

Histogenesis of Myogenic Recapitulation and Myoblasts Autonomous Migration During Lizard Embryogenesis (Reptilia: Sauria)

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Abstract

The article deals the issues of formation, recapitulation (differentiation), morphological changes of myogenic cells in lizard embryos. It has been established that the formation of somites is observed in the oviduct of females. Segmentation of the presomitic mesoderm into numerous metamer units (somites), observed in the cranio-caudal direction, along the anterior-posterior axis. During the formation of somatic muscles of trunk and limbs, 11 stages of myogenic recapitulation are distinguished. All stages of myogenic recapitulation are completed during embryogenesis. Inside the myotome myogenic progenitor cells proliferate and give rise A-type MPCs, apoptotic cells, and myoblasts. During autonomous myoblast migration by chemotaxis, apoptotic cells death promotes the movement of other myoblasts and attracts other myoblasts to the area. Formation of the muscular plate from myosymplasts in the limb bud occurs faster than in the trunk of the lizard embryo. From puberty to the end of reproductive activity, the number of nuclei per myofiber continues to increase, but the number of muscle fibers does not change.

Keywords: recapitulation, embryogenesis, myoblasts, apoptotic cells, muscular plate, migration

1. Introduction

Skeletal muscle comprises in the body of adult lizards 35-40% of total body weight and contains approximately 50% of all body proteins. Skeletal muscles are attached to bones and act to produce and stop movement. Active hyperemia is considered as one of the mechanisms of progressive tissues evolution. Muscles, being the main components of the body, play an important role in the postnatal and prenatal life of lizards. They are also actively participate in physiological and metabolic functions. We studied lizards skeletal muscle myogenesis from the paraxial mesoderm to the formation of myofibers. Knowledge of muscle development models, as well as ways of their transformations in embryonic histogenesis, is necessary to learn the mechanism of their formation. At the same time, the study of embryogenesis of muscle tissue can give useful information about the ways of mastering the land with these animals. Postnatal development involves intricate coordination of embryonic development, When the basis of further growth of the body is established [10]. During the formation of myoblasts, significant morphological changes occur in the embryo. The migration and proliferation of cells is a complex and dynamic process that begins with the stage of gastrulation. At this stage, the first signs of cell differentiation appear using genetic information. The differential of myogenic elements is the process of appearance and increase in structural and functional differences between the cells and parts of the embryo. Cell specialization also has a biochemical essence, which consists in the synthesis of certain proteins that are characteristic only for this type of cell. In early embryogenesis, biochemical specialization of cells is ensured by differential activity of genes. Migration of myogenic stem cells occurs with expression of chemokines and their receptors. We discuss how histogenesis of recapitulation and autonomy of myogenic stem cells migration can guide embryonic studies of vertebrates in the example of reptiles.

2. Materials and methods

This research was carried out on the basis of a scientific work registered at BSU on April 04, 2021 under the number 3/262. The research objects were three widespread species of lizards (*Ophisops elegans* Menetries, 1832; *Lacerta strigata* Eichwald, 1831; *Tenuidactylus caspius* Eichwald, 1831) from the herpetofauna of Azerbaijan. To obtain histological materials during the breeding season (May) of lizards on the territory of Azerbaijan [3], 10 individuals of pregnant females from each species were caught. The research was conducted at the Department of Zoology and Physiology of Baku State University and Medical Biology and Genetics of AMU from 2021 to 2023. Female lizards kept separately before oviposition in special plastic containers. Two individuals from each species were used to study myogenesis during preoviposition. Total 36 pcs eggs are obtained from lizards.

Lizard eggs were artificially incubated at a constant temperature of 28-30°C and a humidity of 50-60%. After the collection of materials captured live lizards were released into the wild. According to the periodization of embryogenesis of reptiles, each stage of morphogenesis corresponds to individual periods of artificial egg incubation. Starting from early embryonic stages, embryos were removed for the preparation of histological preparations. To determine the shape and number of myotomes, total and partial histological slides are prepared. Histology slides are prepared by standard methods. Samples of embryonic muscle tissue were fixed in 10% formalin solution within 3 hours and then the paraffin blocks are prepared for to cutting a ribbon of 3-5 micron-thick sections. Prepared slides are examined under the microscope.

