BIOL20332
GENETICS RSM

BIOL20972
DEVELOPMENTAL BIOLOGY RSM

MODULE 3

MODULE 3: BASIC INFORMATION

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1. GENERAL INFORMATION

1.1. Contacts

The third part of this RSM is organised and taught by Dr. Andreas Prokop and consists of 2 units lasting 3 days each. This module will be taught in 2MUL. LAB COATS are REQUIRED! Please, direct questions regarding the practical to

- Dr. Andreas Prokop (Andreas.Prokop@manchester.ac.uk)
- Dr. Chris Thompson (Christopher.Thompson@manchester.ac.uk).

Course assistants: Yue Qu, Micheline Grillet, Jennifer Heyes, Daniel Howard, Sanjai Patel

1.2. Aims

The aims of this course module are:

1) to encourage self-responsible, systematic and creative approaches to data acquisition and interpretation
2) to introduce good laboratory practice and necessary skills to translate experimental work into high quality scientific publications
3) to develop an understanding of how genetic and cell biological strategies as well as the use of model organism can enhance general scientific knowledge

1.3. Intended learning outcomes

By the end of this course you will...

1) know the principles of genetic cross design
2) have learned and practiced the transferable skills of how to carry out
    a. basic immunohistochemical and histological experiments
    b. filing and documentation of microscopic specimens
    c. present images in scientific publications
3) have a principal understanding of how to use cell biological strategies to study developmental mechanisms.
4) be able to appreciate the use of *Drosophila* as an important model for the study of genetic and developmental mechanisms.

1.4. Additional learning materials to be studied before or during the course

- FlyGenetics-IntroStudents.doc (available on Blackboard)
- FlyGeneticsPresentation.ppt (available on Blackboard)

1.5. Assessment

35% for module 3 that are split as follows:

- 5% for satisfactory maintenance of lab books. To be approved by demonstrators during class.
- 15% for genetic problem solution.
- 15% for submitted figure and legend.

1.6. Time table of the module

Preparation for first day:

- *'Background' (chapter 2 of this manual)*
- *'General guidelines' (chapter 5 of this manual)*
- *Experimentation of first week (chapters 3 of this manual)*
- ***larvae inversion.wmv*** *(AVAILABLE ON BLACKBOARD)*
- ***FlyGenetics-IntroStudents.doc*** *(AVAILABLE ON BLACKBOARD)*

Monday, 1st week (25th of February)

11.00-11.30 Instruction: *Dissection of third instar larvae*
11.30-13.00 Practical work: *1st round of dissections, put on fixative & wash*
13.00-13.45 Lunch
13.45-16.00 Practical work: *2nd round of dissections, put on fixative & wash; transfer to X-Gal*
16.00-16.30 Background: *P-elements*

To be prepared for Tuesday:

- *Embedding (chapter 3.5. of this manual)*
- *Documentation, storage, publication of results (chapters 5 of this manual)*
- *Info on imaginal discs (chapter 3.1. of this manual)*
- *Revisit info on how to organise a figure (chapter 6 of this manual)*

Tuesday, 1st week (26th of February)

11.00-12.30 Self-study of "FlyGeneticsPresentation.ppt" (computer cluster2, bays A & B)
12.30-13.15 Lunch
13.15-14.45 Instructions: *Embedding of imaginal discs, microscopic analysis, documentation & filing of data*
14.45-17.00 Practical work: *Embedding and start of microscopic analysis*

To be prepared for Thursday:

- *Revisit pages on* Drosophila *genetics (chapter 5 of this manual)*

Thursday, 1st week (28th of February)

11.00-12.00 Practical work: *Microscopic analysis and documentation*
12.00-12.30 Transferable skills session: *How to organise a figure for scientific publication (**assessed task - TO BE HANDED IN ON LAST COURSE DAY**)*
12.30-13.30 Lunch
13.30-13.45 Genetic skills: *Identification of genetic markers using fly samples*
13.45-15.00 Genetic skills: *Designing crosses - preparation for assessed team work*
15.00-16.00 Practical work: *Continue microscopic analysis and documentation - start with first crossing tasks (see Appendix B of this manual)*
16.00-16.45 Discussion of results

Task over the weekend:

- *Study 'Experimental work of week 2' (chapters 4 of this manual)*
- *Solve genetic task (see Appendix B of this manual)*

Monday, 2nd week (4th of March)

11.00-12.00 Genetic skills: *Discussing the crossing task of the weekend*
Instructions: *Fixation of embryos*
12.00-13.00 Lunch
13.00-15.30 Practical work: *Dechorionation (15'), heptane/FA fixation (30'), devitellination and transfer to PBT (15'), buffer time: 15'*
in parallel Genetic skills: *Solving genetic tasks (Appendix C)*
15.30-16.00 Instructions: *Immuno-histochemistry*
16.00 *Adding 1st antibody*

Task for Tuesday:

- *Information on Drosophila nervous system (chapter 4.1 of this manual)*

Tuesday, 2nd week (5th of March)

11.00-11.30 Practical work: *Adding 2nd antibody*
11.30-12.30 Genetic skills: *Solving genetic tasks (Appendix D)*
12.30-13.30 Lunch
13.30-13.45 Instructions: *ABC-kit & DAB staining*
13.45-14.15 Practical work: *Adding ABC-solution*
14.15-14.45 Instructions: *Embedding and data analysis/documentation*
14.45-17.00 Practical work: *Carry out DAB staining, embedding & microscopy*

Task for Thursday:

- *Finalise the **ASSESSED TASK** - figure with material from the course and the accompanying figure legend (**to be handed in by Thursday 7th of March**)*

Thursday, 2nd week (7th of March)

11.00-12.30 Genetic skills assessment: *solve genetic crosses (PC cluster 2 bays A and B)*
12.30-13.15 Lunch
13.15-15.00 Practical work: *Continue microscopic analysis and documentation*
15.00-16.30 Discussion of results & general feedback

2. BACKGROUND INFORMATION

2.1. Addressing the genetic basis of two distinct biological processes

All biological processes and phenomena - from development to tissue maintenance, from metabolism to neuronal activity - are essentially regulated through genes and the factors they encode. Which are the genes required in each particular context? How are these genes regulated? Which functions do their products execute? How do they interact with other gene products? Genetic model organisms can be used effectively to give profound answers to these questions. This RSM module will focus on one such model organism, the fruitfly *Drosophila*, and demonstrate how *Drosophila* can be used to identify and study genes in certain biological contexts. We will focus on two biological contexts, the phenomenon of **pattern formation** (week 1) and the regulation of **guided neuronal growth** (week 2) to demonstrate genetic research in *Drosophila*.

