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Mutations in Lamin and How It Causes Multiple Tissue-Specific Disorders

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MUTATIONS IN LAMIN AND HOW THEY CAUSE MULTIPLE TISSUE-SPECIFIC DISORDERS

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83 Pages

Lamins are the major components of the nuclear lamina where they provide a platform for the binding of proteins to the chromatin and confer mechanical stability (Dittmer and Misteli.,2011). Mutations in the human LMNA gene result in at least 15 distinct disorders ranging from muscular dystrophies to neurological disorders to lipodystrophies (Vytopil *et al.*,2003). Interestingly, some mutant forms of lamin protein aggregate, which may be toxic to the cells. However, it is unknown how specific mutations in lamin give rise to tissue-specific disease. I hypothesize that certain tissues are susceptible to specific lamin mutations due to the inability of tissue-specific quality control mechanisms to degrade those mutant forms, leading to protein aggregation and cellular toxicity. Lamin can be post-translationally modified by the addition of a lipid called a farnesyl group that helps anchor Lamin into the nuclear envelope. In addition, some laminopathies are characterized by alterations in the farnesylation state of Lamin. I find that the unfarnesylated form (the predominant form) and the farnesylated form of the different Lam Dm_0 mutant proteins have different expression patterns in the muscle. Future experiments will characterize how these mutant forms of Dm0 affect the functionality of the muscles and neurons in flies and if these forms can be targeted for degradation by p38Kb and the CASA complex.

KEYWORDS: Lamin; Lam Dm_0. Laminopathies; Farnesylation; Chaperone Assisted Selective Autophagy (CASA)-complex.

MUTATIONS IN LAMIN AND HOW THEY CAUSE CAUSES MULTIPLE TISSUE-SPECIFIC DISORDERS

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MUTATIONS IN LAMIN AND HOW THEY CAUSE MULTIPLE TISSUE-SPECIFIC DISORDERS

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B.A.

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CHAPTER I: INTRODUCTION

1.1 Structural Features of Lamin Subtypes

The nuclear lamina is a critical component of the cell nucleus and plays a crucial role in maintaining nuclear structure and function (Moir *et al.,* 2011). It is primarily composed of intermediate filament proteins called Lamins, which are divided into two major subtypes: A-type lamins and B-type lamins (Kapinos *et al.,* 2011). Lamin subtypes have distinct characteristics and functions, and their dysregulation has been associated with various human diseases, including muscular dystrophy, premature aging syndromes, and certain types of cancer (Glass *et al.,* 1993). This chapter aims to provide an in-depth exploration of lamin subtypes, including their structural features, expression patterns, and functional roles.

A-type lamins, also known as lamins A and C, are encoded by the LMNA gene and contain a conserved central α-helical rod domain flanked by globular N-terminal head and C-terminal tail domains (Ascencio *et al.*,2012). The rod domain consists of heptad repeats that form coiled-coil structures, allowing the assembly of lamin dimers and higher-order polymers (Moir *et al.,* 2011). The tail domain contains a nuclear localization signal (NLS) that facilitates the targeting of A-type lamins to the nucleus (Taniura *et al.,* 1995). The tail domain of A-type lamins plays a crucial role in the formation of the nuclear envelope and providing structural support to the envelope (Kapinos *et al.,* 2011). The tail domain of A-type lamins interacts with various inner nuclear membrane proteins, such as emerin and LAP2 proteins (Sullivan *et al.,* 1999). These interactions help to anchor the lamins to the inner nuclear membrane and stabilize the nuclear envelope structure (Guo *et al.*, 2015). The tail domain of A-type lamins also binds to chromatin, the DNA-protein complex within the nucleus (Lammerding *et al.,*2006). This interaction is important for maintaining the overall nuclear architecture and organizing the chromatin within the nucleus by helping to tether

the chromatin to the nuclear envelope and facilitating proper gene regulation and nuclear functions (Guo *et al.*, 2015). It can undergo self-assembly and form higher-order structures, such as oligomers and filaments (Guo *et al.*, 2015). These structures provide mechanical support to the nuclear envelope and contribute to its stability and integrity (Steensel *et al.,* 2017). During the assembly of the nuclear envelope, the tail domain of A-type lamins interacts with other proteins involved in the process, such as nuclear pore complex components (Steensel *et al.,* 2017). This interaction is important for the proper localization and integration of these proteins into the nuclear envelope, ensuring its functional integrity (Taniura *et al.,* 1995) In summary, the tail domain of Atype lamins contributes to the formation and maintenance of the nuclear envelope by interacting with inner nuclear membrane proteins, associating with chromatin, forming higher-order structures, and participating in nuclear envelope assembly (Sullivan *et al.,* 1999). These mechanisms collectively provide structural support to the nuclear envelope and help regulate nuclear functions (Moir *et al.*, 2011).

B-type lamins, encoded by the LMNB1 and LMNB2 genes, include lamin B1 and lamin B2, which share structural similarities with A-type lamins (Steensel *et al.,* 2017). B-type lamins also possess an α-helical rod domain, N-terminal head domain, and C-terminal tail domain (Kapinos *et al.,* 2011). However, their rod domain lacks the extensive coiled-coil structure observed in A-type lamins (Kapinos *et al.,* 2011).). Flies have 2 lamin genes, which arose from a single ancestral lamin gene (Steensel *et al.,* 2017). Lam C, which is a homologue of lamin A/C and Lam Dm_0, which is a homologue of both lamin A, B1 and B2 ((Moir *et al.*, 2011). Lam Dm_0 is the only lamin type expressed in neurons (Lammerding *et al.,*2006).

1.2 Nuclear Stability and Mechanics

Lamins play a crucial role in providing structure and support to the nucleus. They are part of the nuclear lamina, a mesh-like network of proteins located on the inner surface of the nuclear envelope (Sullivan *et al.,* 1999). The nuclear envelope consists of two lipid bilayers, the inner and outer nuclear membranes, which are separated by the perinuclear space. The nuclear lamina lies just beneath the inner nuclear membrane and interacts with it through various proteins (Guo *et al.*, 2015). A-type lamins, with their higher-order polymerization, form a meshwork beneath the inner nuclear membrane, contributing to nuclear rigidity (Kapinos *et al.,* 2011). B-type lamins, although less abundant in the lamina, also contribute to nuclear stability by interacting with other nuclear envelope proteins and chromatin (Guo *et al*., 2015). Lamins provide structural support to the nucleus by forming a meshwork or scaffold-like structure in the nuclear lamina. This network of lamins helps maintain the shape and integrity of the nucleus ((Taniura *et al.,* 1995). Without lamins, the nucleus can become misshapen or undergo structural deformations (Steensel *et al.,* 2017) since lamins interact with other nuclear envelope proteins to provide mechanical strength and stability to the envelope (Glass *et al.,* 1993). Mutations in lamin genes can lead to nuclear envelope defects, such as nuclear envelope ruptures or invaginations, compromising nuclear integrity (Steensel *et al.,* 2017). Lamins contribute to nuclear mechanics by modulating the stiffness and elasticity of the nucleus. This mechanical stability is important for various cellular processes, such as cell migration, mechanotransduction, and nuclear-cytoskeletal interactions (Guo *et al.*, 2015). They undergo phosphorylation and dephosphorylation cycles, allowing for the disassembly and reassembly of the nuclear envelope during cell division and nuclear envelope breakdown and reformation during mitosis (Lammerding *et al.,*2006).

Lamins also play a role in organizing the positioning of the nuclear pore complexes (NPCs), which are large protein complexes responsible for regulating the movement of molecules between the nucleus and the cytoplasm (Lammerding *et al.,*2006). This organization is essential for the efficient transport of molecules into and out of the nucleus (Glass *et al.,* 1993). Additionally, the association between lamins and NPCs contributes to the mechanical strength and flexibility of the nuclear envelope (Steensel *et al.,* 2017).

1.3 Gene Regulation and Chromatin Organization

One of the key functions of lamins is to anchor the nuclear envelope to the chromatin, the complex of DNA and proteins that make up the genetic material (Steensel *et al.,* 2017). Lamins bind to specific regions of the chromatin called lamin-associated domains, providing stability and structural integrity to the nucleus (Sullivan *et al.,* 1999). Lamin subtypes play important roles in gene regulation and chromatin organization. They also play a role in organizing and regulating chromatin within the nucleus (Steensel *et al.,* 2017). lamins anchor heterochromatin (densely packed DNA) to the nuclear periphery, helping to position chromatin domains and influencing gene expression. A-type lamins interact with transcription factors and chromatin-associated proteins, influencing gene expression patterns and chromatin organization (Glass *et al.,* 1993). Btype lamins also participate in chromatin organization and contribute to nuclear architecture by forming interactions with specific DNA sequences and chromosomal domains (Steensel *et al.,* 2017).

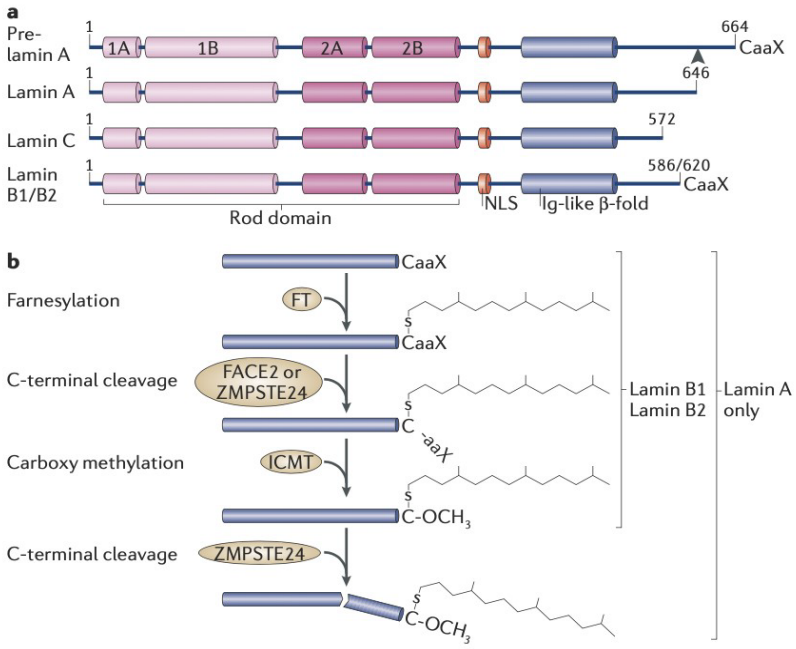
While the different lamin subtypes have distinct functions, they also work together in coordinating nuclear functions and responding to stimuli (Guo *et al.*, 2015). They form a complex network that integrates signals from the external environment and regulates various cellular processes (Steensel *et al.,* 2017).

