

# Introduction

Jennifer Knight

Experiencing the process of scientific discovery is part of training to be a scientist. This book of laboratory exercises is designed to give students an opportunity to explore and carry out experiments that have each made significant contributions to the fields of Experimental Embryology and Developmental Biology over the past 100 years. It is our hope that students will experience the initial thrill of discovery as they learn how to do each experiment, analyze each outcome, and grasp the significance of each conclusion. However, science is not solely about the end discovery but also about the process. This process cannot be appreciated by reading textbooks or scientific journals alone. Rather, a budding scientist must experience firsthand the myriad pitfalls of each experiment. Despite the way this laboratory manual is designed (with step-by-step instructions to accomplish each experiment), students will encounter unforeseen problems in carrying out the experiments. If they are not already intimately familiar with experimental science, students will undoubtedly discover that this process demands a meticulous approach. Designing, setting up, and executing experiments cannot be accomplished in a haphazard way. For this reason, every student must keep a laboratory notebook, a task that many initially regard as “busy work.” In fact, keeping careful record of everything one does in the laboratory is the only way to experience success. At the other end of this process is presenting a finished piece of work to the scientific community. Again, the only way to learn this aspect is to assemble data into a mock scientific “paper,” ready for publication in a journal. If possible, verbally presenting the data to an audience is also a valuable learning experience. Below, we give some suggestions for these two important aspects of scientific discovery: keeping a laboratory notebook and writing a laboratory report.

## KEEPING A LABORATORY NOTEBOOK

A laboratory notebook is a day-to-day record of plans, procedures, results and interpretations. When a scientist refers back to his/her notebook, the notes on procedures, pitfalls and outcomes should help him/her to easily repeat the experiment. In the scientific

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Excerpt

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community, a notebook is essential both for demonstrating integrity and for helping to keep track of each step of an experiment. In a student laboratory setting, a notebook can be just as useful, provided the student takes the time to make it so. A notebook must be bound, and the first two pages should be reserved for a Table of Contents that can be filled out as experiments are completed. When students begin an experiment, they should write down their thoughts on the experiments, questions they may have, and finally, their objective and hypothesis. Next, as students perform an experiment, the actual steps should be recorded, although often this can be done in an outline format rather than in great detail, since the procedures have already been described in the lab manual. It is most important that students make note of problems encountered during the experiment, or of deviations taken from the laboratory manual. These notes will help with trouble shooting if part of the experiment does not succeed. Next, students should record all of their observations and all of their data, both raw and calculated (graphs, tables, etc.). Finally, when the experiment is complete, the students should summarize their results, conclusions, and interpretations in the notebook, before moving on to the next experiment. It is essential that students realize that a laboratory notebook is a work in progress. It is only useful if it is used during the experiment to record the process.

### WRITING A LABORATORY REPORT

A laboratory report should follow the standard format for a scientific paper, described below.

- Abstract: a 4–6 sentence summary of the entire paper, including a brief statement of the hypothesis, the methods used, the outcome, and the relevance of the experiment.
- Introduction: a well-researched description of the topic addressed by the experiment. The introduction gives the reader the context of the experiment. This section should also restate the hypothesis and describe the predictions and goals of the experiment.
- Materials and Methods: a detailed section in which reagents and protocols are clearly described. Often, in a classroom setting, since these details are provided to students in the lab manual, instructors suggest a summary of the materials and methods used. It is still important to write in complete sentences and to accurately state how the experiment was carried out.
- Results: a description of the outcome of the experiments. This section of the paper includes only a description of the data and their presentation – figures, tables, and graphs – but does not discuss the interpretation of the findings.
- Discussion: an interpretation of the experimental data and how it compares to published information about this topic. In this section, students should discuss what their results mean, the implications or significance of these results, and how they might expand or clarify the results. Ultimately, it is important that students put their experiment into the context of other work on this topic.
- References: a detailed citation of each journal article used in writing the paper. There are many different possible formats for references. Students may choose a specific

format by consulting a journal and using that standard format for each article referenced (instructions for authors, often found at the beginning of journals, usually include instructions for referencing other journal articles).

By following the suggestions above, we hope that as instructors and students alike perform the experiments presented in this book, you will find yourselves engaged in and enticed by this exploration of Developmental Biology.

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## SECTION I. GRAFTINGS

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### 1 Two developmental gradients control head formation in hydra

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H. R. Bode

**OBJECTIVE OF THE EXPERIMENT** Two developmental gradients are involved in the axial patterning of the head and the body column of a hydra. One is a morphogenetic gradient of head activation [= head formation capacity], and the other is a gradient of head inhibition. The objective of the experiment is to demonstrate the presence of these two gradients in the body column of adult hydra using transplantation experiments.

