Investigating time & cell fate decisions in the development of the avian posterior body

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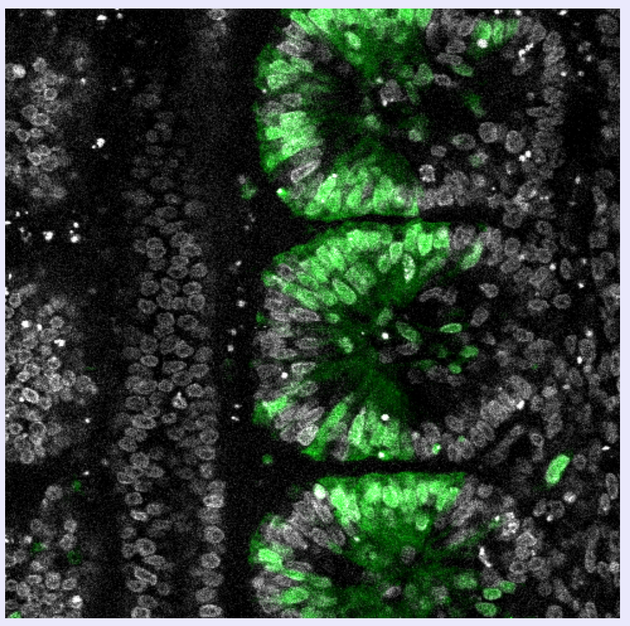
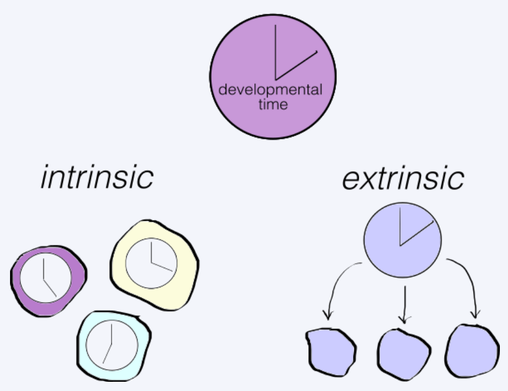
Abstract

The vertebrate posterior body plan is laid down in a sequential manner, with anterior structures being generated before more posterior ones. The pool of cells that contribute to the conserved structures of the anteroposterior axis, including the notochord, somites and neural tube, are termed *axial progenitor cells.* During posterior body development, axial progenitor cells coordinate their cell fate decisions and contributions to the body axis with the overall progression of developmental time. This is necessary for normal morphogenesis. In this project, we will examine the mechanisms underlying how axial progenitor cells "tell the time" during development, in particular focusing on making the distinction between cell-intrinsic and – extrinsic timing mechanisms in controlling *Hox* gene expression.



1. Developmental timer mechanisms may be controlled intrinsically and/ or extrinsically

Previous literature has made the distinction between cell-intrinsic and – extrinsic mechanisms for timing in development[1,2,3,4], with intrinsic mechanisms being those autonomous to a given cell and not impacted by the external environment. Cell-extrinsic timers implicate the importance of the external cellular environment in providing temporal information.



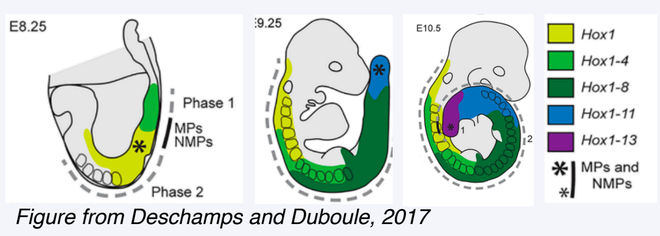
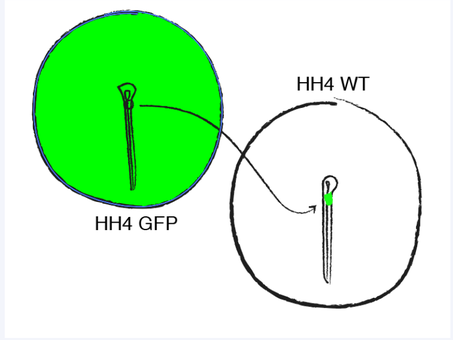


