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Nuclear regulation of mitochondrial functions during oocyte development

Tianqi WANG; Wanxi YANG*

The sperm laboratory, College of Life Sciences, Zhejiang University, Hangzhou, 310058, China

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Abstract: Mitochondria are important in eukaryotic cells due to their functions in energy production and regulation over other cellular activities. Oocytes are produced by a long and precisely controlled process, the dysfunction of which leads to impaired female fertility. As oocytes mature, mitochondria are constantly under the regulation of nuclear genes, the process of which can be modulated by extracellular signals. Understanding how nuclear genes regulate mitochondrial functions is important for studying animal reproduction and human fertility. As more and more genes regulating mitochondrial functions in oocytes are being revealed, new approaches for improving female fertility in both human and animals through mitochondria can be developed.

Introduction

Mitochondria have always been a hotspot for research since first observed in eukaryotic cells in the late 19th century. Later, with the invention of the electron microscope in 1956, scientists observed the pattern of yolk deposition during the development of the frog oocyte, and found unique distribution pattern of mitochondria in the cell (KEMP NE, 1956). In the next few decades, more studies observed changes of mitochondria shape, number and distribution during oocyte development in many different species (Bezzaouia *et al.*, 2014; Calarco, 1995; Dadarwal *et al.*, 2015; Kątska-Książkiewicz *et al.*, 2011; Takahashi *et al.*, 2016; van Blerkom and Runner, 1984; Wischnitzer, 1967). This organelle originated from an intrusive bacterium which then became an indispensable part for the normal development of oocytes.

Before scientists took an interest in the role of mitochondria in oocytes, the function of this organelle has already been widely studied in somatic cells. The most obvious function is related to cellular respiration, but mitochondria are also important for maintaining female fertility besides providing energy for meiosis (Amoushahi *et al.*, 2018; Krisher *et al.*, 2007). Experiments on oocytes showed that the proper functioning of mitochondria is vital for cytoplasmic calcium regulation (Marchant *et al.*, 2002; Tiwari *et al.*, 2017; Wakai and Fissore, 2019; Wang *et al.*, 2018a), reactive oxygen species (ROS) production and intracellular redox potential regulation (Dumollard *et al.*, 2007), and spindle formation during cell division (Liu *et al.*, 2016). Mitochondrial copy number is also relevant to female fertility (May-Panloup *et al.*, 2005; Reynier *et al.*, 2001; Wai *et al.*, 2010). Abnormal mitochondrial functioning can be caused by oocyte aging (Perez *et al.*, 2005) and diseases such as polycystic ovary syndrome (Qi *et al.*, 2020), thus emphasizing the necessity to learn about the mechanism of regulating mitochondrial functions during oocyte development.

The maturation process of oocytes includes meiosis I (MI) and meiosis II (MII), resulting in the formation of one oocyte and three polar bodies (PBs). This process might take from weeks to years in mammals because the development can be paused for a long time. The oocyte enters such a quiescence stage at prophase I and remains there for a long time. In prophase I, mammalian oocytes are trapped at the germinal vesicle (GV) stage until follicle stimulating hormone induce them to continue the pre-ovulatory stage. And then luteinizing hormone triggers germinal vesicle breakdown (GVBD), which is a process of nuclear envelope and nucleoli breaking down, followed by chromosomal condensation. This marks the cell's readiness to continue MI and grow into a mature oocyte. Then as the oocyte proceeds through MI and extrudes the first polar body, the oocyte remains arrested at MII, waiting to become fertilized. In both MI and MII, mitochondria aggregate around the spindle and are asymmetrically divided into two

*Address correspondence to: Wanxi Yang, wxyang@zju.edu.cn
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cells. Most of them are preserved in the oocyte when polar bodies are separated and later degenerated, avoiding the waste of this organelle (Dalton and Carroll, 2013).