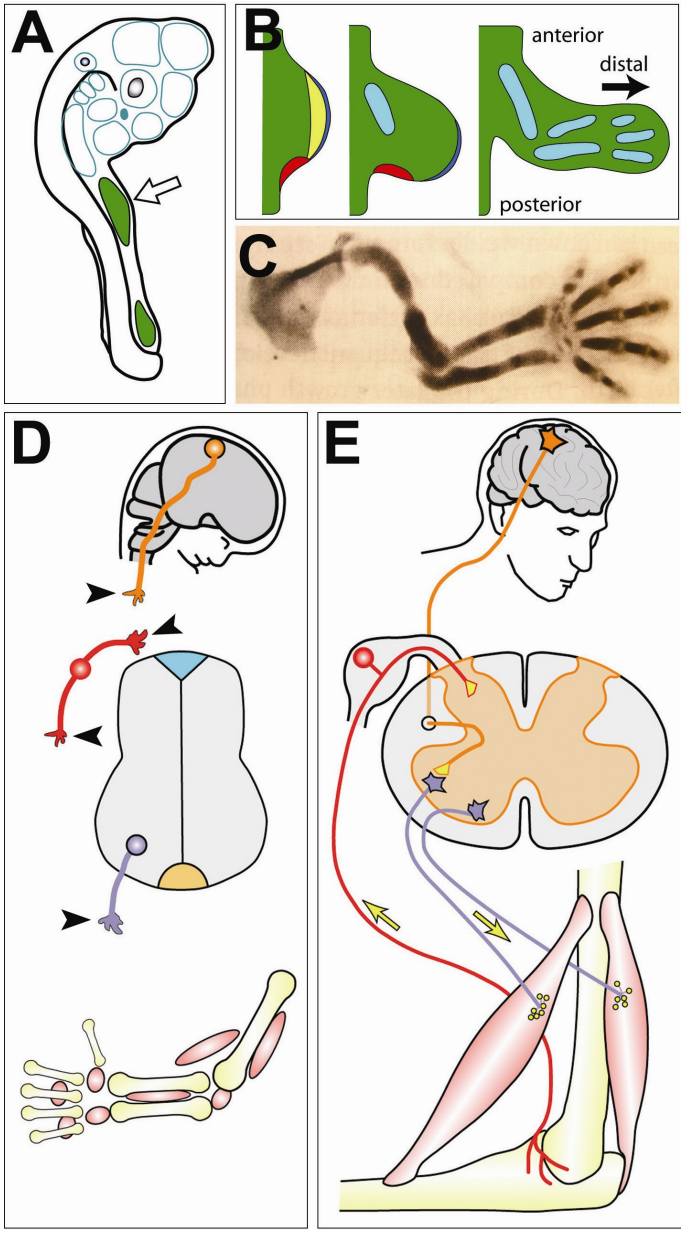


***Figure 1**. Limb formation and guided neuronal growth. **A**) In the developing embryo, limb buds (green) are formed in stereotypic positions. **B**) Determinants in the apical ectodermal ridge (dark blue), the progress zone (yellow) or polarising region (red) pattern the developing limb bud along the anterior-posterior and proximal-distal axis, as illustrated by the cartilage structures (light blue), the precursors of future bones of arm and hand (see **C**). **D**) In the developing nervous system, neuronal axons grow out along reproducible paths via growth cones at their tips (arrow heads). **E**) Stereotypic guidance of axonal growth ensures that sensory neurons (red), interneurons (beige) and motorneurons (blue) arrange into correctly hard-wired circuits that conduct action potentials along slender axons over long distances (yellow arrows) and pass them on to appropriate partner cells at synapses (yellow triangles and circles).*

Week 1: **Pattern formation** is the process in development whereby cells in different positions acquire distinct specifications and arrange into correctly differentiated and proportioned tissues and organs. A simple example is provided by the stripes of a zebra where cells in the stripes accumulate melanin to take on their black appearance and cells in inter-stripe positions do not. These cellular decisions are coordinated by developmental patterning mechanisms across the skin. Similarly, the formation of legs or arms requires that groups of cells in stereotypic positions of the developing body are induced to carry out programmes which lead to the formation of an appendage (Fig.1A). Within these appendages, distal cells have to contribute to the formation of hand or foot, of which anterior cells contribute to a thumb,

posterior cells to a small finger (Fig.1B,C). How can such patterns emerge in an initially unstructured system? How can different genetic information be activated at different positions? As will become clear in this RSM module, cascades of gene-regulatory events are involved in this process of pattern formation.

Week 2: The **guided growth of axons** is a key developmental process that ensures functionality of a nervous system (*Lowery & van Vactor, 2009, Nat Rev Mol Cell Biol 10, 332ff.*). Axons are the extended and slender processes of neurons that form the core of nerves and nerve tracts and constitute the essential information highways of the nervous system, conducting electrical messages in form of action potential. Axons enable neurons to establish synaptic contacts with appropriate target cells (other neurons, muscles or gland cells) that can be up to meters away (Fig.1E). This organisation is the cellular basis for coordinated behaviour. The precision of this wiring process is achieved during development through the guided growth of axons along paths specific to every neuron, a process clearly governed by genetic mechanisms. Like in pattern formation, the actual decision has to be taken by each individual cell, more precisely, by the growing tips of axons called **growth cones**. A growth cone navigates by reading patterned information in the surrounding tissues and making directional choices at many different stages of the entire growth process. This extrinsic guidance information is provided by cocktails of guidance cues in form of diffusible or contact-mediated molecules that are precisely arranged in space and time. Therefore, developmental processes have to ensure that patterned information is provided by all tissues that are to be innervated, and that neurons acquire their specific receptive machinery that enables them to correctly read and interpret extrinsic information and make the right navigational choices during axon growth. In this RSM module we will look at genetic mechanisms governing one particular choice event - the decision of a growth cone to cross or avoid the midline of the CNS.

As will become clear during this RSM module, the genetic mechanisms underlying pattern formation or guided neuronal growth are often of relevance to human disease, and aberration of such mechanisms will cause malformation or malfunction (Fig. 2).

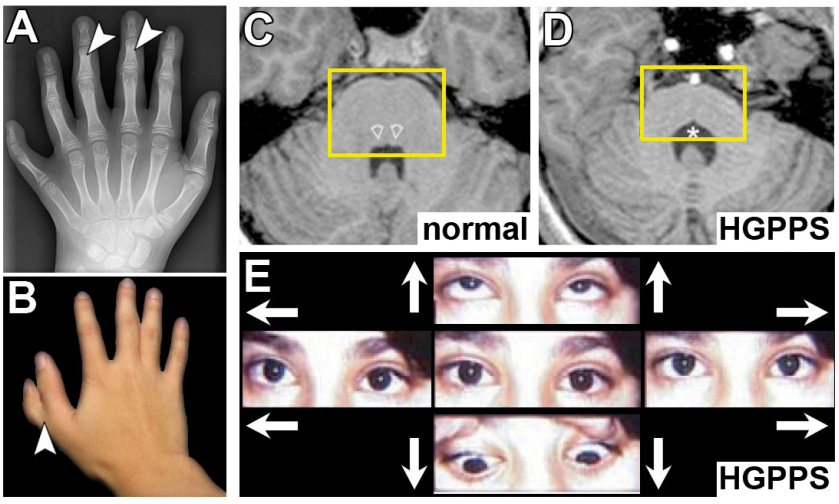


Figure 2. *Examples of inherited human diseases relevant to this Genetics RSM module:*
A,B*) Polydactyly (duplication of fingers; white arrowheads) can be caused through misregulation of Hox genes or of the gene Sonic hedgehog (Shh). As will become clear during the first week of this course, these gene classes and their essential functions in limb patterning were first discovered and understood in flies.* ***C-D****) Mutations of the robo3 gene cause Human gaze palsy with progressive scoliosis (HGPPS). The pathology of HGPPS is caused by a severe reduction of axonal projections across the CNS midline (boxed area) leading to a coordination deficit across the body axis, for example the inability to move eyes horizontally. As explained in detail during this RSM,* robo *genes were first discovered and their principal functions in regulating midline guidance were first understood in* Drosophila*.*