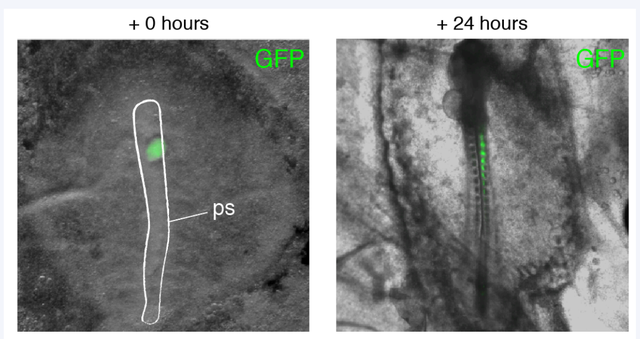
Figure from Deschamps and Duboule, 2017

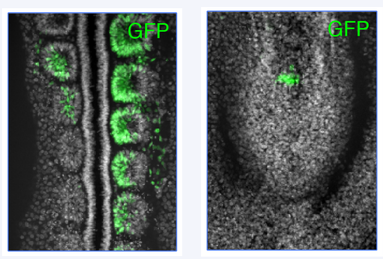
Axial progenitor cells sequentially express *Hox* genes during the elongation of the primary body axis (the Hox Clock[5]), but it is not known how this clock is mechanistically controlled.

2. Grafting allows physical movement of axial progenitor cells between embryonic environments

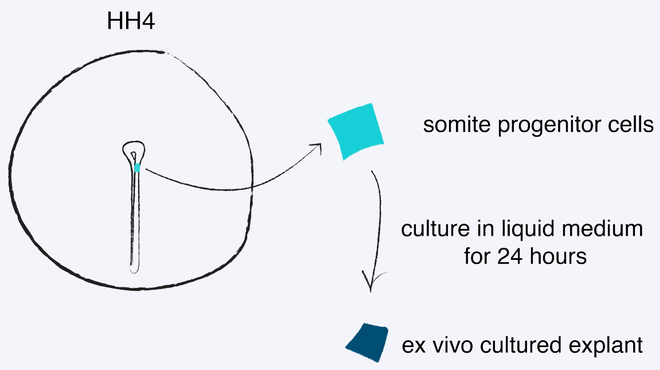
Chickens are a classical model in embryology, being amenable to manipulations such as grafting. Using transgenic GFP chickens produced by the Roslin Institute (Edinburgh),[6] I can isolate donor tissue and graft it into a host embryo.