Importantly, during MI and MII, patterns of mitochondrial distribution in oocytes are different among animal species (Brevini *et al.*, 2005; Dumollard *et al.*, 2007; Yu *et al.*, 2010). For instance, clustered mitochondria associated with smooth endoplasmic reticulum (SER) and lipid droplets are observed in porcine GV oocytes (Cran, 1985; McGaughey *et al.*, 1990). In contrast, mitochondria are evenly distributed in mouse and cattle oocytes before GVBD (Bavister and Squirrell, 2000; Tokura *et al.*, 1993). In human, before GVBD, spherical and oval mitochondria are absent from the cortical part of the oocyte. In mature oocytes, transverse mitochondria form mitochondrial-vesicle complexes and mitochondrial-SER aggregates, both required for fertilization and early embryogenesis (Pereda and Croxatto, 1978). Therefore, extra caution should be taken when using animal models for studying mitochondria in oocytes.

Most oocytes will remain unfertilized and eventually enter a process of apoptosis which is called post-ovulatory oocyte aging, with the remaining small portion of oocytes supporting female fertility. However, mitochondrial dysfunction will lead to pre-ovulatory aging in oocytes (Miao *et al.*, 2009), indicating the important role that mitochondria play in human oocytes. Many nuclear genes can regulate mitochondrial functions in oocytes and several extracellular signaling molecules are shown to have impacts on oocyte mitochondrial function (Abdulhasan *et al.*, 2017; Boruszewska *et al.*, 2020; He *et al.*, 2016). This review is focused on the nuclear control over mitochondria in oocytes on the basis of several different studies on the pathways and nuclear genes regulating mitochondrial functions during the maturation of oocytes.

Mitochondrial Genome

The ancestor of mitochondria in eukaryotic cells is a symbiotic α-proteobacterium. After a long time of evolving and losing more than 99% of its genome, only the necessary genes remain and a large proportion of them are now located in the nuclear genome (Selosse *et al.*, 2001). By contrast, no nuclear genes were found in mitochondria to date.

Starting from the Mitochondrial Eve, the common maternal ancestor of humans today, mitochondrial DNA (mtDNA) haplotypes were evolving over billions of years. Human mtDNA haplogroups A-Z were divided based on studies over different populations from all around the world (Ruiz-Pesini *et al.*, 2000). The two strands of mtDNA called heavy and light strand due to different density centrifugation results (Priesnitz and Becker, 2018) are semi-conservatively replicated just like nuclear DNA. The double-stranded circular DNA in human mitochondria is 16.6 kB long and contains 37 genes, encoding 2 ribosomal RNAs, 22 transport RNAs that are involved in mtDNA translation processes, and 13 essential subunits out of about totally 80 subunits in the electron transport chain (ETC) (Schon *et al.*, 2012). The other mitochondrial proteins, approximately adding up to 1500, are nuclear encoded and are produced in the cytosol.

As a semiautonomous organelle, the replication of mtDNA is not tightly coupled to the cell cycle (Clayton, 1991), and mitochondrial number varies greatly in different cells. Mitochondria are inherited maternally (Dumollard *et al.*, 2007), which means they are transmitted through the female germ line. There are more than 100,000 copies of mtDNA in mature mammalian oocytes, with each mitochondrion containing 1 or 2 copies (Legros *et al.*, 2004). Maternal mitochondria support the energy production of early embryo development, when energy provided by glycolysis is limited (Barbehenn *et al.*, 1974). By contrast, the approximately 100 copies of mtDNA in sperm are ubiquitinated and destroyed during early embryogenesis (Rojansky *et al.*, 2016) to avoid deleterious mutations of the sperm mtDNA, which might be caused by exposure to high ROS levels during spermatogenesis (Sutovsky *et al.*, 1999).

As the blastocyst differentiate to produce germ cells for the next generation, only a small portion of mitochondria can be passed on to the next generation. The number of mitochondria in each cell grows as primordial germ cells migrate and mature. This process is called the “bottleneck” of mitochondria replication, and is believed to reduce the risk of passing pathogenic mtDNA mutations to zygotes that would harm the development of the embryo. Different animal species have different sizes of mtDNA bottleneck (Cao *et al.*, 2009; Floros *et al.*, 2018; Ma *et al.*, 2014; Otten *et al.*, 2016). So far, the exact mechanism of the mtDNA bottleneck is not clear with no regulating gene identified as yet (Cao *et al.*, 2007; Chinnery *et al.*, 2000; Li *et al.*, 2016).

