# S. S. College, Jehanabad

**Department:** Zoology

**Class:** M.Sc. Semester II

**Subject:** Zoology

**Topic:** Myogenesis - Skeletal muscle formation, regeneration and hypertrophy

**Mode of teaching:** Google classroom & WhatsApp

**Date & Time:** 06.10.2020 & 10:30

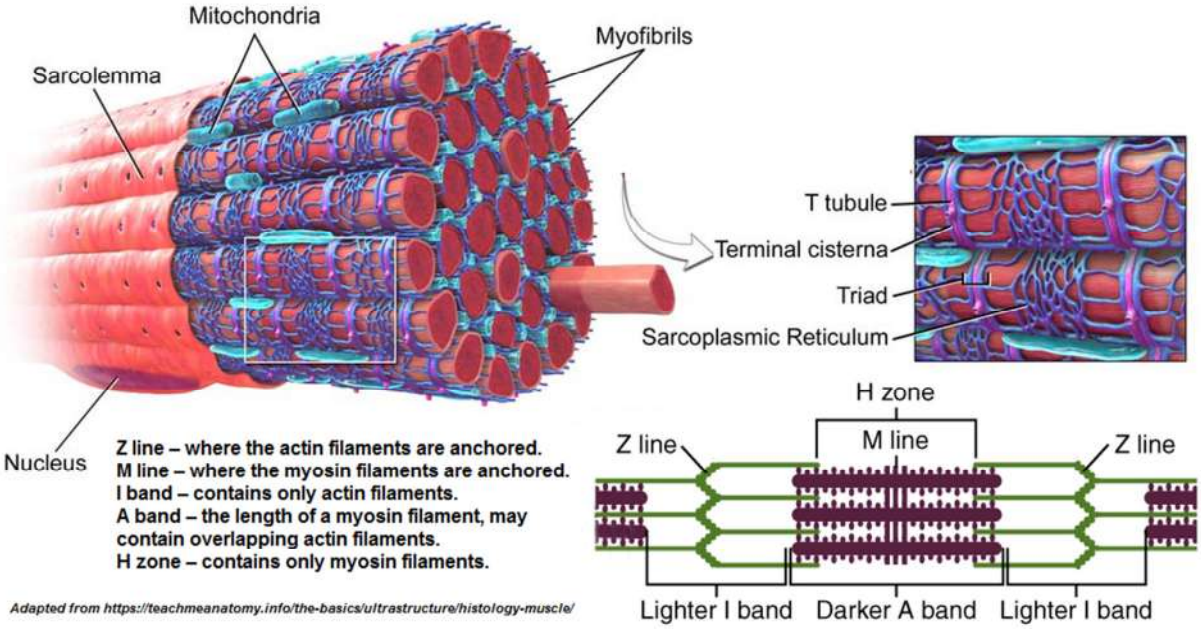
**Teacher:** Praveen Deepak

***To join Department's group, students can use following link***
***https://chat.whatsapp.com/EHuHNfQzoAzJBMFNJvsjQx***
***or scan QR Code***
***WhatsApp No.: +91 75360 68068***

# MYOGENESIS – SKELETAL MUSCLE FORMATION, REGENERATION AND HYPERTROPHY

Myogenesis is the formation of muscle tissue during embryonic development from stem cells in the mesoderm. The formation of muscle tissue takes place through the differentiation of progenitor cells myoblasts into myocytes during the development of an embryo. During the embryonic developmental process, myoblast cells either divide mitotically to give rise to more myoblasts or differentiate into myocytes or muscle cells. Whether myoblast cells proliferate or differentiate into myocytes is strictly dependent on the concentration of certain growth factors. Presence of high level of growth factors have been found to direct the proliferation of myoblast cells to give rise to more myoblast cells, whereas less growth factors in the medium have been found to result in the differentiation of the myoblast cells into myocytes. Myogenesis is actually a developmental process of muscles which involves several stages viz. delamination, migration, proliferation, determination, differentiation, specific Muscle Formation, and satellite cells. *In summary, the myoblasts begin to differentiate into myocytes by leaving the cell cycle and began expressing genes associated with the next stages.* The myoblasts next align to one another and fuse to form muscle tissues.

