Development of feather buds in cultured embryonic chick skin

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Many organs are formed through the interaction between two distinct tissue layers: the epithelium, a sheet of cells that are tightly linked together, and the mesenchyme, a more loosely associated group of cells. The cells of the epithelium and mesenchyme are adjacent, but are not directly connected. They communicate with each other through the production of signaling molecules – secreted proteins produced by one cell that bind to receptors on the other. This communication is essential for the proper formation of organs and the differentiation of the specialized cells within them.

The developing chick feather bud presents a good model system in which to study organ formation (Chuong and Widelitz, 1998). The outer, epithelial layer of skin, the epidermis, is derived from the ectoderm. Under the epidermis lies the dermis, which is derived from the trunk somites (Olivera-Martinez et al., 2001) and forms a loosely-packed mesenchyme. The epidermis initially can form either skin or feathers. The underlying dermis produces and secretes signaling molecules, such as FGF10 (Mandler and Neubüser, 2004; Gilbert, 2010). These proteins bind to receptors on the epithelial cells, causing them to divide and differentiate. The epithelium, in turn, produces sonic hedgehog and BMPs through which it signals back to the mesenchyme. The mesenchyme then condenses under the newly formed feather bud (Chuong and Widelitz, 1998). As a result of this molecular dialogue, the feather bud grows out (figure 1).

Feather formation initiates at Hamburger & Hamilton (1951) stage 28 (between days 6 & 7 of incubation). The earliest feather bud placodes form along the dorsal midline, above the neural tube which may provide a source of Wnt proteins (Chuong and Widelitz, 1998; Olivera-Martinez et al., 2001). Feather bud induction then progresses laterally to form evenly-spaced tracts of feathers (figure 2). After initiation, feather bud development can progress in culture to the long bud stage (Chuong and Widelitz, 1998), suggesting that interactions between the dermis and epidermis are sufficient.

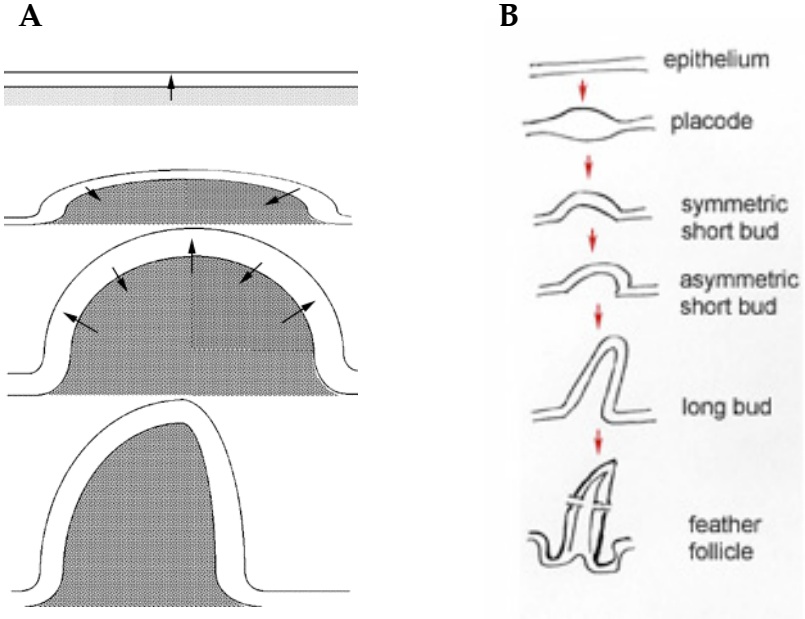

Figure 1. Feather bud formation. **A**. Reciprocal tissue interactions between mesenchyme and ectoderm. **B**. Stages in feather bud formation (from Chuong and Widelitz, 1998).

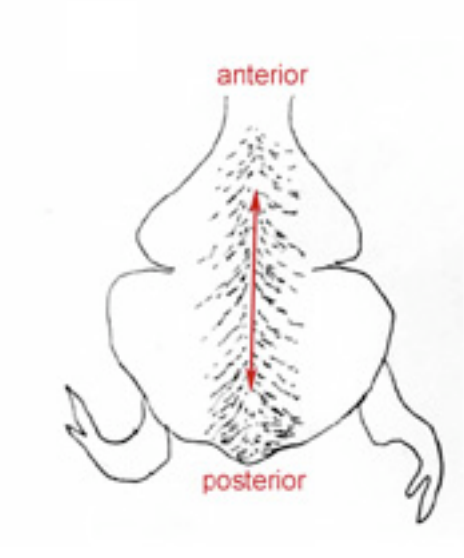

Figure 2. Line drawing diagram of dorsal view of chick body. Arrow highlights location of spinal tract (drawing courtesy of Katy Lewis).

Objectives
Practice microdissection and observe organogenesis *in vitro*. Can be modified to examine the effects of growth factor inhibitors.

Supplies
70% ethanol in squirt bottles
7-day old chick embryos
Dissecting equipment including scissors and fine Dumont forceps
100 mm petri dishes
60 mm petri dishes
Transfer pipets, sterile
Howard's Ringers Solution
Hank's Balanced Salt Solution (optional)
Dulbecco's Modified Eagles Medium (DMEM), 10% fetal calf serum, 50 ug/ml gentamicin
Transwell 3452 (Costar, 24 mm in diameter, 3.0 mm pore size) tissue culture inserts
6-Well tissue culture plate (Costar), optional
CO_2 incubator

Procedure
1. Open blunt end of 7-day chick egg. Dump contents into large (100 mm) petri dish.