Nuclear Control Over Mitochondria

Several well-established cellular pathways were found to control mitochondrial functions. Studies on some individual genes have also revealed their roles in mitochondrial regulation. Understanding the nuclear control over mitochondria can help us to develop clinical applications to improve female fertility. Experiments on human oocytes are scarce due to a lack of material and ethical problems, so we collected researches on animals, mainly focusing on mouse studies (Fig. 1).

AMP-activated kinase

AMP-activated kinase (AMPK) together with MAPK can up-regulate PGC1α regulating mitochondrial biogenesis. AMPK is sensitive to cellular AMP/ATP ratio and also has an impact on cytoskeleton dynamics. In mouse oocytes, knockout of *a1ampk* disturbs the regulation of SIRT1, and the salt-inducible kinases (SIK1 and SIK2) and microtubule associated protein-regulating kinase (MARK) may compensate for some of the functions of AMPK (Bertoldo *et al.*, 2015).

The famous secondary messenger cAMP can regulate oocyte mitochondrial functions through AMPK in mouse oocytes. Concentration of cAMP depends on both the cAMP-synthesizing activities of adenylate cyclases and the degradation of cAMP by phosphodiesterases. Changes in cAMP level affects the GVBD initiator MPF. Increased cAMP in oocytes blocks MPF activation and GVBD, while increased AMP level will stimulate AMPK by phosphorylating T172 in the activation loop of the subunit.