2.2. The fruitfly *Drosophila melanogaster* as a genetic model organism

The use of model organisms is common practice in biological research and, at least since the advent of the genetic code, it has become clear that many insights are translatable between

organisms (Fig.3). In particular, the use of genetically tractable invertebrate model organisms, such as the fruitfly *Drosophila melanogaster* or the worm *Caenorhabditis elegans*, has enormously advanced our knowledge about genetic mechanisms underlying biological processes, with important implications for medical research (Fig.2). Therefore, it is a trend in contemporary biomedical science to view model organisms like *Drosophila* as tools or "test tubes" for human genetics (summarised in Bier E, 2005, *Nat Rev Genet* 6, pp.9ff.). Further advantages of using fly are explained in detail in the accompanying document "A rough guide to *Drosophila* mating schemes". During this RSM module, you will experience two important aspects of work with flies:

- You will learn **how *Drosophila* genetics can be used to decipher principal mechanisms** underpinning biological processes, using pattern formation and guided axon growth as examples. We will discuss how such research is helping to advance our understanding of inherited human diseases.
- Genetics is the common tool used by the majority of researcher working with the fly. To gain a principal understanding how genetic experiments are carried out in practice, you will perform exercises to introduce you to the **design of genetic mating schemes**. This will include learning how classical Mendelian rules are applied, and what genetic tools are available for carrying out genetics as well as experimental work in *Drosophila*. A Manual and a PowerPoint presentation are available on Blackboard as eLearning resources to prepare yourself for this part of the course.

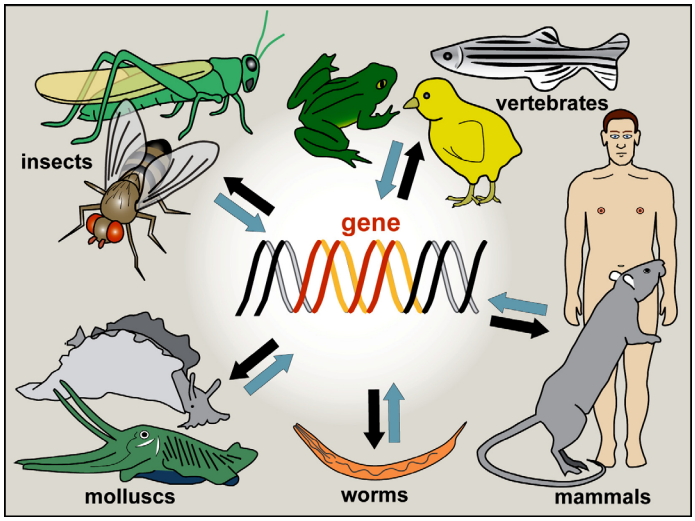


***Figure 3**. The use of model organisms: Many different model organisms are being used to study biological mechanisms and phenomena. Researchers choose the most suitable for their scientific problem by weighing advantages versus disadvantages of each particular model organism. Since organisms across the animal kingdom share a high percentage of their genes, insights obtained in simple model organism can often be translated into higher organisms or humans, speeding up investigation and improving our general knowledge of biological functions and processes.*

EXPERIMENTAL PART

One set of experiments will be carried out in week 1 and a different set in week 2. Experimental procedures to be carried out will be the same for everybody, but the genotypes and antibodies used in individual experiments will vary. Groups of two students will work together throughout the course and will be assigned combinations of genotypes and antibodies for their respective experiments. Stained preparations of all groups will be filed in a common slide box accessible to all students (*please, read the respective general guidelines in Section 11 to assure optimal use of preparations*). All students will be expected to have analysed and documented the outcome of all experiments. Results will be discussed at the end of each week and the documentation will form an essential part of the assessment.

3. EXPERIMENTAL WORK OF WEEK 1

EXPERIMENTAL OBJECTIVE: The aim of this experiment is to make use of enhancer trap lines or promoter constructs to describe genes with specific positional expression patterns in imaginal discs as candidate genes involved in patterning mechanisms.

3.1. Background on imaginal discs

Drosophila is a holometabolous insect transforming from a worm-like maggot into the elaborate shape of the adult fly. To accelerate this process of metamorphosis, all adult tissues are "pre-assembled" in the larva and set aside as defined entities, called imaginal discs and histoblasts (Fig.4). Like in the limb bud of vertebrates, patterning takes place in imaginal discs in the antero-posterior, dorso-ventral and proximo-distal axis, and defined areas of the imaginal discs can be clearly assigned to the future structures of the adult fly, as illustrated for leg and wing discs in Fig.5. In week 1 we will capitalise on the well described leg and wing discs to study genes that regulate their patterning.

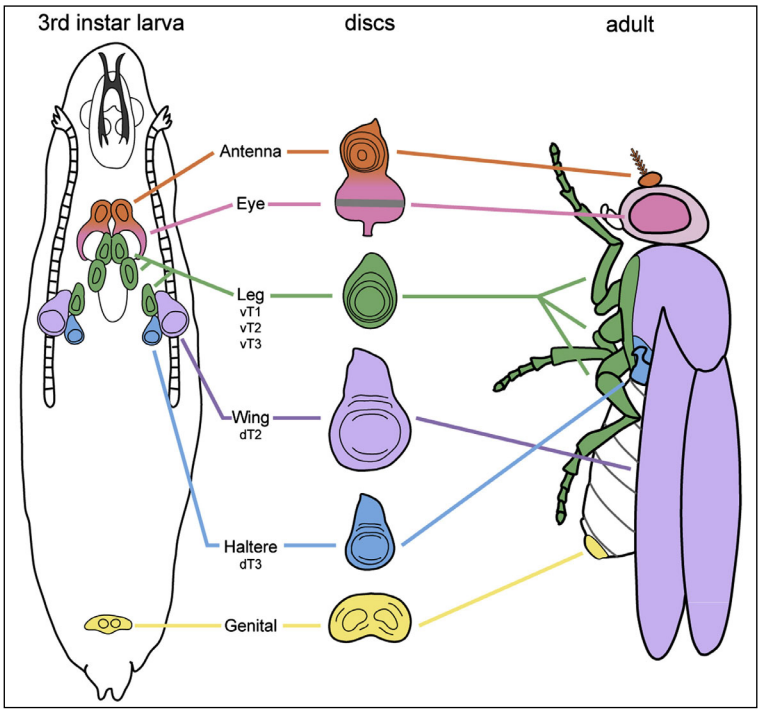


***Figure 4**. Position and prespective fate of the various* Drosophila *imaginal discs. The image shows the position of the various imaginal discs in the late larva (3rd instar larva, left) their shapes and relative sizes (middle) and, following a consistent colour coding, the part of the adult fly these discs contribute to. Image taken from Jory et al., 2012, Cell Reports 2, 1014ff.*

