Emerin and the making of muscle

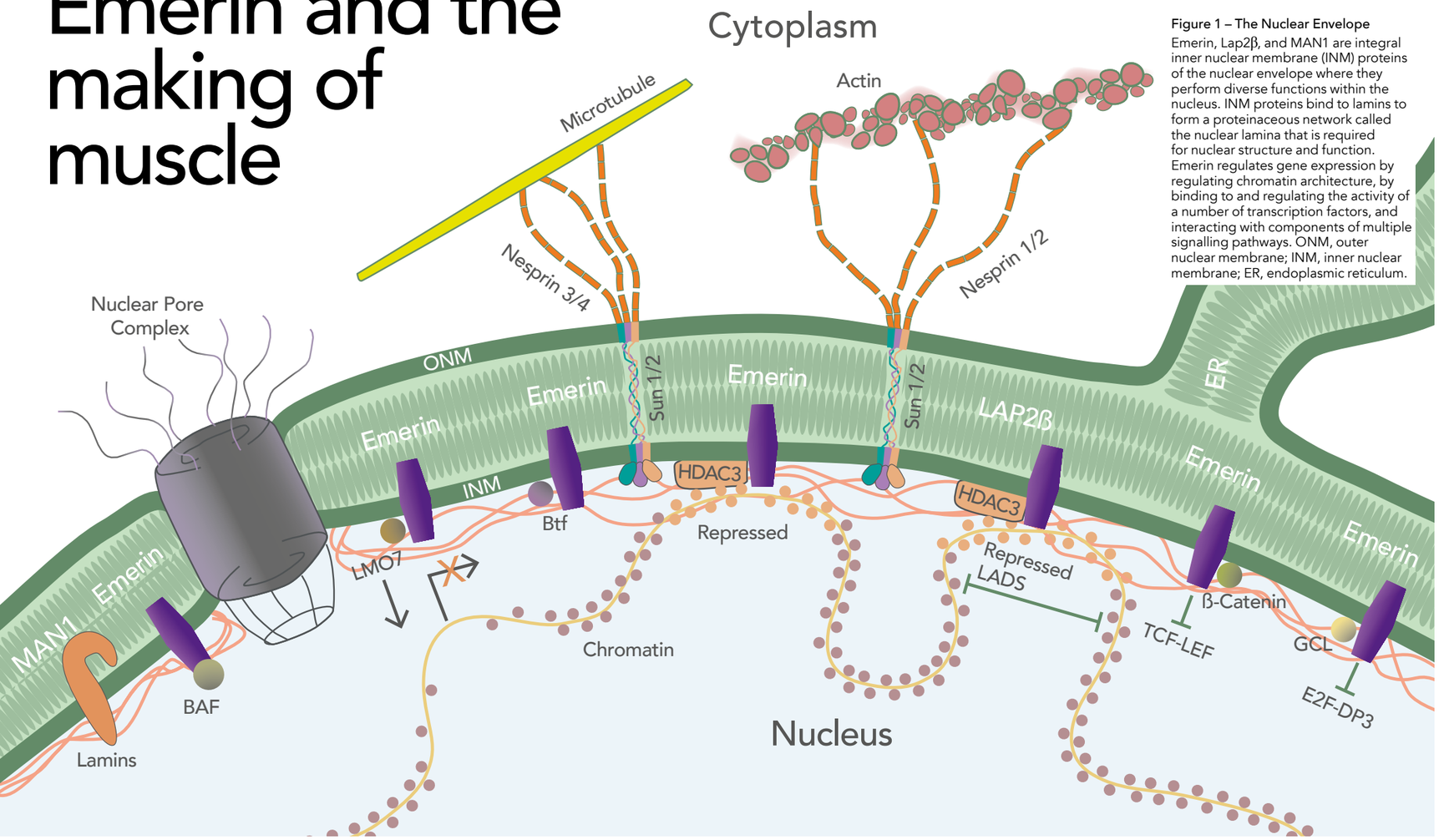

Figure 1 – The Nuclear Envelope
Emerin, Lap2β, and MAN1 are integral inner nuclear membrane (INM) proteins of the nuclear envelope where they perform diverse functions within the nucleus. INM proteins bind to lamins to form a proteinaceous network called the nuclear lamina that is required for nuclear structure and function. Emerin regulates gene expression by regulating chromatin architecture, by binding to and regulating the activity of a number of transcription factors, and interacting with components of multiple signalling pathways. ONM, outer nuclear membrane; INM, inner nuclear membrane; ER, endoplasmic reticulum.

