Use of Transgenic Quail Lines for the Dynamic Imaging of Craniofacial Development

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Abstract

Dynamic time-lapse imaging of explanted fluorescently labeled transgenic quail embryos has proven to be a powerful tool in understanding the cell movements involved in complex developmental processes such as heart tube looping. This model system should be ideally suited to study both normal craniofacial development and malformation. We currently house both cell type specific transgenic lines (neuronal, endothelial) as well as ubiquitously expressing lines. New lines will include photoactivatable and photoconvertable fluorescent proteins which should prove ideal for highly specific cell fate tracking studies. In addition, we are attempting to adapt the powerful CRE-LOX recombination system to allow for more rapid development of novel transgenic quail lines in the future.

Why Study Avian Development?

•Chicken development is a classic model system. Biologists have accumulated a tremendous amount of knowledge studying this system over many years

•Many of the developmental processes found in birds are highly conserved across species

•Avian embryos can be cultured *ex ovo*, and manipulated *in ovo* giving investigators several tools not available for use on mammalian embryos.

Why Make a Transgenic Avian?

•Embryonic cells which stably express a fluorescent transgene allow for detailed tracking of cell fates during embryogenesis without complicated labeling protocols

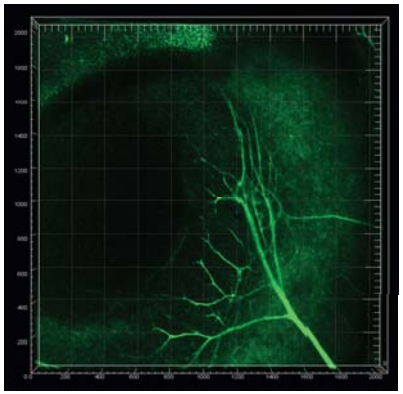
•Tissue-specific expression of a transgene can be used to study the spatial and temporal patterns of these differentiated cells during early development

Why a Transgenic Quail?

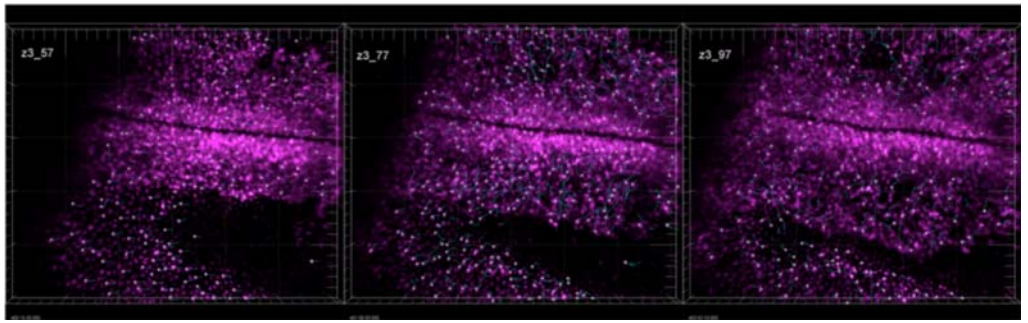
•Japanese quail (*Coturnix japonica*) hatch in 16 days and reach sexual maturity in 10 weeks which shortens the generational time in breeding programs compared to chickens

•Adult quail are small in size (~350g) and can be group housed which leads to cost effective animal husbandry

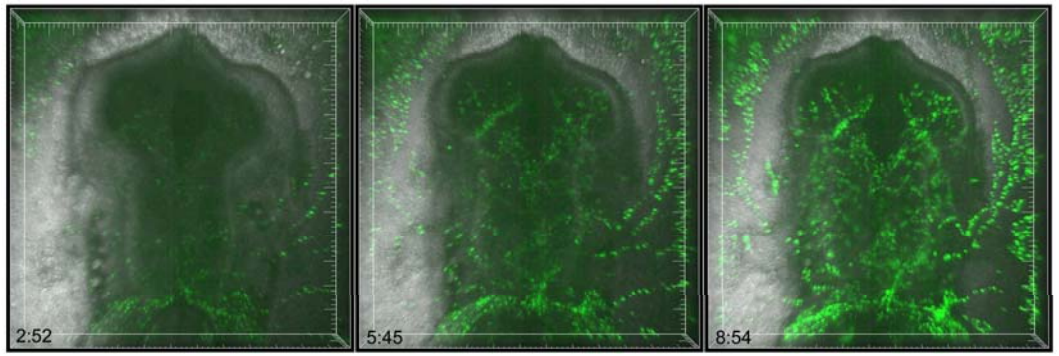
•Quail and chicken are 98% identical genetically allowing for the use of *Gallus* databases



