The Role of the Notch Signaling Pathway in Myogenesis [1]

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Among other functions, the Notch signaling prevents the differentiation [4] of muscle progenitor cells into mature muscle in animals. The Notch signaling pathway is a pathway in animals by which two adjacent cells within an organism use a protein named Notch to mechanically interact with each other. Myogenesis is the formation of muscle that occurs throughout an animal's development, from embryo to the end of life. Throughout the life of an organism, Notch signaling prevents the differentiation [4] of muscle progenitor cells into muscle cells. Such preventions help maintain populations of progenitor cells that can remain dormant until the growth or regeneration of muscle is necessary, at which point the Notch signal to muscle progenitor cells is disrupted, and those progenitor cells differentiate into myotubes and eventually mature muscle. Without Notch signaling, myogenesis proceeds prematurely and dissipates before mature muscle can form.

The phenotypic effects of the Notch signaling pathway were first described in 1914 by John S. Dexter at Olivet College in Olivet, Michigan, who observed a notch in the wings of mutant fruit flies Drosophila melanogaster [5]. In 1917, while working at Columbia University [6] in New York City, New York, Thomas Hunt Morgan [7] identified mutant loci in the flies' chromosomes that correlated with various notched wing phenotypes. This research confirmed the chromosomal theory of inheritance, and for it and similar work Morgan received the Nobel Prize in Physiology or Medicine [8] in 1933. In the late 1930s and 1940s, Donald Poulson at the Carnegie Institute of Washington, Baltimore, Maryland, described the impact of mutations in the Notch locus on the development of various segments in Drosophila [9]. Various mutations in the Notch locus resulted in a wide range of phenotypic abnormalities throughout developing flies, however many of these mutations were lethal early in embryogenesis [9]. In subsequent decades, despite the extensive research on the Notch locus, researchers struggled to identify the function for the Notch gene due to the lethality and broad phenotypic consequences of Notch mutants.

In 1983, Spyros Artavanis-Tsakonas and his research group at Yale University [11] in New Haven, Connecticut, sequenced and analyzed the Notch gene's DNA in Drosophila [9], and they discovered its role in localized intercellular communication. In 1991, Leif Ellisen, a researcher investigating human cancer at Brigham and Women's Hospital and Harvard Medical School [12] in Boston, Massachusetts, identified a gene in humans [13] that was homologous to the Notch gene in Drosophila [9]. Ellisen identified a mutation in this gene in several patients with lymphoblastic leukemia, a cancer of the white blood cells. Ellisen's discovery, that a gene that controls fly embryogenesis [10] also contributes to human cancer, sparked research into Notch signaling in vertebrate development and in human cancer. Since then, researchers have discovered that the Notch signaling pathway has a variety of roles in embryogenesis [10], and that it is disrupted in several cancers.

The Notch signaling pathway in mammals consists of four Notch proteins and several other proteins that modulate various aspects of the mechanism of Notch signaling. Notch proteins span a cell's membrane, extend outward into extracellular space, and function as receptors to receive signals from neighboring cells. The Notch proteins recognize and interact with specific ligand proteins, which are members of the Delta/Serrate/Lag-2 (DSL) family of transmembrane proteins. Because both the Notch and ligand proteins are lodged in the cell membrane, the Notch signal is transmitted between adjacent cells. Activation of the Notch signaling pathway occurs when the Notch receptor physically interacts with its ligand. Once activated, the portion of the Notch protein that is inside the cell is cleaved from the membrane and transported to the nucleus [14]. Once in the nucleus [14], the cleaved Notch protein interacts with various transcription factors and accessory proteins that activate or repress the expression of target genes [15]. In this way the Notch signaling pathway establishes a spatial relationship between adjacent cells, whereby a cell can affect the activity of its immediate neighbors by influencing gene transcription.

One process during embryogenesis [10] is the formation and maturation of skeletal muscle (myogenesis), and the Notch signaling pathway has a role in myogenesis. Myogenesis begins when somitic cells receive intercellular signals from pathways such as the Wnt or Hedgehog signaling pathways, which start the production of proteins that regulate muscle differentiation [4], such MyoD or Myf5. Those proteins are members of the myogenic basic helix-loop-helix (bHLH) family of transcription factors. In somitic cells, those transcription factors activate the expressions of genes [15] that begin in a cell a process called determination [16]. Determination is the first step in a cascade of genetic and morphological changes that results in the differentiation [4] of the cells into their mature, functional states. At this stage these progenitor cells, which have the full potential to develop into muscle cells or fiber, are called myoblasts. The myoblasts migrate to various regions of the developing embryo where they develop into
muscle fiber. As myoblasts increase in number in a region, the myogenic bHLH proteins activate the gene networks that cause the myoblasts to realign into immature muscle fibers called myotubules, and eventually differentiate into mature skeletal muscle fibers.