The role of cellular machinery in the regulation of gene expression is currently a field of intensive study. **Dr James Holaska** of Philadelphia's University of the Sciences is leading the way in uncovering the role of emerin in the development and maintenance of skeletal muscle and pathology of muscle disease. This work has the potential to revolutionise the understanding and treatment of muscle diseases, including various types of muscular dystrophy and cardiomyopathy.

Emerin is a relatively small protein made up of just 254 amino acids. Despite its small size, it is fundamental to the correct formation of muscle tissues and their maintenance and repair. Rich in the amino acid serine, responsible for the catalytic function of many enzymes, it is clearly designed to interact with other molecules. Couple this to the fact that it is highly conserved (the identical protein exists in many other species) and it has all the hallmarks of a vital element of living organisms. Knowing it is important is not enough, however, and Dr Holaska and his team are determined to get to the bottom of its molecular activity and identify the mechanisms by which it exerts its influence on gene expression and molecular pathways.

IT'S ALL IN THE GENES

Every cell of an organism contains the entire genetic code of that organism, stored in the form of the now well-known deoxyribose nucleic acid (DNA). However, not every cell needs all of it. Skin cells need to know how to make the proteins necessary for skin, nerve cells those needed to transmit signals, and muscle cells those needed to make muscles move. For this reason, in any one cell, a vast proportion of the genome is silenced or repressed and will never be translated into proteins. How this is achieved is a major question for modern science, particularly considering recent work with stem cells (undifferentiated cells with the potential to become any cell type).

Much of the regulation is thought to be achieved by structural differences in the arrangement of DNA in the nucleus (the compartment of the cell where DNA is stored and managed). The entire DNA of a human cell, if stretched out, would be about two metres long. For this reason, it is instead tightly wound around support molecules called histones. This histone/DNA complex is called chromatin and is further packaged into units called nucleosomes. DNA cannot be transcribed for translation into proteins whilst it is stored in this way, so which sections are in storage and which unwound is an important regulatory mechanism in gene transcription.

ENTER EMERIN

There are many diverse proteins associated with the regulation of gene expression in the nucleus, some key members of which are identifiable by the disorders caused when they are not correctly expressed. Dr Holaska's research team have found that emerin is key to the expression of a number of genes important in the maintenance of muscle tissue; mutations in the genes which code for emerin have been linked to the development of Emery-Dreifuss muscular dystrophy (EDMD) as well as certain cardiomyopathies (diseases of the heart muscles). How this modest protein exerts these significant effects is the subject of intense research by the Holaska laboratory.

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EMERIN AS THE ARCHITECT

Dr Holaska and his colleagues have identified a number of ways in which emerin regulates the architecture of both the nucleus and the nucleosomes which hold the DNA within it. Perhaps the most important of these is emerin's regulation of histone deacetylase 3 (HDAC3), one of a group of enzymes which alter chromatin structure and regulate transcription factor access to DNA. The team showed that emerin activates HDAC3 and localises it to the wall of the nucleus, where it is able to repress genes by restricting access to the DNA. The researchers have successfully shown that these two molecules work together to localise and activate or repress specific sections of the genome during muscle formation and development. In investigating the structure of emerin itself, Dr Holaska and his colleagues identified the regions of the protein required for specific purposes. These enable the protein to be transported into the nucleus, and for it to then interact with both the nuclear membrane and the underlying matrix of structural proteins called lamins, which supports it. This structural function has been evidenced by showing nuclear membrane dysfunction and increased cell death in emerin-deficient cells.

EMERIN AS THE FACILITATOR

The researchers have repeatedly shown that emerin does not work alone, rather it recruits and regulates a variety of other molecules. The most significant findings in terms of EDMD and related myopathies is emerin's relationship with a specific transcription factor in muscle cell differentiation called *Lmo7*. The process of muscle cell differentiation is complex and requires particular genes to be 'switched' on and off in a time-critical manner. Dr Holaska and others have shown that emerin is able to regulate the activity of *Lmo7* through competitive binding, preventing the factor from initiating expression of genes in a way that is vital for correct differentiation of the cell.