1.4 Expression Patterns of Lamin Subtypes

A-type lamins are expressed in a developmentally regulated manner and exhibit tissuespecific variations (Sullivan *et al.,* 1999). During early development, A-type lamins are expressed at low levels, but their expression increases as cells differentiate (Glass *et al.,* 1993). In most adult tissues, A-type lamins are abundant and widely distributed throughout the nuclear lamina (Kapinos *et al.,* 2011). However, certain tissues, such as skeletal and cardiac muscle, express higher levels of A-type lamins compared to other tissues (Taniura *et al.,* 1995). Unlike A-type lamins, B-type lamins are constitutively expressed in all nucleated cells (Lammerding *et al.,*2006). Lamin B1 is the predominant B-type lamin and is ubiquitously expressed (Pan *et al.,* 2007). Whereas lamin B2 expression is restricted to specific tissues and cell types and is particularly abundant in the central nervous system, including neurons and glial cells (Guo *et al.*, 2015).

1.5 Lamin processing

During mitosis, when the nuclear envelope breaks down and the lamina disassembles, Atype lamins are solubilized and distributed throughout the cytoplasm, whereas B-type lamins maintain close associations with the nuclear membrane (Taniura *et al.,* 1995). The differences in membrane attachment during mitosis are attributed to whether the lamin protein is farnesylated, in which an isoprenol group is added to a cysteine residue following translation to form a thioether linkage. Mature lamin B retains its farnesylation moiety, which anchors B-type lamins to the membrane during mitosis, whereas the farnesylation moiety is removed from lamin A, rendering it more soluble (Kapinos *et al.,* 2011).



Source: Nature Reviews | Molecular Cell Biology, 2012

Figure 1a: Showing the structure and carboxy-terminal processing of A-type and B-type Lamins. a. Each of the Lamins features a small(10–20residues) 'head' domain followed by a central rod domain containing four coiled-coil regions (termed 1A, 1B, 2A and 2B). The large nonhelical 'tail' domain is organized around an immunoglobulin (Ig)-like β-fold. A nuclear localization sequence (NLS) lies immediately downstream of the rod domain.

The proteolytic cleavage of pre-lamin A (indicated by a black arrowhead in figure 1) results in the appearance of mature lamin A (Glass *et al.,* 1993). Lamin A and each of the B-type Lamins contain a carboxy-terminal CaaX motif (where C is Cys, a is an aliphatic residue and X is usually represented by a Met) that defines a site of farnesylation and carboxy methylation (Guo *et al.*,2015). Farnesylation of Lamins on the CaaX Cys residue is done by the protein

farnesyltransferase (FT) soon after synthesis (Ascencio *et al.*, 2012). This is followed by proteolysis of the aaX residues by farnesylated proteins-converting enzyme 2 (FACE2; also known as CaaX prenyl protease) in the case of the B-type Lamins, and by ZMPSTE24, a zinc metalloendoprotease, in the case of lamin A). Processing of the CaaX motif is completed by carboxy methylation by ICMT (isoprenylcysteine carboxyl methyltransferase) of the new C terminus (Sullivan *et al.,* 1999).

Once incorporated into the nuclear lamina, lamin A, but not the B-type Lamins, undergoes an additional ZMPSTE24-mediated cleavage step (in human lamin A this occurs after Tyr646, as indicated by black arrowhead in (**Figure 1a**). which removes an additional 15 amino acids, including the farnesylated Cys, leading to the appearance of non-farnesylated mature lamin A (Burke *et al*, 2012). To facilitate the disassembly of the lamina, lamins are phosphorylated by Protein kinase C and are dephosphorylated by type1 protein phosphatase during reassembly (Taniura *et al.,* 1995).

1.6 Laminopathies: Unraveling the Complexities of Nuclear Lamina Disorders

Mutations in Lamin (LMNA gene) result in at least 15 distinct disorders ranging from muscular dystrophies to neurological disorders to lipodystrophies (Vytopil *et al*.,2003). These disorders are collectively called laminopathies, are a class of uncommon genetic diseases caused by mutations in the lamin genes and other related genes including ZMPSTE24, which codes for a metalloproteinase important in lamin processing (Pan *et al.,* 2007). As dysfunction of the nuclear lamina can impact many organ systems, laminopathies include a broad category of illnesses that have extremely different clinical phenotypic forms, including skeletal muscle diseases like EmeryDreifuss muscular dystrophy, progeria syndromes like Hutchinson-Gilford, cardiac illnesses

including dilated cardiomyopathy, and lipodystrophies (Glass *et al.,* 1993). While these disorders are distinct conditions, they all involve abnormalities in cellular components related to nuclear structure and lipid metabolism (Taniura *et al.,* 1995). Laminopathies primarily affect tissues that require significant nuclear integrity and stability, such as skeletal muscle, cardiac muscle, adipose tissue, and peripheral nerves.

Emery-Dreifuss muscular dystrophy (EDMD) primarily affects the skeletal and cardiac muscles, and symptoms may include muscle weakness, contractures (joint stiffness), and cardiac abnormalities. Hutchinson-Gilford progeria syndrome (HGPS) affects connective tissues throughout the body. This condition causes premature aging, leading to characteristic features like skin changes, joint stiffness, and cardiovascular problems. Autosomal dominant leukodystrophy (ADLD), primarily affects the central nervous system. Symptoms may include progressive motor and cognitive impairment, as well as changes in behavior. Laminopathies can also affect adipose tissue, resulting in lipodystrophies, which are characterized by the loss or abnormal distribution of adipose tissue, leading to metabolic disturbances.

1.7 Treatments for Laminopathies

Treatment options for laminopathies are often focused on managing the specific symptoms and associated complications. These may include physical therapy for muscle weakness and contractures, cardiac medications and interventions for heart-related issues, metabolic management for lipodystrophy-associated metabolic disturbances, and supportive care for peripheral neuropathies. It's essential for individuals with laminopathies to work closely with a medical team specialized in these conditions to receive appropriate care, monitoring, and guidance based on their

specific needs. One interesting aspect of laminopathies is that the mutations causing these disorders do not cluster to a single area of the lamin protein.

Lamin proteins consist of three major regions: the N-terminal head domain, the central αhelical rod domain, and the C-terminal tail domain. Mutations associated with laminopathies can occur in any of these domains, and in some cases, they may affect multiple regions simultaneously (Pan *et al.,* 2007). For example, mutations in the head domain of lamin A/C have been linked to diseases such as Hutchinson-Gilford progeria syndrome (HGPS) (Kapinos *et al.,* 2011). These mutations typically involve a single nucleotide change that leads to the production of a mutant lamin A protein called progerin. Progerin disrupts nuclear structure and function, leading to the characteristic features of HGPS (Glass *et al.,* 1993). In contrast, other laminopathies, such as Emery-Dreifuss muscular dystrophy (EDMD) and dilated cardiomyopathy (DCM), are associated with mutations in various regions of lamin A/C, including the rod domain and the tail domain (Sullivan *et al.,* 1999). These mutations can result in abnormal nuclear morphology, impaired gene expression, and tissue-specific symptoms affecting skeletal muscles or the heart (Taniura *et al.,* 1995).

The wide distribution of laminopathy-associated mutations throughout different regions of the lamin protein suggests that the functional consequences of these mutations can vary depending on their specific location. Mutations affecting different domains of lamin proteins may disrupt distinct molecular interactions, leading to diverse cellular effects and tissue-specific manifestations of laminopathies. Understanding the mechanisms by which specific mutations in lamin proteins contribute to laminopathies is an active area of research. Researchers are investigating how these mutations affect nuclear architecture, chromatin organization, gene expression, and cellular

signaling pathways. By unraveling the molecular basis of laminopathies, scientists hope to develop targeted therapies that can mitigate the effects of these genetic disorders in the future.

Current approaches to treating laminopathies generally concentrate on managing symptoms and consequences. An ongoing investigation is, however, illuminating promising treatment directions. Targeted gene therapies, gene editing methods (such as CRISPR-Cas9), and pharmaceutical interventions to modify the nuclear lamina or its downstream signaling cascades are some examples of these (Glass *et al.,* 1993). Innovative techniques, including those based on small molecules, antisense oligonucleotides, and epigenetic modifiers, have the potential to be used in therapeutic interventions in the future (Pan *et al.,* 2007). Continued study is essential to elucidate the underlying mechanisms and broaden the range of available treatments because the field of laminopathies is developing quickly. Gaining a thorough understanding of laminopathies, including their molecular pathophysiology, genotype-phenotype correlations, and prospective treatment targets, requires collaboration among doctors, geneticists, cell biologists, and bioengineers.

Table 1: Showing Nuclear Lamin Diseases, Function and Phenotypes

Syndrome/disease	Effects on *LMNA* gene and protein	Phenotype
Emery–Dreifuss muscular dystrophy (EDMD)	Autosomal dominant or recessive missense mutations in *LMNA* in various positions. Some may affect assembly of lamin A and interactions with associated proteins.	Progressive muscle weakness, cardiomyopathy
Dilated cardiomyopathy, type 1A	Autosomal dominant missense mutations mostly in exons 1 *or* 3 of *LMNA*.	Cardiomyopathy with minimal effects on skeletal muscle
Limb girdle muscular dystrophy (LGMD)	Autosomal dominant mutations in exon 1 of *LMNA*. The effect on lamin A is not known.	Skeletal muscle weakness and heart defects
Familial partial lipodystrophy, Dunnigan type (FPLD2)	Autosomal dominant missense mutations in exons 8 and 11 of *LMNA*. Mainly affects the Ig-fold domain that may interfere with protein-protein interactions.	Loss of subcutaneous fat, insulin-resistance, diabetes, hypertriglyceridemia and atherosclerosis
Mandibuloacral dysplasia (MAD)	Autosomal recessive mutations R527H, K542N, and A529V in the Ig-fold domain. Compound heterozygous mutations have also been reported. May interfere with protein-protein interactions.	Dental defects, lipodystrophy, atrophy of the skin on hands and feet, mandibular hypoplasia, acroosteolysis, alopecia, insulin resistance, progeroid features.
Hutchinson–Gilford progeria syndrome (HGPS)	Mostly spontaneous mutations (1824 C-to-T) in exon 11 of *LMNA*. This activates a cryptic splice donor site leading to the permanently farnesylated form of mutant lamin A called "progerin" with a deletion of 50 amino acids near the C terminus. Alters lamin functions with respect to nuclear shape maintenance and chromatin organization.	Early onset premature aging with alopecia, loss of subcutaneous fat, severe atherosclerosis, and cardiovascular disease leading to early death.
Atypical progeria syndromes (APS)	Various heterozygous missense mutations in *LMNA*, which are not associated with the production of progerin. These include heterozygous missense *LMNA* mutations, such as, P4R, E111K, D136H, E159K, and C588R.	Associated with different progeroid features including one or more of the following: short stature, partial alopecia, diabetes, lipodystrophy and mandibular hypoplasia, and cardio-vascular disease.
Atypical Werner's syndrome (AWS)	Autosomal dominant mutations A133L mutation in *LMNA*. Effect on protein is unknown, but may lead to changes in protein-protein interactions.	Late onset premature aging, atherosclerosis, sclerodermatous skin, prematurel grey hair.
Restrictive dermopathy (RD)	Mutations in exon 11 of *LMNA* and /or homozygous or compound heterozygous mutations in *ZMPSTE24*. These result in the formation of permanently farnesylated pre-lamin A.	Loss of fat tissue, tight skin, pulmonary hypoplasia, early lethality.
Charcot–Marie–Tooth disease, type 2B1	Autosomal recessive missense mutations in the lamin A rod domain that may affect lamin assembly.	Weakness and areflexia of lower limbs
Generalized lipodystrophy	Autosomal dominant mutations I10T and heterozygous substitution in exon 1 c.29C>T, in *LMNA* with unknown effects on lamin A.	lipodystrophy or lipoatrophy, may include diabetes and a progeroid phenotype