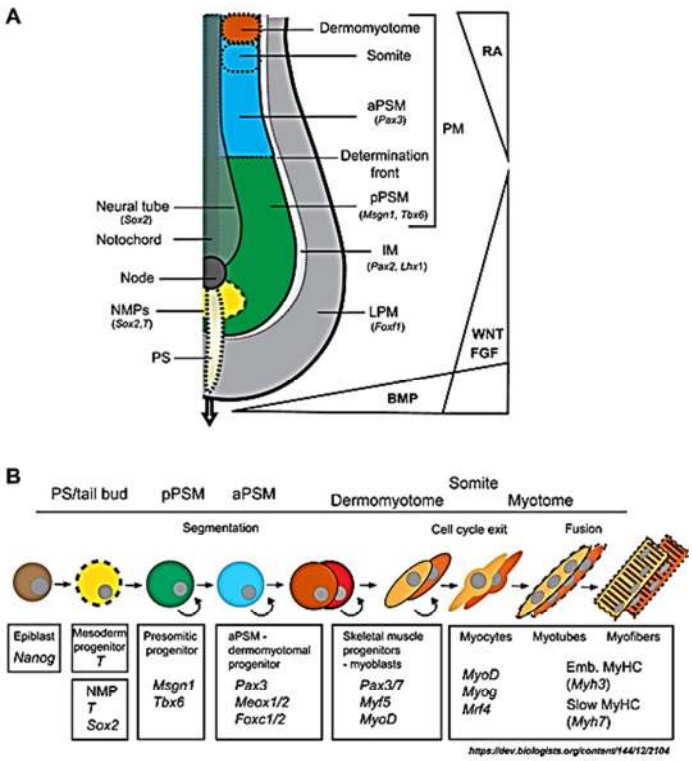
Skeletal muscles form the major portion amongst all the muscles in the organisms, which are more than 600 in humans. These muscles are distinct from both cardiac and smooth muscles in that they can be voluntarily controlled by the organism meant for motion and support. They are composed of bundles of striated myofibers that consist of elongated multinucleated syncytia. These fibers are surrounded by a basal lamina and are filled with a highly organized cytoskeleton composed of myofibrils. Skeletal muscles of the body arise from the somites, transient embryonic structures that originate from the paraxial mesoderm. By contrast, muscles of the head and neck derive from the anterior paraxial mesoderm, which does not form somites.



By understanding how muscle tissue is formed *in vivo* will also help pave the way for recreating muscle tissue *in vitro* from pluripotent stem cells (PSCs), either embryonic (ESCs) or induced (iPSCs) which will definitely help in treatment of different muscles regenerative disease.

**Developmental origin of skeletal muscles**

Skeletal muscles originate from the paraxial mesoderm, a tissue that forms in the primitive streak/blastopore during gastrulation and later in the tail bud during embryonic axis elongation. The nascent paraxial mesoderm constitutes the presomitic mesoderm at the posterior tip of the embryo. The presomitic mesoderm is a transient tissue that can be further subdivided into an immature posterior and a committed anterior region, the latter of which segments to form the somites. It is within the somites that skeletal myogenesis is initiated with the specification of the premyogenic progenitors and skeletal myoblasts. Several phases of proliferation and differentiation lead to the formation of multinucleated myofibers from the fusion of mononucleated myocytes.




**The formation and differentiation of the paraxial mesoderm.** (A) Spatial organization of mesoderm fate in the posterior region of an amniote embryo. Mesoderm forms by ingression of epiblast cells at the level of the primitive streak (PS). Progressively more lateral domains of the paraxial mesoderm (PM), intermediate mesoderm (IM) and lateral plate mesoderm (LPM) are shown and the corresponding marker genes are indicated. The nascent mesoderm is patterned by specific signaling pathways – in particular BMP, Wnt, FGF and retinoic acid (RA) signaling – the activities of which are distributed in gradients in the developing embryo. During axis elongation (arrow), paraxial mesoderm progenitors are, at early stages, located in the anterior primitive streak posterior to the node, and they become incorporated into the tail bud later on. These progenitors include the neuromesodermal progenitors (NMPs). Dorsal view, anterior to the top. (B) Diagram recapitulating the differentiation of paraxial mesoderm toward skeletal muscle. From left to right, the developmental sequence (top) and the intermediate cell types with their marker genes (bottom) are shown. Cell types are color-coded according to the tissue types shown in A. aPSM, anterior presomitic mesoderm; pPSM, posterior presomitic mesoderm; Emb., embryonic.

