Proximo-Distal Sorting-out of Mesenchymal Cells in Recombinant Limbs

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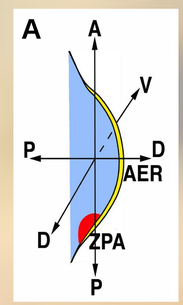
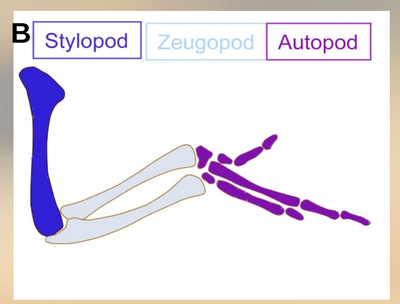
1. Introduction:

The development of the vertebrate limb is one of the premiere systems to study pattern formation and morphogenesis. Emerging limb buds are formed by an external ectodermal hull and a core of mesoderm and display three axes of asymmetry: the proximo-distal (**PD**; shoulder to fingers), the anterior-posterior (**AP**; thumb to little finger) and the dorso-ventral (**DV**; from back of hand to palm) (**A**). Growth and patterning in the PD axis is controlled by the apical ectodermal ridge (**AER**) mainly through the secretion of a battery of fibroblast growth factors (FGFs). The zone of polarizing activity (**ZPA**) controls AP patterning through the production of Sonic Hedgehog (Shh). During limb development the skeletal elements are specified in a proximo-distal manner progressively forming the stylopod (upper arm-thigh) then the zeugopod (forearm-leg) and finally the autopod (hand or foot) (**B**). However, it is not completely known how PD patterning is established and controlled.



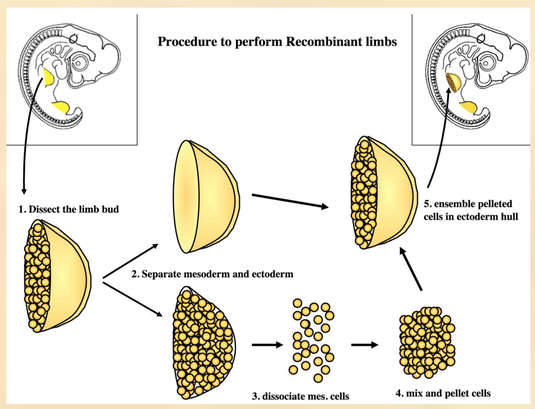


2. Aim of this work

Our goal is to examine whether cells with different proximo-distal origin sort out when they are intermingled in an experimental limb bud situation.

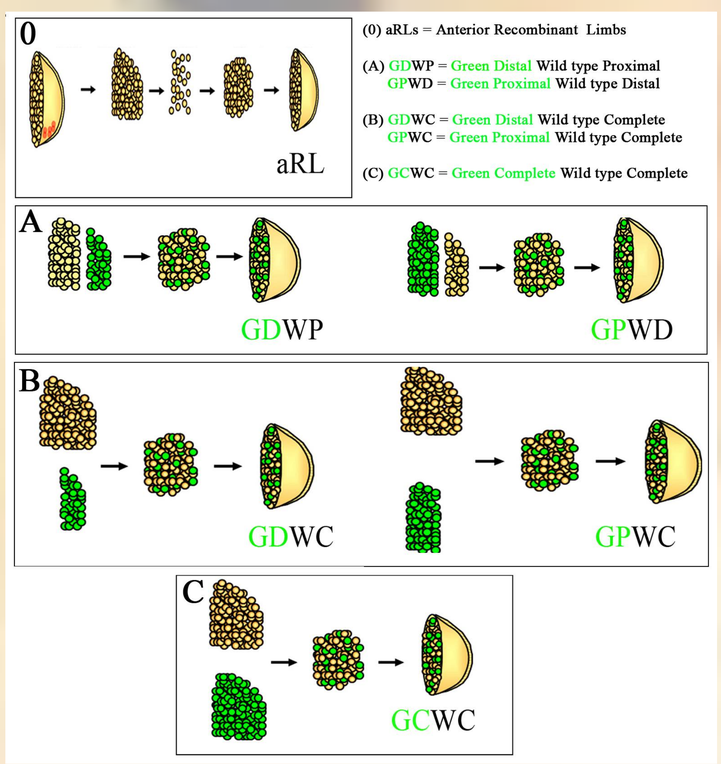
3. Materials and Methods:

3.1 - Recombinant limbs (**RLs).**

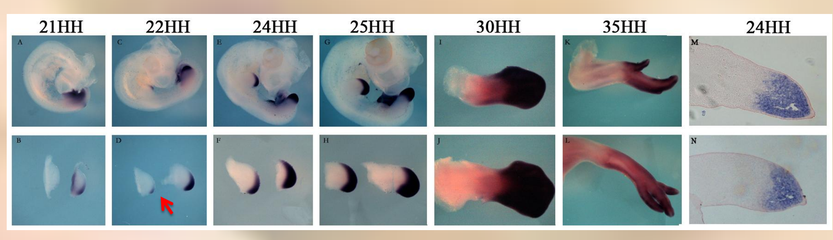


Scheme showing the procedure to perform a RL with mesodermal dissociation

3.2 – Types of RLs used.
The ZPA was never included: anterior RLs.



3.3 – *In situ* hybridization (ISH) in whole mount and in paraffin sections. The expression of several genes involved in limb patterning was analyzed:



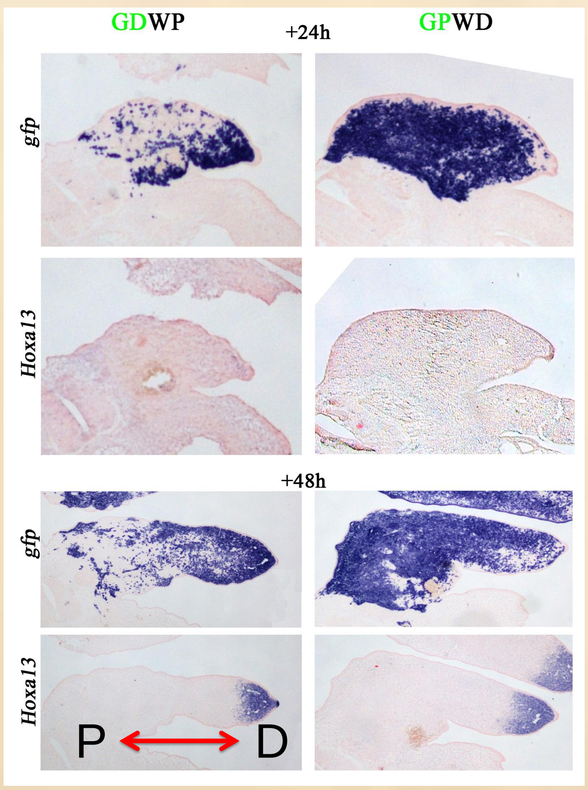
Hoxa13 during limb development. Note that the activation of expression in the wing bud occurs at stage 22HH (arrow). The extension of expression is better appreciated in tissue sections (right panels).

3.4 – *Gfp*-expressing transgenic chickens used for this work

(McGrew et al., 2004)