2. Transfer embryo to clean small (60 mm) petri dish with sterile Howard's Ringers. Remove head close to shoulders and discard.

3. Use fine forceps like scissors to slice skin along flanks, beginning at the neck (figure 3). Try to only cut through the outer layer (skin). Using fine forceps gently peel back skin. Connect cuts behind hind limbs. Try to remove a large intact piece. (Optional: Store skin explants in Hank's Balanced Salt Solution until culture).

4. Set Transwell tissue culture filter inserts in wells of a six well plate and underlay with 1.8 ml culture media each. (Note: do this in sterile hood!).

5. Cut off the end of a sterile transfer pipet to make a large opening. Suck up explants and transfer to top of filter inserts. Explants of dorsal skin should be cultured with the mesenchyme side down. Looking under dissecting microscope, grab one edge of explant and gently pull over filter until explant is flat with dorsal (ectodermal) side up. Generally, the ectodermal surface looks smoother.

6. Remove any media on top of filters. The explants should be cultured at the air:fluid interface, surrounded by a thin film of media that passes through the filter. Incubate at 37°C in a humidified incubator with 5% CO2.

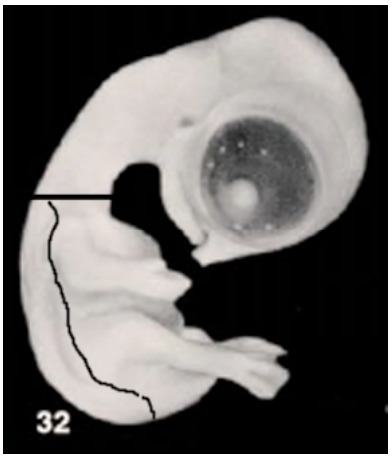

Figure 3. Remove and culture dorsal skin from chick embryo at day 8 of incubation (adapted from Hamburger, V. and H. Hamilton. 1951).

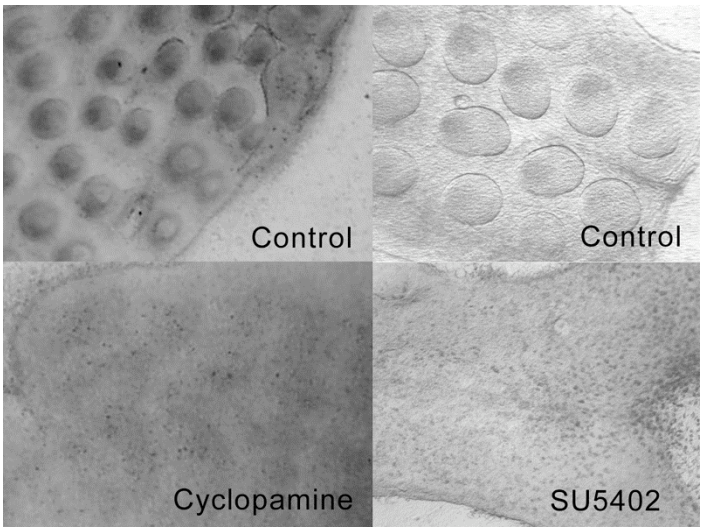

Figure 4. Requirement for SHH and FGF signalling during feather bud formation. Embryonic chicken skin was grown on Transwell filter inserts over tissue culture medium (control) or in the presence of an inhibitor of SHH signalling (cyclopamine) or FGF signalling (SU5402) for 4-6 days.

References

http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Student/katy/chickskinpage.html

Chuong, C.-M. and R. B. Widelitz. 1998. "Feather Morphogenesis: A Model of the Formation of Epithelial Appendages." Molecular Basis of Epithelial Appendage Morphogenesis. R.G. Landes Company. Austin, pp. 57-74.

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Olivera-Martinez, I., J. Thélu, M.-A. Teillet, and D. Dhouailly. 2001. Dorsal dermis development depends on a signal from the dorsal tube, which can be substituted by Wnt-1. *Mech. Dev*. 100: 233-244.

Instructor's notes

1. Fine forceps (e.g. Dumont #5) are very valuable for skin microdissection.
2. Tissue culture supplies are needed which make this lab more expensive. Many reagents shared with chick heart culture lab.
3. Additional 6 well plates allow you to transfer only the Transwell inserts needed (usually 1 per group which will hold 3-4 skin explants each).
4. Pen/strep could be substituted for gentamicin sulfate.

Order information

DMEM (Fisher MT-17-204-CI, 6 x 100 ml, $76)

Fetal calf serum (Fisher BW14507E, 100 ml, $89)

Costar Transwell Clear, 3 μm pore, 24 mm (Fisher 07-200-171, case of 24, $134.52)

Costar 6 well plates (Fisher 07-200-83, case of 50, $145)

Gentamicin sulfate, 10 mg/ml (Fisher 50841714, 10 ml $32; Teknova G3625, 10 ml, $18) or 50 mg/ml (Invitrogen, 15750060, 10 ml, $48.50)

Howard Ringer's solution

14.4 g	NaCl
0.34g	$CaCl_2$ (anhyd.) or 0.46g $CaCl_2.2H_2O$
0.74g	KCl
dH_2O to 2 liter	