**Morphogens and specification of paraxial mesoderm progenitors:** Morphogens are signaling molecules that emanate from a source (possible a restricted region) and spread away from their source to form a concentration gradient. Here signaling factors such as Nodal and BMP4 (Bone Morphogenetic Protein 4) results in the activation of Wnt3 (Wnt stands for **W**ingless related i**nt**egration site) and the early mesoderm marker brachyury (T) that leads to the formation of primitive streak in the embryo. There are four types of mesoderm progenitors; one type, which corresponds to a resident cell population, gives rise to both paraxial mesoderm and neural tube derivatives, known as neuromesodermal progenitors that expresses T (brachyury) and Sox2

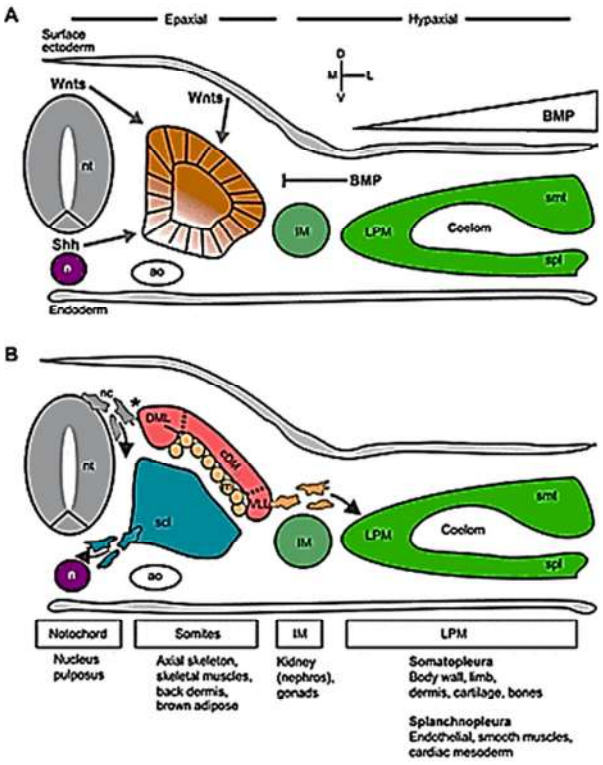
(SRY box 2), a second type of progenitor gives rise to paraxial mesoderm, while a thirs type gives rise to both paraxial mesoderm and lateral plate derivatives, and fourth type gives rise to paraxial mesoderm and notochord. The differentiation of these paraxial mesoderm through these mesodermal progenitors chiefly requires the activation of Wnt3 and BMP 4 signaling cascade, and thus these molecules and intermediates are termed as morphogens of paraxial development in the embryo.

**Differentiation of presomitic mesoderm:** The specification of the future pairs of embryonic segments i.e. the somites is the result of highly dynamic molecular processes within the presomitic mesoderm which involves a molecular oscillator known as the segmentation clock. These molecular oscillators are able to generates pulses of Notch, FGF and Wnt signaling to control the periodic production of somites. The posterior domain of the paraxial mesoderm is composed of four consecutive transcriptional domains: the tail bud, the posterior presomitic mesoderm, the anterior presomitic mesoderm, and the forming somite ($S_0$). The tail bud domain contains the paraxial mesoderm progenitors and is exposed to the highest Wnt/FGF signaling activity, while posterior presomitic mesoderm are characterized by the expression of genes such as *Msgn1, though* Wnt/FGF pathways are still highly active and are essential for maintenance in this domain. Cells of the posterior presomitic mesoderm undergo abrupt signaling, metabolic and transcriptional changes as they enter the anterior presomitic mesoderm including downregulation of *Msgn1* and activation of *Mesp2*, *Pax3*, *Foxc1/2* and *Meox1/2* genes. In the anterior presomitic mesoderm, the posterior Wnt/FGF gradients are counteracted by retinoic acid (RA) produced by the somitic region and the tail bud containing the paraxial mesoderm precursors is protected from the differentiating action of RA by the expression of the RA-degrading enzyme. It is the *Mesp2* gene which defines the anterior and posterior boundaries of the future segment as shown in above figure.