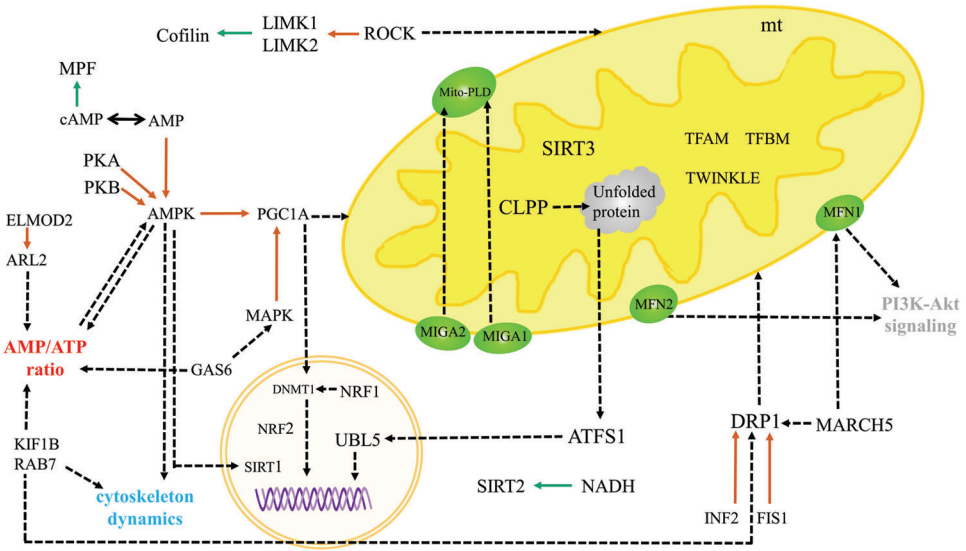

FIGURE 1. Nuclear regulation of mitochondrial functions in mouse oocyte.
All proteins reviewed are encoded by the nuclear genome. Proteins regulating mouse oocyte mitochondrial functions are distributed in cytoplasm, nucleus and mitochondria. Cytoplasmic proteins including AMP-activated kinase (AMPK), dynamin-related protein-1 (DRP1), fission protein 1 (FIS1), sirtuin 2 (SIRT2), engulfment and cell motility domain-containing protein 2 (ELMOD2), growth arrest-specific gene 6 (GAS6), and Ras related protein Rab-7 (RAB7) form pathways to regulate intracellular energy production, cytoskeleton dynamics and other activities related to mitochondrial functions. Nuclear respiratory factor-1 (NRF1), nuclear respiratory factor-2 (NRF2), sirtuin 1 (SIRT1), DNA methyltransferase1 (DNMT1), and ubiquitin-like 5 (UBL5) in the nucleus have functions in regulating mitochondrial activities. Mitochondrial transcription factor A (TFAM), mitochondrial transcription factor B (TFBM), twinkle mtDNA helicase (TWINKLE), sirtuin 3 (SIRT3), caseinolytic peptidase P (CLPP), mitofusin-1 (MFN1), mitofusin-2 (MFN2), mitoguardin 1 (MIGA1), mitoguardin 2 (MIGA2), and Mito-phospholipase D (Mito-PLD) are important nuclear-encoded mitochondrial proteins. Vermilion arrows represent activations, bluish green arrows represent inhibitions, dashed arrows represent regulative or indirect effects on the downstream proteins or other molecules, and solid double arrow represents transformation between two types of molecules.

Protein kinase B (PKB) and cAMP-mediated protein kinase A (PKA) signaling can dephosphorylate an inhibitory site of AMPK, and the activated AMPK promotes the utilization of ATP generating catabolic pathways (Stricker *et al.*, 2010).

Mitochondrial transcription factor A

Mitochondrial transcription factor A (TFAM) is a classic factor controlling mtDNA expression. It belongs to the HMG box family whose function is to package mtDNA (Alam *et al.*, 2003). This mitochondrial matrix protein contributes to the replication and transcription of mtDNA, thus making it critical for mitochondrial biogenesis during oocyte development. TFAM works together with TFBM, POLG and the mtDNA helicase TWINKLE, all of which are also located in the mitochondrial matrix (Novin *et al.*, 2015). TWINKLE, POLG and TFAM together stabilize the structure of mtDNA (Harvey *et al.*, 2007). In a mouse study, expression level of TFAM increases as the oocyte matures and then decreases when full development is achieved (Mahrous *et al.*, 2012). Human studies showed similar results and the corresponding changes in NRF expression level in oocytes (Novin *et al.*, 2015).

In the nucleus, nuclear respiratory factor-1 and -2 (NRF1 and NRF2) guarantee the coordination between nuclear and mitochondrial gene expression by regulating the expression of TFAM and many other nuclear encoded mitochondrial proteins. NRF1 can trans-activate promotors of the *tfam* gene and many mitochondrial genes encoding respiratory subunits and factors involved in the replication and transcription of mtDNA (Machatkova *et al.*, 2012). One example is DNA methyltransferase1 (DNMT1) which can be up-regulated by NRF1 and peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC1α). DNMT1 regulates mitochondrial metabolism by increasing the levels of nuclear-encoded mitochondrial proteins (Sirard, 2019).

Factors regulating mitochondrial fusion and fission

Mitofusin-1 and -2 (MFN1 and MFN2) function in the fusion of mitochondrial outer membrane, while optic atrophy 1 (OPA1) functions in the fusion of mitochondrial inner membrane. And the fission of mitochondria is mediated by cytoplasmic proteins such as dynamin-related protein-1 (DRP1) and fission protein 1 (FIS1). The two balanced processes together maintain the morphology of mitochondria in mammalian oocytes (Wakai *et al.*, 2014).

In mouse studies, *mfn1* and *mfn2* overexpression triggers mitochondrial aggregation and disturbs the distribution of the chromosomes and SER, and decreasing the Ca^{2+} storage in SER. Deletion of *mfn1* down-regulates cadherins and connexins, thus damaging oocyte-granulosa cell communication that eventually results in female infertility (Zhang *et al.*, 2019a). Knockout of *mfn2* causes bigger, rounder and fewer mitochondria and shorter telomeres (usually positively correlated with mtDNA copy number) in mice oocytes but has little effect on female reproduction (Zhang *et al.*, 2019b). Interestingly, although MFN1 alone can support female fertility, MFN1 and MFN2 have nonredundant roles in oocytes. In fact, the loss of MFN2 can partly reverse the negative effect of MFN1 loss on

female fertility. This could be explained by upregulated phosphoinositide 3 kinase-protein kinase B (PI3K-PKB) signaling, which is dysregulated in MFN1 deficient mice, and is of vital importance in the oocyte-granulosa cell communication process (Carvalho *et al*., 2020).

