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The Potential Reprogramming of Trunk Neural Crest Cells in the Red-Ear Slider Turtle

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Abstract

The turtle shell is a novel structure that creates an exoskeleton of bone. The origin of the ventral, or belly-side, plastron is unknown, but it is formed through intramembranous ossification, similar to that of the facial bones. Ossification refers to the formation of bone tissue, which can be completed through a couple methods. Endochondral ossification is most common, and bone is formed from preexisting cartilage. Intramembranous ossification does not require cartilage but rather is formed through nearby multipotent cells. Fluorescence experiments were completed with trunk neural crest cells (NCCs) of T. scripta embryos at two stages of development (Greenbaum stage 11 and 16), corresponding to the two migrations of trunk NCCs from the neural tube, by transfecting plasmids containing enhancers Sox10E1 (a trunk-specific enhancer), Sox10E2 (a cranial-specific enhancer), or RFP (red fluorescent protein, to be used as a control). Each plasmid also contained a green fluorescent protein reporter to visualize the use of the enhancer by the cells. Chicken embryos were used as a control. It was hypothesized that the early stage turtle embryos would employ the trunk-specific enhancer while the late stage turtle embryos would use the cranial-specific enhancer. Results showed no evidence of trunk NCC reprogramming in turtle embryos but did show differences of enhancer use between turtle and chicken NCCs.

The turtle is a unique organism, with severely modified structures as a result of the skeletal integration of its shell (Zangerl, 1969). The shell is made up of two major pieces, the dorsal carapace and ventral plastron (Zangerl, 1969). It should be noted that the plastron and carapace, although both parts of a turtle's shell, develop differently and separately from one another (Rice et. al., 2016). Perhaps the most interesting feature of the plastron is that it forms through intramembranous ossification (Zangerl, 1969). This means that the bones of the plastron do not require a cartilage precursor to ossify, but rather does so through aggregating mesenchymal cells (Rice et. al., 2016). The forming embryo has three basic types of cells that become the wide variety of cells in a full developed organism (Gilbert & Baressi, 2016). These are ectoderm, mesoderm, and endoderm (Gilbert & Baressi, 2016). Mesoderm, or mesenchyme, forms the internal organs and includes those cells that undergo intramembranous ossification

(Gilbert & Baressi, 2016). Because the plastron bones form in the same manner as vertebrate facial bones, it was suggested that the bones are derived from neural crest cells (NCCs) (Gilbert, 2003). Neural crest cells are multipotent cells, meaning that they can have a variety of fates (Martik & Bronner, 2017). During development, these cells migrate away from the forming neural tube as it rounds up from a flat structure to a tube (Gilbert et. al., 2001). For reference, the neural tube is the precursor to the spinal cord (Gilbert et. al., 2001). Generally, NCCs migrating on the anterior side of the neural tube have the potential to fulfill a greater number of fates than those posterior to them (Gilbert et. al., 2001). The length of the neural tube is divided into domains based on the potential of the NCCs within it (Gilbert et. al., 2001). From most anterior to most posterior, the domains are: the cranial, vagal, trunk, and sacral region (Gilbert et. al., 2001). One of the first neural crest abilities to disappear below the cranial region is that which produces bone (Gilbert & Baressi, 2016). In turtle embryos, however, because their plastron forms through intramembranous ossification and has been shown to be formed by NCCs, it appears that the NCCs in the trunk region of this organism are able to form bone as well (Gilbert & Baressi, 2016).

Additionally, there have been shown to be two migrations of these trunk neural crest cells rather than the usual one migration that occurs in other organisms, one during stage 11 and the other during stage 16 (Cebra-Thomas, 2013). These differences in turtles may be due to a number of factors, but ultimately is dependent on the gene regulatory network (GRN) that guides its development (Emmert-Streib et. al., 2014). The GRN is the pathway of genes expressed in the cells that lead them to their eventual fate (Emmert-Streib et. al., 2014). To test whether there is evidence of a large-scale reprogramming of trunk NCCs in turtle embryos, NCCs from the first and second migration will be transfected (meaning that DNA will be inserted into the NCCs) with one of three plasmids, each with a green fluorescent protein reporter for the visualization of the enhancer use. Plasmids are small circles of DNA that contain important sequences (Emmert-Streib et. al., 2014), in this case, enhancers (sequences of DNA that impact the amount a nearby gene will be expressed) important for either cranial or trunk functions will be inserted into these plasmids. Chickens will also be used as a control because they are the closest relative to turtles genetically without the presence of a shell.

Results and Discussion

To visualize whether or not there is evidence of cell reprogramming between the first and second migration of trunk NCCs, plasmids containing relevant Sox10 enhancers (either an enhancer supporting cranial NCC function or trunk NCC function) were transfected into neural tubes from embryos of both the first (G11) and second (G16) migrations. Of these plasmids, one type contained a trunk-specific Sox10 enhancer (Sox10E1) while the other contained a cranial-specific enhancer (Sox10E2). The type of enhancer used by the cell may help to indicate whether the NCCs are acting cranial or trunk-like. To ensure adequate transfection of plasmids into the NCCs, RFP was transfected into a third of the cultured neural tubes as a control. RFP labels nuclei of cells. In the RFP transfected neural crest cells, the nuclei were slightly labeled, but not brilliant. This result may indicate that none of the transfections were complete, impacting the integrity of the results. Between stages G11 and G16 in turtle embryos, there appeared to be very little difference in the fluorescence of GFP. In both cases, the trunk NCCs in the turtle embryos

showed little fluorescence using the trunk enhancer Sox10E1 and visibly brighter fluorescence using the cranial-specific enhancer.

While there was little difference in GFP between the two stages of turtle embryos, there was a visible difference between the fluorescence of GFP in turtles in comparison to chicken embryos. As stated previously, the turtle embryos showed very little fluorescence with Sox10E1 (trunk) inserted and visibly more fluorescence when Sox10E2 (cranial) was inserted. The chicken embryos, however, showed brighter fluorescence with Sox10E1, the trunk enhancer, than with Sox10E2 inserted. In other words, the turtle trunk neural crest cells showed more activity with the insertion of the cranial enhancer in both younger and older embryos, while the chicken trunk neural crest cells showed more activity with the insertion of the trunk enhancer. This would indicate that in chickens, the trunk neural crest cells are acting "trunk-like" or in a manner that would be expected of trunk NCCs. Such a difference between the trunk NCCs of the two organisms supports the idea that those in turtle embryos are acting "cranial-like" in order to form the plastron intramembranously.

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