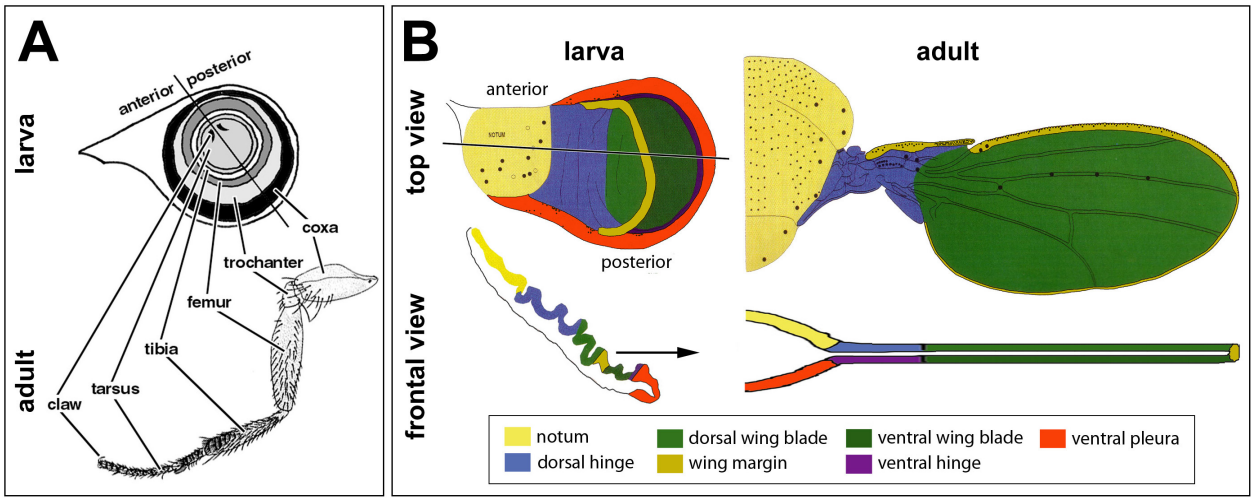


***Figure 5**. Fate maps of* Drosophila *imaginal discs. **A**) Correlation of leg disc areas to structures of the adult leg (colour coded in different shades of grey). **B**) Top and frontal view of larval wing disc and adult wing, both colour coded to illustrate the correlation between leg disc areas and wing structures.*

3.2. Overview over experiments of the first week

In the first week, you will learn to dissect and analyse late larval tissues of *Drosophila* (*Protocol 1*). You will carry out dissections of a number of fly lines carrying specific transposable elements (P-elements; explained in detail in chapter 5 of "A rough guide to *Drosophila* mating schemes"). The P-element insertion of each fly strain mediates expression of the reporter gene *lacZ* in a reproducible pattern, each mimicking the expression of one specific endogenous *Drosophila* gene. The lacZ reporter gene encodes the enzyme ß-galactosidase (see 3.4.1), and its activity can be detected using the X-Gal colour reaction (Protocol 2). Therefore, X-Gal staining makes the spatial expression patterns mediated by the various P-element insertions visible. After staining and embedding of specimens you will use a compound microscopy to analyse and document imaginal discs of the various fly strains provided. You will try to assign expression patterns to imaginal disc regions (Fig.7).

The real names of the analysed transgenic fly lines will not be unveiled before you have completed your documentation. This should inspire you to think about your findings, try to classify phenotypes and speculate about their function and interactions. Note that, in a real laboratory situation, you will never know the outcome of your experiments beforehand. Drawing your own conclusions and developing hypotheses is the key aspect of scientific work and can be trained here.

Table of experiments (week 1):

Groups	larval genotype	staining	comments
	A-lacZ	*2hr 37ºC, then o.N. 26ºC*	
	B-lacZ	*2hr 37ºC*	
	C-lacZ	*2hr 26ºC*	
	D-lacZ	*2hr 37ºC, then o.N. 26ºC*	
	E-Gal4;UAS-lacZ	*2hr 26ºC*	
	F-Gal4;UAS-lacZ	*2hr 37ºC*	
	G-Gal4;UAS-lacZ	*2hr 37ºC*	
	H-lacZ	*2hr 37ºC, then o.N. 26ºC*	

3.3. Dissection of wing and leg imaginal discs (Protocol 1)

Required material per person: 1X PBS solution, 1 Sylgard dish, 1 forceps, 1 dissection needle, 1 razor blade, 1 centrifuge tube, 1 pulled out pasteur pipette, vials with larvae

In order to dissect *Drosophila* at late larval stages we will use the 'inversion strategy' by which all internal organs become exposed and accessible for staining and visualization. Please, watch the movie "*larvae inversion.wmv*" available on Blackboard.

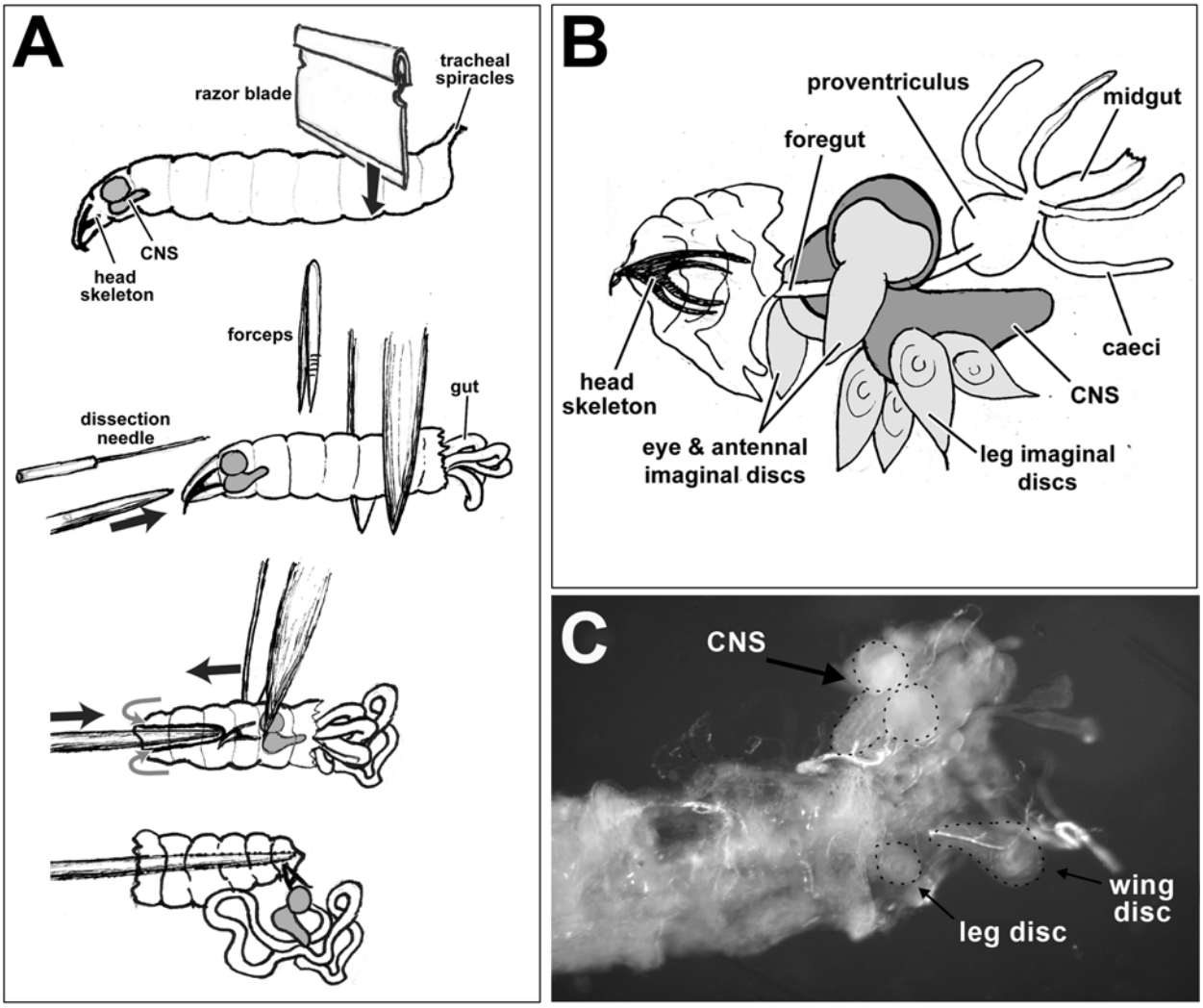


***Figure 6**. Dissection of late Drosophila larvae. **A)** The different steps of the inversion technique: larvae are cut close to their posterior tips with a blade (anterior tip has the dark head skeleton, posterior tip has the prominent spiracles), gently hold the larva with a forceps and use a blunt needle to poke the anterior tip inside until fully inverted. **B)** The CNS has usually two pairs of leg discs attached. **C)** the CNS (with its discs attached), wing discs and the third pair of leg discs can be seen on the inverted larval carcas.*