Mitoguardin (MIGA) is located on the outer-membrane of mitochondria and promotes the mitochondrial fusion. MIGA1 and MIGA2 performs their function by interacting with Mito-phospholipase D (Mito-PLD), which is also located on the outer-membrane of mitochondria and serves as a signaling molecule in the process of mitochondrial fusion. Knockout of MIGA and MIGA2 causes subfertile female mice with decreased mtDNA copy number and mitochondrial membrane potential (Liu *et al*., 2016).

In mouse oocytes, *drp1* knockout leads to aggregated and elongated mitochondria with less contact with SER. Thus Ca^{2+} storage in SER decreases and intercellular communication is impaired, blocking GVBD and oocyte maturation (Udagawa *et al*., 2014). Fission protein 1 (FIS1) recruits DRP1 in the cytoplasm to the outer membrane of mitochondria. INF2 also mediates mitochondrial fission by recruiting DRP1 to mitochondrial division sites (Pan *et al*., 2020).

Membrane-associated RING-CH protein 5 (MARCH5), a mitochondrial ubiquitin ligase, participates in controlling mitochondrial fission and fusion by stimulating mitochondrial elongation. Knockdown of *march5* leads to reduced DRP1 and increased MFN1 levels (Park *et al*., 2010).

Factors mediating mitochondrial distribution

The serine/threonine kinase rho-kinase (ROCK) serves as the effector of the small GTPase Rho. ROCK can phosphorylate LIM kinase (LIMK) 1 and 2 to promote the phosphorylation of Cofilin, thus inhibiting its actin-depolymerizing activity. After GVBD, ROCK is colocalized with mitochondria in mouse oocytes. Disrupting the distribution of ROCK leads to fewer mitochondria around spindles and harms the ATP supply for oocyte maturation (Duan *et al*., 2014).

An important microtubule-dependent monomeric motor, kinesin family member 1B (KIF1B) functions during mouse oocyte development. Loss of KIF1B causes reduced ATP level and disrupts mitochondrial distribution during the metaphase of meiosis (Kong *et al*., 2016).

A small GTPase RAB7 regulates mitochondria distribution and actin dynamics in mouse oocytes. It also maintains mitochondrial membrane potential during meiosis, possibly by phosphorylating DRP1 at Ser616 (Pan *et al*., 2020).

Sirtuin family

There are seven members of the sirtuin family (SIRT1-7) observed in mammals. They are NAD-dependent deacylases with a wide range of histone or non-histone targets. SIRT1, 6, and 7 are found in the nucleus, while SIRT3, 4, and 5 are in mitochondria. SIRT2 exists mainly in the cytoplasm but can also be found in the nucleus.

In mouse studies, SIRT1, SIRT 2, and SIRT 3 have been proven to regulate intracellular ROS, and the inhibition of their activity in aged oocytes lead to dramatic increases in ROS levels and decreases female fertility (Zhang *et al*., 2015; Zhang *et al*., 2016). SIRT1 has overlapping functions with SIRT3, and SIRT1 levels rises when *sirt3* gene is knocked out (Iljas and Homer, 2020).

SIRT3 regulates the acetylation of mitochondrial regulatory proteins and many other proteins in cellular metabolic pathways such as tricarboxylic acid cycles (Zhao *et al*., 2016). Less *sirt3* expression was observed in human oocytes matured *in vitro*, making it a possible target to improve assisted reproductive technology outcomes.

