoskeletal tracks for mitochondrial movement is surprisingly diverse: Mitochondrial movement depends on microtubules in fission yeast *Schizosaccharomyces pombe* [23] and in the filamentous fungus *Neurospora crassa* [24,25], whereas mitochondria move along actin filaments in *Saccharomyces*

Abbreviations: ERMES, ER mitochondria encounter structure; MECA, mitochondria–ER–cortex anchor; mtDNA, mitochondrial DNA; ROS, reactive oxygen species

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cerevisiae [26] and in the filamentous fungus *Aspergillus nidulans* [27]. These cytoskeleton-mediated transport processes are crucial for mitochondrial inheritance during cell division.

Budding yeast *S. cerevisiae* has been used extensively to study the molecular machinery and cellular pathways that contribute to mitochondrial inheritance [12,28–30]. In this review, I highlight past and recent advances that lead to an understanding of mitochondrial inheritance in yeast as a simple eukaryotic model organism.

2. Maintenance of mtDNA

S. cerevisiae is an excellent model organism to genetically dissect the cellular and biochemical pathways required for maintenance of respiratory activity, because it is capable of satisfying its energy requirements with ATP generated by fermentation [31–33]. Thus, oxidative phosphorylation and the presence of the mitochondrial genome are dispensable as long as fermentable carbon sources, such as glucose, are present in the growth medium. Even when oxygen is available, yeast cells generate ATP primarily by glycolysis with ethanol as an end product of fermentation. However, when yeast cells are grown on non-fermentable carbon sources, such as glycerol or ethanol, respiration and the presence of an intact mitochondrial genome become essential. Mutants defective in oxidative phosphorylation form small colonies on media containing limiting amounts of fermentable carbon sources. The term *petite* has been coined to describe this characteristic phenotype [34]. Respiratory-deficient strains carrying mutations in the nuclear genome are referred to as nuclear *petite* or *pet* mutants, whereas mutants with lesions in the mitochondrial genome are referred to as cytoplasmic *petite*. Cytoplasmic *petite* mutants that have long deletions in the mitochondrial genome are termed [rho^-], mutants completely lacking mtDNA are termed [rho^0], and cells containing a functional mitochondrial genome are termed [rho^+] [35].

Most of the mtDNA in *S. cerevisiae* is present as linear molecules of variable length. It is thought that few circular mtDNA molecules serve as templates for amplification by a rolling circle mechanism forming concatemers composed of linear arrays of several genome units [10,36,37]. Some of the key components required for propagation of mtDNA have been identified and characterized (Table 1). Mip1, the ortholog of human DNA polymerase gamma (POLG), is the mitochondrial DNA polymerase in yeast [38]. In contrast to metazoans, where the mitochondrial DNA polymerase consists of a catalytic and two accessory subunits, yeast Mip1 is a single chain enzyme [39]. At least three proteins contribute to mtDNA partitioning by promoting recombination: Mhr1, a protein involved in homologous recombination in mitochondria [40], Cce1, a mitochondrial cruciform cutting endonuclease [41,42], and Ntg1, a base excision repair enzyme [43]. Intriguingly, the activity of Mhr1 and Ntg1 is also required for repair of oxidatively damaged mtDNA [44–46]. Further proteins directly involved in mtDNA metabolism include Hmi1 and Pif1, two mitochondrial DNA helicases [47,48], and Apn1, a DNA repair protein active in the nucleus and mitochondria [49]. For yet unknown reasons maintenance of mtDNA in yeast depends on the integrity of the mitochondrial translation machinery [50–52].

It is still a matter of debate whether the mitochondrial RNA polymerase, Rpo41, plays a direct role in maintenance of mtDNA [52]. The mitochondrial genome contains several origins of replication. It is assumed that these *ori* sites represent transcription start sites recognized by Rpo41, and that transcripts are then further processed to produce primers for replication [39]. However, Rpo41-independent DNA replication mechanisms clearly exist, as some [rho^-] mitochondrial genomes can be stably maintained in Δ*rpo41* null mutants [53,54]. In sum, it appears that mtDNA replication in yeast, at least in some cases, is initiated by transcription at *ori* sites and proceeds by a rolling circle mechanism that is initiated through homologous recombination. It is currently not clear to what extent different pathways of mtDNA replication initiation overlap or complement each other.

