Video Article

Placing Growth Factor-Coated Beads on Early Stage Chicken Embryos

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Abstract

The neural tube expresses many proteins in specific spatiotemporal patterns during development. These proteins have been shown to be critical for cell fate determination, cell migration, and formation of neural circuits. Neuronal induction and patterning involve bone morphogenetic protein (BMP), sonic hedgehog (SHH), fibroblast growth factor (FGF), among others. In particular, the expression pattern of Fgf8 is in close proximity to regions expressing BMP4 and SHH. This expression pattern is consistent with developmental interactions that facilitate patterning in the telencephalon.

Here we provide a visual demonstration of a method in which an in ovo preparation can be used to test the effects of Fgfs in the formation of the forebrain. Beads are coated with protein and placed in the developing neural tube to provide sustained exposure. Because the procedure uses small, carefully placed beads, it is minimally invasive and allows several beads to be placed within a single neural tube. Moreover, the method allows for continued development so that embryos can be analyzed at a more mature stage to detect changes in anatomy and in neural patterning. This simple but useful protocol allows for real time imaging. It provides a means to make spatially and temporally limited changes to endogenous protein levels.

Video Link

The video component of this article can be found at http://www.jove.com/video/307/

Protocol

1. Remove eggs from the incubator

At E2-2.5 (~ St. 17 HH), remove the eggs and prepare them accordingly. They can be worked on immediately or returned to the incubator for a couple hours. The eggs can be placed at room temperature safely for over an hour. Eggs can stay out for the duration of the manipulations. Sometimes these can take an hour or more.

Note: The longer they sit at room temperature, the less likely they are to survive.

2. Preparation of India ink

1. Prepare a fresh solution of 4% India Ink in sterile PBS.
2. Fill a 3 mL syringe with the solution.
3. Place a 27 gauge, ½ inch needle on the syringe.
4. Using a pair of hemostats, bend the needle to a 90° angle.

3. Opening the eggs and injection of India ink

1. Using a pair of forceps, peel back the tape and open the window, securing it by using the reverse side of the tape on the back side of the egg.
2. Guide in the angled needle until it lies just beneath the embryo. Placing the needle under the floating disk insures that the ink, which is toxic to the embryo, will not come in direct contact.
3. You need to only inject enough ink to create a comfortable contrast. This is typically less than ¼ mL.

4. Opening the neural tube

1. Take a pair of fine dissection scissors and cut the thin membrane that lies on top, or sometimes around, the embryo.
2. Take a pair of 55 forceps or a tungsten probe and open the neural tube from posterior to anterior. Make sure the opening is large enough to place a bead into it.

Note: Depending on the age, a fine tungsten probe can be used to do the same thing.

5. Retrieving a coated heparin-acrylic bead

1. Using a pair of 55 forceps, find an intact bead in the solution. Let the bead attach itself to the tip, slowly close the forceps.
2. Remove the bead from the solution and bring it over on top of the egg.
3. Once the bead is out of its solution, it will not likely come off until it has been placed in the albumin.

6. Placing the bead

1. Once the bead has been placed on top of the target area, use a tungsten probe to guide it towards the forebrain or fix it in the hindbrain.
2. Add a few drops of PBS using a 10 µL pipette.

7. Closing the egg

1. Close the egg using the thin piece of tape to shut the window.
2. Seal the egg according to the prepping protocol, available at http://jove.com/index/Details.stp?ID=306.

Discussion

Depending on the protein you wish to use, there are different protocols on how to prepare the beads. In these experiments, we use heparin-acrylic beads, but affi-gel beads can also be used. We have found that the heparin-acrylic beads are easier to manipulate in the aqueous environment of the egg albumin. Beads are soaked in 5 l of protein overnight at 4°C. Control beads are placed in sterile PBS until the time of placement. The advantage in using an in ovo preparation is that the embryo is still alive and free to develop normally [1]. Although the environment in which an explant is kept can be controlled for particular variables, the live embryo allows for an accurate representation of the affect of a protein on developmental patterns [2-3]. All assays that you can perform on an explant, you can with an embryo.

Disclosures

The authors have nothing to disclose.

References

1. Korn, M.J., and Cramer, K.S. Windowing Chicken Eggs for Developmental Studies (10/01/2007) Jounal of Visualized Experiments, 8, http://www.jove.com/index/Details.stp?ID=306

2. Crossley, P. H., Martinez, S., Ohkubo, Y. and Rubenstein, J. L. Coordinate expression of Fgf8, Otx2, Bmp4, and Shh in the rostral prosencephalon during development of the telencephalic and optic vesicles. Neuroscience 108, 183-206 (2001).

3. Alexandre, P., Bachy, I., Marcou, M. and Wassef, M. Positive and negative regulations by FGF8 contribute to midbrain roof plate developmental plasticity. Development 133, 2905-13 (2006).

