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Spemann–Mangold Grafts

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Abstract

In 1924, Hans Spemann and Hilde Mangold (née Pröscholdt) published their famous work describing the transplantation of dorsal blastopore lip of one newt gastrula embryo onto the ventral side of a host embryo at the same stage. They performed these grafts using two newt species with different pigmentation (*Triturus taeniatus* and *Triturus cristatus*) to follow the fate of the grafted tissue. These experiments resulted in the development of conjoined twins attached through their belly. Because of the difference in embryo pigmentation between the two *Triturus* species, they determined that the bulk of the secondary embryo arose from the host embryo while the grafted tissue per se gave increase to the notochord and a few somitic cells. This meant that the dorsal blastopore lip was able to organize an almost complete embryo out of ventral tissue. The dorsal blastopore lip is now called the Spemann–Mangold organizer. Here, we describe a simple yet efficient protocol to perform these grafts using the anuran *Xenopus laevis*.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Ethanol (70%)

H₂O (reverse osmosis [RODI] or distilled [dH₂O])

Modified Barth's Saline (MBS) (1×) <R>

For grafting medium, use 1× MBS containing 50 µg/mL of gentamycin. For recovery medium, use 0.1× MBS containing 50 µg/mL of gentamycin.

Xenopus laevis embryos (stage 10 or 10+, dejellied)

Dejellied embryos are staged according to Nieuwkoop and Faber (1967); see Step 3.

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Equipment

Bridges

Cut coverslips into rectangles (3-mm × 1-cm) using a diamond pen.

Dissecting microscope equipped with a gooseneck lighting system

Eyelash knife

Select a human eyelash with the desired thickness and curvature (Fig. 1A) and thread it through a 23-gauge needle fitted on a 1-cc syringe. For safety purposes, the tip of the needle can be cutoff with scissors before the threading. Fix the eyelash with a drop of nail polish or cyanoacrylate glue.

Forceps (blunt, to manipulate bridges)

Forceps (fine, to remove vitelline envelope)

Glass bead tool

Thin out the end of a Pasteur pipette under a benzene burner and melt the end into a ball roughly the size of gastrula-stage embryo (about 2 mm).

Hair loop

Cut a human hair into 3-inch sections. Thread both ends of a section into a 23-gauge needle fitted on a 1-cc syringe. Push the loop into the needle until the desired stiffness is reached (Fig. 1A). Fix the hair with a drop of nail polish or cyanoacrylate glue.

Petri dishes (60-mm, plastic), coated with a 4-mm layer of 1% agarose

Petri dishes (60-mm, plastic), coated with plasticine

Roll 2 tsp of plasticine (nondrying, toxin free, appropriate for young children) into a ball. Flatten it out into a plastic Petri dish by hand.

Transfer pipette (disposable plastic or glass) with an opening of 2 mm, for transferring embryos

METHOD

The discovery of the Spemann–Mangold organizer (Spemann and Mangold 1924, 2001) earned Hans Spemann the Nobel Prize in Medicine in 1935. Spemann–Mangold grafts are still performed for research purposes (De Robertis 2006; Inui et al. 2012). They also are an incredible teaching experience for students discovering the fundamental principles of developmental biology.

The grafting procedure should be conducted between 15°C and 19°C.

1. Sterilize a plasticine-coated dish with 70% ethanol for 10 min. Rinse for 30 sec with RODI H₂O and then fill with grafting medium.

2. Using the glass bead tool, make two depressions in the plasticine as deep as one-third or one-half the diameter of an embryo (or about 1-mm).
3. Select a donor and a host gastrula between stage 10 (the forming blastopore lip appears as a dotted line with a light gray color) and 10+ (the blastopore lip is now a line of a dark gray color) and transfer the embryos to the plasticine-coated dish. Remove the vitelline envelope from each embryo using the fine forceps. Ensure that the embryos never breach the liquid surface once the vitelline envelope is removed.

See Troubleshooting.

4. Move the donor and host embryos into the plasticine depressions and turn the embryos on their animal (pigmented) sides.

This will ensure that the blastopore lip and dorsal marginal zone (the area between the lip and the pigmentation edge) are clearly visible (Fig. 1B).

5. On the donor embryo, insert the tip of the eyebrow knife at the commissure (corner) of the blastopore lip and thread it all the way to the animal pole. Cut the tissue by pressing the hair loop along the length of the eyelash knife. Repeat the process from the other commissure (Fig. 1B). Cut perpendicular to the first two cuts to free the pigmented edge of the explant (Fig. 1C) and peel the piece of tissue toward the vegetal pole (Fig. 1D).

The peeled tissue is made of superficial pigmented ectoderm with underlying deep mesoderm (Fig. 1D, orange tissue) and some endoderm.

6. To free the explant from the embryo, insert the knife under the mesoderm and cut perpendicularly (Fig. 1D). Lay the explant with its superficial side down (Fig. 1E).