**Compartmentalization of somites:** Soon after the formation of somites, they become compartmentalized along the dorsoventral axis into a dorsal epithelial dermomyotome and a ventral mesenchymal sclerotome. The dermomyotome gives rise to skeletal muscle, brown fat and dermis of the back, whereas the ventral sclerotome produces the axial skeleton and tendons. At the time of their formation, each somite is composed of an anterior *Tbx18*$^+$ and a posterior *Uncx*$^+$ compartment with distinct derivatives (Chal & Porquie, 2009). Cells of the lateral somite give rise to the hypaxial muscles of the limbs or the intercostal, whereas the medial somite forms the sclerotome, dermis of the back and paraxial muscles as in figure below. Wnt, BMP and Shh represent the major signaling pathways for the induction of different somitic fates Dorsally, local inhibition of BMP signaling is also essential for proper dermomyotome specification, while Wnt signals produced from the dorsal neural tube and ectoderm act to maintain the dermomyotome fate (Hirsinger et. al., 2000). Moreover, lineage-tracing studies suggests the presence of Pax7, Pax3 and/or Myf5, which give rise to brown fat. Ventrally, Shh signaling from the notochord and floor plate specifies the sclerotomal compartment, which downregulates *Pax3* and upregulates *Pax1* and *Nkx3.2* expression (Figure below). However, Shh can also stimulate the formation of myotomal cells (Borycki et. al., 1999).

**Myogenesis in the embryo and the fetus**

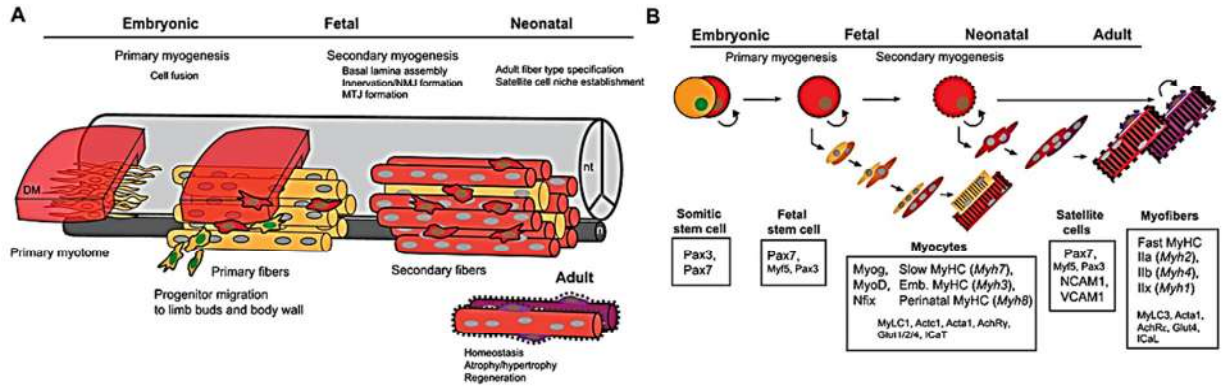
Myogenesis in both embryo and adult is a multi-step process which needs an intricate network of regulatory mechanism. Soon after somite formation, initiation of myogenesis starts with the differentiation of myoblasts cells.




**Somite patterning and myotome formation.** (A) Spatial relationship between the epithelial somite and the surrounding structures. The mesodermal subtypes are shown, as well as the future epaxial and hypaxial domains. Each epithelial somite is patterned into dorsoventral, mediolateral and anteroposterior compartments by signaling factors secreted by the surrounding tissues. Dorsally, Wnt signaling is required for dermomyotome specification, while BMP signaling produced by the lateral plate mesoderm (LPM) inhibits the differentiation of somitic lineages. Ventrally, Shh secreted from the midline plays a major role in sclerotome induction. (B) Spatial relationship between the differentiated somite and the surrounding structures. Dorsally, the somite differentiates into the dermomyotome (DM, red), which can be further subdivided into central dermomyotome (cDM), dorsomedial lip (DML) and ventrolateral lip (VLL). The dermomyotome also gives rise to the myotome (m, orange), which forms beneath from the four DM lips. Cells delaminate from the VLL to give rise to the myogenic progenitors of the limbs that migrate into the LPM. Neural crest (nc, gray) delaminates from the dorsal neural tube and, while migrating ventrally, contacts dermomyotomal cells to promote myogenic induction through Notch activation and β-catenin stabilization (asterisk). ao, dorsal aorta; IM, intermediate mesoderm; smt, somatopleura; spl, splanchnopleura; n, notochord; nt, neural tube; D, dorsal; L, lateral; M, medial; V, ventral.

