2. Review of Literature

2.1 Mitochondria: Emerging role of organelle in development and disease

Organelles in eukaryotic cells provide them unique specificity and variability in function during development in different types of tissue. The study of different organelle and their association during development and pathogenesis have been emerging. The organelles are not independent but their crosstalk orchestrates response to basic cellular requirement in micro-environmental and physiological circumstances. Mitochondria are only organelle in animal cells that are semiautonomous with their own genome. In 1890, Altman described that cellular granules in the cytoplasm showed similar staining as of free bacteria. This lead to hypothesis of endosymbiosis theory and suggested that mitochondria are originated from free living bacteria (Altmann, R., 1890; Martin et al., 2015). The mitochondria are named after Greek word which means thread like organelles. It sizes from 0.75 to 3 microns in diameter. The organelle is highly dynamic with constant fusion, fission and degradation as per localized cellular requirements. The energy demanding cells rapidly increase the shape by fusion dynamics and content of mitochondria. The number of mitochondria is specific to cell type and may vary from none in mature hair shaft and RBCs to thousands in neuronal cell depending on energy and specific requirements of the cells (Linch, 2009; Melser et al., 2015).

2.1.1 Mitochondria and Development

Mitochondria are known to play important role starting from cell division in somatic to meiosis in germ cells. The role of mitochondria after fertilization to differentiation and adult body homeostasis is emerging (McLaughlin and Malik, 2017). During mitotic division, mitochondria are hyper-fused and increase content in G1-S phase whereas these are highly fragmented or decreased in content in M-phase (Horbay and Bilyy, 2016). The mitochondrion contains 2-8 copies of its own circular genome. The mitochondrial copies are ~200 per primordial oocyte, which gains 1000 times when matured for fertilization (Wai et. al, 2008). The content drops down to previous state post fertilization and remains low in perimplatation stages. The mitochondria are formed from pre existing ones by fission events. The fission defects leads to midgestational lethality and improper placental

development, decreasing embryonic viability. The mitochondrial content is replenished back in trophectoderm part in blastocyst while there in decrease in the inner mass cells (Niwa, 2005). After appropriate pre-differentiation, cells obtain different contents of mitochondria depending on the tissue they constitute. The primordial cell destined to form neurons gain highest mitochondrial content followed by muscle, heart and blood while the germ cells obtain the least (John, 2014). Liver harbors 200-5000 mitochondria per cell (Esposti et al., 2012). Mitochondria also play a pivotal role in cell size determination (Yamamoto and Mak, 2017), proliferation and stemness in organ development (Folmes et al., 2012).

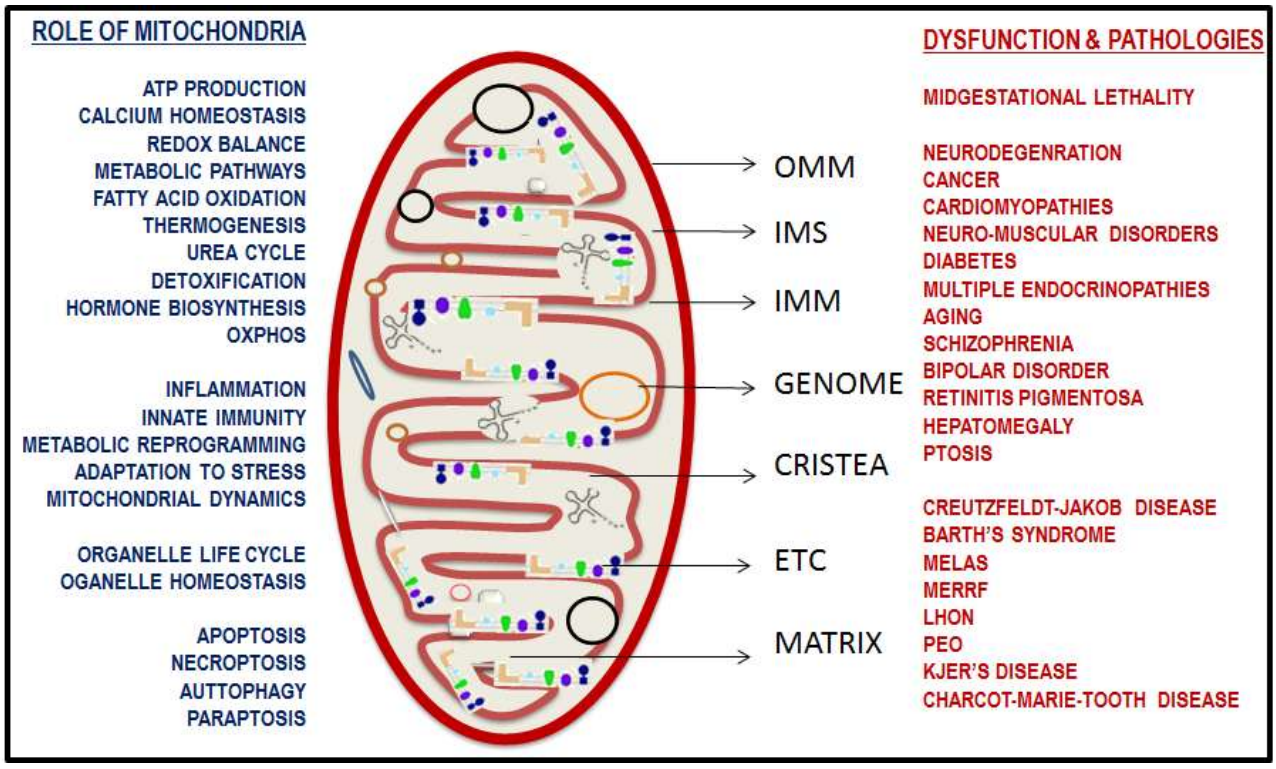

Figure 2.1. Structure and functions of mitochondria and associated physio-pathology. *The components of mitochondria are labeled. The defect in any of these parts leads to severe development and physio-pathological conditions as described. The associated functions are marked in green and pathologies caused by dysfunction are labeled in red.(OMM: outer mitochondrial membrane; IMS: Inter membrane space; IMM: Inner mitochondrial membrane; ETC: electron transport chain)*

2.1.2 Implication in physio-pathology

The mitochondria are involved in numerous cellular processes. The high energy demanding organs like brain, heart, liver and skeletal muscles are mostly affected (El-Hattab et al., 2017). Its dysfunction is observed in numerous neuro-pathological conditions like Alzheimer's disease (Mamelak et al., 2017), Parkinson's disease (Truban et al., 2017), endocrinopathy, hypoglycemia (Cardoso et al., 2013), hypoketosis (Zschocke et al., 2002), acidosis (Goyer et al., 2017), dicarboxylicaciduria, renal failure (Emma et al., 2016), diabetes (Karaa and Goldstein, 2015) and cancers (Guerra et al., 2017).