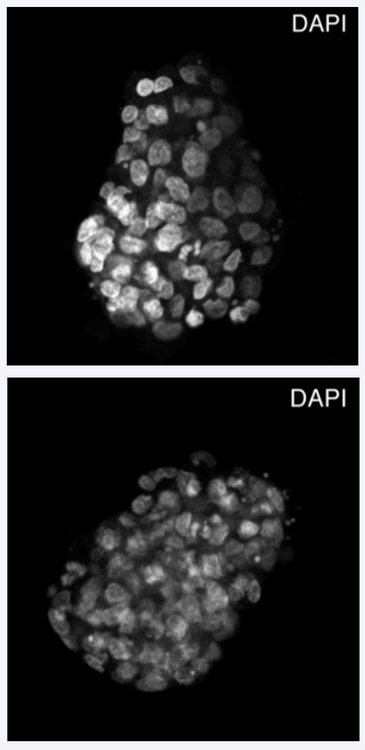






3. Axial progenitor cells may be cultured ex vivo

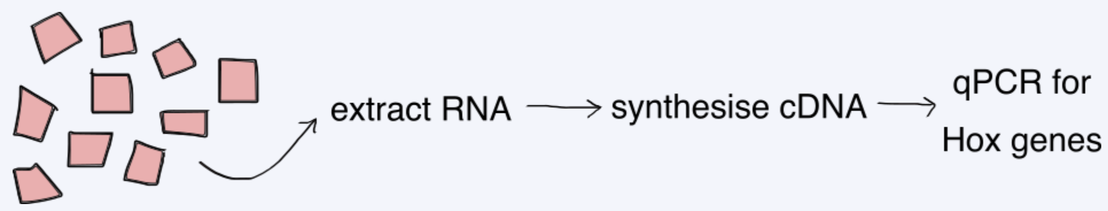


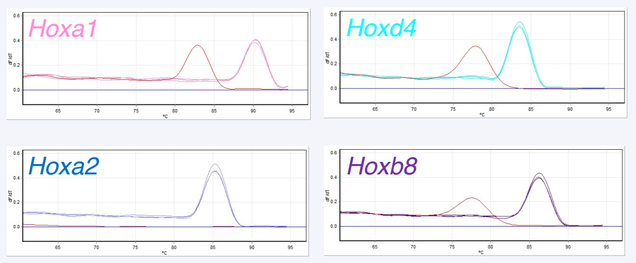


Using a previously-published protocol for epiblast culture[7], regions of the primitive streak can be dissected from HH4 embryos and cultured outside of the embryo for 24 hours.

DAPI-staining shows that cells are healthy after this culture period. These explants will be used to assess whether time progresses in the same way outside of the embryonic environment.

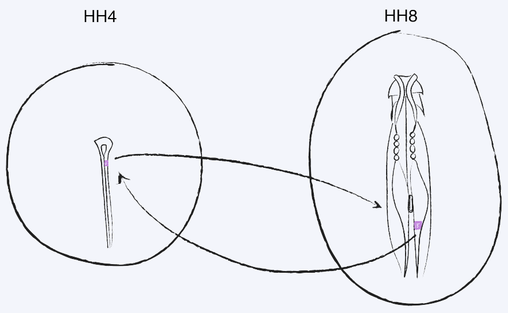
4. Expression of Hox *genes can be examined in explants using RT-qPCR*





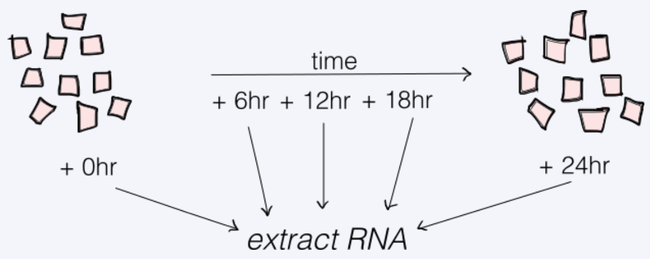
qPCR primers were designed against chicken Hox genes and validated. These primers will be used to assay Hox gene expression in embryonic explants (described in *3).*

5. Experimental plans



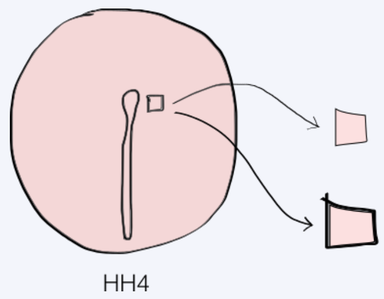
Heterochronic grafts

Forward and reverse (in time) grafts will be performed, to examine whether "time" (assayed through *Hox* gene expression) in donor tissue resets to match the host (extrinsic timing) or is maintained (intrinsic timing).



Explant time series

Hox gene expression profiling will be carried out with explants at various timepoints after culture (and compared with embryonic tissue), to examine 'how time passes' in the explant cells relative to cells within the normal embryonic environment.



Community Effect?

A possible role for Gurdon's Community Effect[10] will be examined using explants and donor tissue for grafts of different sizes, in order to determine whether cell number impacts the outcome of these experiments.

References

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