SIRT2, which can be competitively inhibited by NADH, can suppress the expression of many genes. SIRT2 inhibition disturbs the distribution of mitochondria, SER and cortical granules during meiosis, and can also inhibit mitochondrial biogenesis by downregulation of *tfam* in bovine oocytes (Xu *et al*., 2019).

Proteins in mitochondrial unfolded protein response

The belief that mitochondria lack methods to repair DNA was proven wrong with the discovery of several nuclear-like pathways of mtDNA repairment including homologous recombination (Benkhalifa *et al*., 2014). Even when mutations in mtDNA trigger mitochondrial dysfunction, mtUPR can prevent oocyte aging. Mitochondrial unfolded protein response (mtUPR) is activated under mitochondrial stress such as excessive ROS. Decreased efficiency of transporting mitochondrial peptides leads to accumulated unfolded proteins of various functions in the mitochondrial matrix, which will hamper cell activities (Aldridge *et al*., 2007). So increased folding or degradation, limited import and decreased translation of mitochondrial proteins can reverse this situation. Caseinolytic peptidase P (CLPP) in the mitochondrial matrix activates mtUPR, the depletion of which will activate the mTOR pathway. Misfolded proteins cleaved by CLPP are exported to the cytoplasm to activate the stress activated transcription factor 1 (ATFS1). Then ATFS1 can enter the nucleus to activate ubiquitin-like 5 (UBL5) and affect the transcription of nuclear genes (Wang *et al*., 2018b).

Other factors regulating mitochondria in mouse oocytes

Engulfment and cell motility domain-containing protein 2 (ELMOD2) belongs to the ELMOD family and has the strongest GTPase-activating activity compared to other members within the family. Its main targets are a group of typical GTPases called the ADP-ribosylation factors (ARFs), and particularly the ADP-ribosylation factor-like 2 (ARL2) with multiple aspects of impacts on mitochondria in mouse oocytes. Loss of ELMOD2 reduces ATP level and disrupts mitochondrial distribution during mouse oocyte development (Zhou *et al*., 2017).

Growth arrest-specific gene 6 (GAS6) regulates mitophagy through MTOR and BNIP3 proteins in mouse oocytes. Loss of GAS6 functioning leads to overactivation of mitochondria and impairs oocyte maturation. This process includes increased mitochondrial membrane potential, increased ATP level, increased expression of mitochondrial proteins and tyrosine-protein phosphatase non-receptor type 11 (PTPN11), and decreased expressions of mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK), B-cell lymphoma 2 interacting protein 3 (BNIP3) and other mitophagy-related genes (Kim *et al*., 2019).

Pathways regulating oocyte mitochondria in other animal models

Besides mouse models, scientist have also used many other species for oocyte studies. Some well-established pathways regulating oocyte mitochondrial functions are observed in *Xenopus*, porcines, *Drosophila* (Fig. 2) and other animal models (Paranko *et al.*, 1996). They may provide inspiration for studying mouse and eventually promotes human clinical applications, or can directly benefit modern animal husbandry by helping to improve female fertility.

Xenopus studies helped us understand the mechanisms underlying apoptosis related to mitochondria in oocytes. In mammals, aged unfertilized oocytes and oocytes encountering unfavorable conditions enter apoptosis. Apoptosis also eliminates more than 99% of germ cells in the ovary, which can be triggered by both intrinsic and extrinsic pathways. The former is mitochondria-mediated and the latter is achieved by the cell surface death receptor, and the two pathways are linked by the cleavage of BID (Yue *et al.*, 2015). The cell death process depends on both Cytochrome c (Cyt c) release from the intermembrane space of mitochondria and caspase activation. And the regulation comes from the B-cell lymphoma 2 (BCL2) family including BAX, BAD, BCL extra large (BCLXL) and myeloid cell leukemia 1 (MCL1). Cyt c is released into the cytosol, where it forms a complex with apoptotic protease activating factor 1 (APAF1) to activate procaspase 9, which then activates caspase 3. When the oocyte is stimulated by a death signal, phosphorylated BCL2 associated agonist of cell death (BAD) is translocated to the outer membrane of mitochondria, subsequently activating BCL2-associated X protein (BAX) and inactivating anti-apoptotic proteins. BAD activity is inhibited in prophase oocytes, with mediating phosphatases such as protein phosphatase 2A, protein phosphatase 1, and protein phosphatase 2C controlling the meiosis resumption. When ovulation happens, BAD is phosphorylated on Ser128 by mechanisms controlled by CDK1 and c-JUN NH2-terminal protein kinase (JNK), which will ultimately trigger apoptosis unless the oocyte is fertilized (Du Pasquier *et al.*, 2011). Mitochondrial dysfunction reduces mitochondrial membrane potential through mitochondrial permeability transition pores, triggering the release of Cyt c, apoptosis inducing factor (AIF) and endonuclease G (Tsui *et al.*, 2017).