Having identified *Lmo7* as a target of emerin's activity, the team then demonstrated that a mouse model which lacked the gene to make *Lmo7* exhibited similar symptoms to those of EDMD. The mice exhibited growth retardation, decreased muscle fibre size and impaired skeletal and cardiac muscle function. The team were therefore able to confirm that it is this interaction between emerin and *Lmo7* which is important for regulating the gene transcription and signalling pathways which are the underlying mechanism of EDMD.

In cultures of myogenic progenitor cells (undifferentiated cells which will evolve into skeletal muscle), Dr Holaska and his colleagues have shown that the removal of emerin results in significant perturbation of four critical signalling pathways for cell differentiation. They propose this is caused by emerin interacting with other molecules involved in the regulation of these pathways such as beta-catenin (a dual function protein that regulates gene expression and cell adhesion), and microRNA (short sections of transcribed DNA which interfere with other RNA to prevent translation into proteins). The mechanisms are complex, however, and the team recognise that more work is needed to fully elucidate the pathways involved.

DRAWING OUT THE MERITS OF EMERIN

Dr Holaska's group continues to use the latest molecular techniques to probe the mechanisms underlying nuclear envelope architecture and the role of the proteins that interact with it. To date their work has made a significant impact on the understanding of emerin's function in both gene expression and the architecture of the nucleus which supports it. By observing normal function and comparing it to that in disease states, they have contributed much to the understanding of EDMD in particular and other myopathies generally. Their research so far shows that there is much more still left to discover in this complex field.

