Mitochondrion 11 (2011) 829–838

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Review

Dynamic regulation of mitochondrial function in preimplantation embryos and embryonic stem cells

Alexandra Harvey [a,1], Tiffini Gibson [a,c], Thomas Lonergan [d], Carol Brenner [a,b,*]

[a] Department of Physiology, Wayne State University School of Medicine, Detroit, MI, 48201, United States
[b] Department of Obstetrics & Gynecology, Wayne State University School of Medicine, Detroit, MI, 48201, United States
[c] Carribbean Primate Research Center, Sebana Seca, Puerto Rico
[d] Department of Biological and Environmental Sciences, Western Connecticut State University, Danbury, CT 06810, United States

ARTICLE INFO

Article history:
Received 11 May 2010
Received in revised form 6 December 2010
Accepted 9 December 2010
Available online 17 December 2010

Keywords:
Mitochondria
Preimplantation embryo
Embryonic stem cell
Biogenesis
Dynamics
Development

ABSTRACT

Mitochondrial function is dependent upon regulation of biogenesis and dynamics. A number of studies have documented the importance of these organelles in both preimplantation embryos and embryonic stem cells (ESCs), however it remains unclear how mitochondria respond to their immediate microenvironment through modulation of morphology and movement, or whether perturbations in these processes will have a significant impact following differentiation/implantation. Here we review existing literature on two key aspects of nuclear–mitochondrial cross-talk and the dynamic processes involved in mediating mitochondrial function through regulation of mitochondrial biogenesis, morphology and movement, with particular emphasis on embryos and ESCs.

Contents

1. Introduction

Mitochondria not only provide the energy required to maintain cellular activity through oxidative phosphorylation (OXPHOS) and the provision of intermediates for glycolysis, they participate in a number of pathways that maintain cellular homeostasis, including ion homeostasis, amino acid metabolism, signal transduction and apoptosis. These mitochondrial functions have been shown to be important determinants of early embryonic development, through the regulation of spindle organization, chromosomal segregation, cell cycle and dynamic processes such as compaction, cavitation and blastocyst hatching (Thundathil et al., 2005; Van Blerkom, 2004; Wilding et al., 2001). Pre-existing oocyte components are critical during the interval between fertilization and embryonic genome activation (EGA), during which time the embryo is supported by maternally stored mRNAs, proteins and organelles (Schultz, 2005). Importantly, a significant proportion of genes required for mitochondrial biogenesis, dynamics, homeostasis and regulation of OXPHOS are encoded by

* Corresponding author. Department of Physiology, Wayne State University School of Medicine, Detroit, MI, 48201, United States.
E-mail address: cbrenner@med.wayne.edu (C. Brenner).
[1] Current address: Department of Zoology, University of Melbourne, Melbourne, VIC, 3010, Australia.

1567-7249/$ – see front matter
doi:10.1016/j.mito.2010.12.013

the nuclear genome, necessitating coordination between the two genomes to regulate mitochondrial function (Cannino et al., 2007).

mtDNA is a double stranded, circular molecule consisting of two strands, a heavy (H) and a light (L) strand, encoding 2 rRNAs, 22 tRNAs and 13 subunits of the electron transport chain (Fig. 1). As a result, nuclear encoded genes contribute significantly to mitochondrial metabolic regulation (Garesse and Vallejo, 2001). Proper expression and/or replication of mitochondrial genes is dependent on factors encoded by the nuclear genome interacting with the control region within the hypervariable displacement loop (D-Loop) of the mitochondrial genome (OH; Shadel and Clayton, 1997).

2. Mitochondrial DNA copy number in oocytes and embryos

While most mammalian cells contain between 10^3 and 10^4 copies of mtDNA, the mature mammalian oocyte contains approximately 10^5 copies (Piko and Matsumoto, 1976; Piko and Taylor, 1987), where each mitochondrial organelle contains 2–10 copies of mtDNA. In the human, premigratory primordial germ cells contain approximately 10 mitochondria per cell (Jansen and de Boer, 1998). However, the number of mtDNA copies in metaphase II (MII) oocytes has been reported to differ by over an order of magnitude, ranging from 2×10^4 to 9×10^5 (Almeida-Santos et al., 2006; Barritt et al., 2002; Chen et al., 1995; May-Panloup et al., 2005a; Reynier et al., 2001; Steuerwald et al., 2000). This variability in mtDNA copy number is thought to reflect oocyte quality. Oocytes with higher copy numbers display higher fertilization rates (El Shourbagy et al., 2006; Reynier et al., 2001; Spikings et al., 2006) than those with lower copy numbers. Furthermore, it has also been shown that there is a significantly lower mtDNA copy number in arrested 2-cell embryos compared with embryos that develop further (Almeida-Santos et al., 2006). While it is unclear whether reduced mtDNA copy numbers are a cause or consequence of low oocyte quality, there appears to be a minimum number of mtDNA molecules required for embryo development; approximately 100,000 copies in the mouse and pig (El Shourbagy et al., 2006; Piko and Taylor, 1987). Recently, Wai et al. (2010) reported that while mouse oocytes with as few as 4000 copies of mtDNA could be fertilized and progress normally through preimplantation development to the blastocyst stage, a threshold of 40,000 to 50,000 mtDNA copies was required in the mature oocyte to support postimplantation development (Wai et al., 2010).