3. Research results

The lizards studied by us are oviparous. During the formation of somatic muscles, 11 stages of myogenic recapitulation are distinguished (**Figure 1**). Formation of paraxial mesoderm, somitomers and somites are observed in the oviduct of females. In lizards, muscles of head are derived from the anterior (prechordal) paraxial mesoderm. Anterior paraxial mesoderm does not form somites. Segmentation of the presomitic mesoderm of lizard embryo into numerous metameric units (somites), observed in the craniocaudal direction.

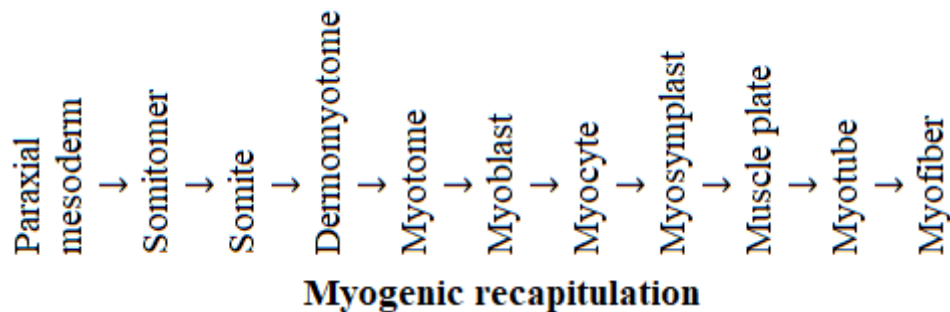


Figure 1. In the embryogenesis of trunk and limbs of lizards, 11 stages of myogenic recapitulation are observed

The reason of formation primary somites near the head is associated with the formation of the brain. Primarily torsion of lizard embryo is caused by cell migration forces. From the dorsal portion of the mature somites formed dermomyotome (**Figure 2**). From cells of the transient epithelium of the dermomyotome, the myotome and dermatome are formed. The myotome of lizard embryo originates from the dorsal medial lip of the dermomyotome. Inside the myotome myogenic progenitor cells are formed. Myogenic progenitor cells (MPCs) proliferate. Differentiation occurs between cells formed as a result of proliferation.