The mitochondrial genome lacks efficient DNA repair mechanism as present in DNA repair mechanisms in nuclear genome. Hence mitochondrial DNA is sensitive to mutations and their accumulation. The mutations in mitochondrial genome and mitochondrial dysfunctions are associated with multiple organ defects like Kearns-Sayre syndrome (Kim et al., 2016), Pearson syndrome (Pagon et al., 1993) and progressive external ophthalmoplegia, Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) (Hsu et al., 2016), Leber's hereditary optic neuropathy (LHON) (Pilz et al., 2016), Friedreich's ataxia (Tamarit et al., 2016), hereditary spastic paraplegia, Bejer's disease, Wilson's disease (Medici et al., 2016) and retinitis pigmentosa (Taylor and Turnbull, 2005).

2.2 Mitochondria: Important in cellular homeostasis

The bioenergetic function of the mitochondria is now well known. The role of mitochondria has been now described other than its conventional regulation in energy metabolism. The organelle precisely balances the cellular redox (the reactive oxygen species, produced as byproduct of OXPHOS) (Korzeniewski, 2016) and calcium homeostasis (Görlach et al., 2015). In synapses of neurons the motile mitochondria are stalled to provide ATP required, buffer the redox and balance calcium levels (Graber et al., 2013). The uncoupling of OXPHOS in brown adipose tissue generates heat to maintain thermogenesis of body (Chang et al., 2017).

The mitochondria are hub of metabolic pathways. The metabolic pathways are integrated to different cellular signaling pathways leading to integrated cellular signaling network. The organelle contains rate-limiting enzymes for purine-pyrimidine biosynthesis, heme synthesis, TCA cycle, and amino acid metabolism. It plays important role in detoxifying ammonia in the urea cycle and various chemicals like environmental pollutants and antibiotics in liver (Adeva et al., 2012). The organelle performs fatty acid oxidation (Houten et al., 2016) and metabolizes cholesterol required for estrogen and testosterone synthesis and neurotransmitter metabolism (Kastaniotis et al., 2017). The functions change in the course of development from embryo to adult, since tissues grow, mature, and adapt to postnatal environment. The role of mitochondria in infections, autoimmunity, innate and adaptive immunity is emerging (Monlun et al., 2017). The mitochondria harbor adaptor protein like MAVS at its outer membrane (Hee and Cresswell, 2017). It is involved in intrinsic innate immune pathways to activate NF-κb and interferon production.

In inter organellar crosstalk

The mitochondria dynamically associates and regulates the homeostasis of various organelles.

2.2.1 Membrane bound organelles: The mitochondria physically interact with membrane bound organelles like endoplasmic reticulum, lysosomes, peroxisomes and melanosomes.

Endoplasmic Reticulum: ER marks sites for mitochondrial fission while mitochondria regulate calcium homeostasis, phospholipid and steroid metabolism. The two organelles tether at various contact sites. These contact site called MAMS (mitochondria associated membranes) serve as signaling platform for inflammatory response, calcium homeostasis and cell death pathways (Simone et. al, 2011; Carlotta et. al, 2011). Emerging evidences suggest that innate inflammatory signaling gene, MITA, PARK genes, presellin and huntingtin are associated with these membrane. The mitochondrial dysfunction or ER stress impairs MAMS associated signaling and leads to neurodegenerative disorders (Tubbs and Rieusset, 2017).

Peroxisomes: These are lipid metabolizing bodies share fat metabolism, ROS homeostasis, protein import, antiviral signaling and cell death mechanisms with mitochondria. Mitochondria are involved in fatty acid oxidation while peroxisomes are involved in anabolism of fats. Both organelles are metabolically interdependent, form dynamic shape and number and respond to stimuli synchronously (Wijk, 2015). The organelles dynamically exchange vesicular bodies for metabolism. Recently mitochondria was demonstrated to pinch off pre-peroxisomes harboring Pex3 and Pex14 which then coalesce with ER derived vesicles carrying Pex16 to initiate peroxisomes biogenesis (Sugiura et al., 2017).

Lysosomes: They contain hydrolytic enzymes and can digest all biomolecules. It fuses with autophagosomes, mitochondria derived cargos, BNIP3/LC3 marked mitochondria to degrade the components. In tumor hypoxic conditions, lysosomes hyper fuses with mitochondria at the sites docked by BNIP3, cleaves mitochondrial VDAC1 at particular residues conferring its closure to cell death signals (Ma et al., 2012). The mitochondrial defects (in Tfam or complex-1) deregulates autophagosome-lysosome fusion dynamics, decreases mitophagy, abnormal lipid trafficking and altered calcium levels (Brahimi-Horn et al., 2015). The lysosomal disorder enhances ROS production, depolarizes mitochondria, reduces ATP production and potentiates cell death (Sumayao et al., 2016).

Melanosomes: The pigment producing organelle has 4 stages of maturation. The stage I and Stage II have less melanin which are frequently embraced by mitochondria. The contact the two organelles is 1.5 fold more than melanosomes themselves. It is connected with mitochondria by fibrillar bridges with mitofusin, MFN2 being crucial component in tethering. The melanosomes depend on mitochondrial ATP, since oligomycin treatment reduces both the contacts and maturation of melanosomes (Huang et al., 2011).

2.2.2 Dynamic membrane-less bodies: Mitochondria dynamically comes in contacts with cellular membrane less bodies for both optimal and stimuli specific regulations like:

P-bodies: The processing bodies are dynamic, ubiquitous 200-500 nm sized RNA rich dense objects. These bodies play crucial role in mRNA life cycle. These bodies are involved in RNA interference since they harbor RNA decaping, RISC components, NMD proteins and decay proteins (Adeli et al., 2011). These bodies supply the instantaneous requirement by supplying mRNA required to reduce genomic transcription load. Interestingly, in the time frame of 3 min, 80% of the cytosolic P-bodies physically interacts with mitochondria dynamically. The interaction remains stable for about 18 seconds. Their association is supported by microtubule networks. Their association is presumed to be involved in dynamic exchange of metabolites, RNA and proteins (Emoult et al., 2012). The mitochondrial dysfunction by drug induced uncoupling leads to mislocalisation of Ago2 and decreases the RNA interference process (Huang et al., 2011a).