**Initiation of myogenesis in the somite:** Myogenesis starts with the activation of the myogenic factor Myf5 in cells of the dorsomedial part of the newly formed. The dorsal epithelial dermomyotome, which expresses Pax3, becomes subdivided into a central domain, a dorsomedial lip, anterior and posterior lips, and a ventrolateral lip. Soon after dermomyotome formation, cells in the dorsomedial lip begin to express Myf5 and to downregulate Pax3. The primary myotome forms as a cell layer sandwiched between the dermomyotome dorsally and the sclerotome ventrally. The first postmitotic skeletal muscle cells formed in the embryo are the myocytes of the myotome. These cells express specialized cytoskeletal proteins including slow (type I, *Myh7*) and embryonic (*Myh3*) myosin heavy chains (MyHC), α-actins [cardiac (*Actc1*) and skeletal (*Acta1*)] and desmin as well as the Notch ligand jagged 2 and metabolic enzymes such as β-enolase and carbonic anhydrase III (CAIII) (Chal & Pourquite, 2017). The newly formed mononucleated myocytes elongate along the anterior-posterior axis to span the entire somite length, a process controlled by Wnt11 signaling. More cells are progressively added to the myotome by the other dermomyotomal lips and fuse to form slow $MyHC^{+}$ myofibers (Figure below). After formation of the myotome, the central dermomyotome loses its epithelial character and its $Pax3^{+}$ cells translocate to populate the myotome, providing the myogenic precursors involved in later phases of myogenesis. Myogenesis progresses as a rostral-to-caudal wave of maturation as the embryo elongates and as new pairs of somites are sequentially added, for example, limb muscles derive from cells migrating from the lateral dermomyotome into the developing limb buds, muscle cells of trunk and limbs particularly express transcription factors

like Pax3 and a set of muscle regulatory factors (MRFs) consisting of Myf5, MyoD (Myod1), MRF4 (Myf6) and myogenin). It is myogenin in the embryo, which controls the terminal differentiation of myoblasts into myocytes.




**Somite patterning and myotome formation.** (A) Spatial relationship between the epithelial somite and the surrounding structures. The mesodermal subtypes are shown, as well as the future epaxial and hypaxial domains. Each epithelial somite is patterned into dorsoventral, mediolateral and anteroposterior compartments by signaling factors secreted by the surrounding tissues. Dorsally, Wnt signaling is required for dermomyotome specification, while BMP signaling produced by the lateral plate mesoderm (LPM) inhibits the differentiation of somitic lineages. Ventrally, Shh secreted from the midline plays a major role in sclerotome induction. (B) Spatial relationship between the differentiated somite and the surrounding structures. Dorsally, the somite differentiates into the dermomyotome (DM, red), which can be further subdivided into central dermomyotome (cDM), dorsomedial lip (DML) and ventrolateral lip (VLL). The dermomyotome also gives rise to the myotome (m, orange), which forms beneath from the four DM lips. Cells delaminate from the VLL to give rise to the myogenic progenitors of the limbs that migrate into the LPM. Neural crest (nc, gray) delaminates from the dorsal neural tube and, while migrating ventrally, contacts dermomyotomal cells to promote myogenic induction through Notch activation and β-catenin stabilization (asterisk). ao, dorsal aorta; IM, intermediate mesoderm; smt, somatopleura; spl, splanchnopleura; n, notochord; nt, neural tube; D, dorsal; L, lateral; M, medial; V, ventral.