Step-by-step procedure:

1) Fill a Sylgard dish 1/3 with 1xPBS
2) Select a few larvae with a forceps (use nice fat ones that crawl up the wall of the vials) and wash them in a small well filled with water
3) Place larvae one-by-one in the Sylgard dish and hold them with the forceps to limit the wiggling movements of the posterior tip; avoid squashing internal organs by applying only gentle pressure.
4) Use a razor blade to cut through the posterior body of the larva (*black arrow in Fig. 6A*); due to muscle contraction part of the internal organs exit at the site of the cut ("*gut" in Fig. 6A*).
5) Position a blunt dissection needle at the anterior larval tip, gently holding the larval body in position with the forceps (*Fig. 6A*); it takes a while until you find a point where the needle does not slip off. Gently push the larval tip in and move the skin over the needle

with your forceps. Move the needle forwards between the shanks of the forceps so that the larval tip is turned inwards and the larva is gradually turned inside-out (*grey curved arrows* in *Fig.9A*). Make sure that the inversion is complete, otherwise it will flip back into its natural outside-in orientation when stripping the carca off the needle.

6) Upon completion of the body wall inversion, all internal organs are exposed (*Fig. 6D*). Pull the digestive tract and fat bodies away whilst the carcass is still propped over the needle. The CNS and imaginal discs should stay connected to the anterior part of the body wall (*Fig. 6E*). Sensory neurons and muscles are closely attached to the body wall in all areas.

7) Dissect as many larvae as possible for about one hour and collect them one by one in a microfuge tube. Then use a Pasteur pipette (the tip of which was pulled out over a Bunsen burner and broken off to obtain a very narrow opening) to remove the PBS. But make sure that specimens do not fall dry!

8) Replace with 0.5-1ml of fixation solution (*1% glutaraldehyde in PBS; see Section 3.1. for an explanation of aldehyde fixation*). Incubate for 10 minutes, then continue with Protocol 2.

3.4. X-Gal staining (Protocol 2)

3.4.1. BACKGROUND

The *lacZ* gene is derived from the *lac* operon of *Escherichia coli* (for details see http://en.wikipedia.org/wiki/Lac_operon). It has been adopted as an effective reporter gene in biological research. The idea of a reporter gene is that it "reports" the transcriptional activation of a gene regulatory element in experimental designs. In our RSM module, we use P-element insertions mediating *lacZ* expression in the pattern of endogenous genes. The *lacZ* gene encodes the enzyme β-galactosidase. In order to detect its pattern of expression in tissues of interest (i.e. cells which display *lacZ* activation) its enzymatic activity can be assayed using X-Gal as substrate (*Fig. 7*). Alternatively, the presence of β galactosidase can be assayed through immunohistochemistry using antibodies specifically directed against this enzyme (*see week 2 and Protocol 5*).

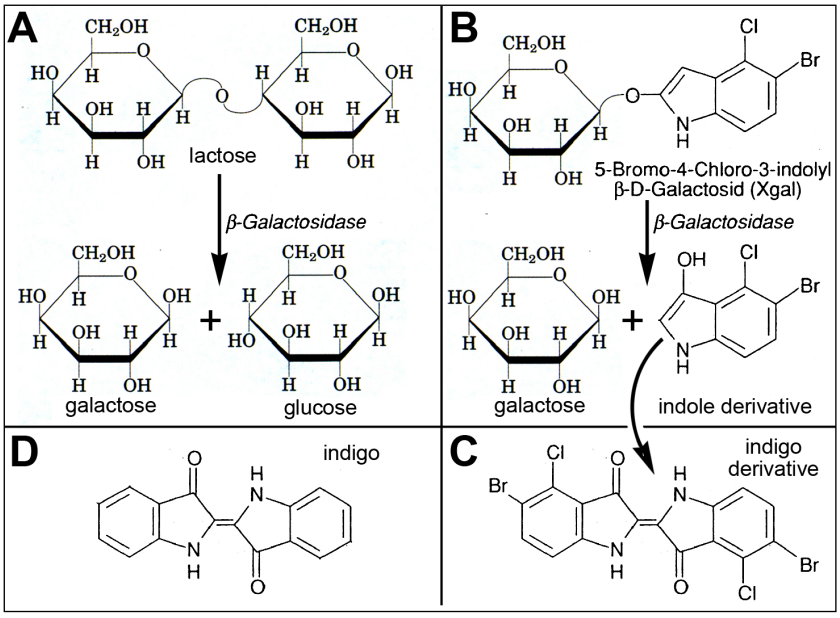


***Figure 7**. The mechanism of X-Gal staining. **A**) The normal function of β-galactosidase is the cleavage of lactose into galactose and glucose. **B**) In a similar manner, it can cleave X-Gal into galactose and an indole derivative (**C**) which, upon oxidation (curved arrow), gives rise to an indigo derivative (compare indigo in **D**) with a system of conjugated double-bonds that absorb light efficiently.*

3.4.2. SOLUTIONS

- X-GAL SOLUTION: 20% X-Gal (5-bromo-4-chloro-3-indolyl-ß-d-galaktoside) in N,N-dimethyl formamid

- INCUBATION BUFFER: 3mM $K_4FeII(CN)_6$ and 3mM $K_3FeIII(CN)_6$ (kept as 30mM stock solutions, respectively), 1mM $MgCl_2$ (kept at 10mM stock solution) dissolved in PBS (0.2M, pH7.2).
- STAINING SOLUTION: prepare 670 parts fresh incubation buffer, warm up to 60-70°C and inject 30 parts of X-Gal solution. Prepare one large batch, then aliquot into centrifuge tubes (one per group).

3.4.3. STAINING PROTOCOL

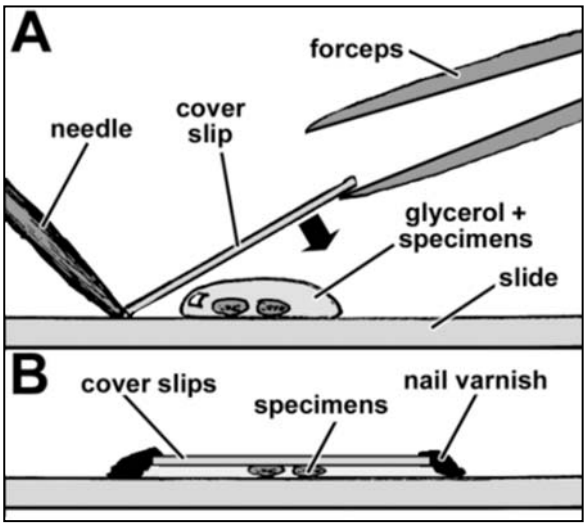
1) Following fixation in 1% glutaraldehyde (*end of Protocol 1*), wash larval specimens once in PBS followed by incubation buffer.
2) Replace incubation buffer with staining solution. The X-Gal component in the staining solution serves as substrate for β-galactosidase, whereas $K_4FeII(CN)_6$ and $K_3FeIII(CN)_6$ provide an oxidative environment catalysing dimerisation of the produced indole derivative (*Fig. 7B,C*). Make sure that the staining solution has cooled down to room temperature, equal to the temperature of the glass well. This will prevent precipitation of X-Gal crystals.