Stress granules: These are dynamic bodies that were first observed in response to stress and now are known to be ubiquitous. The translation stress by drug or inhibitors leads to accumulation translational blocked mRNA, 40S ribosomal subunit, RNA binding proteins and elongation factors into stress granules. These bodies were found to be in close association with P-bodies in stress to export mRNA destined to degradation (Yang et al., 2014). The heat shock stress in yeast induces mitochondrial ROS mediated increase stress granules production (Kedersha et al., 2005). The oxidoreductase function of intermembrane space protein Apoptosis inducing factor, AIF regulates Stress granule formation via glutathione balance (Watanabe et al., 2011).

Nauge Bodies: These are highly dynamic cytoplasmic dense bodies, associated with mitochondria in germ cells. Their differential structure and form in different cells mRNAs lead to numerous nomenclature like PING bodies, nauge bodies, mitochondrial cement, Balbiani bodies or clouds. The bodies store transcriptional components, repressed mRNAs, Ago3, piRNAs and other members involved in piRNA mediated gene silencing PING pong pathway. The MITOPLD a part of mitochondrial cardiolipin at outer surface is core factor for nauge body formation, piRNA pathway and spermatogenesis (Watanabe et al., 2011). MITOPLD, a phospholipase hydrolyses cardiolipin to release signaling

molecule, phosphatidic acid. It also confers a microtubule mediated cellular localization of both nauge bodies (Daniele and Schiaffino, 2014). *These observations suggest that mitochondria are involved in maintenances of mRNA pools via dynamic association with storage bodies required for its optimal and microenvironment specific functions* (Wang and Youle, 2009).

2.2.3 Mitochondria regulates cell death

The process of cell death is important from development to adult body homeostasis. Mitochondria have been known to be important and had been called as central regulator of cell death. Now different types of cell death has been discovered apotosis (Baines, 2010), necrosis (Marshall and Baines, 2014), necroptosis (Cao and Dixon, 2016), ferroptosis (Man and Kanneganti, 2015), pyroptosis (Yumnam et al., 2016), paraptosis (Fox, 2012)), phenoptosis (Valentijn and Gilmore, 2004). All these forms of cell death listed here consume and deplete cellular ATP and NAD. These processes are also dependent on ROS misbalance suggesting the crucial role of mitochondria in all forms of cellular demise.

In intrinsic cell death pathway, the mitochondrial permeability is compromised and proteins from IMS: AIF, cytochrome c and Smac are released. AIF in association with calpains induces genomic DNA degradation. The cytochrome c associates with Apaf and recruit pro-Caspas-9 to form multimeric complex called as apoptosome (Kiraz et al., 2016). It activates Caspase 9 which further initiates proteolytic cascade through activation of executionary Caspase 3 and 7. The cellular stress leads to ROS and NO radicals production. It induces DNA damage that is identified by PARP. It signals to mitochondria to release AIF and leads to cell death. The extrinsic pathway is activated by Fas/TNF binding to respective receptors and initiation of caspase activation cascade along leading to Bid cleavage and transport of its truncated form to mitochondria. At mitochondria it initiates induction of intrinsic pathway by homo and dimerization of pro apoptotic BH3 family proteins forming pores like structure on outer membrane to release of apoptotic proteins (Fox, 2012).

2.3 Dynamic influx of nuclear encoded proteins regulates mitochondrial function

The matrix has its own circular genome with varying copy number. In humans it sizes 16.5 kb, encodes 13 proteins, 22 tRNAs and 2 rRNAs which is not even sufficient for its optimal functions. More than 1500 proteins (corresponds to 99.2% proteins residing at/in OMM, IMS, IMM and matrix) and numerous coding as well as non coding RNA are imported into the mitochondria as per the cellular demand. The imported biomolecules are screened and positioned at particular regions within the mitochondrial compartments. 3% of these genes are required necessary to make ATP synthesis while 95% are involved with other specialized functions (Vega et al., 2015). The mitochondrial functions are regulated by both nuclear genome encoded mitochondrial protein and mitochondrial genome encoded proteins. The varied function of mitochondria requires regulatory mechanism and signals to adapt to the cellular conditions. The mitochondrial functions are controlled by nuclear genome in the following levels:

2.3.1 Nuclear encoded proteins maintain mitochondrial biogenesis and function:

The factors essential for mitochondrial biogenesis, DNA replication, transcription and translation are all dependent on import of nuclear genome encoded mitochondrial proteins (NGEMP). The nuclear encoded transcription factors regulate the genes involved in mitochondria biogenesis, maintenance and its functions. The replication of mitochondrial genome is performed by homo and hetero dimers of POLG and POLG2. The supercoiled DNA is unwound for the process by helicase, TWINKLE. The replication accessory proteins include mitochondrial topoisomerase I, mitochondrial RNA polymerase (mtRNAP), RNase H1, mitochondrial genome maintenance exonuclease 1 (MGME1) and mtSSB. The defects in these nuclear encoded proteins are associated with severe metabolic syndromes like AHS, MNGIE, MEMSA, ANS, ARPEO and ADPEO (Vega et al., 2015). The transcription of mitochondrial genome has three initiation points (2 in heavy chain and 1 in light chain) and is transcribed into 3 polycistronic pre-RNAs. The process involves polymerase POLGRM and MTRPOL, 3 transcription factors TFAM, TFBM1 and

TFBM2 that bind and prime the reaction and mTERF which terminates the transcription. Their defect is associated with neurodegenerative and metabolic disorders like Leigh syndrome. Mitochondrial transcription factor A (TFAM) is nuclear encoded transcription factor that translocates to mitochondria and binds directly to mtDNA. In addition to its role as a transcription factor for mtDNA, mammalian TFAM acts to maintain the copy number of mtDNA and is involved in its replication. The NGEMP controls the cellular energy and homeostasis by co-transcriptional factor like PGC1-α/β. These further regulate cascade od transitional factors :The nuclear respiration factors, NRF1 and NRF2 were first demonstrated to enhance ETC by increasing the production of nuclear genome encode mitochondrial respiratory chain components The peroxisome-proliferator activated receptors α, β, and γ enhances the enzyme productions required for fatty acid oxidation while the estrogen-related receptor (ERR) α, β, and γ regulates gene involved in mitochondrial biogenesis and functions (Richter-Dennerlein et al., 2016). The extracellular micro environment is sensed and these transcriptional factors are activated leading to transcription of the genes required for mitochondrial function adaptation.

