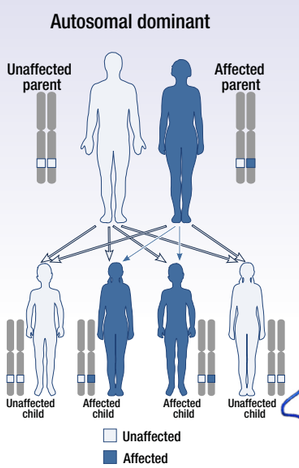
Mutations in lamin gene impair skeletal muscle growth

Dr Catherine Coirault from Sorbonne University investigated the role of lamins in muscle cell proliferation and differentiation. Cells with mutation of lamin type A had an impaired ability for muscle growth in response to functional overload (hypertrophic conditions), shown in cell lines, mouse models and biopsies from LMNA-related congenital muscular dystrophy (L-CMD) patients. In a subsequent experiment, Dr Coirault showed that mutated cells had impaired shuttling of yes-associated protein (YAP), a protein implicated in the transduction of mechanical signal arising from cell-cell contacts and cell adhesions into biochemical signals. As a result, cells are unable to undergo cellular differentiation of muscle stem cells.



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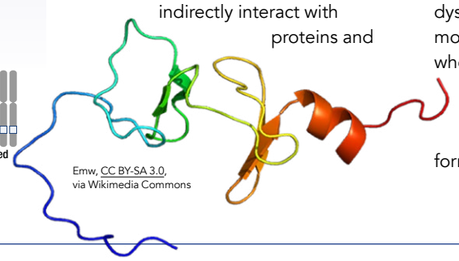
Skeletal muscle is a major component of the human body. It makes up about 38% of body mass in men and approximately 31% in women, and is responsible for movement, respiration and generation of body heat. So when it doesn't function it can cause significant health problems. There is a population of stem cells in skeletal muscles called satellite cells, that are able to differentiate into mature skeletal muscle cells after muscle injury, during postnatal growth, or in response to hypertrophic conditions (functional overload). Biological ageing and many diseases, such as muscular dystrophy, diabetes, and cancer, can decrease muscle size and function. Understanding how and why muscle cells respond in this way has the potential to improve treatments for patients whose muscle cells are unable to grow or regenerate. Dr Catherine Coirault and her research group from Sorbonne University is looking in particular at how mechanical stress affects muscle mass and function.

THE ROLE OF LAMINS

Nuclear lamins are intermediate filaments that provide structural function to the cell nucleus. Intermediate filaments are part of a cell's cytoskeleton, which controls the cell's shape and maintains intracellular organisation. Lamins also directly or indirectly interact with proteins and chromatin, and reorganise in response to mechanical cues affecting the cell, thus modulating the nuclear stiffness (which protects DNA from damage) and cell signalling. For example, lamins affect the nuclear translocation (movement of protein in the nucleus of the cell) of mechanosensitive transcription factor megakaryoblastic leukemia 1 (MKL1). The interaction between lamins and MKL1 consequently determines the specific genes that are turned on, specifically those that control muscle development and function.



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There are two types of lamins, type A and type B, which are encoded by different genes. Mutations to the different types confer different diseases. For example, laminopathies are a diverse group of disorders, including Emery-Dreifuss muscular dystrophies (EDMD), which are caused by mutations in *LMNA*, the gene that encodes type A lamins. EDMD is a rare disease that causes stiffness of the joints, muscle weakness and wasting, and cardiomyopathy, which is weakness of the heart muscle that makes it difficult for the heart to pump and deliver blood to the rest of the body. Symptoms of EDMD usually manifest by the age of ten.

LMNA-related congenital muscular dystrophy (L-CMD) is a genetic and more severe form of laminopathy, where muscle weakness becomes apparent in early infancy or childhood and can worsen very quickly. Infants with the most severe form of L-CMD are unable to develop motor skills, such as sitting or holding their heads up, due to weakness in the neck muscles. L-CMD mutations cause breathing difficulties and severe cardiomyopathy, which can be life threatening. The pathophysiology of L-CMD is still partly unknown and there is currently no therapy for the disease.

LAMIN, YAP AND L-CMD

A protein that is deregulated in L-CMD is yes-associated protein (YAP). YAP is a protein that functions as a transcriptional regulator, which controls if a gene is turned on or off. Transcriptional regulators ensure that genes are expressed in the right cell at the right time and in the right amount throughout the life of the cell. When nuclear, YAP promotes the growth and division of cells and suppresses genes that promote differentiation. Cytoplasmic retention of YAP modulates the activity of different signalling pathways and promotes myogenic differentiation. In consequence, YAP localisation (nuclear or cytoplasmic) is a critical regulator of the cell fate decisions and consequently of development, organ size, and tumorigenesis. Cellular localisation and activation of YAP are determined by cell density, mechanical strength, cell adhesion, energy status, nutrient available, and nuclear shape, to name a few examples.