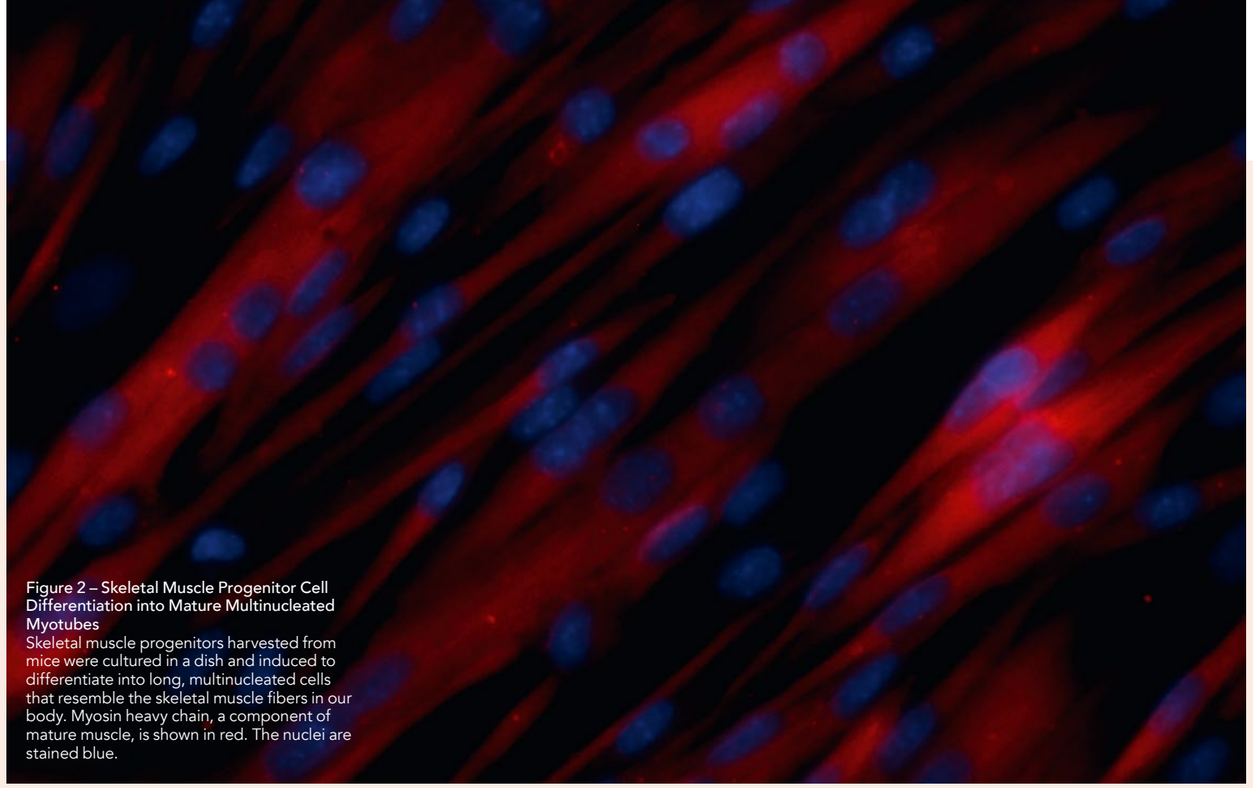

Figure 2 – Skeletal Muscle Progenitor Cell Differentiation into Mature Multinucleated Myotubes
Skeletal muscle progenitors harvested from mice were cultured in a dish and induced to differentiate into long, multinucleated cells that resemble the skeletal muscle fibers in our body. Myosin heavy chain, a component of mature muscle, is shown in red. The nuclei are stained blue.

What led you to begin investigating Emery-Dreifuss muscular dystrophy?
When I first started my post-doctoral fellowship fifteen years ago, it was our initial interest in the function of proteins within the nuclear envelope. The nuclear envelope was then considered by many to be merely a 'bag' that held the genome or a barrier that physically separated the genome from the cytoplasmic machinery. However, the presence of integral membrane proteins on the nuclear side of the nuclear envelope suggested these proteins had functions analogous to proteins on the inner side of the plasma membrane. Evidence over the last 20 years showed that the nuclear envelope is important for regulating many important functions, including cell signalling, mechanotransduction, gene expression, chromatin architecture and cell differentiation.

Mutations in one of these integral membrane proteins caused Emery-Dreifuss muscular dystrophy, demonstrating the importance of emerin in cellular function. We have been studying emerin and its role in normal cellular function and in Emery-Dreifuss Muscular Dystrophy ever since.

What is the role of emerin in this disease and cellular mechanisms generally?
Emerin plays important roles in maintaining nuclear architecture and regulating cell signalling and gene expression. Emerin is thought to be important in regulating the transduction of both chemical and mechanical signals from the plasma membrane to the nucleus. Emerin's role in gene expression regulation is multi-fold, since emerin is important for regulating the coordinated re-organisation of the genome during stem cell differentiation, emerin binds to and regulates the activity of a handful of transcription factors and emerin regulates the nuclear entry and exit of transcription factors. Thus the exact mechanism(s) underlying how emerin regulates gene expression remain ill-defined.

How does the architecture of the nucleus regulate gene expression?
There are two ways to think about nuclear architecture. The first concerns the structure of the nuclear envelope and the biophysical behaviour of the organelle as it relates to its elasticity, rigidity, ability to resist forces when stressed and the geometrical configuration of the proteins and lipids that generate these biophysical properties. The second concerns genomic or chromatin architecture, whereby repressed genes localise to specific regions within the nucleus, including the nuclear lamina at the nuclear envelope, to induce, maintain or propagate repressed chromatin. Loss of emerin causes the nuclei to become more easily deformed and alters the biophysical properties of the nucleus demonstrating its importance in nuclear structure. Emerin also binds to proteins of the 'linker of nucleoskeleton and cytoskeleton (LINC)' complexes, which also play vital roles in nuclear structure and mechanotransduction. Emerin was also found to be required for the temporal localisation of repressed genomic loci to the nuclear lamina when they are turned off during differentiation. Further, the interaction between emerin and HDAC3 was important for positioning repressed genomic loci to the nuclear lamina to inhibit the expression of genes located here. Because nuclear architecture can affect genomic architecture it will be important to determine the mechanisms for how loss of emerin causes altered nuclear architecture and genomic architecture (i.e., the chicken or the egg?).