Source: Semantic Scholars | Butin-Israeli *et al*, 2012

1.8 Drosophila as A Model Organism

The fruit fly *Drosophila melanogaster* has two lamin genes, Lamin C and Lamin Dm_0, that evolved from a single ancestral gene and are homologous to both the LMNA (LamA/C) and LamB genes in humans, and many of the disease-causing mutations in LMNA are conserved in both LamC and Lam Dm_0 (Sullivan *et al.,* 1999). Studies using flies have been instrumental in our understanding of lamin functions. Lamin subtypes in Drosophila interact with various nuclear partners to carry out their functions (Glass *et al.,* 1993). Lamin Dm_0 interacts with a multitude of proteins involved in nuclear envelope organization, nuclear import/export, and chromatin regulation. These actions contribute to the maintenance of nuclear architecture and the regulation of gene expression (Sullivan *et al.,* 1999). Lamin C also interacts with specific nuclear partners, including transcription factors and chromatin modifiers, to regulate gene expression and chromatin organization (Sobotka-Briner *et al*. 1992). The expression and localization of lamin subtypes in Drosophila are tightly regulated at the transcriptional and post-translational levels. Transcription factors and signaling pathways play important roles in controlling the expression of lamin subtypes during development and in response to environmental cues (Pan *et al.,* 2007). Post-translational modifications, such as phosphorylation and proteolytic cleavage, also regulate the stability, localization, and function of lamin subtypes (Taniura *et al.,* 1995).

The expression patterns of lamin subtypes in Drosophila vary across different developmental stages and tissues. During early embryonic development, lamin Dm_0 is abundantly expressed in the nuclei of all somatic cells (Burke *et al.,* 1999). As development progresses, lamin Dm_0 expression becomes restricted to the somatic cells of the developing central nervous system and epidermis (Sobotka-Briner *et al.,* 1992). In contrast, lamin C expression is initially low but increases during later stages of embryonic development. Furthermore, Lam Dm_0 is expressed in both muscles and neurons

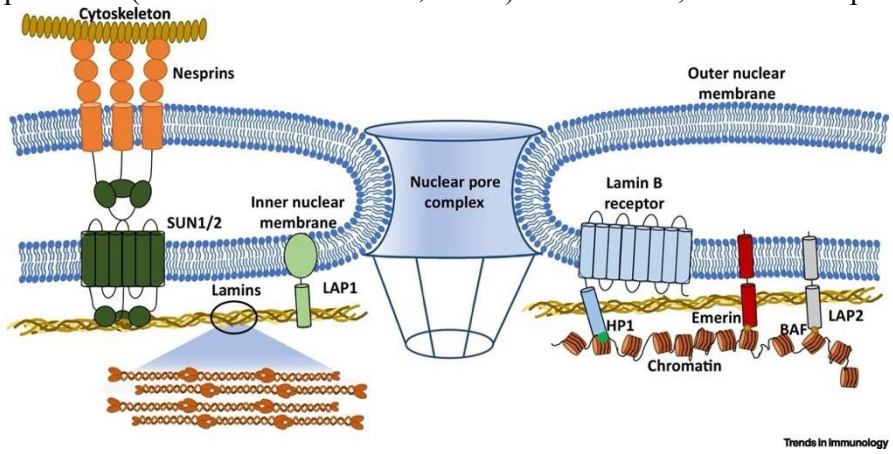


Figure 1b. Showing the nuclear pore complex and lamin interaction together with other lamin associated proteins.

while LamC is not expressed in neurons (Andres *et al.,*2007), suggesting that Lam Dm_0 in muscles and neurons has both A- and B-type lamin functions. Loss of function studies using genetic mutants and RNA interference techniques have revealed distinct roles for lamin Dm0 and lamin C in nuclear organization, nuclear morphology, and chromatin organization (SobotkaBriner *et al.,* 1992). Lamin C has been well studied in nuclear organization and gene regulation in both embryonic, larval, and adult tissues (Sobotka-Briner *et al.,* 1992). Additionally, lamin C has been implicated in the modulation of chromatin accessibility and the regulation of gene expression during development and aging (Sobotka-Briner *et al.,* 1992). Lamin Dm_0, which is less well studied, has been shown to be essential for maintaining nuclear shape and integrity during early embryonic development (Lombardi *et al.,*2011). Further investigations into the precise mechanisms underlying the

functions of lamin subtypes in Drosophila will deepen our understanding of nuclear architecture and its impact on cellular processes. Studies in both humans and flies have found that mutant lamin proteins aggregate (Glass *et al.,* 1993), which may be toxic to the cells. Recently, the Vrailas-Mortimer lab has found that wild-type Lam Dm_0 aggregates with age and that this is regulated by the aging gene p38 MAPK (p38Kb) (VrailasMortimer *et al.,* 2011). In addition, p38Kb regulates Lam Dm_0 degradation through an interaction with the Chaperone-Assisted Selective Autophagy **(**CASA**)** complex, a quality control mechanism that targets damaged/misfolded proteins for degradation through the autolysosomal pathway (Taniura *et al.,* 1995).

1.9 The p38Kb Pathway and The Chaperone Assisted Selective Autophagy (CASA) complex.

The p38K pathway is a signaling cascade that is activated in response to cellular stressors, including oxidative stress, heat shock, and endoplasmic reticulum (ER) stress ((Davis *et al.,* 2008). The pathway consists of a series of protein kinases that are activated sequentially, ultimately leading to the phosphorylation of downstream targets and the activation of various cellular responses (Kapinos *et al.,* 2011). In the context of protein aggregates, p38K signaling can be activated by several mechanisms. One mechanism is through the activation of the unfolded protein response (UPR), which is a cellular stress response pathway that is triggered by the accumulation of misfolded proteins in the ER ((Davis *et al.,* 2008).). The UPR can activate p38K signaling, which in turn regulates the expression of chaperone proteins, such as heat shock proteins, that help to refold or degrade misfolded proteins (Berman *et al.,* 2001). Additionally, p38K signaling can be activated by oxidative stress, which is commonly associated with protein aggregation (Berman *et al.,* 2001). Oxidative stress can result in the production of reactive oxygen species (ROS), which

can damage proteins and promote their misfolding and aggregation ((Davis *et al.,* 2008).). The activation of p38K by oxidative stress can lead to the phosphorylation and activation of transcription factors that regulate the expression of genes involved in protein quality control and clearance (Kapinos *et al.,* 2011). Furthermore, p38K signaling can modulate the activity of molecular chaperones and proteolytic systems involved in protein aggregate clearance (VrailasMortimer *et. al.,* 2011). For example, p38Kb can phosphorylate and activate heat shock factor 1

(HSF1), a transcription factor that regulates the expression of heat shock proteins (Berman *et al.,* 2001). Heat shock proteins are molecular chaperones that assist in protein folding and can also promote the clearance of protein aggregates. Overall, the p38K signaling pathway plays a critical role in the cellular response to protein misfolding and aggregation. By regulating the expression of chaperones, proteolytic systems, and other stress-responsive genes, p38K signaling helps to maintain protein homeostasis and prevent the accumulation of toxic protein aggregates in tissues. Dysregulation of this pathway can contribute to the pathogenesis of neurodegenerative diseases and other protein aggregation-related disorders (Vrailas-Mortimer *et. al.,* 2011).

The CASA complex is made up of a network of proteins that work together to identify and deliver cargo for degradation via autophagy (Berman *et al.,* 2001). The Chaperones Hsp70 and HspB8 can detect abnormal protein structures or misfolded proteins and refold them. The cochaperone BAG3, improves chaperone action and makes it easier to recognize client proteins. The adaptor protein p62/SQSTM1, functions as links between chaperones and the autophagosomal membrane, enabling cargo engulfment and subsequent destruction (Kapinos *et al.,* 2011). CASA complex-mediated selective autophagy encompasses several distinct pathways, including aggrephagy (Berman *et al.,* 2001). Aggrephagy attacks protein clumps that have developed because

of cellular stress, mutation, or improper protein folding ((Davis *et al.,* 2008).). Toxic aggregates are removed and sequestered with the help of CASA complex proteins, such as p62/SQSTM1 and BAG3, which reduce proteotoxic stress and preserve cellular homeostasis (Kapinos *et al.,* 2011). For cellular health to be maintained and the buildup of harmful substances to be avoided, the appropriate operation of CASA complex-mediated selective autophagy is essential. Numerous neurodegenerative conditions, such as Alzheimer's, Parkinson's, and Huntington's illnesses, and even laminopathies have been linked to dysregulation of this process, where abnormal protein aggregates are a part of the pathology of the illness (Davis *et al.,* 2008). Aside from that, alterations in mitophagy have been linked to cellular aging and illnesses of old age (Kapinos *et al.,* 2011).

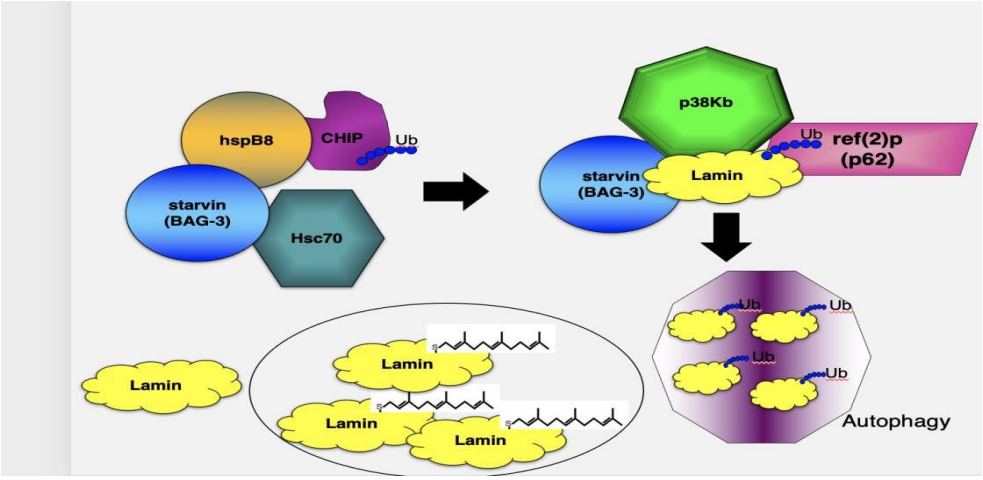


Figure 1c.: Model of chaperone assisted selective autophagy (CASA) complex. The CASA complex works in protein homeostasis through the identification and targeting of misfolded or damaged proteins for lysosomal degradation. We hypothesize that p38Kb interacts with the CASA complex to regulate aggregation of Lamin within the adult muscle of Drosophila.