Several factors mediating oocyte mitochondrial functions are revealed in porcine studies. One example is POLG, the dominant mutations of which lead to premature ovarian failure in clinical studies (Pagnamenta *et al.*, 2006). Both mitochondrial DNA polymerase subunit gamma 1 and 2 (POLG1 and POLG2) are linked with the proper functioning of the mitochondrial genome. The catalytic subunit encoded by POLG1 and the accessory subunit POLG2 compose a complex involved in mtDNA replication (Harvey *et al.*, 2007). The expression level of *polg2* keeps increasing during oocyte development and decreases when the oocyte comes close to maturation. Knockdown of *polg2* causes abnormal spindle and actin distribution and inhibits ATP synthesis in porcine oocytes (Lee *et al.*, 2015). TFAM initiates a process to enable POLG1 to copy mtDNA. Other factors involved in this process include POLG2, mitochondrial single stranded binding protein (mtSSB), mitochondrial-specific DNA topoisomerase I (TOP1MT), and twinkle mtDNA helicase (TWINKLE) (Korhonen *et al.*, 2003). Other proteins regulating mitochondrial functions in porcine oocytes include mitochondrial permeability transition pore (mPT), a nonselective channel in the inner membrane of mitochondria, VDAC, an anion channel in the outer membrane of mitochondria, and cyclophilin-D (Cassará *et al.*, 2009; Schatten *et al.*, 2014).

The insulin pathway studied in *Drosophila* oocyte has also been studied in mouse models but focused on other cell types. According to the fruit fly oocyte studies, activated JNK enhances insulin receptor expression and initiates the feedforward insulin-MYC signaling loop, promoting mitochondrial respiration and biogenesis by increasing the levels of electron transport chain (ETC) subunits and other mitochondrial functional proteins. It is assumed that the insulin signal stabilizes the MYC protein by inhibiting GSK activity of phosphorylating v-myc myelocytomatosis viral