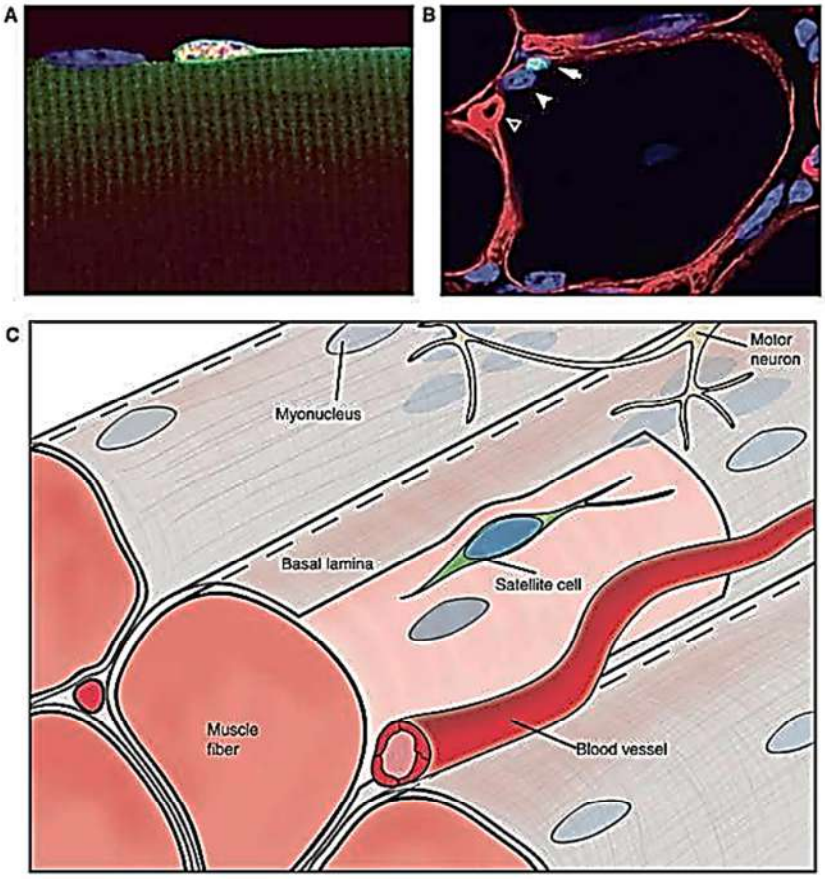
**Primary and secondary myogenesis:** Myogenesis in the embryo can be separated into two phases; an early embryonic or primary phase and a later fetal or secondary phase. Primary phase results in the production of the primary myofibres, which derive from $Pax3^+$ (mouse) or $Pax3^+/Pax7^+$ (chicken) dermomyotomal progenitors. These primary myofibers form the early myotomes and limb muscles, providing the templates upon which adult muscles are built up. They express a specific set of proteins, such as the slow MyHC and myosin light chain 1 (MyLC1, Myl1). During the second phase of myogenesis, a subset of the $Pax3^+$ myogenic progenitors begins to express Pax7 and downregulates Pax3 which fuse among themselves or to the primary fibres to give rise to secondary or fetal fibres that express specific markers such as β-enolase, Nfix or MyLC3 (Myl3). During secondary myogenesis, muscle growth is sustained essentially by cell fusion and the addition of myonuclei from proliferating $Pax7^+$ progenitors. However, postnatal muscle growth mostly results from individual fiber hypertrophy through the addition of novel myofibrils. These $Pax7^+$ progenitor cells also form the pool of adult muscle stem cells, known as satellite cells, which are responsible for growth and development of muscles in adult.

## Myogenesis in the adult

Unlike de novo embryonic muscle formation, muscle regeneration in higher vertebrates depends on the injured tissue retaining extracellular matrix scaffolding that serves as a template for the formation of muscle fibers except amphibians, certain fishes, and some lower organisms that has the ability to regenerate muscle without an instructive template. It requires the recruitment of an undifferentiated progenitor cells to the site of injury. The satellite cells acts as a progenitor during myogenesis in the adult. These cells have been shown to use asymmetric divisions for

self-maintenance and, at the same time, give rise to more committed myogenic progeny. These cells are capable of entering several different mesodermal lineages such as muscle, bone, and brown fat. Therefore, the satellite cells act as stem cells in true sense and like other stem cells; they also require a specific compartment/environment known as "stem cells niche" that supports self-renewal of stem cells by simultaneously preventing them from differentiation.