The mechanisms of gene expression are very complex; how can you be confident that your interpretation is correct?
We can't really be sure our interpretations are 100% correct, but we are confident we are on the right path. We are confident that emerin regulation of genomic organisation during differentiation via its regulation of HDAC3 is important for the impaired differentiation in emerin-null myogenic progenitors. First, disruption of the coordinated expression of differentiation genes inhibits myogenic differentiation; this is true for most developmental programmes, whereby transcriptional programmes are tightly regulated and coordinated. Genomic regions containing the differentiation genes *Pax3*, *Pax7*, *MyoD* or *Myf5* are all mispositioned during differentiation resulting in their expression at inappropriate times during differentiation of emerin-null cells to impair myogenic differentiation. Approximately 35% of the genome associates with the nuclear lamina and we think emerin plays a key role in this localisation by interacting with and activating the activity of HDAC3 and other chromatin-repressive enzymes. Based on our results, we further predict emerin may preferentially interact with more dynamic genomic regions containing developmental genes that are activated or repressed in a temporally coordinated fashion. We plan on testing these predictions in the near future.

However, we do not think this is the sole function of emerin in terms of gene expression regulation, given the many studies showing emerin functions directly in transcriptional regulation. First, evidence suggests emerin regulates nuclear accumulation of transcription factors in response to cell signalling, including β-catenin and MKL1, to regulate their activity. It is unclear whether emerin directly regulates nuclear import, nuclear export, or interactions of transcription factors with its binding proteins or DNA targets in the nucleus; these are active research areas in many labs around the world. Second, we and others showed emerin binding to transcription factors inhibited their ability to bind directly to their promoter or enhancer elements on DNA to inhibit expression of their target genes. Thus the direct inhibition of transcription factor activity by emerin binding is another mechanism for emerin regulation of gene expression. How this functions in differentiating muscle cells or in other cell types in the body remains to be seen. Lastly, emerin can be post-translationally modified on more than 15 different residues. Post-translational modifications are well established in molecular biology to regulate the binding of proteins to their partners, to regulate the activity of proteins and to alter the structure of the protein. Thus, how these many post-translational modifications, individually and combinatorially, regulate emerin's functions in nuclear structure, chromatin architecture, gene expression and myogenic differentiation will be important areas of future research in our field. For example, is there a post-translational modification in emerin that blocks HDAC3 binding to emerin to regulate the association of genomic regions with the nuclear lamina to repress their expression? If so, then the dynamic addition or removal of this modification may occur during myogenic differentiation to regulate the association of MyoD genomic loci during myogenic differentiation to regulate its coordinated expression.

How might your work lead to treatments for skeletal- and cardio- myopathies?
Muscle regeneration is impaired in EDMD. By studying the mechanisms by which emerin regulates skeletal muscle stem cell differentiation it is predicted therapeutic targets for treatment will be identified. Recently, we identified a number of molecular pathways misregulated during differentiation of emerin-null progenitors, for which a few inhibitors exist. We are currently creating myogenic progenitors expressing selected EDMD-causing emerin mutants to confirm these findings. We also plan on using unbiased approaches to identify molecular pathways disrupted in emerin-null progenitors and all EDMD-causing emerin mutant progenitors. Using this approach we predict we will identify pathways implicated in EDMD. Validation of pathway involvement in the disease mechanism will be followed by small-molecule screening for inhibitors or activators of these pathways to rescue the impaired differentiation seen in the EDMD mutants. Other laboratories, notably Howard Worman's lab at Columbia University, showed that similar molecular pathways may be involved in the skeletal muscle phenotype and cardiomyopathy. Thus we predict that molecular pathways identified by studying myogenic differentiation will also be important for cardiomyopathy pathogenesis and any inhibitors identified in our studies will be tested for treating cardiomyopathy.

Detail

RESEARCH OBJECTIVES
Dr Holaska's work focuses on emerin and its role within the cell. His work has multiple applications, including a better understanding of Emery-Dreifuss muscular dystrophy.

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COLLABORATORS
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BIO


Dr Holaska majored in biology at St. Joseph's University and graduated in 1995. After working for a year at a molecular biology start-up company, he joined the graduate program in Microbiology at the University of Virginia. Working under Dr Bryce Paschal he received his PhD in 2001. He then started a post-doctoral fellowship in the laboratory of Dr Katherine Wilson, where he began studying the structure and function of the nuclear lamina, which is still the focus of his research today.

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