Literature Review

One outstanding question is how distinct mutations in the Lamin genes result in such different disorders, as these proteins play a critical role in nuclear shape and are expressed in most cell types. In addition, these different lamin mutations can result in proteins that aggregate (Vrailas-Mortimer *et. al.,* 2011), I hypothesize that certain tissues are susceptible to specific lamin mutations due to the inability of tissue-specific quality control mechanisms to degrade those mutant forms, leading to protein aggregation and cellular toxicity. I also hypothesize that the CASA complex will degrade the muscular dystrophy mutant proteins but not the CMT mutants when expressed in neurons resulting in CMT.

However, the CMT mutant proteins will be degraded but not the muscular dystrophy mutants when the lamin mutations are expressed in muscles resulting in diseased muscles or muscular dystrophy. This is because the CASA-complex changes the folding of the mutant lamin proteins and how they interact with the CASA-complex. Thus, the tissue specificity may be due to tissue-specific quality control mechanisms that are responsible for degrading lamin, and the inability of these specific mechanisms to degrade certain mutant forms of lamin leads to the formation of toxic aggregates and disease symptoms. Therefore, I will test the muscles and neurons of flies, if p38Kb can also regulate the degradation of lamin mutations that give rise to muscular dystrophy and/or neuropathy. I also hypothesize that in the muscle, the muscular dystrophycausing mutant Lamin proteins will aggregate while the neuropathy mutant proteins will be degraded. In addition, in the neurons, the neuropathy mutant proteins will aggregate, and the muscular dystrophy proteins will be degraded in the neurons of the flies. Thus, it explains why some mutations give rise to muscle disorders and others give rise to neurological disorders.

Human	lam C	lamDm0	Disease
R298C	K313C	R320C	CMT
L162P	A177P	L184P	primary dilated cardiomyopathy
R190W	R205W	R212W	CMT and cardiomyopathy
G449V	G489V	G481V	CMD (Congenital muscular dystrophy)
L489P	V528P	I522P	Striated muscle laminopathy

Table 2. Showing the various mutant forms of lamin in Flies (LamC and LamDm0) and in Humans with their corresponding Tissue-specific Disorders. However, we do not know the molecular mechanisms underlying how mutations in lamin affects these individuals.

To test if different lamin mutations aggregate in the muscle and motor neurons I used western blotting to quantify the levels of accumulation of lamin in mutant lamin. I also tested how lamin mutations affected locomotive functions and longevity of the lamin mutant flies. Results from western blotting revealed increased levels of lamin in mutant flies. Result from western blotting showed that mutations in lamin affect the ratio of post-translational modification (farnesylation) in flies. Mutant lamin flies with increased levels of farnesylation had the worse locomotive function and reduced lifespan after performing behavioral and longevity assays, respectively.

CHAPTER II: MATERIALS AND METHODS

The UAS-GAL4 System

In Drosophila research, the UAS-GAL4 system was a potent instrument that transformed the fields of genetics and developmental biology. This technology enables the study of gene function and the exploration of numerous biological processes by allowing researchers to control gene expression in a precise and spatiotemporal manner. It has been essential in increasing our knowledge of Drosophila development and has laid the path for numerous other organisms' findings as well. The upstream activation sequence (UAS) and the GAL4 transcription factor are the two primary parts of the UAS-GAL4 system. A DNA sequence that has the ability to bind to the GAL4 transcription factor makes up the UAS element. GAL4, on the other hand, is a yeast transcription factor that, upon binding to the UAS element, can initiate gene expression. Researchers have created a flexible mechanism to regulate gene expression in Drosophila by fusing these two elements.

The UAS element is positioned upstream of the target gene in the UAS-GAL4 system, and a tissuespecific promoter regulates the expression of the GAL4 transcription factor. The GAL4 protein attaches to the UAS element when these two components are together, activating gene expression. By regulating the expression of GAL4, this approach enables researchers to selectively activate or repress gene expression in particular tissues or at developmental stages.

The versatility and specificity of the UAS-GAL4 system are two of its key benefits. Researchers can focus on cell types or tissues of interest because GAL4 expression can be regulated by several tissue-specific promoters. This specificity allows for the research of gene function in a specific setting, revealing valuable information about the function of genes in many biological processes.

The molecular processes underpinning development, behavior, and disease can be uncovered by carefully regulating the expression of genes.

The UAS-GAL4 system also enables temporal regulation of gene expression. Researchers can precisely control when gene expression is activated or repressed by employing temperature sensitive GAL4 variations or the Gal80 repressor, which can limit GAL4 activity. This temporal control is crucial for investigating dynamic processes during development or increasing gene expression at stages to get around potential developmental flaws. In research on Drosophila, the UAS-GAL4 system has been extensively utilized to examine a variety of biological issues. The patterning of the body plan, the formation of organs, and the determination of cell destiny are just a few examples of how it has shed light on developmental processes. Additionally, the system has proven crucial in revealing the genetic underpinnings of behavior, including learning and memory, circadian rhythms, and social interactions. Furthermore, the UAS-GAL4 system has been used to model diseases, enabling researchers to learn more about the molecular causes of numerous human diseases. The UAS-GAL4 system has been modified for use in research on zebrafish, mice, and even plants in addition to Drosophila. This illustrates the extensive use and significance of this potent genetic weapon. The UAS-GAL4 system has sped up scientific advancement and paved the door for the creation of innovative medicines and interventions by enabling precise control over gene expression. In conclusion, the UAS-GAL4 system in Drosophila has transformed genetics and developmental biology. Researchers have been able to explore gene activity and elucidate the molecular mechanisms behind many biological processes thanks to its flexibility, selectivity, and temporal control. The UAS-GAL4 system has significantly impacted numerous fields of biological study and advanced our understanding of Drosophila development. This technology holds enormous potential for new discoveries and uses in the years to come as it develops.

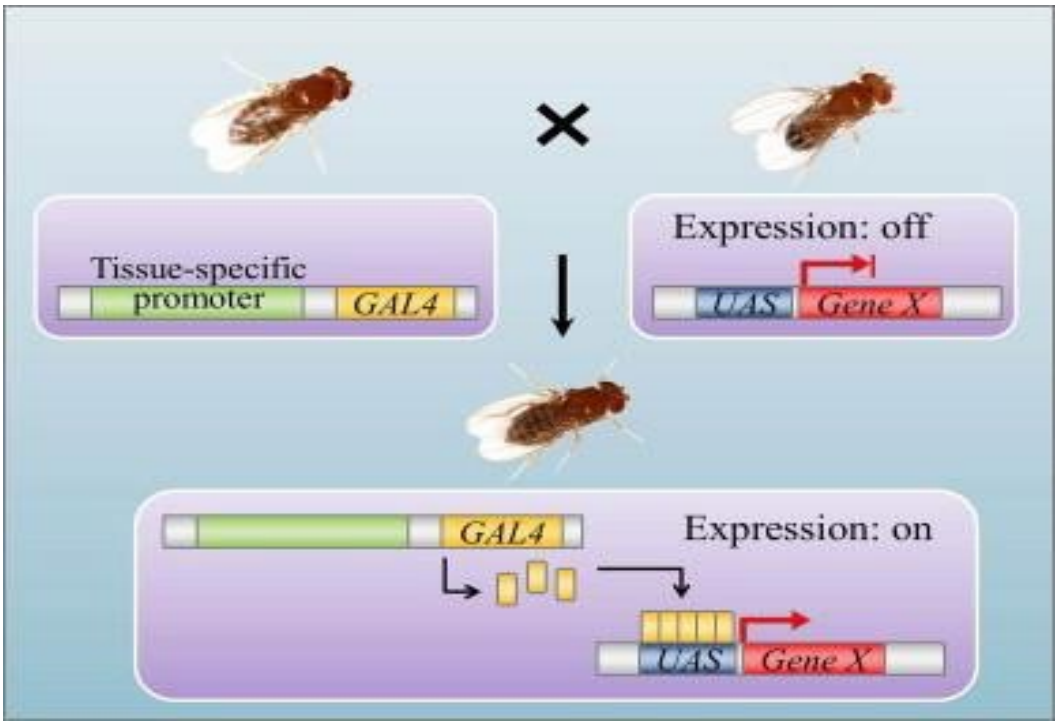


Figure 2: UAS-GAL4 system. To activate the desired transgene, the GAL4 serves as a driver. A tissue-specific promoter that is located upstream of GAL4 enables transgene expression at a specified time or location. A second transgene upstream of the sequence for a gene of interest or

RNAi for the gene of interest contains the UAS site (Upstream Activator Sequence, or UAS). To promote production of the transgene encoding the desired gene of interest, the GAL4 protein must first bind to the UAS site in a particular tissue.

Western Blotting

To quantify accumulation of main forms of lamin (75kDa) and farnesylated (100kDa) forms of lamin in the mutant flies. Western blots were performed on lysates from thoraxes or heads of three virgin female flies of each lamin mutant genotypes. As each of the lamin mutant proteins

have been tagged with HA, I dissected three thoraxes each for every mutant lamin flies. I homogenized the tissues in 30ul of SB buffer, and I probed with anti HA to detect differences in lamin mutant expression as compared to expression of wild type lamin. The specific HA antibody I used was the mouse HA 12CA5 with a catalogue number of MA1-12429 from thermos fisher. Alpha tubulin loading control was used to compare the expression of the mutant forms of lamin in the flies. These experiments were performed in triplicate.

Fly Climbing (Behavior Assay)

To determine if increased accumulation and aggregation of lamin in the muscle and neurons affects locomotor functions in the flies, I performed the fly climbing behavior assay. This assay specifically employed the negative geotaxis approach, so I tapped flies to the bottom of a chamber, then calculated the proportion of flies that reach the top within a predetermined time. Flies were aged for 1, 3 and 5 weeks respectively. 10 duplicates of flies per genotypes were collected into vials containing food and kept in an incubator at 25C. Food were changed at least twice each week. The climbing abilities of each duplicate per genotype were tested after weeks 1, 3 and 5. Proportion of flies were calculated by counting the number of flies at the top after 5, 10 and 20 seconds I tested 20 replicates of 10 flies at 1 week, 3 weeks, and 5 weeks of age for each genotype. in mutant forms of lamin will restore the locomotive functions and abilities in Mutant lamin flies.