The following will be performed by course assistants:

3) Incubate at room temperature or at 37°C for several hours or over night.
4) Once staining can be seen, tip the content of the microfuge tube over into a glass well. Fill it up once more with staining solution and hold against light to make sure no tissue remains in the tube. If so, tip over into the same glass well.
5) Stop staining reaction by removing staining solution and adding 70% glycerol.

3.5. Embedding of larval discs (Protocol 3)

Required material: 1 forceps, 1 dissection needle, X-Gal stained specimens, slides with frosted edges, pencil, 22mm x 22mm cover slips, 70% glycerol



1) Stained specimens are transferred with a forceps to a big drop of 70% glycerol on a slide which is placed under a dissection microscope (white side of the base plate facing up).
2) Using needle and forceps, remove wing and leg discs from the stained carcasses and separate them to one side of the drop.
3) Using a laboratory pipette, place a small drop of 70% glycerol (40 µl) on a slide (*with frosted edge*). Transfer discs with the tip of the needle or forceps into this drop.
4) When a couple of leg and wing discs have been collected in this drop, carefully place a cover slip using a forceps; put down one edge first (*use needle to keep it in position; A in Fig.*), then slowly lower the whole cover slip (*arrow*). With this procedure you prevent inclusion of air bubbles.
5) Seal all fringes of the cover slip with nail varnish (*B in Fig.*).
6) Make sure that your slides are properly labelled and filed according to the instructions given under 'General guidelines'.

4. EXPERIMENTAL WORK OF WEEK 2

EXPERIMENTAL OBJECTIVE: The aim of this experiment is to describe pathfinding phenotypes caused by a set of Drosophila *mutations and hypothesise their potential function and interrelation.*

4.1. Background: The midline of the *Drosophila* CNS

The spinal cord of vertebrates and the ventral nerve cord of *Drosophila* show certain commonalities in their organisation (Sánchez-Soriano, N. *et al.*, 2007, *Neural Develop* 2, 9). One such common principle is that the axons of most neurons cross the midline of the nervous system, whereas axons of a defined sub-group of neurons never crosses but stays ipsilateral (on the side of their cell body; Fig.8). Mechanisms underlying this simple choice are shared between vertebrates and *Drosophila*. They have been discovered first in *Drosophila* induced by genetic screens. On this RSM module you will get to know some of these genes and how their phenotypes can be studied.

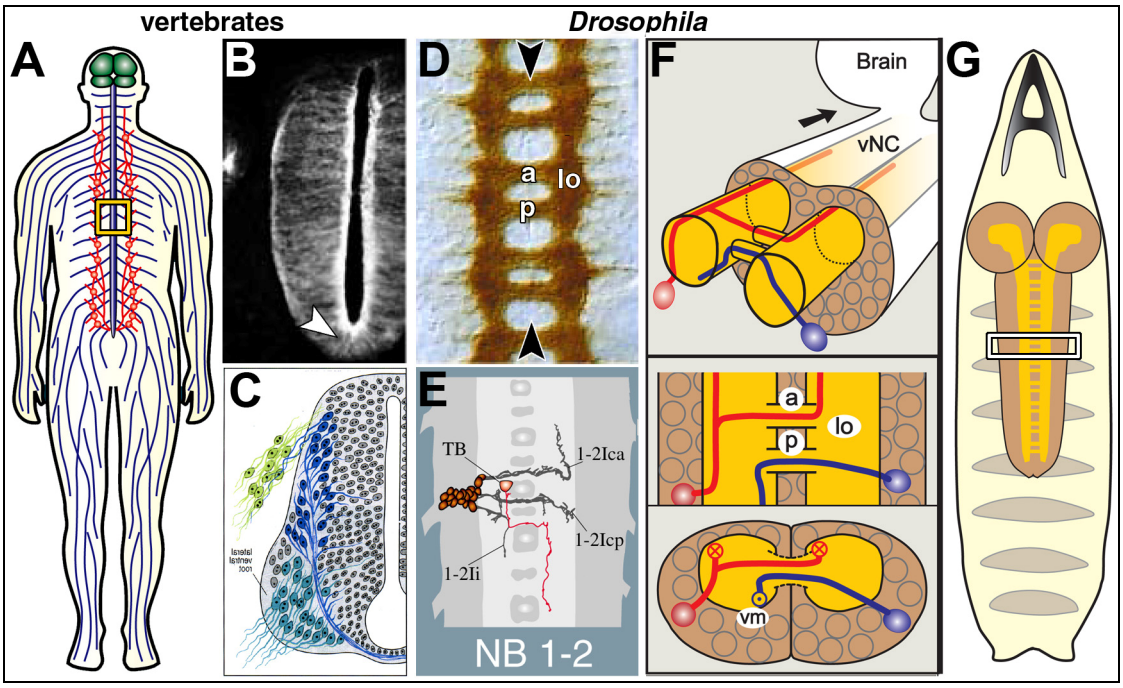


***Figure 8**. Midline crossing at the CNS midline. **B**) Cross section through the developing spinal cord of vertebrates (corresponding to boxed area in **A**); white arrow head indicates the floor plate. A similar frontal view of the developing* Drosophila *ventral nerve cord is shown at the bottom of **F** (corresponding to boxed area in **G**); it shows that commissural axons in the fly CNS cross the midline in a more dorsal position; two commissures (a, anterior; p, posterior) per segment communicate between the longitudinal connectives (lo). **D**) shows a CNS stained with BP102 antibody (brown) which highlights the axonal area (called neuropile, orange in F,G). **E**) shows traces of real embryonic neurons of the neural cell lineage NB1-2 crossing the midline through various commissures.*

4.2. Overview over experiments of the second week

In the second week you will study the functions of genes involved in the guidance of neuronal growth in the developing nervous system of *Drosophila*. To this end, you will analyse embryos in which the 'pathfinding or guidance genes' A, B and C are mutated. To visualise nervous system defects caused by mutations in these genes, we will use antibodies (*BP102, α-Fas2*) which mark preferentially the neuropile (*Figs. 8 and 9*). In one example (C-lacZ), a ß-Gal-expression

pattern of an enhancer trap line will be compared to the expression of the endogenous gene product (anti-C). Like in week 1, gene names will not be disclosed until the final discussion.

