

# Guidance of Avian Neural Crest Cells by ephrin-B Ligands

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## ABSTRACT

The neural crest is a transitory population in the vertebrate embryo that emerges in the dorsal neural tube and migrates out to give rise to a range of derivatives, including neurons, glia, bone, and cartilage. We are interested in understanding the molecular mechanisms underpinning their migration guidance.

Neural crest cells migrate hundreds of microns in a twenty-four hour period and exhibit interesting cell behaviors during migration on the millisecond, second, minute and hour time scales. In order to understand how events at each of these time scales contributes to the control of migration, we have developed a suite of migration assays capable of presenting potential guidance molecules to the neural crest cells in a spatially and temporally defined manner. By visualizing the resulting cellular responses to the guidance molecules, using time-lapse confocal microscopy, we are able to build up a quantitative picture of the normal response of neural crest cells to environmental signals.

Eph receptors and their ligands, the ephrins, have been implicated in neural crest migration guidance by several laboratories. In an attempt to understand Eph/ephrin signaling at a cell biological level, we have developed a novel migration assay to observe neural crest cells' responses to ephrin-B ligands. We photolithographically immobilize ephrin-B on glass against a background of fibronectin. We then culture primary neural crest cells on these patterns and visualize their membrane and cytoskeletal dynamics as they interact with bound ephrin using a modified confocal microscope. We made several improvements to our confocal microscope that resulted in a ten-fold gain in sensitivity and now allows imaging of single cells for hundreds of

frames without photobleaching. Using this approach, we have established a detailed record of normal responses to ephrin-B1 and ephrin-B2 ligands. To determine the signal transduction requirements for ephrin-B signaling we expose the cells to various pharmaceutical perturbations and compare the resulting behavior against untreated cells. We are using a related assay to explore the effects of transiently applied, spatially defined ephrin stimulus and have observed response times markedly different than the published literature.

To enable us to correlate these in vitro observations with in vivo cell migration, we are mapping the normal neural crest migration pathways in somites 10-20 of the chick trunk using confocal and two-photon microscopy. With this normal map as reference we have begun over-expression studies with ephrin-B1 to enable us to determine whether the in vitro effects of ephrin-B ligands correlate with responses in the intact embryo. This whole embryo work enables us to test our in vitro hypotheses in the context of the full complexity of the chick embryo. We believe that neural crest cell migration is best approached as a multi-timescale, multi-resolution problem and that a true understanding of the control of migration will come when we can iteratively loop through in vitro and in vivo experiments, first formulating, then testing hypotheses at different length and time scales.