Lifespan Assays

As described in (Vrailas-Mortimer et. al., 2011), all lifespan experiments were conducted with virgin females and males at 25 degrees Celsius using Genesee molasses formulation food. All lamin mutant crosses were completed in bottles at 25 degrees Celsius. Flies were transferred to

new vials atleast twice each week. Vials were organized in replicates of 10 flies. 10 duplicates of flies per genotypes were collected into vials containing food and kept in an incubator at 25C. Food was changed at least twice each week. The longevity of each duplicate per genotype was tested after weeks 1, 3 and 5. The longevity of flies was calculated by counting the number of flies alive after each week. Death was recorded weekly and plotted.

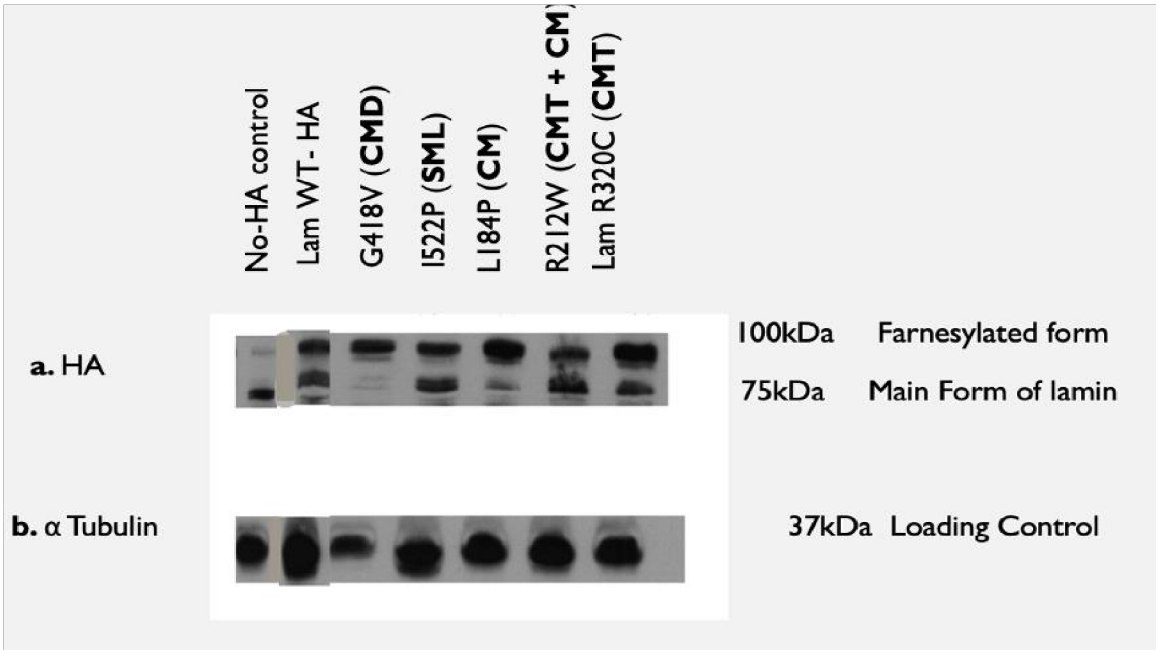
Sucrose Gradient Fractionation

To determine the exact location in the tissues where Farnesylated and main forms of lamin are aggregating in the cell. 30 adult muscles and thoracic ganglion tissue from three-week-old females of each genotype were tested.10% to 50% sucrose gradients were made and Lysate samples contain a mixture of different size macromolecules were layered on the surface of a gradient whose density increases linearly from top to bottom and then placed in ultracentrifuge at 55000rpm for 20hours at 4C. The pellets were, however, resuspended in 200ul of the buffer.

CHAPTER III: RESULTS

Mutations in Lamin affect the ratio of farnesylated to unfarnesylated lamin when expressed in the muscle.

Wildtype or mutant lamin tagged with HA was expressed in the muscle and flies were collected at 1 week of age. Lysates were probed for the presence of lamin. 2 forms of lamins (Figure 3.1a). Probing with HA antibody revealed the main forms of lamin at 75kDa and the farnesylated form of lamin at 100kDa (Figure 3.1a). This suggests that mutations in lamin undergo a form of post-translational modification known as farnesylation which could also contribute to tissue-specific disorders (Sobotka-Brine *et al.,* 1992) and subsequently influencing the longevity and behavior of flies. Densitometry analysis was done using photoshop and ImageJ to quantify the ratio between the main forms and farnesylated Lamins in flies (Figure 3.1b). The CMD, CM and CMT mutations had the highest levels of farnesylation. This indicates that more of the Lamins at the 75kDa are getting farnesylated in the muscles as a result of these mutations.



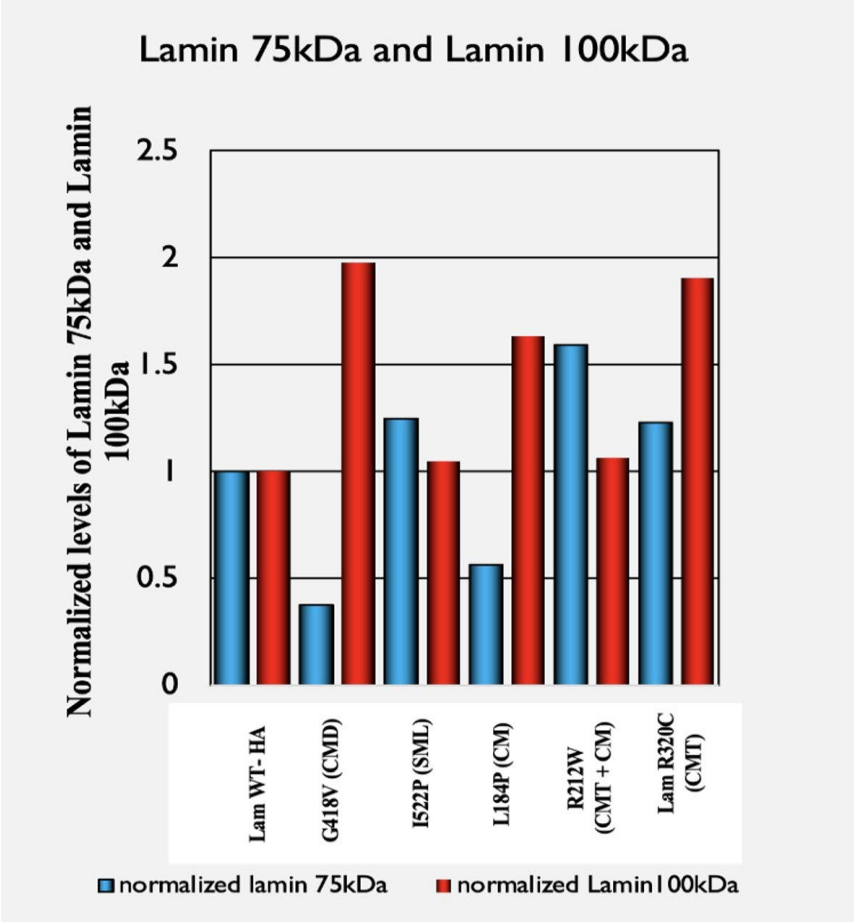
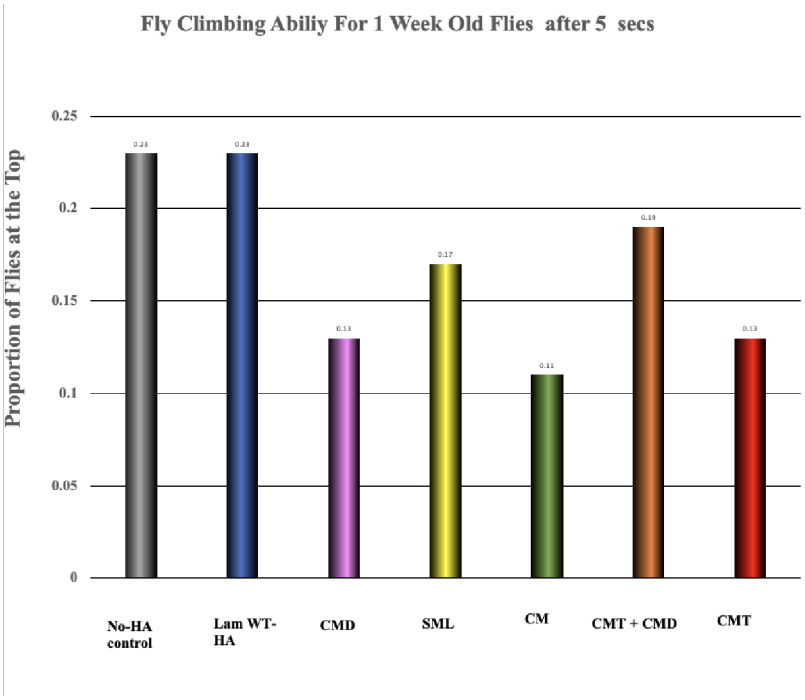
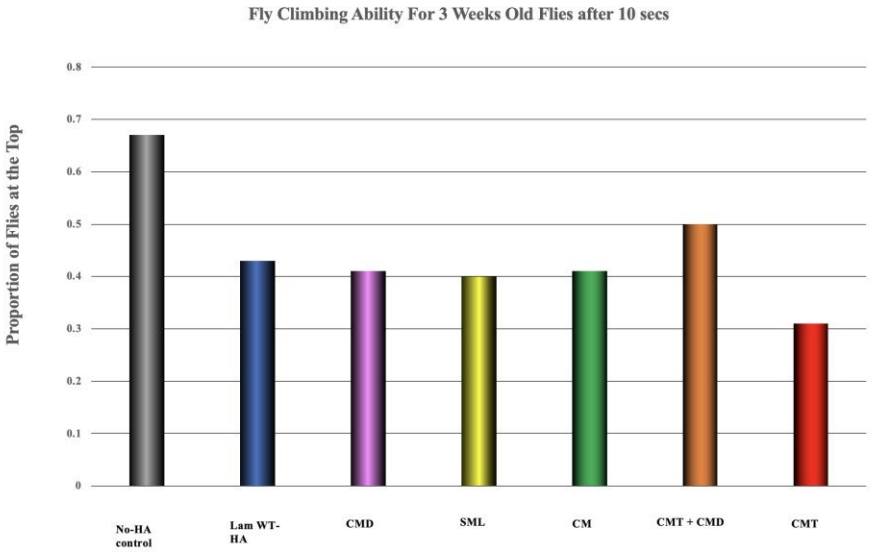


Figure 3. 1a: Mutant Forms of Lamin Have Different Protein Expression Levels in The Muscles
Immunoblot analysis of muscle lysate from control (w1118) or transgenic expression of HA-tagged WT or mutant Lam Dm0. The main species of WT Lam Dm0 is ~74kDa. The Lam Dm0 R212W mutation results in a slightly higher molecular weight band. A longer exposure reveals the 100kDa form of Lam Dm0**.** Alpha tubulin loading control. These data suggest that disease mutations in Lam Dm0 that cause CMT, cardiomyopathy, and skeletal myopathies affect the protein in different ways when expressed in muscle tissue. n= pooled thoraxes of 3 flies per genotype of 1 week old female flies.
1b. Densitometry analysis of western blotting.

