Nicole Le Douarin and Charles Ordahl's Experiments on the Developmental Lineages of Somites [1]


Through various studies developmental biologists have been able to determine that the muscles of the back, ribs, and limbs derive from somites [5]. Somites are blocks of cells that contain distinct sections that diverge into specific types (axial or limb) of musculature and are an essential part of early vertebrate development. For many years the musculature of vertebrates was known to derive from the somites [5], but the exact developmental lineage of axial and limb muscle progenitor cells remained a mystery until Nicole Le Douarin and Charles P. Ordahl published “Two Myogenic Lineages within the Developing Somite” in 1991. This paper describes their experiment, which used chick [6]-quail chimeras [7] to demonstrate the exact lineage of the limb and back musculature.

Axial, or back musculature, and limb musculature have many developmental differences. For instance, axial muscles form from precursor cells within somites [8] while limb muscle arises from precursor cells that migrate away from the somites [9] and invade lateral regions of the body. Another key difference between these two types is that the cells of axial muscle are mononucleate (each contains one nucleus [10]) while those of limb muscle are multinucleate (each contains many nuclei). Axial muscles express determination [8] factors approximately two days before limb muscles begin to form in chick [8] embryos, a characteristic Le Douarin and Ordahl were quite familiar with. Unlike limb muscle cells, the continued existence of the earlier differentiated axial muscle cells depends on the presence of the neural tube [10] and notochord [11]. These and other developmental differences between the axial and limb muscles led Le Douarin and Ordahl to question whether the axial and limb muscle progenitor cells could be microsurgically separated prior to their specialization and if their lineage could then be traced.

In order to answer this question, Le Douarin and Ordahl used chick [8] and quail embryos with 16 to 21 developed somites [5] each. Early Gallus gallus (chick [8]) somites [5] were surgically separated into either lateral (horizontally cut) or medial (vertically cut) halves. Half somites [5] in chick [8] embryos were replaced with half-somite counterparts from Coturnix coturnix japonica (quail) embryos. The distinctive nucleus [8] from the quail cells allowed for easy determination [9] of fate from the donor half. The chick [8] (donor) embryos that survived four days of postoperative incubation were embedded in wax, sectioned with a microtome [12] at 5 μm, and stained by the Fuelgen method. Previous work had shown that wing muscles derive from the myogenic precursor cells that migrate from somites [5] 16–21 of the developing chick [8] embryo. This fact led Le Douarin and Ordahl to manipulate somites [5] 16–21 in order to determine if the lateral or medial halves were capable of contributing wing myogenic cells. The lateral or medial halves of somites [5] 16–21 in two-day-old chick [8] embryos were replaced with half somites [5] from the quail counterpart in attempt to demonstrate their migratory pattern and developmental lineage.

In the medial chick–medial quail half-somite replacements, quail cells densely populated both the myotomal (blocks of tissue that develop into body wall muscle) and sclerotomal (blocks of tissue that develop into the vertebrae) structures within the somite. Structures on the operated side appeared to develop normally in both size and in shape. The two colleagues determined that the transplantation did not disrupt somite development and that the transplanted half participated in somitogenesis [13] (the production of subsequent somites [5]) normally. In every case, the forelimbs had no quail cells, which convinced the researchers that the cells of the transplanted medial somites [5] did not migrate to the limbs. In the lateral chick–lateral quail half-somite replacements, the forelimb muscles were densely populated with quail cells and there were no quail cells in the non-muscle tissue (i.e., cartilage, mesenchyme [14]). To the surprise of Le Douarin and Ordahl, the somatic structures did not contain any quail cells.

In another study published in 1991, researchers Mark Selleck and Claudio Stern reported that the medial and lateral halves of the somites [5] are derived from different precursor populations during gastrulation [15]. This piqued the interest of Le Douarin and Ordahl and helped them to determine the degree to which the different fates of the medial and lateral somite halves had properties intrinsic to each half. To reach this conclusion, they completed a series of heterotopic half-somite experiments in which donor half somites [5] were always taken from the left side of the quail and implanted into the right side of the chick [8]. This way, the position of the donor somites [5] could be altered without changing the anteroposterior or dorsoventral orientation.

In the medial–lateral half-somite heterotopic replacement experiments, chick [6] lateral somite halves were replaced with quail medial somite halves. This resulted in the presence of quail cells in the forelimb muscles. The myotome and sclerotome were predominantly composed of chick [6] cells; however, there were more quail cells in the somatic regions in this experiment than in the lateral–lateral replacement experiments. The researchers concluded that the cells of the medial-lateral replacement showed a drastic reduction [16] in their capacity to populate forelimb musculature via migration, but some cells may have been capable of responding to migratory cues.
Le Douarin and Ordahl also decided to do a series of experiments that involved removing the neural tube and notochord from the embryos in order to see their effect on the surgically altered somites (via medial half replacement). Removal of the notochord and neural tube led to the disintegration of the somite and disappearance of myotomal musculature, but surprisingly, the limb muscles developed normally. Somites were absent from the embryo and no quail cells were found in the developing limbs or in somatic areas. The researchers assumed that the cells of the transplanted medial half somites underwent cell death.

After analyzing their results, it was clear to Le Douarin and Ordahl that two muscle precursor populations existed in newly formed somites of the early embryo. They determined that the precursor of limb musculature arose exclusively from the lateral half of the somite whereas the precursors of the axial musculature arose exclusively from the medial half of the somite. This suggested that axial (myotome) is derived solely from precursor cells located in the dorsomedial quadrant of the somite.

These experiments led Le Douarin and Ordahl to set out to identify the lineages for lateral and medial halves earlier in development. This also led them to investigate the timing of gene expression in the two lineages leading to the development of somatic and limb muscle. After these and subsequent experiments, the two colleagues observed that the fate of somite cells can be determined by their position in relation to three axes of the somite. The positioning along the anteroposterior axis contributes explicit properties to the rostral and caudal halves of the somite that affect segmentation of the adjacent nervous system. The position along the mediolateral axis determines which somite cells will migrate out to form wing muscle and which will remain in situ to form sclerotome, myotome, and dermatome. The position along the dorsoventral axis determines the commitment to arrangement of sclerotome and dermomyotome. Although each of these points is important in the development of an embryo, only the establishment of the anteroposterior axis appears to be an intrinsic property of the mesoderm from which the somites derive while the other two appear to be influenced by external signals. Therefore, Le Douarin and Ordahl regard somites as structures in development that receive and impose differentiative and morphogenetic information.

Sources


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