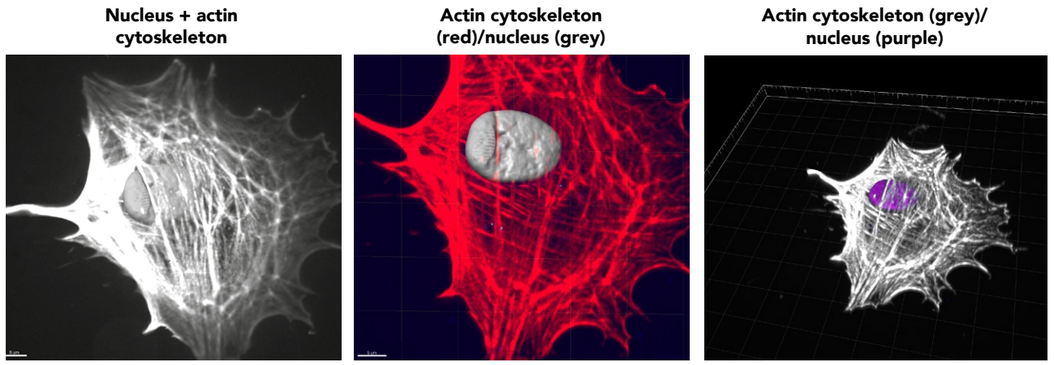
Dr Catherine Coirault and her research group from Sorbonne University is interested to determine how mechanical stress affects muscle mass and function in normal and pathological conditions. In their 2021 paper published in the *International Journal of Molecular Sciences*, they found that human muscle stem cells (MuSCs) with a mutation in the lamin gene, *LMNA*, had impaired skeletal muscle growth when exposed to functional overload (an experiment to ablate muscles or cut tendons of muscles to stimulate muscle growth/muscle hypertrophy). The same result was shown in mouse models of L-CMD, as there was no skeletal muscle growth after being exposed to functional overload, as their satellite cells were unable to differentiate and mature into skeletal muscle cells.

Lamins directly or indirectly interact with proteins and chromatin in response to mechanical cues.

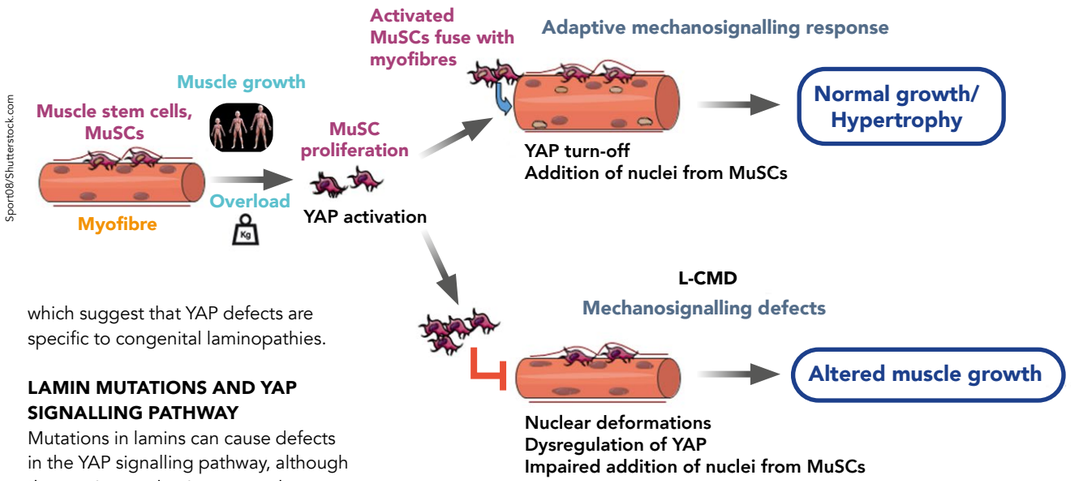
Interestingly, there was abnormal regulation of YAP translocation to the nucleus in response to different mechanical stimuli in *LMNA* mutated cells. Previously, there was no conclusive result that suggested defects in cellular response to mechanical stimuli (mechanotransduction) in lamin mutations could cause abnormal skeletal muscle growth in laminopathic patients.