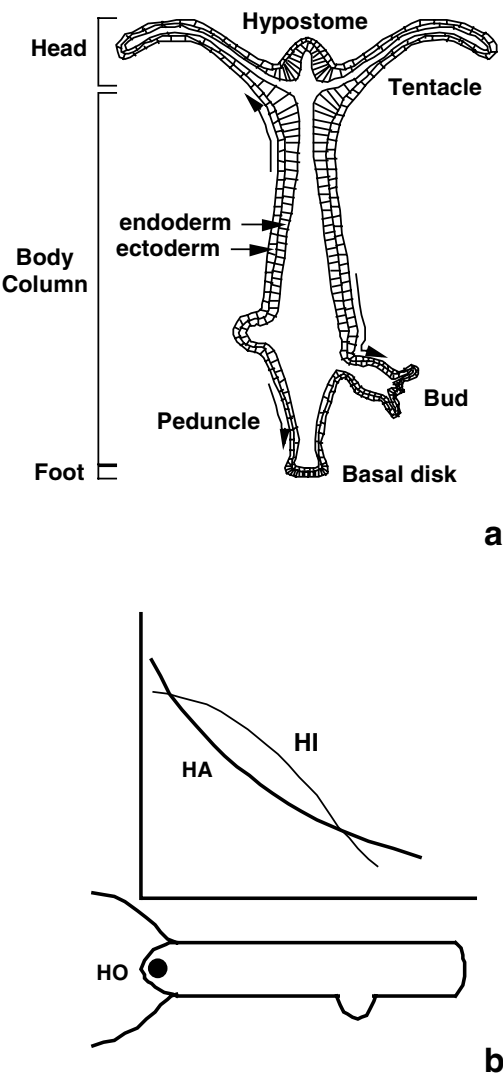
**DEGREE OF DIFFICULTY** The experiments involve the isolation of a piece of the body column and transplantation to the body column of a second animal. Although this appears difficult at first sight, with a little practice, almost all students learn to carry out these grafts at the rate of 6–10 successful grafts/hour.

#### INTRODUCTION

In animals, the developmental processes associated with axial patterning occur during early stages of embryogenesis. One example involves the processes governing head formation at the anterior end and tail formation at the posterior end of the anterior–posterior axis. In hydra, a primitive metazoan, this type of axial patterning occurs not only during embryogenesis, but also in the adult. This is due to the tissue dynamics of an adult hydra.

As shown in Figure 1.1a, a hydra has the shape of a cylindrical shell. Along the single axis are the head, body column and foot. The head at the apical end consists of a mouth region, the hypostome, and beneath that the tentacle zone, from which tentacles emerge. The protrusions on the lower part of the body column are early [left] and advanced [right] stage buds, hydra’s mode of asexual reproduction. The wall of the shell is composed of two epithelial layers, the ectoderm and endoderm, which extend throughout the animal. Among the epithelial cells (not shown in Figure 1.1a) are smaller cells such as neurons, secretory cells and nematocytes, the stinging cells of cnidaria.

The tissue dynamics is the following. The epithelial cells of both layers are continuously in the mitotic cycle (e.g. Bode, 1996). Yet, despite the ever-increasing number of



**Figure 1.1.** (a) Cross section of an adult hydra showing the three regions and the two tissue layers as well as two stages of bud formation. (b) Diagram of the developmental elements that control head formation. HO = head organizer; HA = head activation gradient; HI = head inhibition gradient. (a) is adapted from *Amer. Zool.*, 41, 621–8 (2001).

epithelial cells, the animal remains constant in size. This occurs because the tissue of the upper body column is apically displaced onto the tentacles and eventually sloughed at the tentacle tips (Bode, 1996). Tissue of the lower body column is displaced down the body column and sloughed at the foot (Figure 1.1a). Tissue from the middle of the column is primarily displaced into developing buds, which eventually detach from the adult. Thus, the animal is in a steady state of production and loss of tissue.