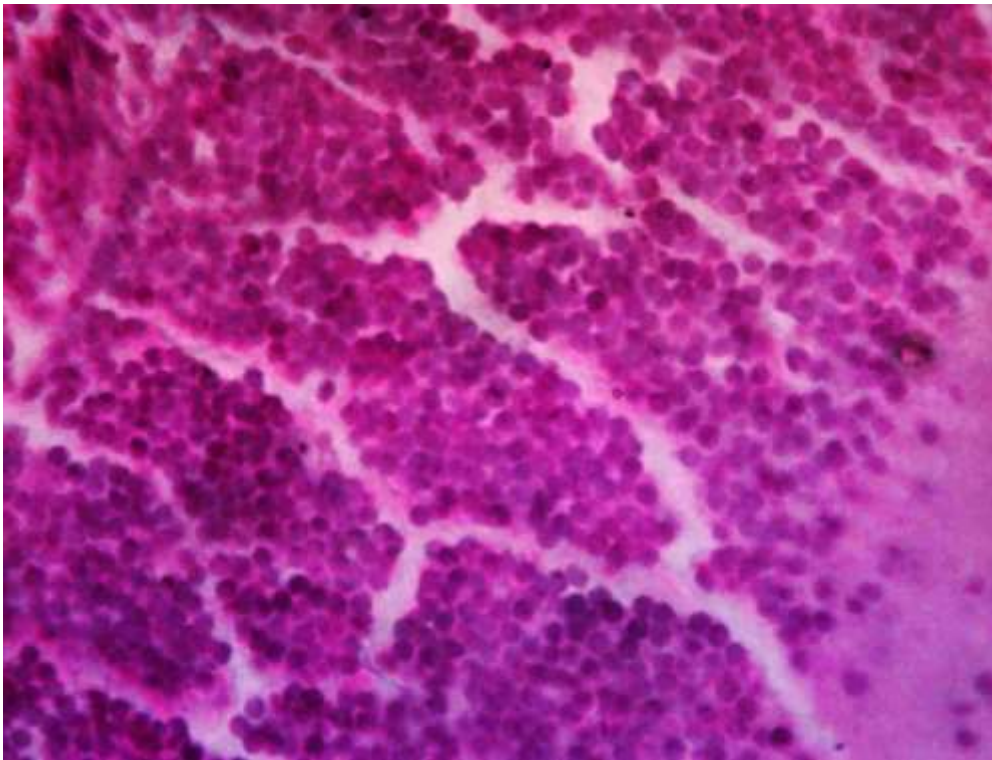


Figure 2. Cross section of the dorsal part of the somites of Snake-eyed lizard embryo 1 day after oviposition. Hematoxylin and eosin stain, original magnification x 100.

From cells of the transient epithelium of the dermomyotome, the myotome and dermatome are formed. Inside the myotome myogenic progenitor cells (MPCs) proliferate. Differentiation occurs between cells formed as a result of proliferation. During differentiation, A-type MPCs, apoptotic cells, and myoblasts are formed. A-type MPCs stay in their place and continue proliferation. Apoptotic cells die. Myoblasts migrate from the myotome to sites of future muscle differentiation. Myoblast migration is beginning of histogenetic recapitulation. During the autonomous migration of myoblasts, between them other tissue elements are not observed, and some migrating cells die. This means that the autonomous migration process takes place by chemotaxis. Dead cells promote the movement of other myoblasts and attract other myoblasts to the area. Adult myoblast are withdrawal of the cell cycle. The adult myoblasts elongate along the anterior-posterior axis, forming mononucleated myocytes. Before the fusion of myocytes with myoblasts, myoblasts fusion-competent must fuse with cells. Myocyte fuse with myoblasts at the target place to form myosymplast. We also observe this stage in the myogenesis of the Caspian sturgeon. After formation of the primary myosymplast, myocytes and myoblasts join to this complex. The fusion of myoblasts with the myosymplast in the limb bud occurs faster than in the trunk of the lizard embryo. Following this process, the formation of a muscular plate occurs from adult myosymplasts. At this intermediate stage, the shape and location of the nuclei are different. Blastomeres

and fusion-competent are attached to the muscle plate. This is followed by formation of myotube (**Figure 3**). Myofibrils rapidly form along the periphery of the myotube.