Importantly, Dr Coirault analysed muscle biopsies of L-CMD patients and found consistent defects in satellite cells activation and YAP signalling, which suggest that defects in mechanotransduction can impair skeletal muscle growth in humans. Overall, the experiment exemplified the importance of lamins in regulating the activation of satellite cells through mechanoresponsiveness and, as a consequence, skeletal muscle growth. It also showed that lamins are important for normal YAP signalling. In models for EDMD, these results were not seen,

Images of human muscle stem cells from confocal microscopy



Confocal images of nuclear DNA (stained with DAPI in blue) and lamins in human muscle cell. Lamins are subdivided into A- and B-types. They form a filamentous layer between the inner nuclear membrane and DNA. With a confocal microscopy, which makes 'slides' as images, the lamin staining looks like a ring around DNA.



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The study results indicate that defects in the nuclear envelope can cause abnormal YAP signalling.

which suggest that YAP defects are specific to congenital laminopathies.

LAMIN MUTATIONS AND YAP SIGNALLING PATHWAY

Mutations in lamins can cause defects in the YAP signalling pathway, although the precise mechanisms are unknown. It has been hypothesised that altered lamins can change the shape of the nucleus, due to its importance in maintaining the structural architecture of the nucleus, which in turn changes the rate of YAP import into the nucleus by opening up nuclear pores.

Dr Coirault investigated this hypothesis by looking at the subcellular distribution of YAP in mutated MuSCs. In low cell density, YAP is primarily localised in the nucleus to promote activation of target genes and cell proliferation. When cells reach a critical high density, YAP is then translocated to the cytoplasm and phosphorylated by another protein, LATS1/2 kinase, which leads to its degradation. This is part of the Hippo pathway, which regulates cell proliferation and death. When YAP is translocated to the cytoplasm, cells are no longer directed for cellular growth, and the balance is tipped towards the differentiation of satellite cells. In cell culture, the balance between proliferation and differentiation occurs when satellite cells are confluent and make cell–cell contacts. In vivo, activated satellite cells fuse with existing myofibres after YAP is inactivated.

In *LMNA* mutant cells, cell–cell contact is unable to induce relocalisation of YAP into the cytoplasm. There is persistent accumulation of YAP in the nucleus in confluent cells, in contrast to normal cells, where YAP is mainly localised in the cytoplasm when cells contact each other. This means that mutant cells are unable to undergo satellite cell differentiation as seen during normal physiological response, such as during muscle injury or in response to hypertrophic conditions. When the nuclear pore import is inhibited, the accumulation of YAP in the nucleus is abolished, which establishes a causative relationship between nuclear pore complexes and YAP signalling. This study provides supporting results to indicate that defects in the nuclear envelope can cause abnormal YAP signalling, which is seen in muscular dystrophies.

Moreover, there is also a loss of environmental sensing for mechanical stimuli in mutant cells, as the reduction of cell spreading – which is a cue for changes in cellular behaviour – does not induce relocation of YAP to the cytoplasm. This shows that the defect shuttling of YAP between the nucleus and cytoplasm is a hallmark of the most severe muscular dystrophies related to nuclear envelope mutations.

KEY TAKEAWAYS

Through these experimental efforts, the mechanisms underlying the relationship between lamin mutations and muscle-specific defects are better understood. Specifically, Dr Coirault has shown that, for at least three different lamin mutations, cells with a mutation in the lamin gene had impaired skeletal muscle growth when exposed to hypertrophic conditions. Moreover, mutated cells have the impaired ability of shuttling YAP, a protein involved in cellular growth, between the nucleus and cytoplasm, which affects the balance between cellular proliferation and differentiation. This has major implications for muscular dystrophies patients, as their muscle cells are unable to grow, regenerate damaged muscles and differentiate their satellite cells. With this knowledge, researchers can develop more effective therapeutic approaches for L-CMD patients that specifically target YAP proteins or other key players in the story.

Through these experimental efforts, the mechanisms underlying the relationship between lamin mutations and muscle-specific defects are better understood.

Behind the Research

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Research Objectives

The effects of mechanical stress on muscle mass and function.

Detail

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Bio
Catherine Coirault has a PhD in Physiology and is a medical doctor specialising in cardiology. She is a permanent senior researcher at INSERM. Her research group is currently focusing on how mechanical stress affects muscle mass and function in normal and pathological conditions. In parallel, she is committed to promoting the interconnections between science, culture and society.

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Personal Response

What other areas of the YAP signalling pathway are being investigated by your research group in relation to muscular dystrophies?

"We aim to test the possibility that increased nuclear YAP signalling in muscle, leading to increased proliferation and defective differentiation, is a common pathophysiological feature of the different myopathies characterised by mechanotransduction defects. These are centronuclear myopathies (CNM) – a group of congenital myopathies characterised by the presence of centrally located nuclei associated with muscle weakness and atrophy – and desminopathies, one of the most common intermediate filament human disorders associated with mutations in close interaction proteins, desmin and alpha-B-crystallin. We are also looking at the implication of YAP in the muscle atrophy that occurs in the elderly (muscle sarcopenia)."