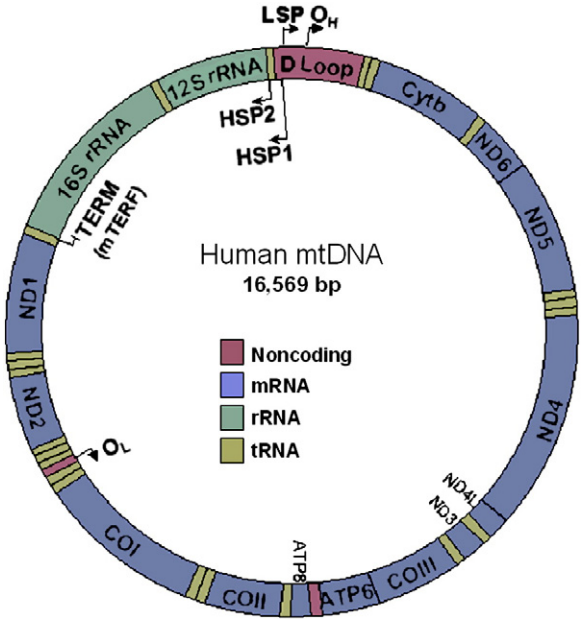

Fig. 1. Circular double-stranded mitochondrial genome (mtDNA), which encodes 13 proteins, 22 tRNAs and two rRNAs. The two strands of mtDNA are termed heavy and light. Heavy-strand transcription is initiated from two sites, HSP1 and HSP2. HSP1 produces a transcript that terminates at the 3′ end of the 16S rRNA gene (TERM). The HSP2 site is close to the 5′ end of the 12S rRNA gene and produces a polycistronic molecule, which corresponds to almost the entire H strand. Light-strand promoter (LSP) transcription produces the ND6 mRNA molecule and primers for initiation of DNA synthesis at OH. The tRNA genes encoded on each of the two strands are indicated with the standard one-letter symbols for amino acids. COI, cytochrome c oxidase subunit I; COII, cytochrome c oxidase subunit II; COIII, cytochrome c oxidase subunit III; Cytb, cytochrome b; HSP, heavy-strand promoter; ND1, NADH dehydrogenase subunit 1; ND2, NADH dehydrogenase subunit 2; ND4, NADH dehydrogenase subunit 4; ND6, NADH dehydrogenase subunit 6; OH, origin of H-strand DNA replication.
Modified from Asin-Cayuela and Gustafsson (2007) and Falkenberg et al. (2002).

In the mammalian embryo, prior to the blastocyst stage of development, mtDNA copy number remains constant (Piko and Taylor, 1987; Thundathil et al., 2005) or decreases (May-Panloup et al., 2005b), while inhibition of mtDNA replication, with ethidium bromide (Piko and Chase, 1973), or through the generation of knockout mouse models, does not prevent development until the time of implantation (reviewed by Harvey et al., 2007; Larsson et al., 1998). As a result, it is assumed that preimplantation embryos are dependent upon energy produced by oocyte-inherited mitochondria due to a lack of a mtDNA replication event occurring before the blastocyst stage (May-Panloup et al., 2005b; Piko and Taylor, 1987; Thundathil et al., 2005). Contrary to this dogma, it has been suggested that mitochondrial replication may also occur during a very short period from the 1- to 2-cell stage in the mouse embryo after fertilization (McConnell and Petrie, 2004), where inhibition of the mitochondrial polymerase (polymerase gamma), or mitochondrial ATPase resulted in decreased mtDNA content. Further modulation of mtDNA copy number was observed in fertilized oocytes through the addition of homocysteine, resulting in an increase in mtDNA. Similarly, Kameyama et al. (2007) reported that in vitro culture induced an increase in mtDNA copy number in 8-cell stage rat embryos relative to their in vivo derived counterparts. Significantly, these data suggest that culture conditions regulate mtDNA replication events, where suboptimal conditions may drive replication or alter expression of nuclear components regulating biogenesis as a means to increase metabolic activity to support further development.

3. Regulation of mitochondrial DNA replication

The nuclear contribution to mitochondria includes genes encoding catalytic and auxiliary proteins, protein import and assembly, and transcription and replication factors required to regulate the processes of mtDNA transcription and replication. Polymerase gamma (PolG), the only known polymerase responsible for mtDNA replication, consists of two subunits, a large catalytic subunit (PolGA) that harbors the 3′–5′ exonuclease repair and 5′ deoxyribose phosphate (dRP) lyase activities, and a smaller accessory subunit (PolGB) which increases both the catalytic activity and processivity of the catalytic subunit (Kaguni, 2004). PolG alone is not sufficient for mtDNA replication to occur. Twinkle (PEO1), a mtDNA helicase, appears to be the rate limiting step in mtDNA replication initiation (Spelbrink et al., 2001; Tyynismaa et al., 2005; Korhonen et al., 2003). Mutations in Twinkle lead to multiple mtDNA deletions, while inhibition of Twinkle expression in cultured cells results in rapid mtDNA depletion, and overexpression results in increased mtDNA copy number (Tyynismaa et al., 2004). Twinkle helicase activity is stimulated by mitochondrial single stranded DNA-binding protein (mtSSB), which acts to stabilize the mitochondrial genome and nucleoid structure (Korhonen et al., 2003). Together with PolG, these proteins form a minimal replisome (Kaguni, 2004; Korhonen et al., 2003). Two models of mtDNA replication exist; the strand-asymmetric model, and the synchronous leading- and lagging-strand (or strand-displacement) model, which are reviewed elsewhere (Bowmaker et al., 2003; Clayton, 1991). While no consensus on the model of replication has