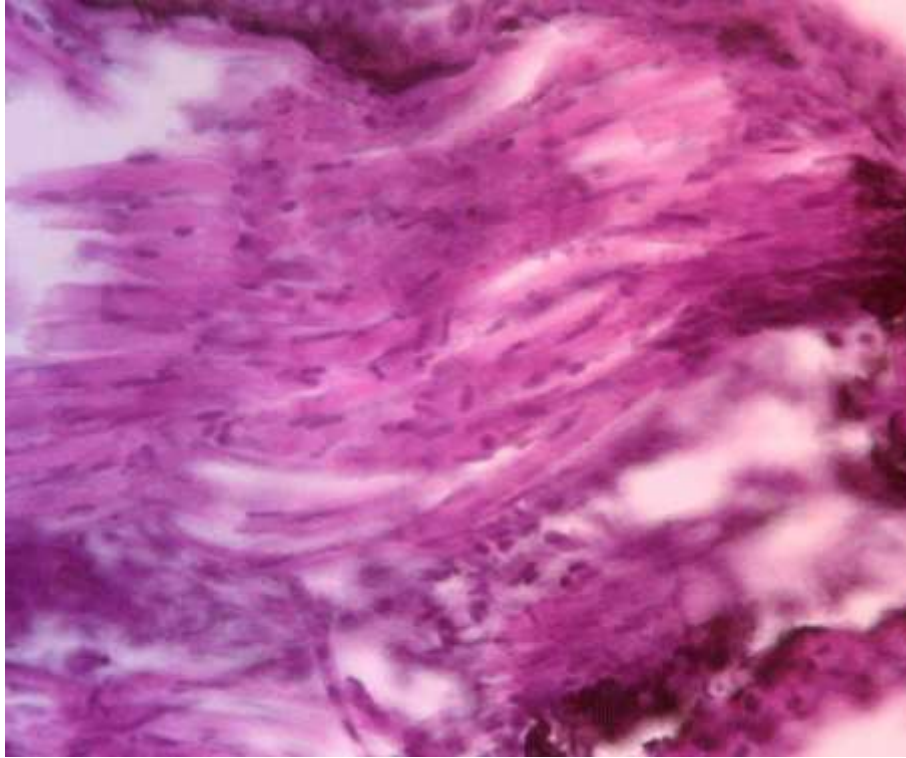


Figure 3. Formation of the myotube from the muscle plate of *Lacerta strigata* embryo. Hematoxylin and eosin stain, original magnification x 50.

Some myoblasts are not involved in the formation of myofibers. These myoblasts, attached to sarcolemma receptors, remain outside the myofiber and are called myosatellite. The adult myotube and myosatellites are surrounded by basal lamina, to form myofiber. During the further development of the embryo, formation of myofibrils occurs in the centre of myotube and nucleus are pushed to the periphery. During the recapitulation process, important changes occur related to the location of the nucleus. The myoblast and myocyte nucleus are located in the center of the cell. During the formation of the myosymplast, nucleus are also located in the center of bead-like structures. But with formation of the muscle plate, place of the nucleus changed. At this stage, not only location of the nucleus differs, but the shape of the nucleus also changes. In the early myotube, nucleuses are located in the centre. With the formation of myofibers, the nucleuses move to the periphery (**Figure 4**).