**Satellite cells niche:** Satellite cells also require such microenvironment that maintains its functional integrity and instructs its commitment for muscle cell formation. The satellite cells are more sensitive to their microenvironment compared to the hematopoietic stem cells (HSCs), which after release can home back to their niche relatively efficiently while maintaining their stemness (Cosgroye et. al., 2009). In their niche, satellite cells sit closely apposed to the myofiber and are covered by the extracellular matrix of the basement membrane (figure below). They are furthermore often localized in close proximity to capillaries, which supply them with essentials nutrients. Unless activated by muscle injury or other stimuli, their niche allows adult satellite cells to persist in a quiescent, non-proliferative state, which is critical for their lifelong maintenance (Shea et. al., 2010). It is due to which they cannot be isolated from their niche; upon isolation, they readily differentiate into myoblast cells. They are found to be outnumbering hematopoietic stem cells (HSCs).



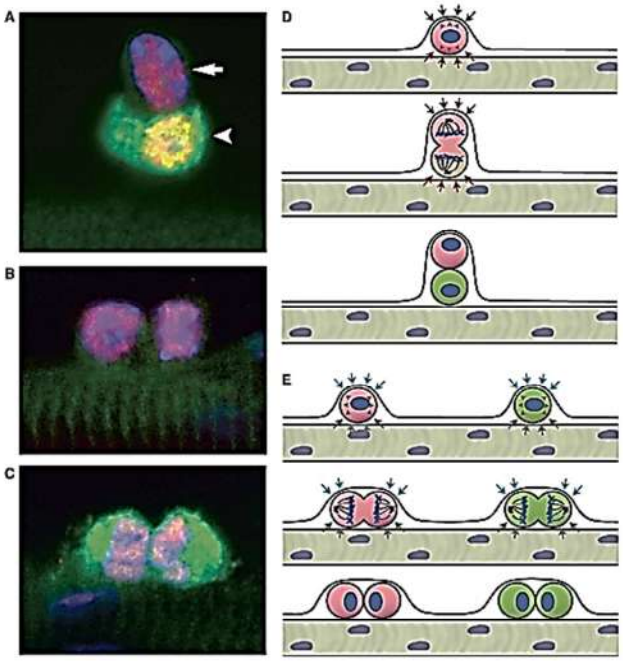

**Schematic of skeletal muscle and the satellite cell niche.** (*A*) Satellite cells reside along the host muscle fiber and are marked by *Pax7* expression (red); nuclei (blue); cytoplasm (green). (*B*) Satellite cells (arrow) marked by Pax7 (green) are found beneath the basal lamina (red) that surrounds each muscle fiber. In mature muscle, they are always associated with a myonucleus (arrowhead) and are in close proximity to local capillaries (empty arrowhead). (*C*) Representation of skeletal muscle and the satellite cell niche. Molecular signals within the niche govern the behavioral response of satellite cells in maintaining quiescence or activation during injury.

Source: *Bentzinger, C. F., et. al. , 2012*

**Origin of satellite cells:** As mentioned above satellite cells are originated from pluripotent cells of the embryonic somite. When diphtheria toxin-mediated cell death is induced in *Pax3/Pax7*-expressing cells in the developing mouse somite, no muscle progenitors are found in the limbs

(Hutchenson et. al., 2009). Bone marrow–derived progenitors, skeletal muscle side population cells, mesoangioblasts, pericytes, CD133 (Prom1)$^{+}$ progenitors, and PW1 (Peg3)$^{+}$ interstitial cells have also been found to participate in the formation of multinucleated myotubes (Mitchell et. el., 2010). Only Pax*7*-expressing satellite cells are capable of replenishing the satellite cell pool or repair the tissue following injury thus they are proved to be a major or only mediators of myofiber regeneration in the adult. All satellite cells, whatever origin, express *Pax7*, and in some muscles also *Pax3*, throughout the life of the organism (Kuang & Rudnicki, 2008). These cells provide remarkable regenerative capacity to the skeletal muscles and therefore even after multiple injuries, they are able to maintain at a constant size.

These cells replenish themselves and develop into muscle cells by adopting stochastic differentiation and asymmetric division (Kuang et. el. 2008). In the stochastic paradigm, one stem cell divides to give rise to two committed daughter cells, while another stem cell gives rise to two identical daughter stem cells to maintain a constant progenitor pool, while in asymmetric division, a stem cell gives rise to a father cell that is identical to the original stem cell and a committed daughter cell (see figure below).