2.3.2 NGEMP signals release of stalled mitochondrial translation:

The nuclear genome and mitochondrial genome encoded mitochondrial transcription orchestrates in synchrony. The mitochondrial respiratory chain is composed of 4 complexes coupled with F_1F_0 particles. 7, 0, 1 and 4 subunits are encoded by mitochondrial genome out of 45, 4, 11 and 14 structural subunits of Complex I, II, III and IV respectively. Hence synchronous synthesis of subunits from both genomes is required to maintain stoichiometry of electron transport chain and to save/balance energy in translation. Recently, mitochondrial translation plasticity of mitochondrial complex-IV was observed. The mitochondrial genome encoded COX-1 nucleates the assembly into intermediate called MITRAC. The subunits from both genomic origins assemble via this platform sequentially and synchronously. The component of MITRAC changes dynamically with progression of influx and availability of subunits to assemble into complex-4. Recently COX-1 mRNA was observed to be primed with mito ribosomes at

inner mitochondrial membrane. Its translation was stalled until the influx of nuclear encoded subunit of COX-4 (Lesnik et al., 2015). This suggests the occurrence of yet un-dicephered nuclear control on mitochondrial functions possibly for other 3 complexes.

2.3.3 Mitochondrial outer surface: as a PLATFORM for local translation of NGEMP

The translation of proteins destined inside mitochondria were previously believed to be synthesized in cytosols. Emerging evidences suggest that nascent mRNA, ribosomes (Lesnik et al., 2015) and mRNA storage bodies (as described above) are in close vicinity with mitochondria. Recent studies suggest that nuclear encoded mitochondrial electron chain subunits are synthesized at mitochondrial outer membrane (Golani-Armon and Arava, 2016). The mitochondrial outer membrane is decorated with ribosomes in flight muscles cells of drosophila. The process involves the core components of mitophagy pathway, PINK1 and PARKIN. These proteins recruit nascent mRNA of nuclear encoded respiratory chain components in repressed form at OMM to provide de novo synthesized protein at mitochondrial outer surface (Schatton et al., 2017). It had been recently observed that the nascent nuclear encoded mitochondrial targeted mRNAs are also transported by an evolutionary conserved protein, CLUH to mitochondria at the OMM (Couvillion et al., 2016). The absence of this protein leads to mitochondrial respiratory chain defects. Its loss also recruits mitochondria near the periphery of nucleus. These observations suggest the carrier mediated nuclear encoded mitochondrial respiratory chain mRNA transport and local protein translation at outer mitochondrial membranes.

2.3.3 NGEMP regulates mitochondrial transcript processing

2.3.3.1 The processing of mitochondrial pre-mRNAs:

The mitochondrial genome comprises of two strands. The purine rich heavy strand and pyrimidine rich light strand, termed so based on their respective densities. The light strand encodes 1 protein out of 13 and 8 tRNAs amongst 22. The heavy strand is encoded into polycistronicpre-mRNA with 2 rRNAs and 10 mRNA (2 of them are bicistronic: ND4/ND4L and ATP6/8) which is interspaced with 14 tRNAs. This polycistronic pre-

mRNA lacks intron. The tRNAs and mRNAs from the heavy strand are further processed into individual components by tRNA excision and release of adjacent pre-mRNA. The process involves 3' cleavage of tRNA by RNase P complex and RNase Z/ELAC at 5' end. The individual mRNAs are polyadenylated and capped subsequently. The half lives of ND1-3, 5 and CYTB are shorter of about 90 min while the rest have longer span which correlates with OXPHOS (Wolf and Mootha, 2014). This corresponds to level of complexes formed. The complex-1 is least abundant among 5 complexes while complex IV and V are constitutively high. This suggests precise regulation of individual components is required for ensuring proper OXPHOS complex stoichiometry (Wolf and Mootha, 2014).

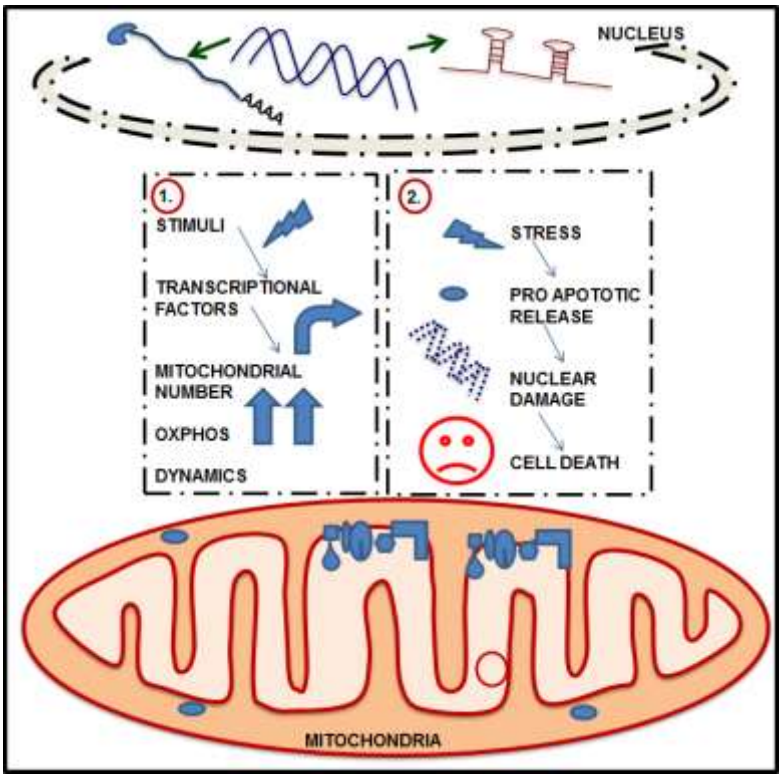

Figure 2.2. Nuclear – Mitochondrial crosstalk. *The figure depicts the interdependence and mutual co regulation of nucleus and mitochondria in various conditions as described. The nucleus reprograms mitochondrial biogenesis, replication and transcription in stimuli dependent manne. The mitochondrion communicates the cell death signals by releasing the killer proteins like AIF towards nucleus to amplify the cell death cascade.*

2.3.3.2 pre-mt-mRNA processing in mitochondrial granules:

The processing was recently observed to be conducted in highly dynamic foci in mitochondrial inner matrix called mitochondrial RNA granules (MRG). 10-20% of MRG are distinct from mitochondrial nucleoids lacks mitochondrial DNA while the majority are BrdU positive and lacks DNA. GRSF1 was first define component, while 6 members of FASTK family, MRPS family and DHX family of proteins were identified as constituents later. Out of nearly 1500 NGEMP, 107 surely are RBPs by Mitostring approach. The proteins involved in tRNA processing, transcriptional enhancement and stabilization of the heavy strand precursor mRNAs and light strand degradation were reconfirmed (Wolf et al., 2014).