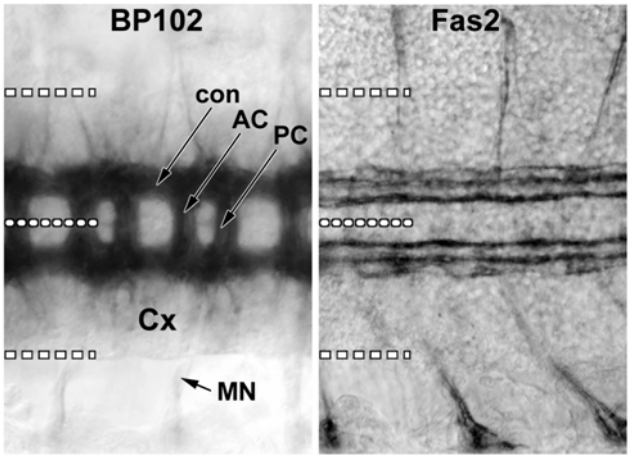


***Figure 9**. Staining patterns of BP102 and Fas2 in the embryonic CNS (compare Fig.8, D-G). Horizontal view of CNS (lateral borders indicated by dashed lines, dotted line indicates midline); AC, anterior commissure; con, connective; Cx, cortex; MN, motornerve; PC, posterior commissure. BP102 labels the synaptic neuropile, Fas2 a set of anteroposterior axon bundles within the neuropile and motornerves.*

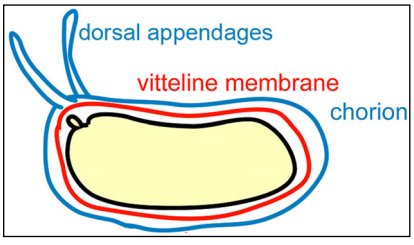
Table of experiments (week 2):

Groups	mutant gene	BP102 (mouse)	anti-A (mouse)	anti-C (mouse)	anti-lacZ (rabbit)	anti-Fas2 (mouse)
	A	+				+
	B	+				
	C	+				+
	C-lacZ	+	+	+	+	

4.3. Fixation of embryos (Protocol 4)

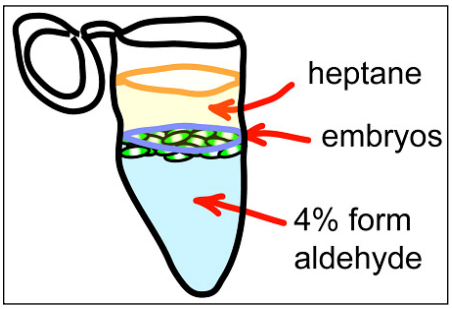
We will provide agar-filled petri dishes onto which flies have laid their eggs over night (i.e. embryos have had time to develop for maximally 13 hr and minimally 4-5 hr (depending on the time point an egg was laid). Embryos should not be older than stage 16 (*see Appendix A*), i.e. the stage at which cuticle starts being secreted. The cuticle forms the exoskeleton providing a protective sheet that renders the animals impenetrable to the aqueous fixation solution. Thus, only embryos up to an age of about 13hr (and slightly older) can be fixed and analysed as whole mount preparations. The only obstacles are two extra-embryonic membranes (vitelline membrane and chorion) which can be removed by straight forward chemical procedures:

1) Hand-pick all flies and bigger dirt particles off the agar plates.
2) To remove the chorion of developing eggs/embryos, fill the petri dishes with 50% household bleach for 1 min.; under the dissection microscope you can observe that embryos float to the surface and shed their chorion (dorsal appendages no longer visible).



3) Pass embryos with bleach through a funnel with sieve; wash any remaining ones off the plate with water.
4) Wash embryos thouroughly with water (tap water is fine) to remove all residues of bleach.
5) Using a small painting brush, transfer the embryos from the sieve into a microfuge tube containing 500µl of heptane and 500µl of a 4% formaldehyde solution in PBS. Embryos float at the interface of hydrophobic heptane (upper phase) with the PBS solution (lower phase).
6) Shake/rotate for 20 minutes. Heptane penetrates the vitelline membrane thus allowing the fixative to access the embryonic tissue.

7) Use a pulled-out Pasteur pipette to remove most of the lower phase.
8) To remove the vitelline membrane from the embryos, add 500µl of methanol, close the cap immediately and shake rigorously for one minute; the vast majority of embryos should turn white (alcoholic denaturing) and sink to the bottom of the cap; if not, try to remove as much of the lower methanol phase as possible and repeat the procedure..

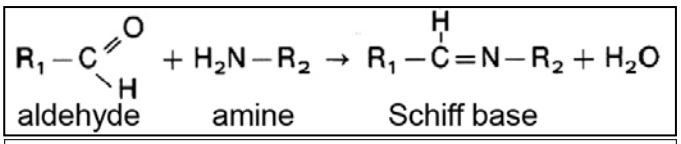


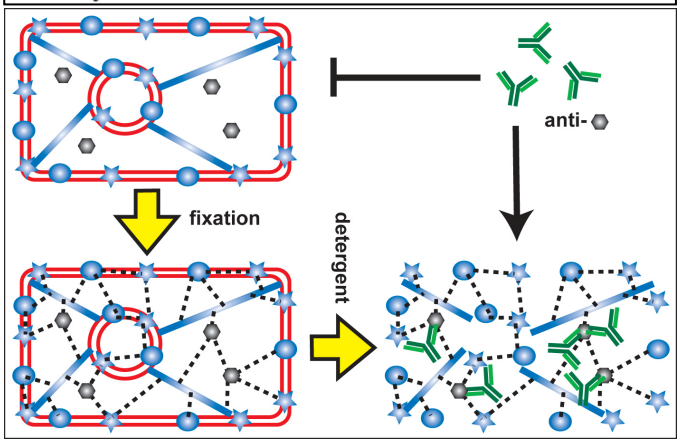
9) After successful removal of the vitteline membrane, remove all liquid (including heptane) and replace with methanol.
10) Wash once more with methanol.

4.4. Immunohistochemistry (Protocol 5)

4.4.1. BACKGROUND

Immunohistochemistry provides the possibility to visualise proteins in their natural position in tissues or organisms. It is based on the fact that we can produce antibodies against specific epitopes/proteins by injecting these peptides into animals of a different species than the one the peptide originates from (*commonly used are rabbits, goats, horses, donkeys, sheep, rats, guinea pigs, mice*). After a few weeks, their natural immune response usually provides amplified amounts of peptide-specific antibodies which can be harvested and purified from their blood. As you will remember from your immunology lectures, each antibody is composed of two longer heavy chains (*darker green in image*) and two shorter light chains (*brighter green*).

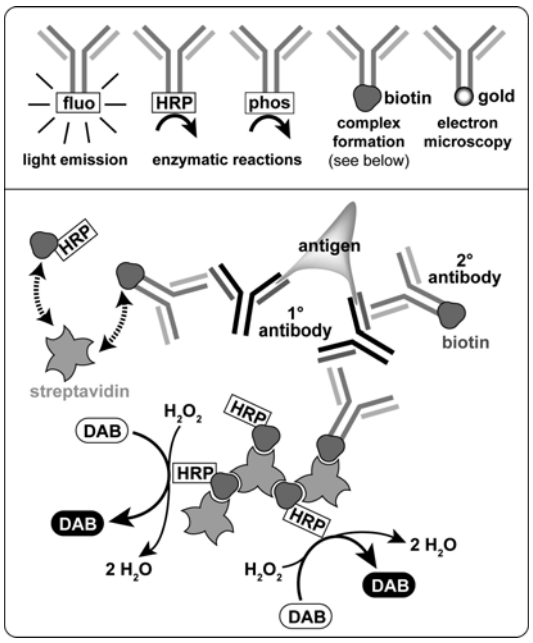




We will study protein localisation in the *Drosophila* nervous system. To this end, we will use antibodies specific to either endogenous proteins or to products of specifically expressed 'reporter genes'. In order to carry out the staining, *Drosophila* embryos will be fixed with aldehyde (*usually formaldehyde; see Protocol 4*). Aldehyde fixation is based on a chemical reaction (Schiff condensation; *see image*) which cross-links amino groups (*black dashed lines in image*) to preserve tissue in natural shape and proteins in their typical sub-cellular positions. Subsequently, specimens will be treated with detergent to strip cell membranes of their lipid membranes (*red double lines*) and make them penetrable to antibodies. These antibodies will bind and thus localise at specific tissue regions where their target epitopes/proteins localise. But how do we make these antibodies visible?

