Development of the Heart Field in the Axolotl Embryo
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Background

Embryogenesis is a broad term used to describe the stages of development between fertilization, the fusing of gametes, and hatching, the birth of an organism into the world (Gilbert, 25-26). In most animals, these stages include cleavage, gastrulation, organogenesis, and gametogenesis. In many species there is a larval period in the life cycle that follows hatching and is required for the young organism to reach sexual maturity. Organogenesis is the period following gastrulation where the three germ layers are further organized into tissues and organs. This process often requires the rearrangement and interaction of disparate and distant cells. These cells often must migrate long distances from their original positions to their final locations (Gilbert, 25-26). Such is the case for the Axolotl heart.

The heart is the first functional organ in the amniote vertebrate. Presumptive heart cells migrate through the primitive streak and form two groups of cells at the same level as Hensen's node. The cardiogenic mesoderm layer is specified by the adjacent endoderm through the BMP and FGF signaling pathways. These signals direct the two groups of cells to differentiate separately while they migrate anteriorly toward the gut tube. The two tubes of endocardia fuse when they are brought together by the infolding of a superficial layer called the splanchnic mesoderm. (Gilbert, 492-494)

The Axolotl embryos used in this experiment will be stage 15 of development. Embryos in this stage have gone through cleavage, the mid-blastula transition, and begun gastrulating. They have a neural plate and the beginning formation of neural crests. However these have not begun to invaginate or fold toward each other. (Professor Judy Cebra-Thomas, personal communication, February 25, 2004)

Regulative development generally occurs in early gastrulation that lead to the conditional specification of a cell's fate. A cell undergoing regulative development can be transplanted to another part of the embryo and form whatever structure belongs in that

area instead of the structure that it would have originally formed because it is competent to receive the different signals from the new cells around it.

The second mode of development is mosaic development. Mosaic development results from the autonomous specification of a cell's fate. These cells, instead of depending on cell-cell interactions, are determined by cytoplasmic factors contained within the cell itself. These cells will form a given structure even if they are moved to a new location and are exposed to cell-cell interactions and signals that differ from their original position. Most organisms contain different types of tissues, each of which may undergo one of these developmental mechanisms at a given time (Gilbert, 2003).

All embryos undergo both Mosaic and Regulative development at some point in their growth. Regulative development generally occurs in early gastrulation when cells are induced to form different structures according to their location in the embryo. This kind of development is dependant on cell-cell interactions. Mosaic development occurs when cytoplasmic factors within a cell induce it to become a certain structure. Cells undergoing mosaic development will form a given structure even if they are moved to a new location and are exposed to different cell interactions. This lab will investigate whether the heart undergoes mosaic or regulative development by bisecting the heart primordial field of axolotl embryos with non-heart tissue donated by older embryos and observing how the bisected embryos grow. If the heart undergoes mosaic development, we would expect that the cells will develop normally even if the two sides are not in physical contact with one another. This will lead to half a heart growing on either side of the non-heart tissue graft. However, if instead the heart undergoes regulative development we would expect that the cells on the two sides will "sense" the absence of the other cells and compensate for them. This will lead to the development of two distinct hearts. (Gilbert, 319)

The objective of this lab is to determine whether the heart primordial cells of a stage 15 axolotl embryo undergo mosaic or conditional development.

Materials

- stage 15 and 20 *Ambystoma mexicanum* axolotl embryos
- Hair loop embryo holder
- Eyebrow microscalpel
- Tungsten microscalpel
- 1% Agar-coated operating dish
- Small glass stirring rod
- 100%, 50, and 20% HEPES-Buffered Steinberg's Solution (HBSt)
- 70% Ethanol
- Dissecting Microscope
- Still and Video Camera

Procedure

1. Preparation of microtools

   Hair loops: Insert both ends of a hair into 5 mL glass capillary tube and push through until loop is 1-1.5mm in width. Hair loop will used to hold embryos still, so compare to embryos to ensure appropriate size. It is a good idea to make hair loops in a range of sizes. Reinforce loop by immersing in wax. Remove excess wax by blotting onto Kimwipe on hot plate. CAUTION: Do not burn yourself!

   Eyebrow knives: Embed an eyebrow hair in a 5 mL glass capillary and seal with wax as above.

   Tool Sterilization: Rinse all tools with 70% Ethanol followed by sterile HBSt before use.

   Operating dish: Heat small rod or the tip of a Pasteur pipette in flame until tip is rounded and press on surface of agar to form embryo-sized wells

2. Manually dejelly embryo in Steinberg's solution. Use fine forceps to break a hole in the gooey jelly coat as shown on video. Embryo can then be maneuvered out.
3. Transfer embryos into well of operating dish containing increasing concentrations of HBSt and antibiotics. Remove half the solution with pipetteman and fill with 100% HBSt. After a minute remove half the solution and fill with 100% HBSt. Allow embryo to sit 2 minutes in high salt solution. This will cause the vitelline membrane to swell and make it easier to see and remove as shown in video. Demembranate embryo in 100% HBSt by carefully grabbing near neural crest cells with both tweezers and pulling to form a hole as described in the dejellying step above.

4. Position donor embryo on lateral side and recipient embryo on dorsal side (neural crest) exposing ventral side in separate wells of operating dish.
5. Hold donor embryo with hair loop instrument and use Tungsten microscalpel to remove small chunk of tissue from lateral, gill-forming surface.
   CAUTION: Do not remove a chunk of neural crest cells. These have the tendency to naturally migrate to the exterior of the gastrula and will therefore not heal well in the recipient embryo.
6. Hold recipient embryo ventral side up with hair loop and pick a small slit down the midline of the embryo with eyebrow microscalpel or tungsten microscalpel.
   CAUTION: Be gentler than when cutting the donor gill chunk. Do not slice through the entire embryo and do not cause inner cells to spew out of embryo!
5. Imbed donor gill-forming piece in the incision on the recipient embryo. Pat down and ensure its position with eyebrow or tungsten microscalpel.
6. Leave one recipient embryo with only the incision but no graft, and one embryo uncut as controls.
7. Observe the development of the embryos for 7 days.
8. While the high Ca^{2+} concentration of the HBSt enhances healing by saturating the Ca^{2+} binding sites of cadherin molecules, a high salt concentration causes developmental abnormalities. Therefore, after embryos heal from surgery, gently replace 100% HBSt with 50%, then 20% HBSt. Change solution concentrations after 12 hrs.
9. Collect pictures and movies daily using a microscope equipped with a camera and recording cameras.

Literature Cited

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