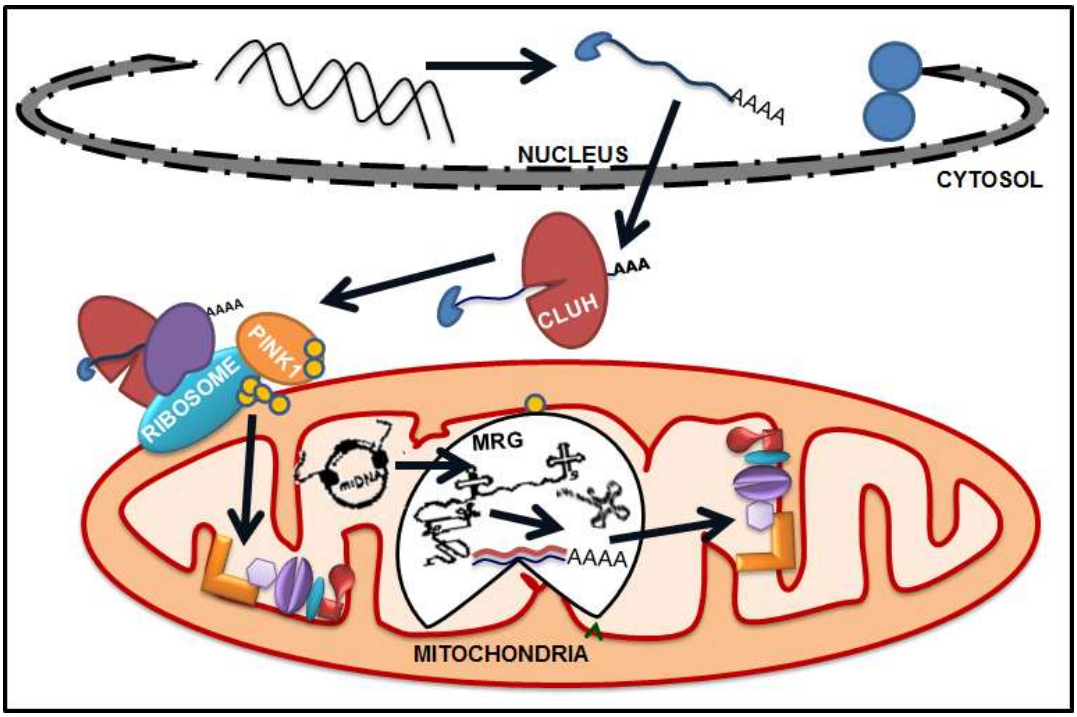

Figure 2.3. Nuclear control over mitochondrial proteome. *The nucleus regulates both nuclear genome encoded and mitochondrial genome encoded proteins that are destined to be a part of mitochondrial component. The NGEMP, OXPHOS subunits are synthesised at mitochondrial outer surface while the processing of mitochondrial polycistronic transcript occurs in mitochondrial RNA granules (MRG) in the matrix.*

The MRG components, FASTKD4, FASTKD2, GSRF1, FASTK, RPUSD, DHX30 appeared to shield a specific set of mt-mRNAs from degradation (Zaganelli et al., 2017, Jourdain et al., 2015; Antonicka et al., 2015). For instance, GRSF1 and FASTK specifically regulated enhances ND6 processing and tRNA punctuation by interacting with RNaseP. On the other hand, FASTKD5, FASKD2, DDX28, RPSUD3 regulates rRNA processing, maturation and ribosomal incorporation. COX1 and COX3 were regulated by RPUSD3 and TRUB2 pseudouridine modification MRG. These examples again suggest that MRGs as site for stoichiometric balance of tRNA(s), rRNA(s), ND6 and rest 12 subunits by yet unknown mechanisms (Antonicka et al., 2015).

These evidences suggest the existence of novel mechanism of site specific regulation of nuclear encoded mitochondrial proteins at its outer membrane and within mitochondria. However the fine tuning of these processes by ncRNAs specifically miRNA remains elusive. It will be an interesting area for intensive research.

2.4. Transport of ncRNA inside mitochondria

The import through double membrane is not restricted to proteins, there is massive export and import of RNA. The export-import of ncRNA into mitochondria is conserved from protozoans, plants, fungi, yeastand animals (Sieber et al., 2011). Recently, it was observed that polynucleotide phosphorylase (PNPASE), an inter membrane space (IMS) protein has key role in import of small RNA. The enzyme has 3′–5′ exoribonuclease activity and degrades RNA trying to enter mitochondria. However, RNA with a stem loop structure and 0–4 nt overhang at 3′ end is inaccessible to catalytic domain thereby protecting from degradation. This may enhance specific structural ncRNA import into the organelle. PNPASE is conserved in bacteria, plants, flies, mice, and humans. In plants, it is localized both in the intermembrane space of mitochondria and chloroplast. Arabidopsis lacking chloroplast targeted PNPASE, cpPNPase (pnp 1–1) showed 98% difference in ncRNAs expression levels in chloroplast as compared to wild type (Hotto et al., 2011).The similar investigation of PNPASE in mitochondria may provide more clues of small ncRNA import. PNPase KO cells have severe OXPHOS deficient phenotype. These cells are devoid of mitochondrial endoribonuclease, RNase MRP (which processes mitochondrial