As tissue is displaced apically, it is converted into head tissue, whereas tissue displaced basally becomes foot tissue. What are the axial patterning processes that control

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the changes in the fate of these moving epithelial cells? A body of transplantation and regeneration experiments have provided insight into these processes (Browne, 1909; Wolpert, 1971; MacWilliams, 1983*a, b*; Bode and Bode, 1984). Bisection of the body column leads to the regeneration of a head at the apical end of the lower half. This indicates that body column tissue has the capacity to form a head. Transplantation experiments have shown that a head organizer region is located in the hypostome (Figure 1.1b) (Broun and Bode, 2002). This organizer transmits a signal, or morphogen, to the body column which sets up a gradient of head formation capacity, commonly referred to as the Head Activation Gradient (Figure 1.1b) (MacWilliams, 1983*a*). With this capacity, what prevents regions of body column tissue from forming heads? The head organizer also produces and transmits an inhibitor of head formation, which is also graded down the body column (Figure 1.1b), thereby preventing body column tissue from forming heads (Wolpert, 1971; MacWilliams, 1983*b*). These two gradients control the fate of the body column tissue as it is displaced apically. When the tissue reaches a point where  $[HA] > [HI]$ , the body column tissue is converted into head tissue. This mechanism maintains the axial patterning at the upper end in the context of the tissue dynamics of the animal. These gradients and their behavior have been incorporated into a model that provides a useful overall view of axial patterning in hydra (Meinhardt, 1993; see Chapter 27).

MATERIALS AND METHODS

In this section the equipment and materials required for carrying out transplantation experiments are described using a procedure developed by Rubin and Bode (1982). The culture of hydra and the source of specific pieces of equipment or materials are presented in the Appendix.

EQUIPMENT AND MATERIALS

Per student

- Dissecting microscope with 10 × oculars and, optimally, variable magnification of 1–4 ×.
- Pasteur pipette with rubber bulb (Fisher Scientific).
- Two pairs of fine-tipped forceps (Fine Science Tools) to handle pieces of fish line and “sleeves.”
- Scalpel (Fine Science Tools). An ordinary razor blade will work equally well.
- Medium-sized [60-mm diameter] plastic or glass petri dishes (Fisher Scientific).

**Per practical group.** If available, an 18°C incubator with a light that can be set with a timer so that it is on a cycle for 12 h on and 12 h off. If an incubator is not available, experimental samples can be left in the lab if the temperature is in the 15–25°C range.

**Biological material.** One-day-starved adult hydra (see Appendix) without buds. Two adult hydra are needed for each graft: one is the donor, and the other is the host. Determine how many grafts will be made and obtain twice that number of adult animals.

For each transplantation choose two adults that are the same size. Thus, 250–300 hydra are necessary for all four experiments [see Appendix on Maintenance of a Hydra Culture].

**REAGENTS**

CaCl <sub>2</sub>	NaHCO <sub>3</sub>
MgCl <sub>2</sub>	KNO <sub>3</sub>
MgSO <sub>4</sub>	Glutathione

**PREVIOUS TASKS FOR STAFF**

**Preparation of fish lines and “sleeves”**

- 1–1.5 cm pieces of fish line [8 lb; diameter = 0.3 mm; local store for fishing supplies].  
Using a scalpel and forceps, cut 1–1.5 cm long pieces of fish line.
- “Sleeves”: 2–3 mm pieces of polyethylene tubing (VWR Scientific). [As the ends should be pointed, cut the fish line at a 45 degree angle. Cut as many as are needed for an experiment. For the “sleeves,” cut two for each piece of fish line. Make the cuts perpendicular to the axis of the tubing.]

**Maintenance of hydra culture.** During this and the previous experiment the hydra must be maintained under standard conditions (see Appendix).

**Solutions**

- Hydra medium: 1.0 mM CaCl<sub>2</sub>, 1.5 mM NaHCO<sub>3</sub>, 0.1 mM MgCl<sub>2</sub>, 0.08 mM MgSO<sub>4</sub>, and 0.03 mM KNO<sub>3</sub>; pH 8.0.
- 1 mM glutathione (Sigma) in hydra medium.

