Synopsis

Muscle development involves concerted action of a repertoire of mechanisms governing myoblast proliferation, migration, fusion and differentiation. Subsequently, there are cellular events administrating proper muscle function and maintenance of muscle integrity. **Chapter 1** covers what is known about muscle development, building up of mass and maintenance in vertebrates and Drosophila, highlighting the myogenic programs and factors that play a role in them. The formation of vertebrate skeletal muscles can be recapitulated in Drosophila indirect flight muscles (IFMs), making IFMs an interesting model to dissect and understand the mechanisms of muscle development and maintenance. The cellular and developmental events that occur during IFM development have been discussed in detail along with their genetic control which encompasses both cell autonomous and cell non-autonomous mechanisms. The fly resources and tools used for experimentations have been described in **Chapter 2**.

One of the hallmark events during muscle development is myoblast fusion. Myoblasts are kept in undifferentiated state until they fuse through a balanced action of anti-differentiation and pro-differentiation factors. The swarming myoblasts are in semi-differentiated state and just prior to fusion should exit cell cycle to achieve terminal differentiation. The mechanisms of cyclin/CDK complexes and their regulation via CKI (CDK inhibitor) are known in a cell. However, tissue specific factors exerting additional control on molecules that participate in cell cycle have been proposed but have not been shown *in vivo*. **Chapter 3** uncovers a novel role played by the transcription factor, Erect wing (Ewg) in IFM development and patterning. Despite the fact that Ewg is known to express in fusing myoblasts and nuclei of developing IFMs and has long been used as a nuclear marker for IFMs, the mechanism(s) behind Ewg's function has remained enigmatic. Historical perspective of Ewg has been presented in **Chapter 1**. One set of IFMs; dorsal longitudinal muscles (DLMs) require larval templates for their formation and the other set; dorsal ventral muscles (DVMs) form *de novo*. **Chapter 3** shows that Ewg is required in a spatio-temporal fashion to initiate myoblast fusion process. In the absence of Ewg, the

number of fusion events in a given time is reduced. In addition *de novo* fusion is observed in the region of DLM development just like DVM and overall IFM development is delayed resulting in an aberrant adult IFM pattern. Genetic studies undertaken reveal a requirement for Ewg in exerting a temporal control on myoblast fusion. This is achieved by down-regulating Cyclin E levels, as a result of which the myoblasts exit cell cycle at G1/S stage. Through this study the proposal for DLM development and pattern has been put forth as follows: i) appropriate progression of DLM development commences on synchronous exit of myoblasts from cell cycle. This function is facilitated by Ewg expression in fusing myoblasts assisting symmetrical DLM formation in hemithoraces. ii) DLM pattern of six muscles in each hemithorax is dependent on template survival which requires fusion of enough myoblasts and further subsequent fusion events to support the splitting of three larval templates or presumptive DLM.

The muscles that develop should preserve their structural integrity for efficient functional output. Muscles perform extensive activities warranting high energy requirements. IFMs are widely utilised for thorax movements that aid flight. IFMs are exclusively oxidative in nature with upto 40% mass contributed by large mitochondria themselves. **Chapter 4** describes yet another novel finding for Ewg function in IFM maintenance. Vertebrate homolog of Ewg is nuclear respiratory factor 1 (NRF1) known for its role in mitochondrial biogenesis. This prompted an investigation on the role of Ewg, if any, in mitochondrial function and IFM maintenance. In this chapter, Ewg null adult IFMs are shown to undergo degeneration. Mitochondria in these muscles show rounder and smaller phenotype. Mitochondria morphology is traced throughout Drosophila pupal DLM development and extensive fusion is observed in last one-fourth of pupal phase. In Ewg null condition transcripts of *Opa1-like* required for inner mitochondrial membrane fusion is found to be absent, suggesting lack of mitochondrial fusion behind the smaller mitochondrial morphology. This emerged as an intriguing problem since Ewg expression follows until sarcomerogenesis (formation of sarcomeres) initiates at mid pupal stage. Developmentally extending Ewg's expression beyond mid pupal stage is not observed to increase *Opa1-like* levels pointing an indirect regulation by Ewg. However, *Opa1-like* knock-down beyond mid pupal stage is not observed to result in any muscle or flight defect. It is thus proposed

that Ewg expression early during muscle development helps to up-regulate *Opa1-like* levels needed to support mitochondrial growth and fusion. In addition, this chapter provides additional data on requirement of Opa1-like for maintenance of mitochondrial as well as muscle integrity. This is the first ever report of tissue specific temporal regulation of *Opa1-like* by Ewg.

Chapter 3 and **Chapter 4** conclude spatially segregated functional requirements of Ewg which are also mechanistically distinct. Expression in fusing myoblasts channelizes fusing myoblasts to exit cell cycle and undergo timely fusion saving the larval template, subsequent fusion assists template splitting thus forming the appropriate adult DLM pattern. On the other hand expression until mid pupal stage up-regulates *Opa1-like* expression, facilitating mitochondrial fusion during the late pupal stage. This as a result helps maintain structural integrity of muscles in the adult.

Vertebrate skeletal muscle contains multiple muscle fibres that provide appropriate mass and size to muscles. As DLM share similarity in development to that of the vertebrate skeletal muscle, DLM organisation is studied to get insights into the mechanisms which regulate the process. **Chapter 5** discusses role of nuclear number and nuclear activity in determining the DLM organisation. In order to alter nuclear number, myoblast population is reduced to amounts lesser than that of the wild type and to alter nuclear activity, two nuclear encoded genes *Opa1-like* and *Marf* , involved in inner and outer mitochondrial membrane fusion respectively have been knocked down in IFMs. First, the DLM organisation is established by comparing it to the vertebrate skeletal muscle organisation. This organisation is affected on lowering the number of myoblasts destined to fuse and form IFMs, without affecting the differentiation. On the other hand, when nuclear encoded mitochondrial fusion genes are knocked down, not only DLM organisation but their differentiation is also affected. A proposal for achieving DLM organisation has been presented which should also apply to vertebrate skeletal muscle given their developmental similarity.

In conclusion, the studies decipher a novel mechanism by which a transcription factor, Ewg exerts a temporal control on myoblast fusions directly influencing progression of DLM formation, and thereby, symmetry and pattern.

Moreover, Ewg is also shown here to regulate mitochondrial fusion during later pupal stages helping muscles to attain greater function and maintain structural integrity. Discovery of such regulatory mechanisms controlling mitochondrial dynamics in vertebrates can open up new avenues to understand and design new therapeutic approaches to tackle mitochondrial diseases. Additionally, myoblast fusion and hence myonuclear number and their efficient functioning are shown to be important determinants of muscle organisation. This has further implications in understanding and using stem cell science in dystrophic or atrophic or ageing related muscle loss and therapy.