leading strand transcript to form primers for DNA replication) and RNase P (which cleaves polycistronic transcripts to generate individual mtRNAs and processes 5′ end of tRNA) (Puranam and Attardi, 2001; Wang et al., 2012a). The nuclear-encoded 5S rRNA is the most abundant RNA in mitochondria (Entelis et al., 2001; Smirnov et al., 2008) which is a crucial component of mitochondrial ribosomal assembly. The import of 5S rRNA was recently found to be dependent on mitochondrial enzyme rhodanese (thiosulfate sulfurtransferase), which interacts with both α-domain and γ-domain regions of 5S rRNA and mitochondrial ribosomal protein L18 (MRP-L18), a member of the L18/eL5 family (Smirnov et al., 2011). Similarly, the impairment in mitochondrial encoded tRNALys and tRNALeu due to A8344G and A3243G mutations in mitochondria causes MERRF and MELAS syndrome respectively. The pathologies were rescued in vitro by partial resumption of these tRNA (Karicheva et al., 2011; Kolesnikova et al., 2004). Similarly, the cytosolic tRNA(Gln) is transported to mitochondria in ATP dependent manner (Rubio et al., 2008). The mitochondria imports nuclear encoded tRNA (Alfonzo and Soll, 2009; Duchene et al., 2009). The number of tRNA imported into mitochondria ranges from one in yeast, to all in trypanosomes. The tRNA import in yeast occurs by both protein import machineries (TOM-TIM complex) and non-canonical import systems (Rubio et al., 2008). Mammalian mitochondria are known to import/export tRNA both in vitro and in vivo (Kolesnikova et al., 2004; Rubio and Hopper, 2011). Interestingly, the mitochondrial tRNAMet, tRNAIle, tRNALys, and tRNAGlu were found in the cytosol (Maniataki and Mourelatos, 2005). However, the significance of this and mechanisms of RNA transport in context of mitochondria is not yet well understood.

Interesting, Ago2, core component of RISC assembly (co-localised with mitochondria) was found to be involved in the transport of a 70 nt tRNA(Met) from mitochondria to cytosol (Maniataki and Mourelatos, 2005). This suggests that other components involved in miRISC, Ago2 and miRNAs may also be associated or imported to fine tune the previously discussed processes.

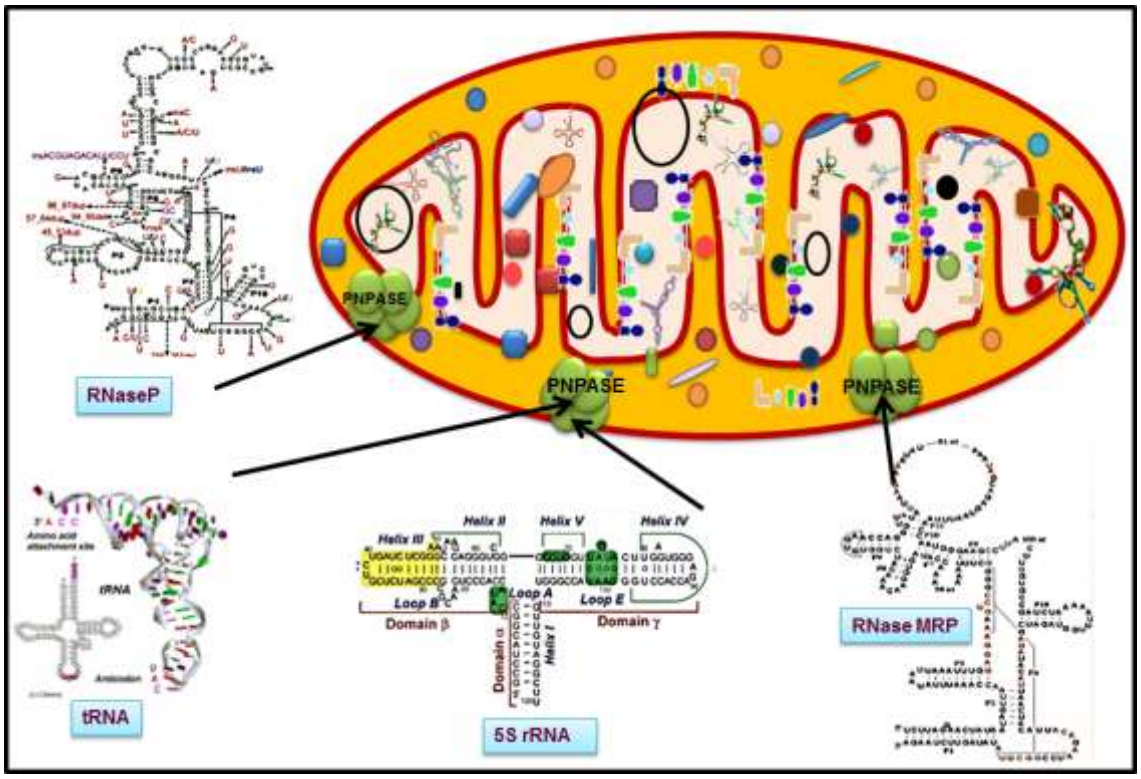

Figure 2.4. Import of nuclear encoded non coding RNA into the mitochondria. *The import of nuclear genome encoded non coding RNAs with 3' 2-5 nt overhang into mitochondria via pnpt1 is been depicted as per the description in the text.*

2.5. MiRNA

Micro RNAs (miRNA) are endogenous non coding RNAs highly conserved across the genomes of animals, plants, fungi and viruses (Ambros, 2004; Bartel, 2004). miRNAs account for ~ 1% of the human genome. The human genome is composed 3 million base pair of DNA organised in 23 sets of chromosomes; <2% of which are coded into functional proteins. The same primary genomic message builds differential transcriptome, assigns different function and structure in more than 200 types of cells in humans (Bentwich et al., 2005). The 98% of genome was presumed to be junk until the discovery of let-7 by Victor Ambros in early 19th century (lethal 7 – a small ncRNA regulating developmental switch in *C. elegan*) (Lee et al., 1993). miRNAs are transcribed from genomic DNA by canonical or non-canonical pathways.