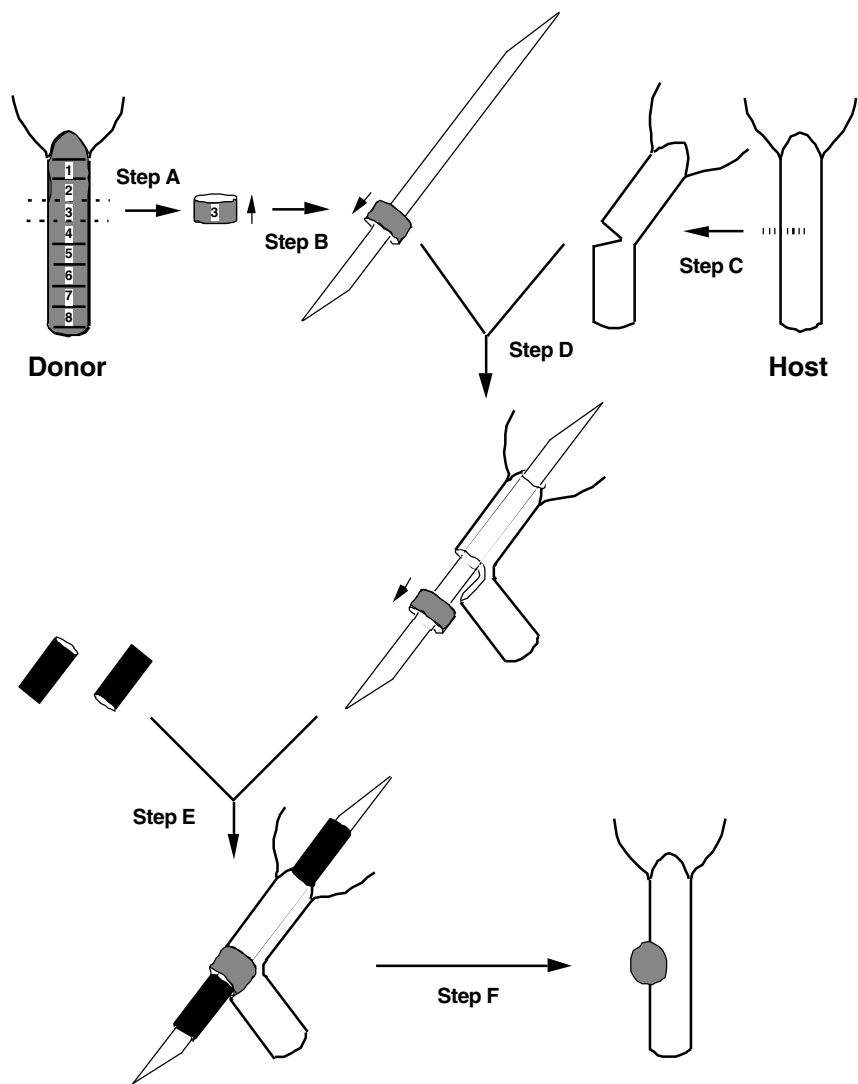
**TRANSPLANTATION PROCEDURE**

An individual transplantation, or graft, involves the following:

**Isolation of a ring of body column tissue** (Figure 1.2: Step A). Usually the ring of tissue isolated consists of 1/6 – 1/8 of the body column. Figure 1.2 shows a body column divided into 8 regions. To isolate a region do the following: Place a hydra in a medium-sized petri dish containing hydra medium, and let it stretch out. Determine the location of a region to be isolated. For example, for the 3-region, let the animal stretch out and estimate the location of the middle of the body column. Then estimate the location of the point half way between the middle and the top of the body column [where the tentacles emerge]. This location would be the top of the 3-region. With a pair of forceps in one hand, cradle the hydra. Using the scalpel in the other hand, gently bisect the animal at the apical end of the region you intend to isolate. Let the contracted animal extend, and bisect once more at the point below the apical end which will result in a ring of tissue approximating 1/8 of the length of the body column.

**Thread the ring of tissue onto a piece of fish line** (Figure 1.2: Step B). When grafting a ring of tissue into a host, it is important that the basal end of the isolated ring be

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**Figure 1.2.** Detailed procedure for transplanting a ring of tissue from the body column of a donor hydra to the body column of a host hydra. The six steps for the procedure are described in the text.

brought into contact with the host. To ensure that this happens be certain that the ring of tissue is threaded onto the fish line in the appropriate orientation (as indicated by the arrow in Figure 1.2: Steps A and B). Using two pairs of forceps, gently cradle the ring with one pair, and holding a piece of fish line with the second pair, gently slide the piece of fish line through the ring. Make sure that the apical end of the ring of tissue is facing the end of the fish line. Slide the ring along the fish line until it is about 3–4 mm from the end.

**Graft the ring of tissue to the host** (Figure 1.2: Steps C and D). Place an adult hydra, which will serve as the host, into the petri dish with the ring of tissue and let

it stretch out. Using the scalpel make a cut perpendicular to the body axis that extends about 1/2 way through the body column (Figure 1.2: Step C). For all the experiments described below the location of where the cut will be made will be indicated in terms of the body length [BL]. Thus, when grafting into a location that is 75% of the distance down the body column from the head, the location will be identified as "75% BL."