The mitochondrial genome is packaged into protein–DNA complexes. These structures are called nucleoids by analogy to DNA-organizing structures in bacteria, even though mtDNA packaging proteins probably are of eukaryotic origin [55]. *S. cerevisiae* has about 10–40 nucleoids per cell which are anchored to the mitochondrial inner membrane and evenly spaced along the mitochondrial reticulum (Fig. 1). Each nucleoid contains several mtDNA copies [3,12,52,55]. The major DNA-binding protein of yeast nucleoids is the non-histone high mobility group protein Abf2 [56]. Abf2 plays a major role in packaging of mtDNA, protects it against nuclease attack and chemical damage, and binds and stabilizes recombination intermediates [57–59]. Additional nucleoid components are the proteins required for DNA replication, transcription, repair, and recombination [55]. Other proteins that were found in nucleoids include the mitochondrial chaperonin Hsp60, which was proposed to be required for nucleoid division [60], the citric acid cycle enzyme aconitase, which was suggested to couple mtDNA maintenance with cell metabolism [61], and various other heat shock proteins, metabolic enzymes, and proteins of unknown function [3,55].

Surprisingly little is known about the cellular mechanisms of mtDNA segregation in yeast cells. During its sexual life cycle two haploid yeast cells of opposite mating type fuse to form a diploid zygote. If the parental cells contribute different mitochondrial genomes the zygote contains a mixture of mtDNAs with different genotypes, a state termed heteroplasmy. However, within few cell divisions the mtDNAs unmix, and cells become homoplasmic [3,62]. Genetic evidence suggests that only a small fraction of the mtDNA pool is transferred from the zygote to the bud, and that the position of the bud determines which parental cell contributes its mtDNA. Cells that bud from the mid-point of the zygote inherit mtDNA from both parents, whereas those that bud from either end preferentially inherit mtDNA from only one parent [3,63]. Furthermore, examination of fluorescently labeled nucleoids in zygotes indicated that nucleoids are anchored within the organelle and remain localized in distinct parts of the cell [64,65]. Thus, it is thought that diffusion of mtDNA within the organelle is limited. Instead, it is actively transported into the bud by a yet poorly characterized nucleoid segregation apparatus [3,66]. Presumably, similar mtDNA segregation mechanisms are active in zygotes and vegetatively growing cells.

3. Bud-directed mitochondrial transport

S. cerevisiae has been used extensively to study the molecular mechanisms of organelle inheritance [28,29,67–69]. During mitotic growth yeast cells multiply by asymmetric cell division, a process termed budding. At the beginning of each cell cycle cells become polarized and select a site for bud emergence. Growth is initially restricted to the bud tip and then switches to even expansion over the entire bud surface. As the bud reaches the size of the mother cell, growth is directed to the bud neck, and a septum is formed that separates the daughter cell from its mother. Correct organelle partitioning is achieved by active and directed transport of organelles to the growing bud concomitant with retention of a portion of the organelles in the mother cell [69]. Actin cables that consist of bundles of actin filaments provide the tracks for directed transport processes during cell growth. These cables are assembled by formins, conserved proteins that are located at the bud tip or bud neck and associate with the plus ends of actin filaments. Thus, polarized actin cables initially extend from the growing bud deep into the mother cell. When the bud grows larger formins are relocated from the bud tip to the bud neck and assemble cables that emanate from the bud neck and extend into the mother and daughter [69,70].

Immediately after bud emergence mitochondria enter the bud to ensure inheritance of the organelle (Fig. 1). Mounting evidence suggests that bud-directed mitochondrial movement along actin cables is driven by myosin motor proteins. Already in 1994 an ATP-sensitive, reversible actin-binding activity was detected on isolated yeast mitochondria [71]. As this interaction displayed all characteristics of actin–myosin interactions and could be blocked by pretreatment of actin filaments with

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