Fly climbing assay was performed to determine how the mutant lamin forms affected the longevity of the flies. It was observed that the CMD, CM and CMT mutants, which had increased farnesylation, were impaired at earlier ages and expression of the other mutant lamins become impaired later (Figure 3.2).




Fly Climbing Ability For 3 Weeks Old Flies after 10 secs
Proportion of Flies at the Top
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
No-HA control
Lam WT-HA
CMD
SML
CM
CMT + CMD
CMT

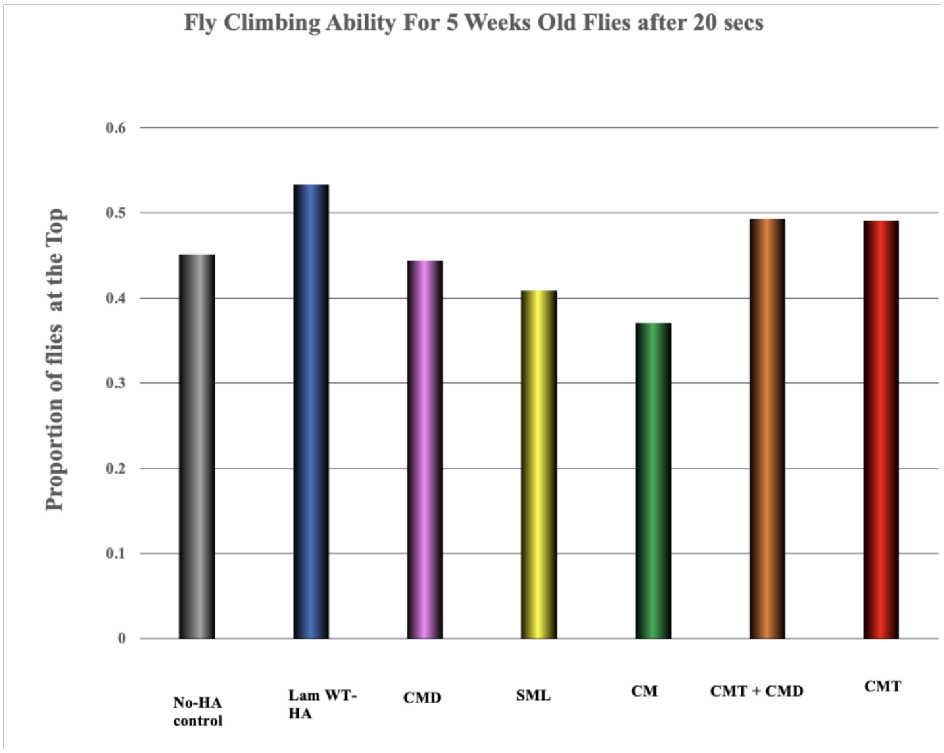


Figure 3.2 : Lamin mutation affects Locomotive and Flight functions in Female flies.

A. Negative geotaxis analysis showing the proportion of 1 week old Female flies at the top after 5 seconds.
B. Negative geotaxis analysis showing the proportion of 3 weeks old Female flies at the top after 10 seconds.
C. Negative geotaxis analysis showing the proportion of 5 weeks old Female flies at the top after 20 seconds.

Lifespan analysis was performed to determine how the mutant lamin forms affected the longevity of the flies. And also, to test if increased levels of farnesylation further reduced longevity. This data suggests that the farnesylated mutants are impaired more quickly and the other mutants catch up later (Figure 3.3).

Lamin Mutation affects longevity in female flies with HA-tag

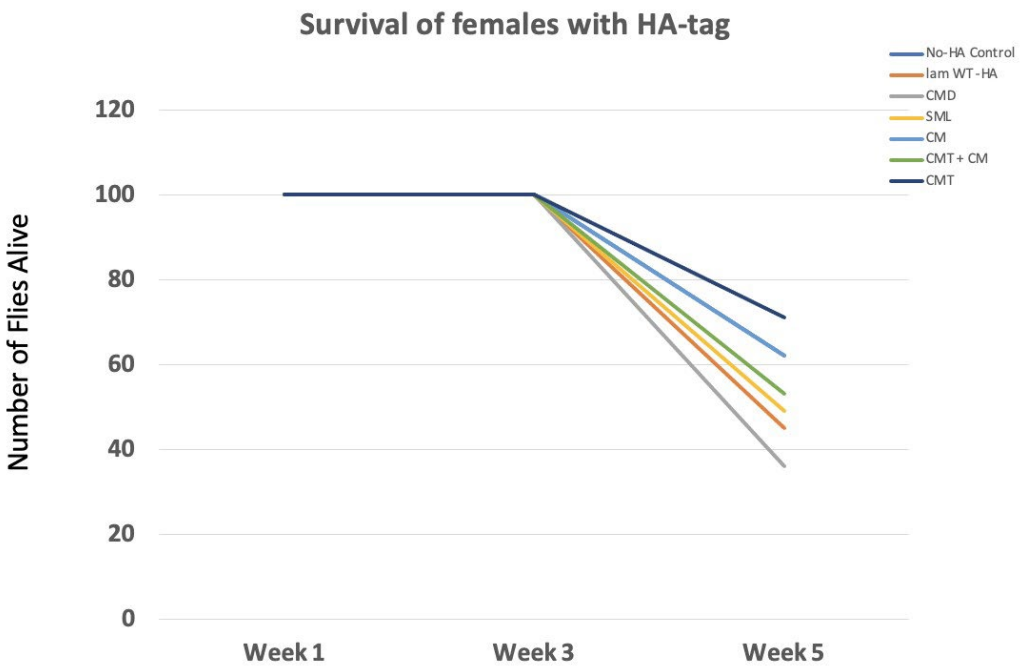


Figure 3.3: Lamin Mutation affects longevity in female flies with HA-tag.

Mutant lamin female flies tagged with HA started showing reduced lifespan after 3.

Mutations were deleterious after week 5, further reducing lifespan of flies. 10 flies per genotypes were collected in 10 replicates. Death was observed and counted each week.

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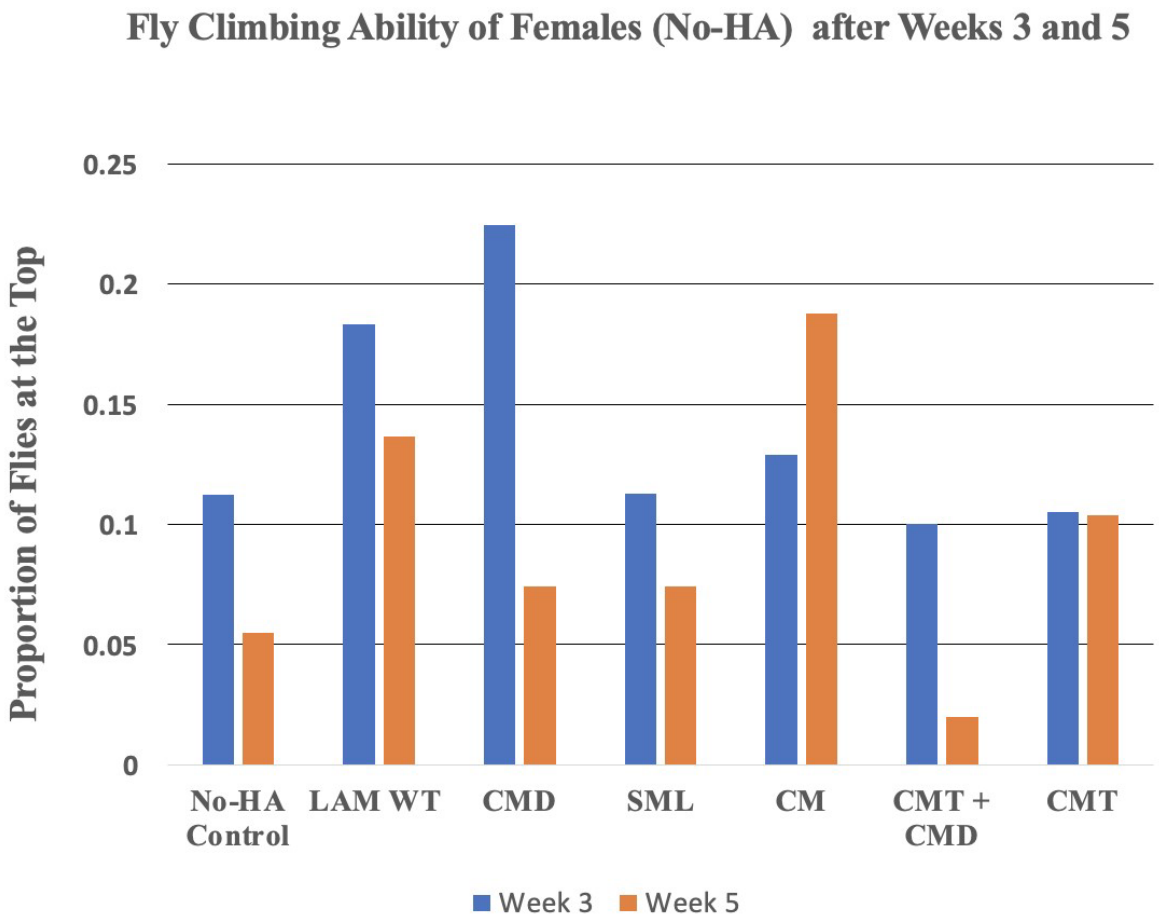
Distribution of fly climbing is affected by Lamin mutants without HA-tag