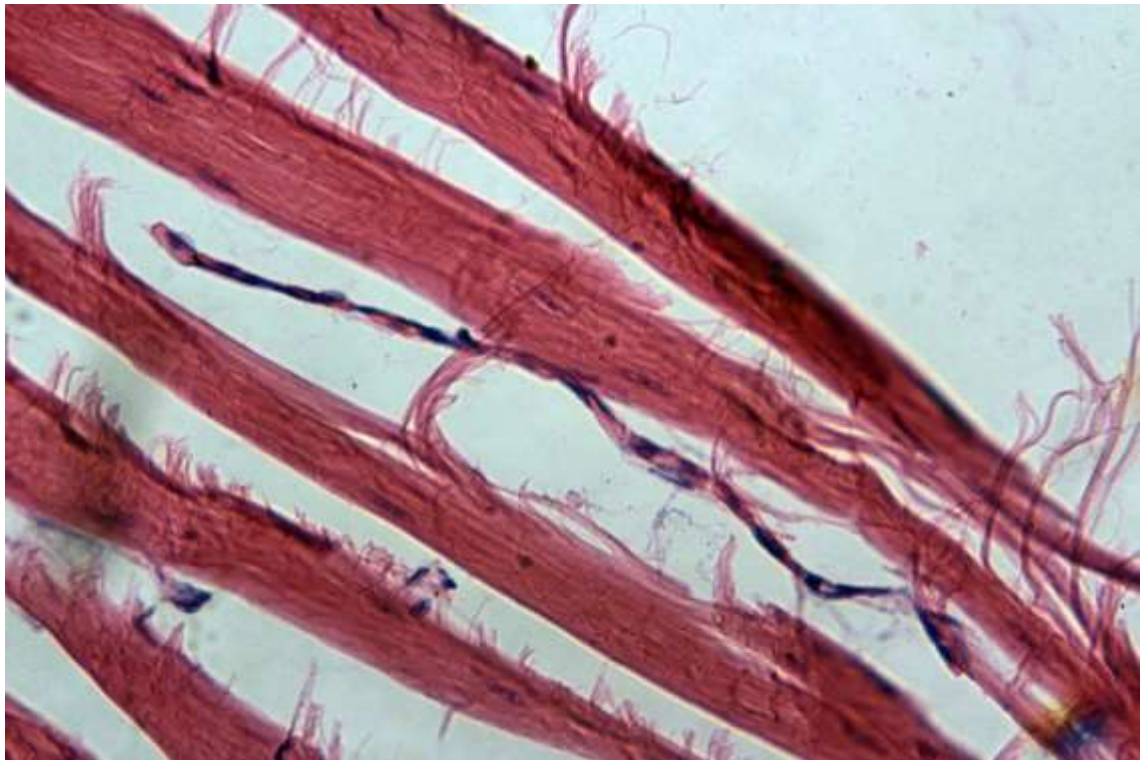


Figure 4. Completely formed myofibrils of *Tenuidactylus caspius*. Hematoxylin and eosin stain, original magnification x 40.

When myofibers are formed, they are covered with the external lamina and reticular fibers. The external lamina and reticular fibers form the endomysium. Endomysium separates one myofibril from another. The endomysium of each myofibril connects with others and forms a network of connective tissue. The perimysium assembles myofibrils into fascicles and the epimysium surrounds the muscle. The muscle connective tissue connects muscles with tendons by myotendinous junctions. Muscle formation in lizard embryos does not occur simultaneously. The formation of muscles in lizard embryos occurs in different parts of the body at different times of embryogenesis. After birth, skeletal muscle myofibers develop by adding new myonuclei from myosatellite cells. From puberty to the end of reproductive activity, the number of nuclei per myofiber continues to increase, but the number of muscle fibers does not change.

4. Discussions

Mesoderm is the middle germ layer that develops during gastrulation of the embryo [21]. Origin of skeletal muscles of trunk and limbs are in the paraxial mesoderm, which is formed in the blastopore. Skeletal muscles of the head are derived from the prechordal paraxial mesoderm [11]. Somitogenesis is divided into periodicity, epithelialization, specification and differentiation stages [23]. Mature somites arise

from the presomitic mesoderm [17]. Mature somites contain the mass of ventral sclerotome and dorsal dermomyotome cells. After several migration of the dermomyotome epithelial cells, myotome and dermatomes arises [18]. Pioneer muscle cells form within the myotome in response to signals from the lateral plate of mesoderm [9]. After formation of the myotome, the central dermomyotome loses its epithelial character and cells translocate to populate the myotome, providing the myogenic precursors involved in later phases of myogenesis [7]. Myoblasts, moving away from myotomes, begin to migrate singly or in small groups to the location of future muscles, where they proliferate and then differentiate to form muscles [4]. Myoblast proliferation occurred under the control of fibroblast growth factors [13]. The myogenic regulatory factors control the determination and differentiation of muscle cells [8]. In the process of differentiation, the first postmitotic skeletal muscle cells that form in the embryo are myocytes. The fusion of muscle cells begins after the formation of myocytes [22]. Alignment of the myoblasts together into chain is mediated by cell membrane glycoproteins, including several cadherins [6]. Myosimplasts grow by adding new myonuclei, which are supplied by resident muscle precursors. Myosimplasts progressively organize into muscle masses and form myotube [20]. Myofiber is formed from myotube [16]. The myosatellite cells are situated underneath the basement membrane of the myofibers [19]. Like myoblasts, myosatellite cells can proliferate, differentiate, and fuse into myofibers [18]. Each fibre is innervated by a single motor neuron, via the neuromuscular junction [2] and supplied by a network of blood capillaries [5]. The basement membrane of myofibers [15], composed of extracellular matrix materials, surrounds each fibre and is linked to the sarcolemma via integrin proteins [12]. The basement membrane is responsible for most of the muscle's tensile strength and elasticity [1]. It is also acts as a scaffold anchoring the myofibers to the endomysium and to the muscle tendons [14].

Conflicts of Interest. The authors declare that there are no conflicts of interest regarding the publication of this paper.

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