**Asymmetric versus stochastic modes of satellite cell division.** As determined by lineage tracing, 10% of adult satellite cells have never expressed *Myf5* and are referred to as "satellite stem cells." (*A*) Satellite stem cells (arrow) undergo asymmetric division in an apical–basal orientation in which the daughter cell that is detached from the basal lamina up-regulates *Myf5* and the fluorescent lineage tracer *YFP* (arrowhead). Pax7 (red); YFP (green); nuclei (blue). (*B*,*C*)
In the stochastic mode of division, both types of satellite cells divide planar along the host fiber and give rise to two identical daughter cells. (*D*) Model of apical-basal divisions leading to an asymmetric outcome. Opposing signals from the basal lamina and the myofiber control the orientation of DNA spindles and the asymmetric cosegregation of proteins and DNA strands. Post-cytokinesis, daughter cells continue to be subjected to different signals leading to asymmetric cell fates. (*E*) Planar divisions lead to the symmetric expansion of cells. Signals such as the Wnt7a–PCP pathway drive the planar orientation of DNA spindles. Daughter cells in this outcome remain attached to the host fiber and the basal lamina, thus receiving similar signals, and maintain identical cell fates.
Source: *Bentzinger, C. F., et. al. , 2012*

It has been found that a small subpopulation, ~10% of satellite cells, never express *Myf5* and thus they divide in an asymmetrical apical–basal manner with respect to the muscle fiber membrane, giving rise to a more committed *Myf5* negative cell that self-renewed. These data suggest that the satellite cell pool in adult skeletal muscle contains an asymmetrically dividing, self-renewing population of satellite stem cells that is responsible for maintenance and homeostasis of the satellite cell population. In contrast, expression of *Pax7/MyoD* in the satellite cells results in hypertrophy of *Pax/MyoD* double positive cells and progression of an elaborate self-renewal mechanism that prevents lineage progression and terminal differentiation. This mechanism of development of skeletal muscles preserve throughout the life.

## References


1. Gilbert, S. F. Myogenesis: The Dvelopment of Muscles. In "Developmental Biology. 6th Edition. Sunderland M. A.: Sinauer Associates. 2008.
2. Pestronk, Alan. "Myogenesis & Muscle Regeneration". WU Neuromuscular. Washington University. Retrieved 2013-03-16.
3. Chal, J & Pourquite, O. Patterning and differentiation of the vertebrate spine. In The Skeletal System: Cold Spring Harbor Monographs Vol. 53, Ed O. Pourquie. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 2009, pp. 41-116.
4. Hirsinger, E., et. al. Somite formation and patterning. Int. Rev. Cytol. 2000; 198: 1-65.
5. Johnson, R. L., et. al. Ectopic expression of Sonic hedgehog alters dorso-ventral patterning of somites. Cell 1994; 79: 1165-1173.
6. Chal, J & Pourquite, O. Making muscle: skeletal myogenesis in vivo and in vitro. Development 2017; 144: 2104-2122.
7. Cosgrove, B. D., et. al. A home away from home: challenges and opportunities in engineering in vitro muscle satellite cell niches. Differentiation 2009; 78: 185-194.
8. Shea, K. L., et. al. Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration. Cell Stem Cell 2010; 6: 117-129.
9. Bentzinger, C. F., et. al. Building muscle: molecular regulation of myogenesis. Cold Spring Harb Perspect Biol. 2012;4(2):a008342.
10. Hutchenson, D. A., et. al. Embryonic and fetal limb myogenic cells are derived from developmentally distinct progenitors and have different requirements for beta-catenin. Genes Dev. 2009; 23: 997-1013.
11. Mitchell, K. J., et. el. Identification and characterization of a non-satellite cell muscle resident progenitor during postnatal development. Nat. Cell Biol. 2010; 12: 257-266.
12. Kuang, S. and Rudnicki, M. A. The emerging biology of satellite cells and their therapeutic potential. Trends Mol. Med. 2008; 14: 82-91.
13. Kuang, S., et. al. Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell 2008; 2:22-31.


ooooooooo