3.1 Rationale of hypothesis

Mitochondrial dysfunctions are one of the major hallmarks of neurodegenerative diseases, including FXTAS. Various studies had shown mitochondrial dysfunctions in cell and mouse models of FXTAS as well as in fibroblasts and brain tissue of premutation carriers [1]–[3]. However, the cellular and molecular mechanisms triggering mitochondrial dysfunctions in FXTAS are not well understood. As reviewed in the literature there are two major mechanisms contribute to the FXTAS pathogenesis: *RNA gain of functions and protein toxicity mediated by FMRpolyG* (Fig. 3.1). RNA gain of function leads to sequestration proteins of miRNA biogenesis (DROSHA/DGCR8) and results into altered miRNA biogenesis in FXTAS (Fig. 3.1). While RAN translation of expanded CGG repeats forms toxic protein FMRpolyG and causes decreased level of functional FMRP. The sequestration of RNA binding proteins is known to modulate miRNA biogenesis and affect their trafficking to different subcellular organelles including mitochondria. Further, the FMRpolyG that forms aggregated may interact with mitochondria and sequester mitochondrial proteins which may cause mitochondrial dysfunctions (Fig. 3.1). In the current study, we investigated the FMRpolyG interaction with mitochondria and its role in modulation of mitochondrial functions using different *in vitro* cellular systems and animal model.

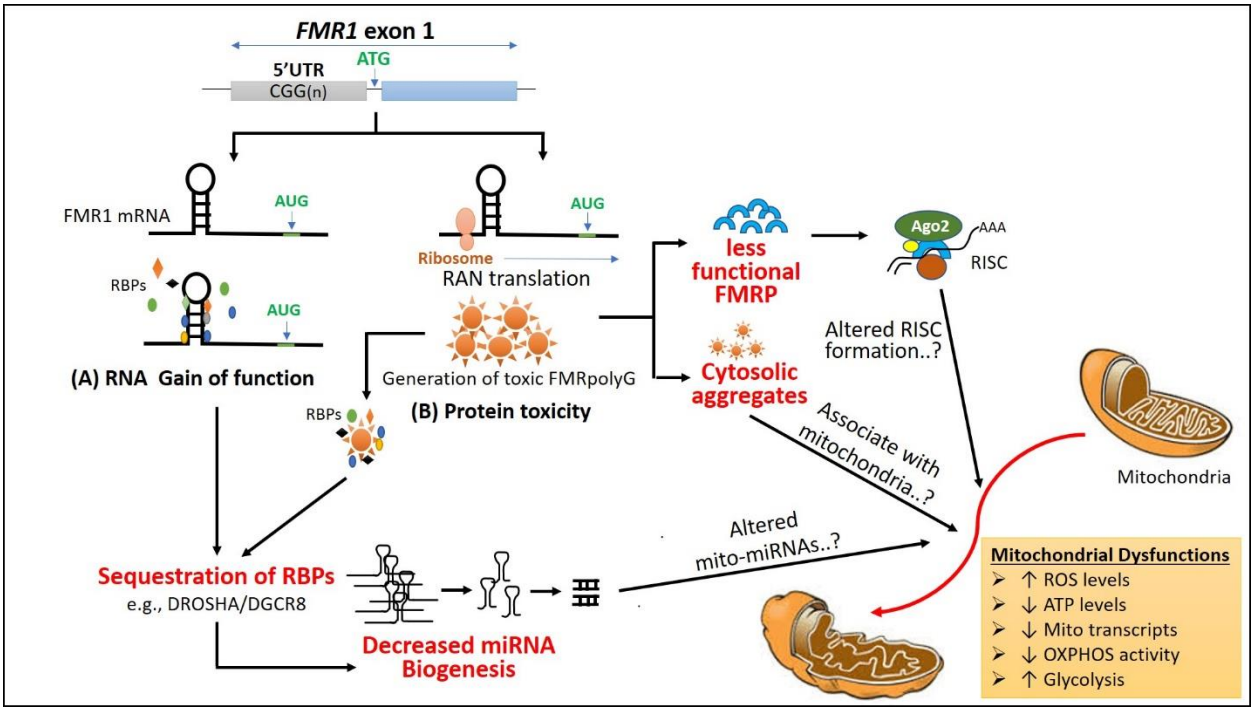

Figure 3.1. Schematic representation of possible molecular mechanisms for mitochondrial dysfunctions in premutation condition. (A) RNA gain of function: The 55-200 CGG repeat within the 5′UTR of FMR1 is transcribed into FMR1 mRNA, which can form higher-order structure such as hairpins, which have the ability to sequester several RNA binding proteins leading to altered splicing and decreased miRNA biogenesis. Decreased expression of miRNAs leads to their altered translocation to the mitochondria. (B) Protein toxicity: CGG repeats present

at 5′UTR of FMR1 mRNA translated by noncognate codon (ACG) resulting in formation of toxic protein, FMRpolyG. (1) FMRpolyG forms intranuclear inclusion and ruptures nuclear membrane and may affect transport of miRNAs/mRNAs and NEMP (nuclear encoded mitochondrial proteins) to the mitochondria. (2) Less amount of functional FMRP may lead to altered RISC formation and translational regulation of their target mRNAs. (3) Formation small inclusion of FMRpolyG may localize to mitochondria and possibly involve in mitochondrial malfunctions.

miRNAs belong to a class of noncoding RNAs important for post transcriptional regulation of mRNAs by partial base-pairing of miRNA-mRNA mediated by the RNA-induced silencing complexes (RISC) [4],[5]. Studies from last decade have shown evidences of organelle specific localization of miRNAs and presence of crucial RISC component like Ago2 in the mitochondria [6],[7]. As miRNAs expression and mitochondrial functions are altered in FXTAS, we tend to investigate the altered trafficking of miRNAs at mitochondria and the potential role of mito-miRs in regulation of mitochondrial bioenergetics and cell viability in FXTAS *in vitro* cellular model.

3.2 Specific objectives

- **Objective-1:** Analysis of FMRpolyG induced toxicity in FXTAS condition (in vitro cell line model system.)

- **Objective-2:** Alteration in mitochondrial function, dynamics and physiology due to expressed expanded CGG repeats.

- **Objective-3:** Study the miRNA dysregulation at cellular levels and its association with mitochondria.

- **Objective 4:** Understanding the roles of identified miRNAs in mitochondrial dysfunctions in FXTAS condition.