2.5.1 Canonical miRNA biogenesis:

RNA polymerase II transcribes ~1000 nt long primary miRNA (pri-miRNA) with stem loop structures, 5' cap and 3' tail (Ambros, 2004; Bartel, 2004). 38% of murine miRNAs are encoded from intronic regions while 61% of mammalian miRNA are encoded as polycistrons (H.R. Chiang, 2010). The stem loop structure of pri-miRNA is then recognised by microprocessor complex by RNaseIII enzyme, Dorsha and its dsRNA binding partner, Pasha (also known as DGCR8). Pasha binds at the juncture of dsRNA stem and Dorsha cleaves off into ~ 60 -100nt long precursor miRNA (pre-miRNA) at 1 helical turn. The pre-miRNA has a ~2-nt overhang at 3' end (Han et al., 2006). The 2-nt overhang of pre-miRNA is recognized by Exportin-5 and exported out of nuclei in Ran-GTP dependent pathway. In the cytosol, pre-miRNA is cleaved by another RNaseIII enzyme, Dicer with the help of its dsRNA binding partner, TRBP. The pre-miRNA is cleaved off at 3 helical turn from base within the stem, to release paired ~20 nt miRNA/miRNA* duplex with a 3' overhang in both the strands (Park et al., 2011). The asymmetric assilmilation of thermodynamically stable strand called as guide strand occurs in Ago2, miRISC (RNA induced silencing complex) complex. The mature miRNA bound Ago2 complex screens complimentary 3' UTR region in target mRNA and recriuts acessory proteins to fine tune the protein levels. The binding complementarity of seed sequence and mRNA regulates the fate of mRNA. The perfect complementarity between seed sequence (6-8 nt sequence beginning from first or second nt at 5') and target mRNA leads to mRNA degradation whereas imperfect complementarity causes translation repression or ribosome falling off from polysomes (Saraiya et al., 2013). The process is also governed by associated RNA binding proteins at miRISC complex.

2.5.2 Non canonical processing:

The non-canonical pathway involves either splicing off miRNAs from intronic regions, other ncRNAs like tRNAs (Lee et al., 2009) and snoRNAs (Martens-Uzunova et al., 2013) or by bypassing the enzymatic cleavage processes. ~ 1/3 miRNAs are encoded from

intronic regions of protein coding genes. The hairpin structured miRNAs are first cleaved off, forming smaller pre-miRNAs before maturation of mRNA.

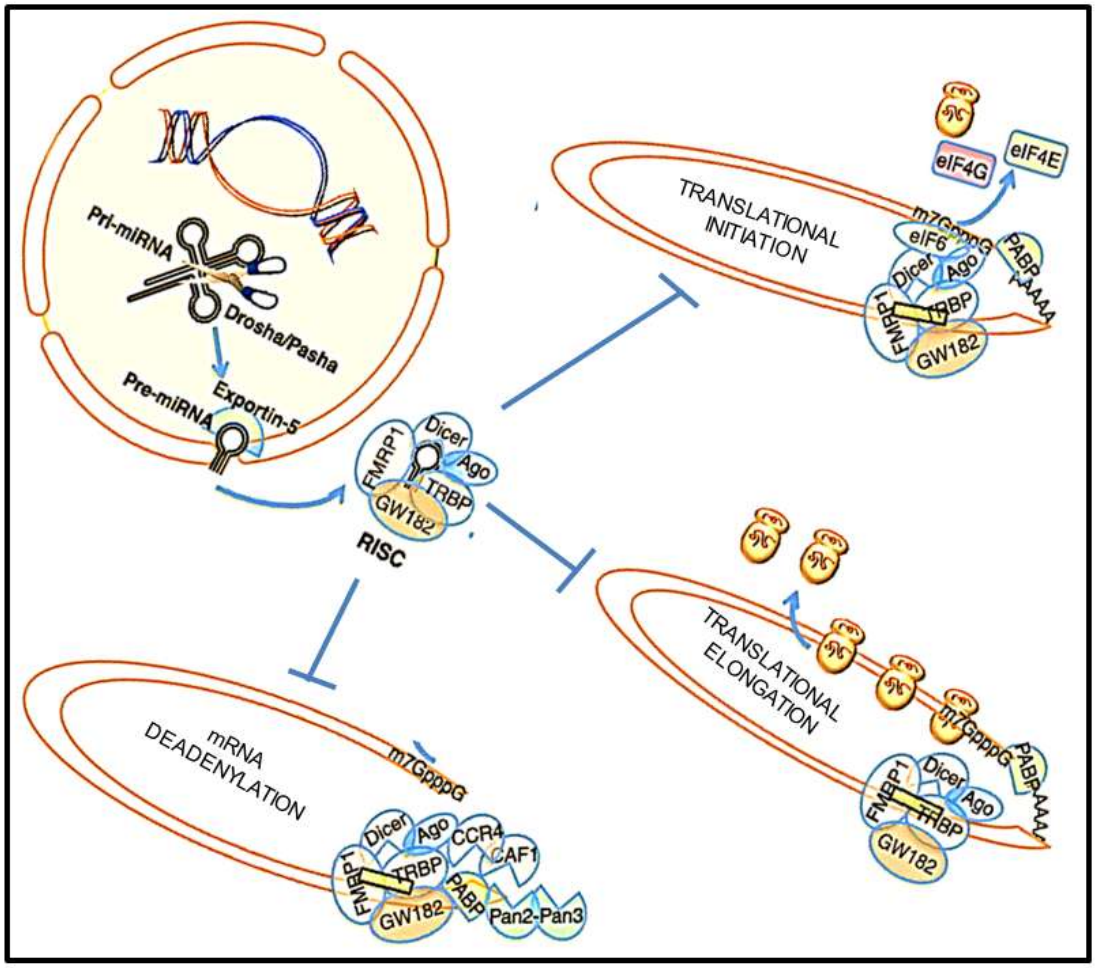

Figure 2.5. miRNA biogenesis pathway, associated factors and the outcome. *The figure depicts mode of action of miRNAs. Based on complimentarity of seed sequence with target mRNA, the miRNA translation and elongation are atteneuated or the mRNAs are degraded.*

These miRNAs are known as mirtrons (Westholm and Lai, 2011). The deletion of microprocessor components, DGCR8, DORSHA and DICER induces lethality/ pathology. However, some miRNAs are identified to be processed independent to these core factors. Nearly, 7% of miRNA are matured in Dorsha independent pathway involving shorter pri-

miRNA cleavage in Dicer recognisable format by RNase P enzyme. On the other hand, pre-miR-451 a sole Dicer independent miRNA undergoes processing by Ago2 (Cheloufi et al., 2010). However, the miRNA biogenesis from mitochondrial DNA encoded ncRNA will be interesting prospective to be elucidated. It may have import implication especially in mitochondrial genomic disorders.

2.5.3 Association of miRNA processing components with mitochondria:

Emerging evidences suggests the association of RNA interference proteins Ago2, DICER1 and FMRP associates with mitochondria (Li et al., 2008; Wang and Springer, 2015). On the other hand, component of mitochondrial RNA granule GRSF1 was demonstrated to regulate processing of miR-138 and miR-346 (Song et al., 2015). These observations stongly suggest obvious crosstalk between miRNA processing proteins with mitochondria. However the dynamic cross talk of other accessory proteins and interacting partners with mitochondria remains elusive.

