CHAPTER 6

SUMMARY
1. The area of muscle study is one of the most interesting research avenues where different fields of studies can work together to address various questions. Biochemically and structurally, much is known about the muscle however, there is an uphill amount of work that needs to be done, especially in the field of developmental biology, to understand the tissue in greater depth.

2. Transposon P-element, with the intervention of molecular advancements, has helped in revolutionizing research in *Drosophila*. Enhancer trap Gal4 technique is one of the most powerful and versatile technique for genetic manipulation studies in *Drosophila*.

3. The area of muscle study is one of the most interesting research avenues where different fields of studies can work together to address various questions. Biochemically and structurally, much is known about the muscle however, there is an uphill amount of work that needs to be done, especially in the field of developmental biology, to understand the tissue in greater depth.

4. A large gap remain unfilled due to lack of Gal4 strains that spatio-temporal expresses in different organs and tissues of *Drosophila*. In addition, ageing is a global issue and there is a need to identify markers which can be used for assessing proper diagnosis and prognosis purposes. Whether arthrin, the mono-ubiquitinated actin in flight muscles, is necessary for any kinds of physiological functions in insect flight is not known. These issues were investigated and presented in fourth chapter.
5. Chapter 2 presents an enhancer trap Gal4 screen conducted by mobilizing \( P\langle GawB \rangle \) within the genome with the objective to isolate Gal4 strains that express in different subsets of flight muscles and neurons innervating them in adult and during development. The findings are as follows:

a) A total of 30 Gal4 strains were isolated from the screen.

b) The isolated lines showed different expression patterns including few which exclusively showed expression in different subsets of thoracic muscles and together in flight muscles and neurons innervating them.

c) The physical insertion sites for five of the strains were localized through inverse PCR and sequencing technique and found to be localized at different regions of the genome.

d) The driver Gal4 in each of the selected five strains were found to be expressed in essential cell lineages as indicated by the expression and misexpression of toxic and cell cycle inhibitor genes.

e) Knock down studies of muscle differentiation factor, \( Mef2 \) and integral component of troponin complex gene, \( TnT \) indicated that the Gal4 in all the five strains are expressed in the muscle cell lineage.

f) Knock down studies of muscle structural gene followed by behavioural studies indicated that \( UH4-Gal4 \) is expressed in the tubular jump muscles of \( Drosophila. \)
g) Further characterization of the isolated strains is necessary to understand their expression patterns better and identify the genes, if any, is disrupted by the Gal4 insertion. It will allow the potential use of the drivers in their respective tissues they express. Characterization of the genes involved will also help to understand its functions.

6. Chapter 3 deals with the enhancer trap Gal4 strains which can also be used to screen genes whose activity increases with age and identified a gene involved in ageing processes. The findings are:

   a) From a screen of enhancer trap Gal4 strains, an insertion line was identified where the Gal4 expression level increased in IFMs with age.

   b) Physical localization through inverse PCR confirmed the insertion to be located at *Egg-derived tyrosine phosphatase (EDTP)*. However, the strands encoding the P-element and *EDTP* were opposite to each other.

   c) Validation through real time PCR in aged flies confirmed the significant increased of *EDTP* expression in IFMs.

   d) The increased expression of *EDTP* was also found in muscle mutant alleles and E3 ligase mutant which affect myofibrillar organization in the early life of the flies.

   e) The study indicated that EDTP can be used as a potential marker for ageing and muscle degeneration assessment purposes after thorough studies in higher vertebrates including human.
7. Chapter 4 presents \textit{UH3-Gal4} expression in the IFMs initiating from 34-36 hrs APF onwards and continue to express in adult. The findings are:

a) \textit{UH3-Gal4} is inserted in the \textit{hyperkinetic} gene region.

b) Knock down of muscle structural genes under \textit{UH3-Gal4} showed severe IFM defective phenotype without affecting the jumping and walking behaviours. This indicated the exclusive expression \textit{UH3-Gal4} in the IFMs among thoracic musculatures.

c) When the Gal4 activity is suppressed by bringing Gal80\textsuperscript{ts}, the muscle defect could be avoided. This highlighted the ability to use the strain for isoform switching studies in IFMs.

d) Studies of different troponin complex genes knock down confirmed the essential function of each troponin isoforms except for \textit{TnC1}.

e) Rescue of \textit{Tnl} null allele by the overexpression of embryonic \textit{Tnl} isoform could rescue the myofibrillar formation but not flight.

f) The study proves \textit{UH3-Gal4} to be a potential driver for IFM studies.

8. Chapter 5 deals with Arthrin, a mono-ubiquitinated form of Act88F that accumulates only in the IFMs of asynchronous flight insects. The findings are:

a) In \textit{Drosophila}, arthrin initiates its accumulation during mid-pupal stage when adult specific structural protein isoforms switching occurs in the IFMs.
b) Lysine at position 118 of Act88F is the ubiquitin ligating residue to form arthrin.

c) Arthrin is essential for increased flight endurance but not for achieving high wing beat frequency.

d) Rescue of null alleles for troponin complex genes by non-IFM protein isoforms allows arthrin formation in the IFMs indicating that irrespective of troponin isoforms, it is the presence of proper troponin complex which is necessary for arthrin formation. This established an associative link between arthrin and troponin complex proteins.

e) Despite the conservation of ubiquitin ligating residue, arthrin cannot be formed with other actin isoforms.

f) Further studies are required to understand the mechanism of arthrin formation and other possible functions through genetic approaches.

Therefore, the isolated enhancer trap Gal4 strains under study, expressed in different subsets of flight muscles in adult as well during their early developmental stages of *Drosophila*, revealed many new understanding in the muscle biology in general and *Drosophila* in particular. Further studies on many more such specific Gal4 strains can unravel fine tuning of many biological processes.