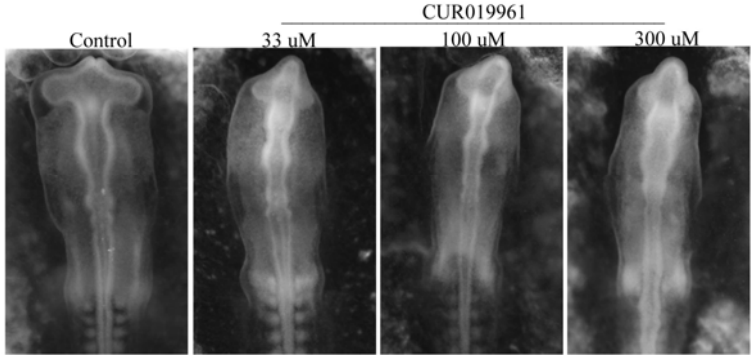
Neuronal processes in the eye of an E4 Tg(*syn*:eGFP) quail embryo. The fine morphology of the neuronal network is apparent in this tiled confocal z-stack with eGFP expression driven by the human Synapsin I promoter. The ganglia and soma of the nervous system can be imaged using the Tg(*syn*:H2B-eGFP) quail line. Image and tile stiching by C. Canaria.



Dynamic imaging and analysis of cephalic neural crest (CNC) cells delaminating from the midbrain level neural tube of an HH10 Tg(*pgk1*:H2B-chFP) quail embryo. Static images taken at section z3 (out of 20 z sections) with the CNC cells trailed by "blue dragon tails" that represent a 2-hour travel history of each cell. Squares=100 mm^2. 4D imaging data of brain development was collected every 5 minutes for 18 hrs and quantitatively analyzed using Imaris cell tracking software. PGK is a ubiquitous promoter driving expression of mCherry in all cells. Images taken by J. Yang.



Dynamic imaging of a HH9-10 Tg(*tie1*:H2B-eYFP) quail embryo. The proliferation and organization of early blood vessels in the head region was imaged in a live Tg(*tie1*:H2B-eYFP) quail embryo for six hours. The embryo was cultured according to Chapman et al. Dev. Dynamics 220: 284-289 (2001) in the heated chamber of a Zeiss 710 laser scanning confocal microscope. The nuclei of differentiated endothelial cells stably express eYFP in this transgenic line. Images taken by C. Canaria.



Increasing concentrations of an inhibitor of the Shh pathway, induces dose-dependent HPE (holoprosencephaly) in developing quail embryos. The use of transgenic quail will allow detailed cell tracking during chemical and genetic perturbation of normal brain development, making them an excellent animal model for the study of craniofacial malformation. (Frank-Kamenetsky et al. J Biol 1:10. 2002. CUR019961 Genentech, Inc.), Images taken by Y. Sato.

mRNA expression pattern of FoxD3 and Msx1 in HH11 quail embryos using HCR (hybridization chain reactions). Probe sets specific for the genes of interest and metastable hairpins labeled with Alexafluor 647 were hybridized to fixed quail embryos and imaged using a Zeiss 510 upright confocal microscope. The area of the embryo containing rhombomere 4 is shown. Quail embryos have been used successfully in the HCR procedure (Choi et al. Nat. Biotech 28: 1208-1212 (2010), which should allow for simultaneous, highly sensitive analysis of multiple RNA targets. Images taken by D. Huss.