Synopsis

Myofibrillogenesis is a complex process involving assembly of many structural proteins in an orchestrated spatio-temporal manner to form a highly ordered contractile sarcomeric unit. Mutations in the proteins involved in muscle contraction and function lead to myopathic conditions in human. Hence, understanding the etiology of these diseases and genes involved may help in accurate diagnosis, prognosis and exploration of possible therapeutics.

Molecular players and signaling pathways of myogenesis are highly conserved across phyla, enabling us to exploit indirect flight muscles (IFM) of *Drosophila melanogaster* as a model to study muscle development and function. IFM is the only fibrillar muscle which has considerable functional similarity to vertebrate cardiac muscles. It also enables the analyses of all stages of muscle development from its earliest stages of fusion of the imaginal myoblasts to fully differentiated muscle with its assembled contractile apparatus. Perturbance of developmental process in IFM leads to flightless flies with dysfunctional muscle. High throughput mutant screens, designed to isolate flightless flies have led to the identification of large number of genetic loci which are involved in muscle patterning and myofibrillogenesis, thus giving useful insights into the structural and functional aspects of fibre formation.

One such classical mutant, *flightless H* (*fliH),* isolated during mutagenesis screen leads to IFM degeneration after fibres are formed normally. This interesting phenomenon is designated as muscle hypercontraction and is comparable to hypertrophic cardiomyopathies in humans. The muscle hypercontraction phenotype in this mutant was found to be temperature dependent and development of the process initiated at later stages of pupation. Cellular events associated with the IFM hypercontraction were followed up through development using this mutant. Further, interaction of *fliH* allele with other genetic backgrounds gave valuable insights on mechanisms of causation of muscle hypercontraction. Genetics played a pivotal role in identifying the mutant locus. The mutation was genetically mapped to the regulatory region of the *wupA* gene which was confirmed by sequencing data. The *wupA* gene codes for Troponin I (TnI), an inhibitory component of the troponin-tropomyosin complex of thin filaments. The mutation leads to reduced level of *TnI* transcript and hence reduced amount of protein, as a consequence, troponin complex formation is impeded leading to uninhibited

acto-myosin interactions, thus causing muscle fibre breakdown. Our study reveals that *fliH* is a unique allele which confers temperature sensitive muscle phenotype. This is the first mutation found in the regulatory region of any structural gene which is temperature dependant and leads to muscle hypercontraction. This study also emphasizes that stoichiometry of structural proteins is important for proper functioning of muscle.

Apart from mutations in sarcomeric genes, perturbations in calcium signaling also affect muscle functioning and lead to development of cardiac hypertrophy and failure. Hence, the role of calcineurin β-subunit (*canB2*), a calcium dependant protein phosphatase, in muscle was analyzed. Studies involving overexpression of *canB2* in IFM showed that it leads to muscle hypercontraction. In addition, characterization of one of the new allele generated for the present study confirmed presence of muscle tearing and sarcomeric structure abnormality. *canB2* alleles genetically interact with other hypercontracting alleles and enhance the hypercontraction phenotype. Overall, present study will help us to understand how genetic predisposition can enhance or suppress muscle hypercontraction.

In a reverse genetics approach, role of muscle LIM protein, *Beadex (Bx)* in IFM was analyzed, as point mutations and loss of function alleles of LIM genes are associated with cardiomyopathies in humans. Immuno-histochemistry showed that Bx is expressed in myoblasts associated with wing imaginal disc which gives rise to IFM. Expression is also seen in developing IFM and in the neurons innervating the IFM. However, unlike the other known LIM proteins in *Drosophila*, *Bx* was not adhered to muscle fibre and showed predominant cytosolic localization. Targeted knockout and over-expression in muscles showed fibre rupturing and Z-disc deformities. Our results suggest that Bx may be involved in mechano-sensory stress signaling pathway like the other LIM proteins in humans and proper maintenance of the sarcomeric structure.

Thus, present study elucidates the role of three loci namely: *wupA*, *canB2* and *Bx* in proper muscle development and function. All the three loci code for proteins having orthologues in higher vertebrates and have been implicated in the pathogenesis of cardiomyopathies and/or skeletal myopathies in humans. Overall, such studies involving analyses of genes implicated in muscle development and function will help in exploring disease pathways which may help in derivation of new therapeutic strategies.