

## **Evidence for a "Myogenic Determination Front" in the Segmental Plate Mesoderm Revealed by Loss of Ectoderm Membrane Rafts in Chicken Embryos**

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It is still unclear where and when embryonic myogenic determination occurs in higher vertebrates like the chicken embryo. One proposal is that a "myogenic determination front" is present for muscle specification in the segmental plate mesoderm (SPM) and depends on molecular signals to activate the muscle program for myotome formation in somites. In contrast, a different proposal suggests that myogenic specification occurs before the gastrulation event and that special cells with the G8 antigen identify determined cells with the myoD muscle transcription factor. To address the issue of a "myogenic determination front" in the segmental plate mesoderm, we previously showed that membrane raft (MR) destruction in the ectoderm by a 6-hour methyl beta-cyclodextrin (MBC) treatment resulted in absence of myoD expression in nascent somites and loss of primary myotome formation in older somites. In similar cases of MBC treated embryos allowed to develop overnight (~17 hours), all 12 nascent somites formed from the SPM in the experimental time frame showed abnormal myotome formation by titin antibody labeling and confocal microscopy. However, in 20% of cases, titin labeling was observed but only in a few somites (2-3 somites) arising from the most anterior positions in the SPM. In 80% of cases, somites did not show titin labeling to reveal a loss of myotome formation. The absence of myotome in somites could be due to loss of G8 cells in the paraxial mesoderm and nascent somites in consequence to ectoderm MBC treatment. G8 antibody treatment of MBC and control embryos revealed that G8 positive cells were present in both embryo types and that G8 was abundant in SPM and nascent somites, but not in the lateral plate mesoderm or other mesodermal tissues. The absence of myotome in somites is not the result of loss of G8 cells that have been reported to signal for the myotome. Furthermore a "myogenic determination front" is revealed in the SPM as a result of myotome differentiation only in somites arising from the most cranial positioned areas of the SPM. We conclude that the ectoderm provides important signals possibly mediated through rafts for the regulation of the SPM for myotome formation.