After cutting, a gap will appear (Figure 1.2: Step C). Using two pairs of forceps, cradle the host with one pair and slide the fish line, holding the ring of tissue into the cut, up through the gastric cavity, and out the mouth (Figure 1.2: Step D). When reaching the mouth, gently push, and the animal will open its mouth. Then slide the ring of tissue along the fish line so that it is in firm contact with the cut edges of the host.

**Thread "sleeves" onto the ends of the fish line** (Figure 1.2: Step E). It is important to keep the ring of tissue firmly in place as well to keep the animal from moving along the fish line. To do this, pieces of polyethylene tubing, referred to as sleeves, are threaded onto the two ends and brought into contact with the ring of tissue and the head respectively (Figure 1.2: Step E). The 2–3 mm pieces of polyethylene tubing are the "sleeves." With one pair of forceps hold the fish line extending out of the mouth. Use the second pair of forceps to slide a sleeve onto the piece of fish line extending from the ring of tissue, and use it to push the ring of tissue so that it is firmly in contact with the host tissue. Repeat this step with a second sleeve so that it is firmly in contact with the hypostome. Do not push so hard that the tissue folds.

**Healing of the graft and removal of the fish line** (Figure 1.2: Step F). With a pair of forceps gently transfer the graft to another medium-sized petri dish containing hydra medium. It is not a problem if the graft and fish line float on the surface. When all the grafts for a sample have been completed and transferred to this dish, place the dish [as is, or on a tray] in the 18°C incubator, or on the lab bench at 15–25°C.

The cut edges of the ring of tissue and the host will fuse together and heal within 1–2 h. At any time thereafter, remove the sleeves from each graft. Do this by holding one end of the fish line firmly with a pair of forceps, and gently removing the sleeve from the opposite end. Repeat this step for the second sleeve. Then, firmly holding the end of the fish line protruding from the mouth with one pair of forceps, place the other pair of forceps so that it gently cradles the fish line extending from the mouth. Now, slowly pull the fish line through the mouth until it is free of the host animal and the grafted ring of tissue. Or, gently push the animal down the fish line until the animal and the fish line are separated.

**Examination of the grafts.** Once the sleeves and fish line have been removed from all the grafts in the sample, the grafts should be incubated at 18°C. Thereafter, the grafts should be examined daily to determine the fate of the grafted ring of tissue.

OUTLINE OF THE EXPERIMENTS

Two pairs of experiments can be carried out to demonstrate the presence of the head activation and head inhibition gradients in hydra.

A. A HEAD ACTIVATION GRADIENT IN THE BODY COLUMN

These simple experiments demonstrate that tissue of the body column has the capacity to form a head and that this property, termed head activation, is graded down the body column.

**1. Tissue of the body column has head formation capacity.** Head formation capacity can be shown simply by bisecting an animal in the middle of the body column and letting the lower half regenerate a head at its apical end (Figure 1.3a).

PROCEDURE

1. From the stock culture pick out 10 1-day-starved adults of similar size.
2. Place them in a 60-mm petri dish containing about 10 ml hydra medium so that the dish is half full with medium.
3. Using a scalpel, bisect each animal in the middle of the body column resulting in an upper half with a head, and a lower half with a foot.
4. Using a Pasteur pipette with a bulb, remove the lower halves, and transfer to a second 60-mm petri dish with hydra medium.
5. Incubate the dish at 18°C.

**DATA RECORDING.** The head of a hydra consists of two parts (see Figure 1.1a): The dome-shaped upper half is the hypostome, which contains the mouth. The lower half is the tentacle zone from which a ring of tentacles emerge. Head regeneration will occur as follows (Figure 1.3a): Following bisection, the wound at the apical end of the lower half heals over. At an early stage, a ring of small protrusions, or tentacle bumps, forms below the apical cap. Subsequently, the bumps grow into short tentacles, and later into long tentacles. As the tentacles are forming, the mouth is developing in the hypostome. A fully formed mouth will open widely in response to glutathione treatment, which provides an easy way to assay the formation of the mouth. The analysis of head regeneration should be carried out in the following steps:

- Examine each of the 10 regenerating lower halves daily for 4–5 days with respect to tentacle formation and mouth formation using a dissecting microscope. When the daily analysis is complete, return the samples to the incubator.
- Determine the extent of tentacle formation. Start this examination on the day after decapitation, and carry out every 1–2 days until the end of the experiment.
  - (a) Number of regenerates with a healed apical cap.
  - (b) Number of regenerates with a ring of small protrusions, or tentacle bumps, which form a ring at the base of the apical cap.