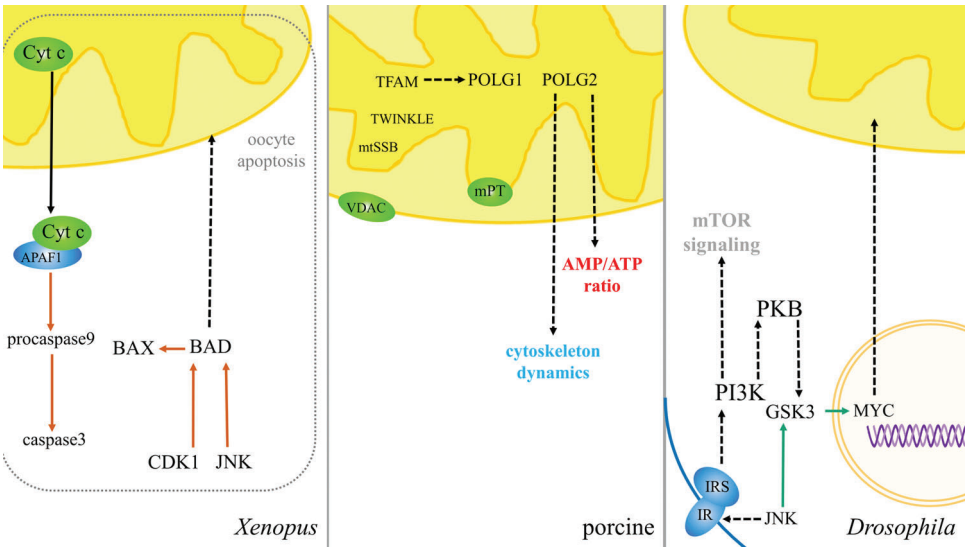

FIGURE 2. Nuclear regulation of mitochondrial functions in oocytes from different animal models. On the left is a *Xenopus* oocyte presenting the mitochondrial-related apoptosis mechanism. In the middle is a porcine oocyte representing nuclear-encoded mitochondrial proteins with regulating functions identified so far. On the right is a *Drosophila* oocyte presenting the insulin pathway regulating mitochondrial functions. Vermilion arrows represent activations, bluish green arrows represent inhibitions, dashed arrows represent regulative or indirect effects on the downstream proteins or other molecules, and solid arrow represents the transferring process of molecules.

oncogene homolog (MYC) and leading to its ubiquitination and degradation (Wang *et al.*, 2019). The insulin pathway inhibits glycogen synthase kinase 3 (GSK3), a target gene of PKB, and represses mitochondrial respiratory quiescence by adjusting the protein content in mitochondria through protein import, export and synthesis. The ecdysone signaling pathway, another pathway linked to insulin function, represses PI3K and its downstream mTOR functioning (Sieber *et al.*, 2016). Mitogen activated protein kinase kinase 2 (MAP2K2) is also identified as part of the insulin receptor-signaling pathway (Laskowski *et al.*, 2017).

Clinical Application of Mitochondria-Controlled Oocyte Development

With the development of global economy and subsequent changes in modern life styles, infertility is becoming a more and more severe threat to the wellbeing of humans and human societies. According to relevant data, female infertility is the leading cause of more than 15% couples in developed countries and 25% couples in developing countries who fail to have children in the first five years of marriage (World Health Organization 2016). With several important functions in regulating oocyte maturation discussed in the above sessions, mitochondria are currently a research hotspot for improving oocyte quality.

Scientists have found several factors that harm the quality of mitochondria in human oocytes including delayed childbearing age (Schwartz and Mayaux, 1982; Sugimura *et al.*, 2012; van Noord-Zaadstra *et al.*, 1991; Wang *et al.*, 2009), environmental heat stress (Gendelman and Roth, 2012), abnormal blood glucose level, and exposure to cigarette smoke (Grindler and Moley, 2013). For instance, exposure to cigarette smoke leads to abnormally high levels of BAX expression and ROS in oocytes. The popular assisted reproductive technology, oocyte *in vitro* maturation (IVM) also harms mitochondrial functioning. IVM procedures lead to lower mtDNA copy number and decreased Cyt c oxidase activity in oocytes, which have negative effects on reproductive outcome (Amoushahi *et al.*, 2018; Zeng *et al.*, 2009).

Mitochondrial transfer was suggested as a therapeutic strategy for infertility treatment in 1998 but was suspended by the US Food and Drug Administration in 2002 due to uncertain downstream effects (Brown *et al.*, 2006). Culture media additives such as coenzyme Q10 (Heydarnejad *et al.*, 2019), melatonin (An *et al.*, 2019), nitric oxide, and antioxidant molecule cocktails (Labarta *et al.*, 2019) can improve the embryonic development in animal models. Mitochondria-targeting transcription activator-like effector nucleases (mitoTaLens), mitochondrially-targeted zinc finger nucleases (mtZFNs), and mitochondria-targeted adeno-associated virus (mTS-aav) aimed at altering mtDNA sequences to enhance female fertility are also currently explored (Gammage *et al.*, 2014; Kleinstiver *et al.*, 2016; Yu *et al.*, 2015). Such approaches to achieve ovarian rejuvenation are still at early stage, allowing us to expect more advances and achievements in the future.

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