2.5.4 MiRNA regulate mitochondrial functions

The miRNA may bind to different cognate mRNAs and regulate numerous protein levels from same or different pathways. For example, miR-155 regulates various components of various signalling (Faraoni et al., 2009). On the other hand, seed sequences of numerous miRNAs may have complementarity at 3' region of same mRNA, suggesting existence of numerous regulators and complicated inter connected pathways for same (Pasquinelli, 2012). The aberrant expression of miRNA occurs in different pathophysiological conditions. The aberrant expression of miRNA also affects mitochondrial functions and cell death because mitochondria imports 99% of its protein. The miRNAs regulate nuclear encoded mitochondrial proteins at post transcriptional level consequently affect mitochondrial homeostasis, OXPHOS, ATP synthesis, apoptosis, ROS generation or fatty acid metabolism in several pathologies as summarized briefly in Table 2.1.

2.6. Potential association of MiRNA and MITOCHONDRIA……??

The mitochondria dynamically move along with microtubules and stalls at specific regions like lamellipodium of migrating cancer cells (Zhao et. al, 2013) and synapses in neurons to balance calcium and ROS levels and to provide ATP (Wang et. al, 2016). The mitochondria were proposed harbour nascent mRNA. The miRNAs on the other hand are transported to various sub-cellular localisations and enriched in sub cellular compartments like synapses (Sheng, 2017; Wan et al., 2012). Interestingly, the contemporary studies supported some of these presumptions. The experimental evidences suggest few nuclear encoded miRNAs translocates to mitochondria from various sources: from rat liver (15 miRNAs) (Kren et.al, 2009), mouse liver (20 miRNAs) (Bian et al., 2010), myotubes (20 miRNAs) (Barrey et al., 2011) and 143B (3 miRNAs) (Mercer et al., 2011) and human muscles (46 mature miRNAs and 2 pre-miRNAs). The majority of them are found to target nuclear encoded mitochondrial genes whereas some regulates mitochondrial genome encoded transcripts like hsa-miR-133a targets ND1 (Kren et.al, 2009) and hsa-miR-130 targets COX3 (Kren et.al, 2009).

However the association of miRNAs in mitochondrial compartments and their functions was not elucidated systematically. Hence, the systematic characterisation of mitochondrial association with ncRNA specifically miRNA was elucidated. Further the impact on association was determined in cell death signals. The molecular pathways were deciphered and physiological impact was further elucidated.

Table 2.1 miRNAs affecting mitochondrial functions

miRNA[1]	**Traget[2]**	**Physiological Role[3]**
rno-miR-220c	holocytochrome-c synthatase	Diabetes
hsa-miR-122	PGC-1α	Lipid metabolism
hsa-miR-125b	p53	CML
hsa-miR-125b	Bak1	CML
hsa-miR-125b	Mcl1	CML
hsa-miR-133a	ND1	Muscular activity
hsa-miR-133a-1/2	dynamin2	Centronucleur myopathy
hsa-miR-143	ERK5	Colon cancer
hsa-miR-15a	UCP-2	Insulin production
hsa-miR-15a	Mcl	Apoptosis in CLL
hsa-miR-15a	Bcl-2	Apoptosis in CLL
hsa-miR-15b	Arl-2	ATP depletion, mitochondrial impairment
hsa-miR-16-1	Mcl	Apoptosis in CLL
hsa-miR-16-1	Bcl-2	Apoptosis in CLL
hsa-miR-16	Arl-2	ATP depletion, mitochondrial impairment
hsa-miR-17-92	MnSOD	Cancer
hsa-miR-17-92	TrxR2	Cancer
hsa-miR-17-92	GPX2	Cancer
hsa-miR-181	BCl-2-L11	Primary astrocyte culture
hsa-miR-181	Bim	Primary astrocyte culture
hsa-miR-181	Mcl-1	Primary astrocyte culture
hsa-miR-181	BCl-2	Primary astrocyte culture
hsa-miR-181c	COX IV	Cardiac infarction
hsa-miR-195	Arl-2	ATP depletion, mitochondrial impairment
hsa-miR-199	HIF-1	Cardiac myocytes in oxygen
hsa-miR-199	Sirtuin1	Cardiac myocytes in oxygen
hsa-miR-21	PTEN	Inhibits breast cancer
hsa-miR-210	COX IV	Chronic hypoxia
hsa-miR-210	ISCU	Cancer, ICU patients
hsa-miR-210	Cox 10	Cancer
hsa-miR-210	AIFM3	ROS production, cardiomyocytes
hsa-miR-214	Ncx1	Cardio protective, intracellular Ca++
hsa-miR-214	Bim	Cardio protective, intracellular Ca++
hsa-miR-214	CypD	Cardio protective, intracellular Ca++
hsa-miR-214	CamKII	Cardio protective, intracellular Ca++
hsa-miR-223	Glut4	Insulin resistence in diabetic heart
hsa-miR-23a/b	GLS	Promotes cardiac hypertrophy

hsa-miR-23a/b	Fas	Protects H_2O_2 induced apoptosis in ARPE-19
hsa-miR-27b	MMP13	Promotes cardiac hypertrophy
hsa-miR-27b	Col I/III	Promotes cardiac hypertrophy
hsa-miR-30	p53	Mitochondrial dynamics in Heart
hsa-miR-34a	TrxR2	Aging of kidney
hsa-miR-355	SOD2	Aging of kidney
hsa-miR-375	Atg7	Mitophagy in HCC
hsa-miR-388	Cytochrome c oxidase IV	Impairs OXPHOS and reduced ATP
hsa-miR-424	Arl-2	ATP depletion, mitochondrial impairment
hsa-miR-499	Calcineurin	Cardio protective
hsa-miR-696	PGC-1α	Aerobic metabolism in skeletal muscles
hsa-miR-743a	Malate dehydrogenase	Oxidative stress in Alzhimers

Table 2.1 miRNAs affecting mitochondrial functions. *The physiological impact as a consequence of miRNA affecting mitochondrial proteome by binding to respective mRNA has been summarized at the beginning of the study.* [1] *Name of miRNA;* [2] *the mitochondrial target of each miRNA; 3 the observed role in physiology;*