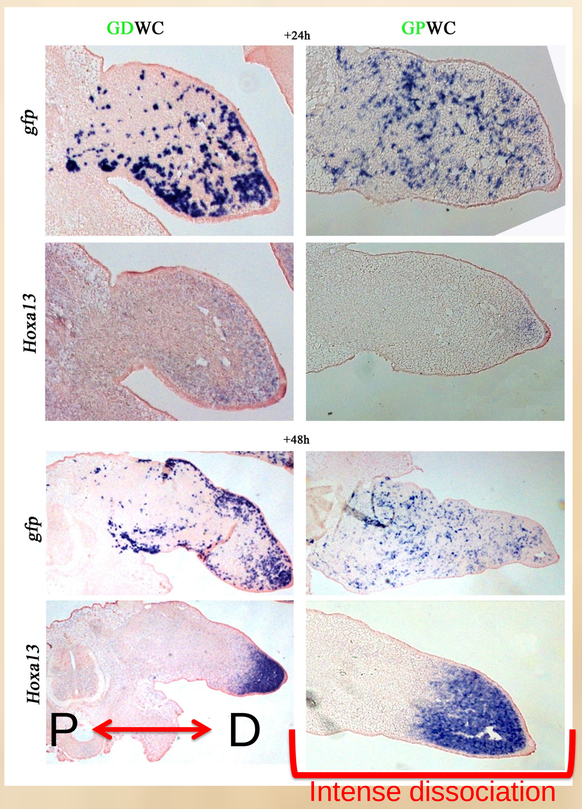
4. Results:

A Proximal and distal cells sort out in the RL



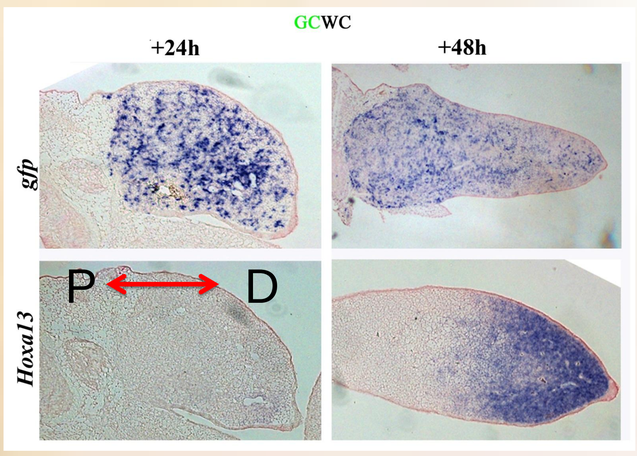
The ISH for *Gfp* revealed segregation of cells with similar PD origin. Furthermore, distal cells moved distally while proximal cells occupied the majority of the PD axis except the subectodermal area. This reorganization occurred within 24h after grafting and was later maintained. Activation of *Hoxa13* was normal disregarding the sorting out.
Proportion of marked cells: 1:3

B The distribution of cells correlates with their PD origin



Identical results were obtained when the marked cells were diluted with WT cells of similar PD origin. However, when the dissociation to perform the RL was profuse, as was here the case for the GPWC RL, cell aggregates were not observed. Note normal activation of *Hoxa13*.
Proportion of marked cells: 1:7

C Uniform mixing of *Gfp*-expressing and WT cells



Note uniform distribution of distal and proximal cells all along the PD axis of the RL indicating that the expression of *Gfp* has no effect *per se*. Note also normal *Hoxa13* expression.
Proportion of marked cells: 1:7

5. Conclusions:

1. ***Gfp*-expressing cells are easily tracked in the RL situation**
2. **In the RL situation, cells sort out according to their PD origin. The sorting out is more evident for distal cells**
3. **The distal relocation of distal cells indicate active cell movements that occur very rapidly after the formation of the RL. They may respond to a positive signal from the AER (FGFs) or to a negative signal from the flank (Retinoic acid).**
4. **It is possible that the level of initial dissociation may influence the subsequent formation of cell aggregates.**
5. **Further experiments are required to determine the molecular basis of these two different behaviors.**
6. **Disregarding sorting-out, PD patterning is normally established in the RL, accordingly to the expression of the autopod marker gene *Hoxa13*.**

6. Acknowledgements:

I am grateful to all members of the Ros' lab for support and helpful discusions. I also thank Mª Félix Bastida and Patricia Saiz for critical reading of this project, Endika Haro and Carlos Garrido-Allepuz for their ideas and Marisa Junco for her every day help. This work has been supported by the Spanish Government Grant BFU2011-24972 to M.A.R. Thanks are also due to the Roslin Institute, University of Edinburg, for providing transgenic animals.

7. References:

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