The freed explant now contains ectoderm (thin, long and pigmented tissue), head mesoderm (Fig. 1D–F, orange tissue) and the involuting bottle cells (Fig. 1F, purple) that created the blastopore lip. Some endoderm cells (much larger cells than the other tissues) may also be present.

7. Trim the explant so that it contains the head mesoderm and the bottle cells.
 - i. To trim the ectoderm, slide the length of the eyebrow knife under the dorsal side of the head mesoderm mass (i.e., cleft of Brachet) and press firmly down until the tissue is separated.
 - ii. To trim the mass of endoderm cells, insert the knife between the head mesoderm and endoderm and press firmly down until the tissue is separated (Fig. 1E,F).

8. On the host embryo, cut out a piece of tissue roughly the size of the newly trimmed explant from the ventral side of the embryo using the same cutting techniques described in Steps 5–6 (Fig. 1G).

Ideally, the vegetal-most incision should coincide with the outline of the future blastopore that will form once the embryo reaches stage 10.5 or 11 (Fig. 1G, blue line). The blastocoel cavity should be seen on the equatorial side of the incision (Fig. 1H).

9. Graft the explant obtained in Step 7 into the cavity created in Step 8, ensuring that the bottle cells are aligned with the projected line of the blastopore and respecting the superficial/deep orientation of the explant (Fig. 1I).

See Troubleshooting.

10. Using the hair loop, gently move the grafted embryo within the plasticine depression so that the grafted blastopore lip is located above the plasticine line (Fig. 1J, top). Using blunt forceps or a pipette tip, dig up some plasticine to form a fulcrum close to the graft (Fig. 1J, top).

11. Using blunt forceps, put a coverglass bridge on the fulcrum such that two-thirds of the bridge's length is behind the plasticine fulcrum and the other one-third covers the grafted area (Fig. 1J, middle).

12. Using blunt forceps, push gently down on the part of bridge that contacts the fulcrum. Stop pushing once the bridge touches the graft and slightly flattens it (Fig. 1J, bottom).

13. Allow the epidermal ectoderm to heal for 20 min.

See Troubleshooting.

14. Remove the bridge with the blunt forceps and transfer the grafted embryo into an agarose-coated dish filled with recovery medium. Grow the grafted embryo between 15°C and 18°C.

Secondary axes are visible as soon as the early neurula stage (the day after the graft).

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 3): The plasticine is sticky and wounds the embryo once the vitelline envelope is removed.

Solution: This problem will occur if the plasticine is new, but will disappear after a few uses. Until then, 1% BSA (w/v) can be added to the grafting medium to coat the surface of the plasticine. Alternatively, dishes coated with agarose can be used. When bridges need to be applied, a fulcrum made of silicon grease can be applied directly onto the bridge. It is best to fill a 5- to 10-mL syringe with the silicon grease and fit the syringe with a P200 micropipette tip for easier application.

Problem (Step 9): The embryos develop too fast and the experimenter does not have time to perform the grafts.

Solution: Keep the embryos, media and plasticine for at least a few hours at 15°C before grafting is scheduled. Perform the grafts on a cooling table set up at 15°C if available.

Problem (Step 13): The host embryo disaggregates shortly after the graft is completed.

Solution: The medium or the type of plasticine used could be the culprit. Make fresh media, add gentamicin and avoid using HCl to adjust the pH (as excess Cl⁻ ions may affect the healing of the explant). Make sure the plasticine used is nondrying and adequate for young children. Plasticine commonly found in art supplies stores contains chemicals that will kill the embryos.

Problem (Step 14): The secondary axis is incomplete (lacks the head).

Solution: *Xenopus laevis* develops faster than the urodele embryos used by Spemann and Mangold. Consequently, the window of time available to perform good quality Spemann–Mangold grafts in *Xenopus* is quite narrow (1 to 1.5 h, depending on the temperature). It is worthwhile to select embryos at stage 10 so that by the time the embryos are prepared for surgery, they are at stage 10+. Operating at the lower range of temperature (15°C) slows development and gives more time to the experimenter. Spemann–Mangold grafts can be performed at stage 10.5 or even later, but will result in incomplete twinning (a secondary embryo lacking the anterior-most structures such as the head).

RECIPE

Modified Barth's Saline (MBS) (1×)

CaCl ₂	0.41 mM
CaNO ₃	0.3 mM
HEPES-NaOH	15 mM
KCl	1 mM
MgSO ₄	0.82 mM
NaCl	88 mM
NaHCO ₃	2.4 mM

Adjust pH to 7.6. Store at room temperature for up to 1 mo.

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FIGURE 1.

Key tools and steps for transplantation of the Spemann–Mangold organizer. (A) Eyelash knife (*left*) and hair loop (*right*). (B–J) Diagram representing the key steps of the grafting procedure.