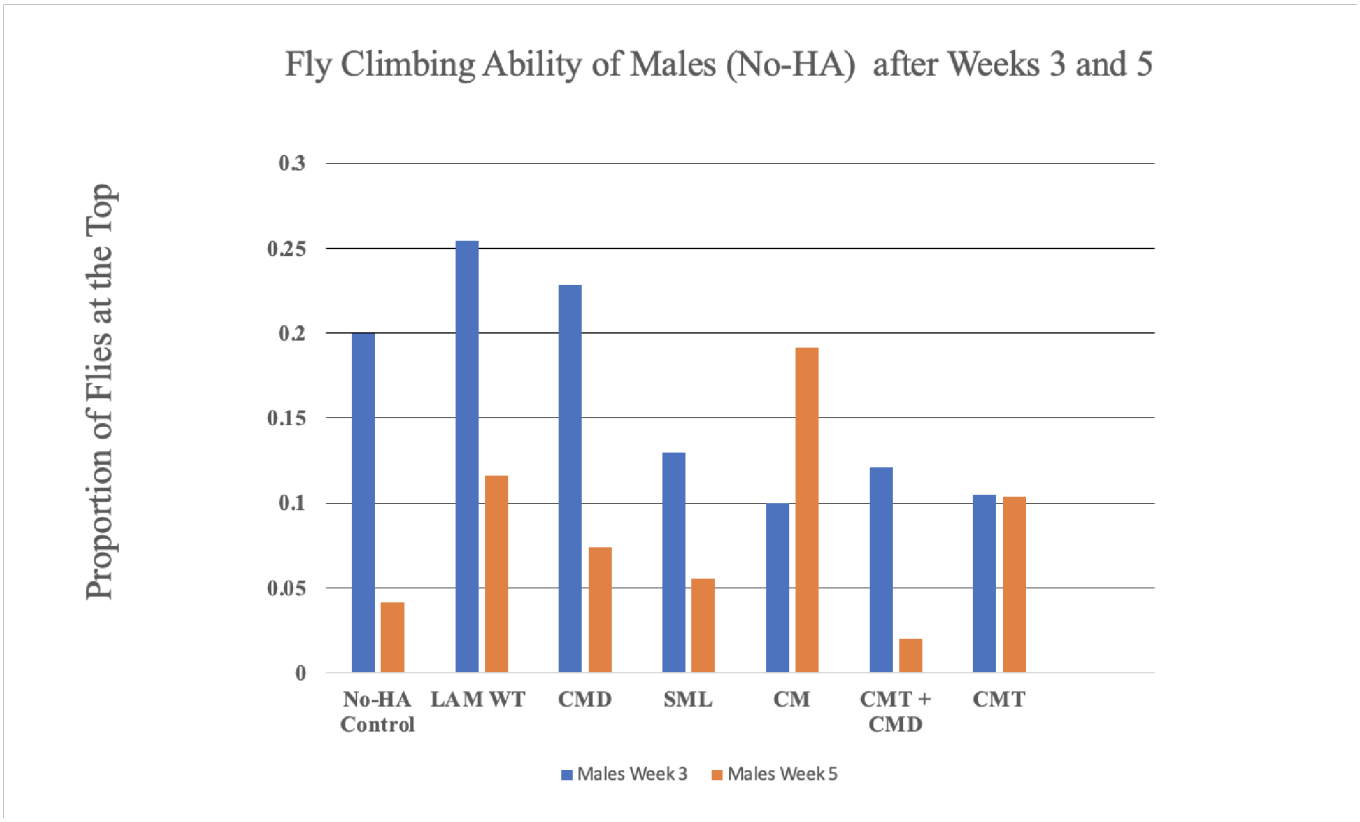


Figure 3.4: Distribution of fly climbing is affected by Lamin mutants without HA-tag.

a. Mutant lamin female flies without HA-tag started showed reduced locomotive functions after 3 and week 5. 10 flies per genotypes were collected in 10 replicates. Climbing ability was tested and calculated each week.

b. Mutant lamin male flies without HA-tag started showed reduced locomotive functions after 3 and week 5. 10 flies per genotypes were collected in 10 replicates. Climbing ability was tested and calculated each week.

Lamin Mutation reduces longevity in female and male flies with no HA-tag

Fly Climbing ability was also tested on lamin mutant flies with HA-tag removed. This was done because tags are known to cause proteins to aggregate when expressed in tissues so I performed this experiment to determine if the tags causes further impairment in the muscles of

flies. Most of the genotypes had slow climbing activities when HA-tag was removed (Figure 3.4). It changed the behavior of the flies (Figure 3.4) Apart from slowing down the rate at which flies climbed, the climbing patterns were opposite to what was seen in the flies with the HA-tag. The CMD, CM and CMT mutant lines recorded some of the best climbing. Figure 3.2. This data suggests that the HA-tags might be located at a site very much important for the Lamin protein's activity, and its addition can cause changes in the protein's behavior or lead to loss of function.

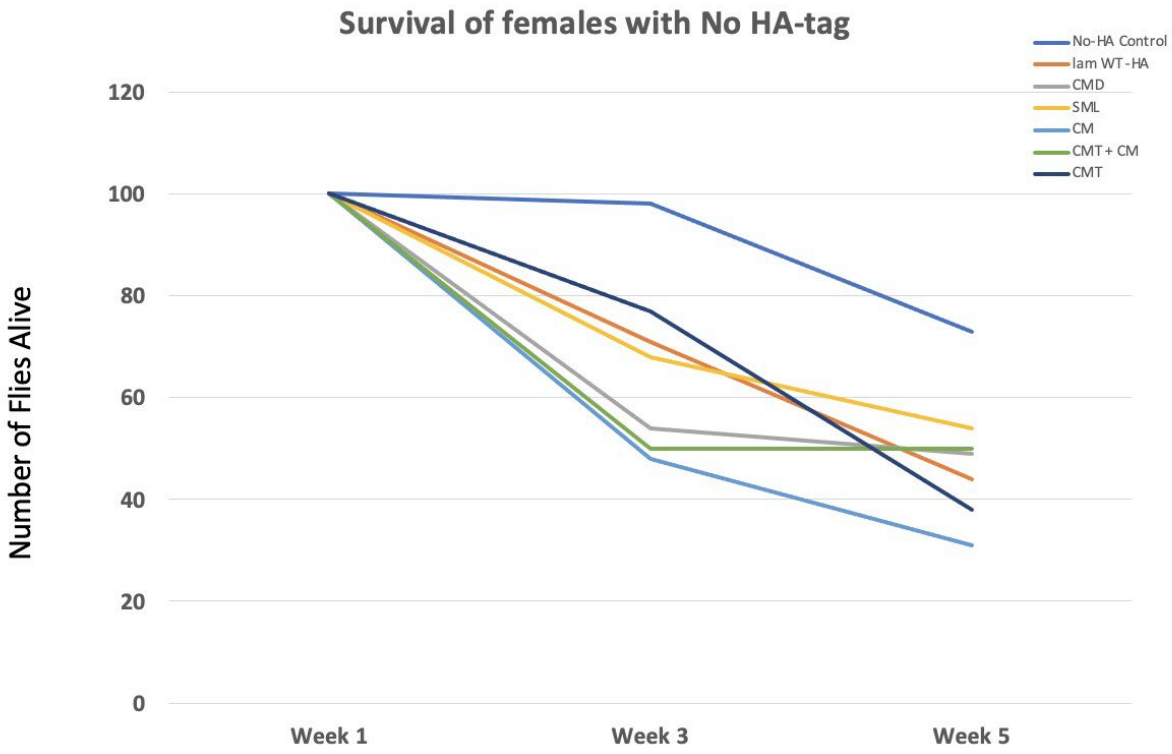


Figure 3.5a: Lamin Mutation reduces longevity in female flies with no HA -tag
Mutant lamin female flies without HA-tag started showing reduced lifespan after 3. Mutations were deleterious after week 5, further reducing lifespan of flies. 10 flies per genotypes were collected in 10 replicates. Death was observed and counted each week.

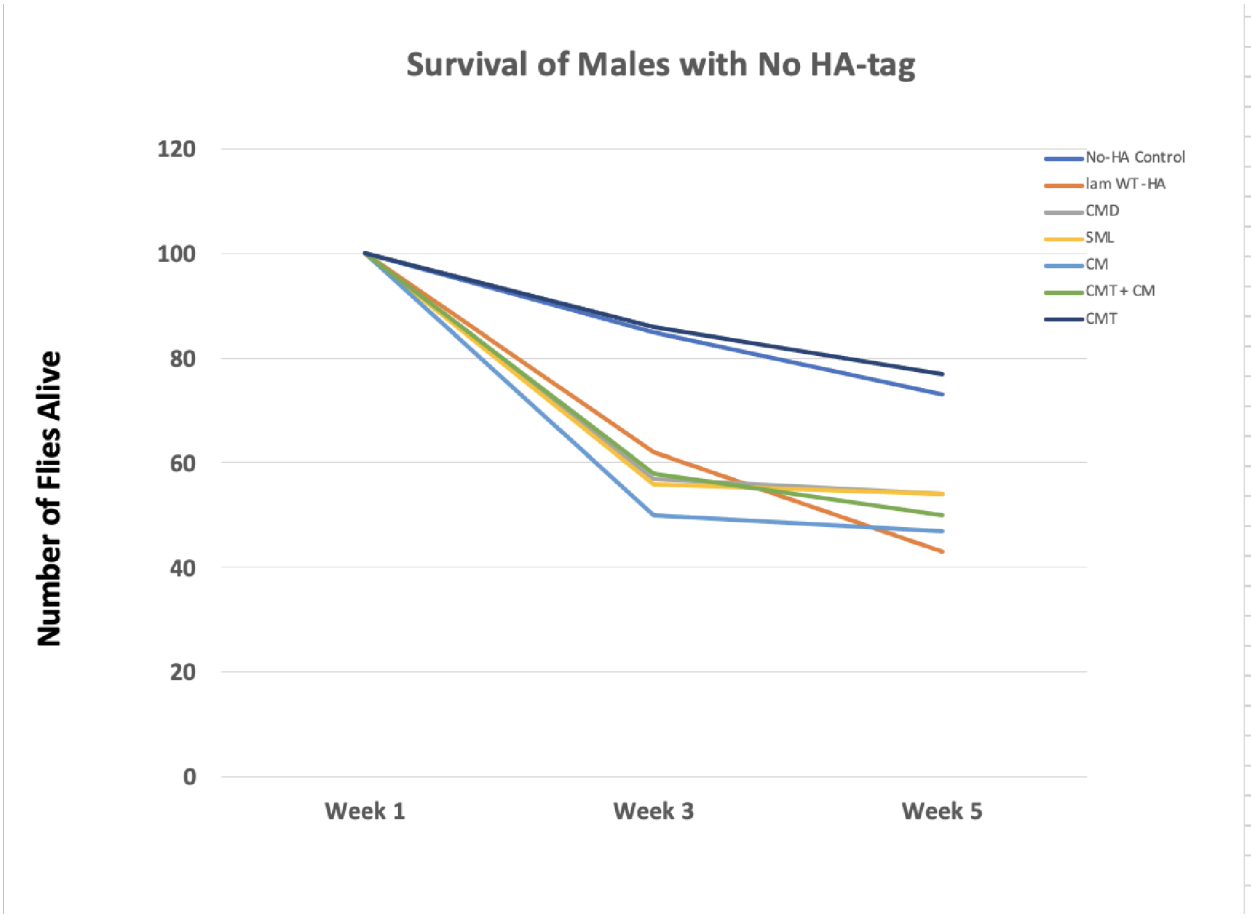


Figure 3.5b: Lamin Mutation reduces longevity in male flies with no HA-tag.
Mutant lamin male flies without HA-tag started showing reduced lifespan after 3. Mutations were deleterious after week 5, further reducing lifespan of flies.

Survival analyses were also performed on lamin flies without HA-tag. Lamin wild-type together with the Cardiomyopathy mutant flies had the worst survival when HA-tag was removed. This data suggests that the HA-tags may be in a region critical for the Lamin protein's activity, and its addition can cause changes in the protein's behavior or lead to loss of function. That was probably why the wild-type flies had the worst climbing when HA-tag was removed. This was the exact

opposite of what was observed in lamin mutant flies with HA-tag. The best survival was shared between the W^{1118} and lam WT control flies, followed by SML and CM + CMT mutant flies.

