ABSTRACT

The muscular system is a highly complex and important system in the body. Proper muscle physiology is critical for locomotion, digestion, circulation, reproduction as well as for metabolic and immune homeostasis. Defects in muscle development, structure or function result in muscle disorders and diseases. Chapter 1 reviews the important events of muscle development and growth as well as the various processes that are involved in the regulation of the same. The muscle disorders that occur due to the mis -regulation of these processes are discussed. Specifically, the significance of microRNAs in muscle development and function in the context of cardiac hypertrophy has been described. Chapter 1 also explains the importance of mitochondrial morphology and function for normal tissue functioning along with the dynamic processes that mediate changes in mitochondrial shape and size, namely fusion and fission. Thus, the first chapter discusses what is known and unknown about the roles played by microRNAs in and the regulators of mitochondrial dynamics during muscle development, and highlights questions being addressed in the present thesis. The advantages of using the Drosophila indirect flight muscle (IFMs) as a model system to address the unanswered questions are also enumerated in this chapter. The main features of IFM development and the similarities with vertebrate muscle development have been highlighted.

Chapter 2 details the various Drosophila melanogaster lines, genetic tools and the experimental techniques used in this study. In the context of muscle function, the spatial and temporal regulation of the expression and assembly of structural proteins into structural units (sarcomeres) is crucial. The derailing of this process has been shown to result in number of muscle defects. One among them is cardiac hypertrophy which is characterized by mis-regulation of structural protein levels. Recently, miR-9 was shown to be involved in cardiac hypertrophy, however the role played by miR-9 in the regulation of muscle proteins is not known.

Chapter 3 explains the novel findings regarding the role of miR-9a (Drosophila homolog) in the regulation IFM development. Results from IFM-specific over-expression of miR-9a during early muscle development indicate that miR-9a may have a role in repressing the regulators of dorsal longitudinal muscles (DLMs – a subset of the IFMs) patterning. The results discussed in this chapter also reveal that the over-expression of miR-9a exclusively in the IFMs during myofibrillogenesis rendered the flies flightless and the muscles showed hypercontraction -an auto-destructive process resulting from mis-regulated acto-myosin interactions. Bioinformatics analysis predicted 27 putative targets of miR-9a in muscles and Troponin-T (TnT), a structural protein component of the thin filament complex required for regulation of muscle contraction, was identified as putative target of miR-9a . Based on the observations that TnT levels are reduced when miR-9a is over-expressed and that overexpression of TnT, which lacked the miR-9a binding site, resulted in rescue of miR-9a over-expression phenotype, Chapter 3 concludes by stating that Troponin T is a major target of miR-9a in the IFMs. This finding along with the fact that human cardiac Troponin T (TNNT2) possesses a miR-9 binding site indicates that miR-9 could be involved in regulating the Troponin T levels during cardiac hypertrophy. Maintenance of mitochondrial quality and quantity is vital particularly in an energetically active tissue such as muscle. Mutations in the genes encoding regulators of mitochondrial dynamics have been shown to result in degenerative diseases. However, the process of mitochondrial fusion and fission are not well studied in vivo , especially during tissue development. In

Chapter 4, the changes in mitochondrial morphology across IFM development have been described for the first time. Since all the major events of myogenesis during IFM development have been well demarcated and can be spatio-temporally tracked, it serves as a good model to investigate the mitochondria dynamics and roles of molecular players. Mitochondrial morphology was observed to be thin and continuous in the early stages of development, circular during mid-pupal phase and large and tubular during late pupal stage, indicating the occurrence of both mitochondrial fusion and fission during myogenesis. Further, Chapter 4 details the effect of knock down of the regulators of mitochondrial fusion and fission, namely Mitochondrial associated regulatory factor (Marf) and Dynamin related protein 1 (Drp1) during development of the IFMs. Genetic studies that revealed the importance of these regulators in mammalian development and human diseases are also mentioned. The results presented in Chapter 4 show that the knock down of

Marf during development of the IFMs resulted in abnormal mitochondrial morphology and dysfunctional mitochondria that undergo mitophagy. While, Marf expression was found to be vital during early in IFM development, it did not appear to be as necessary during later in development. Knock down of Marf during the myofibrillogenesis phase of IFM development did not result in any defect in mitochondrial morphology function and myofibril ultrastructure. Importantly, it is shown in Chapter 4 that when Marf was depleted from early in development, adult flies exhibited abnormal sarcomeric structures, were incapable of flight and had greatly reduced life span. On the other hand, knock down of Drp1, the regulator of fission did not affect the mitochondrial morphology, muscle function and myofibril ultrastructure. Therefore, for the first time, this study reports that the spatiotemporal regulation of mitochondrial fusion and not fission appears to be critical for IFM development, maintenance, and function. In conclusion, the present study offers the following novel insights into the regulation of IFM development and the how specific developmental events influence IFM function; I) The major target of miR-9a in IFMs is Troponin T, whose levels must be regulated during myofibrillogenesis in order to achieve stoichiometric balance essential for muscle contraction. II) The expression of Marf, a mediator of mitochondrial fusion is crucial during a window of time in IFM development in order to achieve normal mitochondria morphology and function as well as structurally sound, functional muscle fibres in adult.