Myogenesis research first appeared in the late 1950s and early 1960s by researchers such as Howard Holtzer at the University of Pennsylvania [17], in Philadelphia, Pennsylvania who described the stages of myogenesis and some of the proteins that define muscle. Much of that research was done \textit{in vitro} [18] with cultured cells. In 1994, after the discovery of the mammalian homolog of the Notch receptor gene, Raphael Kopan and colleagues at the Fred Hutchinson Cancer Research Center [19] in Seattle, Washington, discovered that the \textit{Notch} and \textit{Myf5} genes [15] are expressed in early progenitor cells of skeletal muscles in mice. They demonstrated \textit{in vitro} [18] that the Notch protein prevented the \textit{determination} [16] of the myoblasts, even in the presence of the pro-\textit{determination} [16] protein Myf5. They hypothesized that the Notch protein suppressed the \textit{differentiation} [4] of myoblasts during their migration to the appropriate region of the embryo. They further hypothesized that the Notch proteins suppressed the \textit{differentiation} [4] of myoblasts to maintain populations of stem cells [20] in organisms as a source to regenerate damaged muscle.

Just as those in the Poulson lab struggled to work with \textit{Drosophila} [8] with lethal Notch mutations, researchers working with vertebrate species struggled to investigate the role of Notch signaling during myogenesis \textit{in vivo} [21]. In 2007, two groups of researchers used different techniques to overcome this obstacle: Cre-Lox recombination, and generation of a hypomorph allele for a specific Notch ligand.

Elena Vasyutina, working in Carmen Birchmeier's lab at the Max Delbrück Center for Molecular Medicine in Berlin, Germany, and colleagues used the Cre-Lox recombination technique on mice to silence Notch signaling only in myoblasts, and to observe the roles of the Notch signaling in myogenesis without disrupting other aspects of embryogenesis [110]. Researchers use Cre-Lox recombination to remove a particular region from a chromosome in specific tissue at any given time. Vasyutina and colleagues knocked out the \textit{RBP-J} gene in myoblasts, a critical component in Notch signaling.

The second strategy, the generation of a hypomorph, was employed by Karin Schuster-Gossler and colleagues at the Hannover Medical School in Hanover, Germany. They used mouse [22] embryos to generate a hypomorph allele, a form of a gene that contains a mutation that allows only partial function, of Dll1, a ligand for the Notch2 protein. These two experimental methods allowed researchers to overcome the obstacle of \textit{embryonic lethality} [23] to Notch mutants, and to investigate the role of Notch signaling in myogenesis in developing organisms, revealing novel functions not observable with previous techniques utilizing myoblasts in cell culture.

Consistent with the \textit{in vitro} [18] studies, the experiments by Schuster-Gossler and Vasyutina confirmed the roles of the Notch signaling pathway in the suppression of proliferation and \textit{differentiation} [4] of myoblasts. However, these \textit{in vivo} [21] results revealed additional roles for Notch signaling: interrupting Notch signaling during myogenesis results in the premature \textit{differentiation} [4] of myoblasts, and the loss of populations of progenitor stem cells [20]. Embryos with dysfunctional Notch signaling rapidly and abnormally develop muscle early in embryogenesis [110]. However, the muscle formation process soon stalls, resulting in decreased amounts of normal musculature. These results indicate that Notch signaling is critical to maintain a healthy population of progenitor cells, the state of which will dictate the ability of an organism to develop and regenerate damaged muscle.

Because muscle damage is common throughout the life of an organism, its body must maintain populations of muscle progenitor cells that can undergo myogenesis and replace damaged muscle. Groups of dormant myoblasts called satellite cells are adjacent to nearly all fully differentiated muscle groups in the adult organism. The Notch signaling pathway holds these satellite cells in a quiescent state by inhibiting the myogenic bHLH genes [15]. These genes [15], when expressed, strongly influence a cell's fate. Even in cells that are not muscle progenitor cells, if the \textit{MyoD} or \textit{Myf5} genes [15] are abnormally expressed, they can activate gene networks that convert the cells to muscle. Thus, these genes [15] must be regulated to restrain cells from differentiating into mature muscle. Biologists have shown that without negative \textit{regulation} [24] of the myogenic genes [15] by the Notch signaling pathway, satellite cell populations quickly deplete by rapid myogenesis. Such depletions result in the loss of muscle groups during development, and in the loss in adults of the ability to regenerate damaged muscle.

Research organisms, like \textit{humans} [13], show a decrease in the ability to regenerate muscle as they age. Researchers have correlated decreased expression of the Dll1 gene, a ligand of the Notch receptor, with older myoblasts, indicating that as muscle progenitor cells age they exhibit a \textit{reduction} [25] in the activity of the Notch signaling pathway. As the activity of Notch signaling declines with age, populations of muscle progenitor cells decrease in size and number, which results in the poor regenerative capabilities of older individuals. When researchers experimentally increase Dll1 expression in older muscle cells, the regenerative ability returns to levels associated with younger cells, leading to the potential of Notch signaling as a target to combat degenerative muscular diseases and muscular aging in general.

Such results have led to further exploration into the molecular mechanisms and gene targets of Notch signaling during...
myogenesis. In 2009 Matthew Buas, working in the lab of Thomas Kadesch at the University of Pennsylvania School of Medicine in Philadelphia, Pennsylvania, and colleagues activated the Notch signaling pathway by treating cultured myoblasts with the Dll4 protein, and using microarrays, they measured changes in gene expression shortly after. They identified eighty-two genes as likely targets of Notch signaling. Subsequent investigations into the individual effects of each of these genes discovered that many of them appeared to inhibit genes that promote myogenesis, but that silencing the expression of many of these genes fails to induce myogenesis. This result indicates that the Notch signaling pathway targets many genes in myogenic networks, resulting in a complex and cumulative mechanism of myogenic repression.

Sources

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