CHAPTER IV: DISCUSSION

Farnesylation affects lamin mutant flies leading to tissue-specific pathologies (Azibani *et al.,* 2014). Farnesylation is a post-translational modification process that involves the addition of a farnesyl lipid group to a protein (Sobotka-Brine *et al.,* 1992). One protein undergoing farnesylation is Lamin, an important component of the nuclear lamina (Azibani *et al.,* 2014). Mutations or alterations in the farnesylation of Lamin can lead to tissue-specific disorders, primarily affecting tissues with high rates of cell division and mechanical stress (Azibani *et al.,* 2014). It is important to note that tissue-specific disorders resulting from farnesylation abnormalities in Lamin may also involve other factors, such as cell type-specific gene expression patterns, tissue-specific mechanical stress, and interactions with other proteins (Sobotka-Brine *et al.,* 1992). The precise mechanisms underlying these disorders are still being investigated, and further research is needed to fully understand the complex relationship between Lamin farnesylation and tissue-specific pathology. I found out that increased farnesylation in flies resulted in reduced longevity and slower fly climbing behavior. However, based on this existing knowledge, we can hypothesize that increased levels of farnesylation could potentially exacerbate the effects of lamin mutations or alterations in tissue-specific pathologies. If lamin is excessively farnesylated due to increased farnesyltransferase activity or altered farnesylation regulation, it could lead to the accumulation of abnormal lamin proteins and further compromise nuclear structure and function.

The HA tag is a commonly used epitope tag that is added to proteins to facilitate their detection and purification in laboratory experiments. It consists of a short amino acid sequence derived from the influenza hemagglutinin protein. When the HA tag is added to a protein, it allows researchers to specifically target and study that protein using antibodies or other

affinitybased methods. The addition of an HA tag to a protein does not usually interfere with its normal function or stability However, the tags might be in a region critical for the protein's activity, and its addition can cause changes in the protein's behavior or lead to loss of function. This manipulation can potentially impact the expression level or regulation of the protein, leading to unintended consequences. The addition of the HA tag could also affect the protein's half-life, cellular localization, or interactions with other molecules. Fly climbing assay was also performed on lamin mutant flies without HA-tag (both male and female adults). It was also observed that the male lamin mutant flies without HA-tag had better climbing activities as against their corresponding females (Figure 3.4). However, there was a similar climbing pattern across males and female mutant flies without the HA-tag.

Mutant flies with the highest levels of farnesylated lamin (Congenital muscular dystrophy, cardiomyopathy, and Charcot Marie Tooth disease, Figure 3.1a and b) had impaired climbing after week 1. The rest of the mutant lamin forms caught up with the impairment as flies aged to 3 and 5 weeks. This data suggests that the farnesylated mutant impairs quickly and the rest catches up later. A similar pattern was seen in their lifespan as well (Figure 3.4). The precise mechanisms by which Lamin mutations affect longevity are not yet fully understood. It was also interesting to see that the control (w^{1118}), CMT and CM mutant flies survived longer regardless of their slow climbing behavior (Figures 3.5a and 3.5b).

Given that these proteins play a crucial role in nuclear structure and are expressed in the majority of cell types, one unanswered puzzle is how diverse mutations in the Lamin genes cause such a variety of illnesses. The fact that these various lamin mutations can cause proteins to aggregate further supports my hypothesis that some tissues are more vulnerable to lamin mutations than others because tissue-specific quality control mechanisms cannot effectively degrade those

mutant forms, resulting in protein aggregation and cellular toxicity. Additionally, I propose that when produced in neurons and leading to CMT, the CASA complex will not degrade the CMT mutations but will instead degrade the mutant proteins for muscular dystrophy. This could be due to the lack of machinery to degrade lamin with neuropathy-causing lamin mutations in neurons, but not in muscle cells. This can be attributed to the differences in the cellular functions and requirements of these two cell types.

In the neurological system, neurons, which are highly specialized cells, are in charge of communicating and transferring electrical impulses. They differ from other cell types due to their particular traits and functional needs. One of the characteristics that sets neurons apart is their post-mitotic nature, which means that once they have fully differentiated, they do not divide or go through considerable cell division. When compared to other cell types, neurons typically have lower turnover rates for lamin proteins (Sobotka-Brine *et al.,* 1992). In contrast to other cells, they do not actively destroy lamin proteins regularly (Azibani *et al.,* 2014). As a result, neurons may lack the required equipment to effectively break down and destroy the altered lamin proteins when lamin protein mutations, such as those that cause neuropathy, arise. This may cause the nuclear structure to be disturbed, an accumulation of abnormal lamin proteins, and ultimately neuropathy.

On the other hand, muscle cells, including skeletal, cardiac, and smooth muscle cells, are highly dynamic and undergo regular cycles of cell division, growth, and regeneration. They possess robust protein degradation machinery, including the ubiquitin-proteasome system and autophagy, to maintain cellular homeostasis and eliminate damaged or misfolded proteins (Sobotka-Brine *et al.,* 1992). These degradation systems help muscle cells clear out any mutated or abnormal lamin proteins that may arise (Azibani *et al.,* 2014), reducing the potential for lamin-related diseases. Overall, the differences in the degradation machinery between neurons and

muscle cells reflect their distinct cellular functions, requirements, and metabolic activities. These variations in protein degradation pathways contribute to the differential susceptibilities of different cell types to lamin mutations and associated diseases.

Mutations in the human lamin A gene (*LMNA*) cause muscular dystrophies, type 2 Charcot-Marie Tooth disease, and premature aging diseases, among others, were studied in both humans and flies. (Muñoz-Alarcón *et al,* 2007). No viable mutations in human lamin B genes were known until recently when three single nucleotide mutations within the *LMNB2* locus were found in lipodystrophy patients (Ascencio *et al.*, 2012), and a duplication of the chromosomal region containing *LMNB1* was correlated with human leukodystrophy (Bertrand *et al.,* 2012). It remains a mystery how changes in proteins expressed in all cells selectively affect certain tissues and what molecular functions are performed by the differently expressed lamins. Since 1999, mutations in *LMNA* have been shown to cause several different inherited diseases. (Worman *et al,* 2004). Before these discoveries, the predominant functions of lamins A and C were thought to be to provide mechanical support to the nucleus and to anchor "silent" heterochromatin to the inner nuclear membrane (Sobotka-Brine *et al.,* 1992). The discoveries linking lamins to inherited disorders have led to a new question: How do mutations in these proteins, expressed in nearly all differentiated somatic cells, cause different diseases, some of which are tissue-specific? This motivated and inspired my thesis topic to elucidate the mechanisms of how different lamin mutations affect tissues differently.

Investigators in the field have proposed two nonexclusive hypotheses to address this question. The "mechanical stress" hypothesis states that abnormalities in nuclear structure, which result from lamin mutations, lead to increased susceptibility to cellular damage by physical

stress. This hypothesis is supported by observations that fibroblasts from patients with lamin A/C mutations and transfected cells expressing the mutant proteins often have severe abnormalities in nuclear morphology and that fibroblasts from subjects with FPLD (familial partial lipodystrophy) are susceptible to damage by heat shock. (Worman *et al,* 2004). The "gene expression" hypothesis, which proposes that the nuclear envelope plays a role in tissue-specific gene expression that can be altered by mutations in lamins, is based primarily on observed interactions between the nuclear envelope and chromatin components (Ascencio *et al.*, 2012) . With reference to my data obtained, the mutation causes changes in the degradation of lamin protein and the way the proteins fold in various tissues. This could possibly be a combination of both the "mechanical stress" and "gene expression" hypotheses. These factors can, however, cause changes in the structure and function of lamin resulting in why the same lamin mutation aggregates differently when expressed in muscles and neurons of flies.

CHAPTER V: CONCLUSION

Lamin proteins interact with chromatin and contribute to the spatial organization of the genome within the nucleus (Azibani *et al.,* 2014). They help establish distinct nuclear compartments, such as heterochromatin and euchromatin domains, which can have different effects on gene expression (Bertrand *et al.,* 2012). The organization of chromatin within the nucleus can vary between different cell types, leading to cell type-specific gene expression patterns (Sobotka-Brine *et al.,* 1992). Lamin proteins directly interact with transcription factors and other regulatory proteins involved in gene expression (Azibani *et al.,* 2014). These interactions can affect the accessibility of specific genomic regions to transcription factors, influencing the transcriptional activity of genes (Azibani *et al.,* 2014). Lamin-mediated regulation of gene expression can differ between cell types, allowing for cell type-specific gene expression patterns in different contexts (Bertrand *et al.,* 2012). I found out that mutations in lamin expressed in the muscles of flies resulted in increased levels of farnesylation. Farnesylation is a post-translational modification that involves the addition of a 15-carbon farnesyl lipid group to the cysteine residue near the C-terminus of a protein.

Farnesylation plays a crucial role in protein localization and membrane association (Bertrand *et al.,* 2012). It is involved in the regulation of various cellular processes, including signal transduction, cell cycle progression, and protein-protein interactions (Sobotka-Brine *et al.,* 1992). Farnesylation is particularly important for proteins that are targeted to the cell membrane, such as certain small GTPases, which play a role in intracellular signaling pathways (SobotkaBrine *et al.,* 1992).

In humans, the abnormal farnesylation leads to the accumulation of a truncated and toxic form of Lamin A called progerin (Beck *et al.,* 1990). Progerin disrupts nuclear structure and

function, leading to premature aging symptoms and affecting various tissues, including skin, bone, and cardiovascular tissues (Azibani *et al.,* 2014). The abnormal accumulation of prelamin A affects the nuclear structure, leading to severe skin abnormalities and restrictive lung disease (Beck *et al.,* 1990). Emery-Dreifuss Muscular Dystrophy (EDMD) is also a muscular dystrophy characterized by muscle weakness and wasting joint contractures, and cardiac abnormalities (Beck *et al.,* 1990). Mutations in the LMNA gene disrupt Lamin A/C farnesylation, leading to nuclear envelope abnormalities and altered gene expression in muscle and cardiac tissues (Beck *et al.,* 1990). The tissue-specific effects primarily manifest in the skeletal and cardiac muscles. All these scenarios explain how farnesylation could potentially affect fly climbing activities and longevity. Therefore, an increased level of farnesylation from my data suggests that specific proteins involved in signaling pathways or membrane association are being modified with farnesyl groups at a higher rate. This can potentially impact the functionality and activity of these proteins, leading to alterations in various cellular processes and developmental pathways in the fruit fly resulting in reduced lifespan and longevity. Future experiments will focus on expressing lamin mutations in p38Kb mutant and p38Kb wild-type flies. I expect that overexpression of p38Kb in lamin mutant flies will result in rescuing behavioral and phenotypic functions in flies. I also anticipate that p38Kb through the interaction with the CASA-complex will help degrade the muscular dystrophy mutant proteins when expressed in neurons and vice versa when expressed in muscles. Likewise, the CMT mutant proteins will also be degraded when expressed in muscles.

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