In order to visualise antibodies in tissues (*or in Western blots*) they can be associated with specific detection agents (*see image next page*). Such agents can be (a) fluorescent markers (*fluo in upper panel*) which can be detected directly in fluorescent microscopes, (b) enzymes like horseradish peroxidase (*HRP*) or alkaline phosphatase (*phos*) which can be detected via enzymatic reactions, (c) biotin which links to other avidin into larger complexes and (d) gold particles which are impenetrable for electrons and are therefore visible as sharp black dots in electron microscopes. In order to improve visibility and sensitivity of antibody stainings amplification steps can be used. To this end, the target-specific antibodies (*1° in lower panel*) are not directly coupled to detection agents but are detected by secondary antibodies (*2° in*

lower panel). Secondary antibodies can be obtained by injecting constant regions of antibodies of one animal species (*e.g. from rabbit*) into animals of another species (*e.g. goat*). As a result, 'anti-animal-specific' antibodies can be obtained (*i.e. goat-anti-rabbit*) which can be used to detect any target-specific primary antibodies produced in the respective animal species (*i.e. all primary antibodies produced in rabbits can be detected*). Since several secondary antibody molecules (*themselves coupled to detection agents*) can bind to one primary antibody, the signal will be enhanced, i.e. sensitivity increased. A second advantage of this system is that primary antibodies can be detected without having to link them directly to detection agents in time consuming biochemical procedures. Instead libraries of secondary antibodies are kept in the laboratory which can be used straight away for immunohistochemical experiments in any combination of choice.



Here we will use the ABC-detection system (*streptavidin-biotin-complex; lower panel of the image*) which provides a further step of enhancement. In this system, secondary antibodies are coupled to biotin. Biotin forms complexes with streptavidin at a ratio of 3:1. By adding streptavidin molecules and free biotin molecules during the staining procedure streptavidin-biotin-complexes bind and cluster at biotin-coupled secondary antibodies. If the added free biotin is itself linked to a detection agent (*here HRP*) this leads to a significant enhancement of this agent at sites of target epitope/protein. HRP is a peroxidase enzyme derived from horseradish plants. Its natural task is to catabolise H_20_2 and thus to protect cells from this harmful molecule. This procedure represents a redox reaction in which H_20_2 is reduced. For our immunohistochemical experiments we add therefore H_20_2 and diaminobenzidine (*DAB*) which serves as electron donor for the HRP reaction. Oxidation of DAB produces a conjugated double bondage system which absorbs light and therefore produces a brownish stain which precipitates in the area of the streptavidin-biotin-complexes.

4.4.2. PROTOCOL FOR IMMUNOHISTOCHEMICAL PROCEDURES

1) Remove methanol and add PBT for 1hr (*PBS containing 0.1-0.3% of the detergent Triton-X*) to dissolve cell membranes and make them penetrable for antibodies.
2) Use a pulled-out Pasteur pipette to remove as much PBT as possible without loosing specimens and add the appropriate antibody solution in PBT (*will be handed out*).
    - anti-Robo (1:5; mouse)
    - anti-Slit (1:5; mouse)
    - anti-Fas2 (1:10; mouse)
    - anti-BP102 (1:10; mouse)
    - anti-ßGal (1:1000; rabbit)
3) Keep agitating at either room temperature for 3-5hr or at 4°C over night.
4) Rinse about 3 times with PBT over a period of 10-15 min.
5) Remove PBT and add secondary biotin-coupled anti-mouse or anti-rabbit antibody (*1:400 in PBT; will be provided*). Make sure your secondary antibody matches your primary antibody with respect to the animal it was produced in!
6) Keep agitating for 1-3hr at room temperature or at 4°C over night.
7) Prepare the streptavidin-biotin-complex (ABC) solution: add a 1:100 solution of streptavidin in PBT to a 1:100 solution of HRP-coupled biotin in PBT and shake/rotate for 30 min (use within one hour).

8) Rinse 2 times with PBT over a period of 10 min, and add the ABC solution.
9) Keep agitating for 1hr at room temperature.
10) Rinse 2-3 times with PBT over a period of 10-15 min.
11) Remove PBT and add a solution of PBT containing 0.02% H_20_2 and 0.1% DAB which will be handed out in a 15ml Falcon tubes. You must wear gloves to avoid skin contact!
12) Immediately transfer the content of your centrifuge tube with the gradually staining specimens into a glass well.
13) Observe the progressing staining reaction under the dissection microscope (white face of bottom plate up).
14) Within 1-5 minutes, specimens should take on a strong region-specific brown stain. Ask course assistants for advise when to stop the reaction. It should latest be stopped, when a general unspecific brown background stain starts developing.
15) Stop the reaction by removing the H_20_2/DAB solution with a pulled-out Pasteur pipette and replacing it with PBT. The H_20_2/DAB solution is transferred back into the original Falcon tube.
16) Rinse at least one more time with PBT.
17) Through slow rotating movement of the glass well collect embryos at the bottom of the well.
18) Use a laboratory pipette with a blue tip to transfer embryos back to a fresh centrifuge tube.
19) Remove PBT (dispose off into the Falcon tube), fill up with 90% glycerol.
20) Centrifuge at 3000 rpm for 2 minutes.
21) Cut off the very tip of a yellow pipette tip with a razor blade, set the pipette to 100 µl, and pick up as many embryos as possible into this volume; to achieve this, place the pipette tip in the area of the tube wall with the highest embryo density, then slowly release the button of your pipette moving the tip gently forward.
22) Transfer to a microscope slides (100 µl per slide) and embed as explained in Protocol 3.
23) All DAB-litter (Falcon tubes, pipette tips, centrifuge tubes) will be collected by the course assistants in yellow bags for incineration.

GENERAL GUIDELINES

5. DOCUMENTATION OF EXPERIMENTS AND FILING OF DATA

5.1. Why bother? A short introduction

Understandably, this RSM can reflect scientific laboratory work only to a degree. For example, the outcome of your experiments is known beforehand (*at least to the assistants*) whereas, during later stages of your career (*e.g. in the context of your final year project or PhD project*), you will carry out experiments of which no one knows the results. Hence, you will have to invest considerable time to reproduce your results, determine their statistical significance, design and carry out control experiments. You will have to interpret your data and extract statements or working hypotheses. This is the essence but also the fun of experimental work! To make you aware of these scientific procedures and train you as well as possible during this RSM, we will encourage you to interpret your specimens and data independently, to speculate, discuss and hypothesise. Based on your observations, we will discuss the outcome at the end of each section.

Essential to good laboratory practice is that you take notes about your experimental procedures, document your results and file your data and documentations in efficient and reliable ways. You should be able to understand your notes, reproduce the experimental procedures and be able to access your documentation many years later. Keeping good protocol

notes is essential part of our assessment in this RSM and requires discipline and care. Thus, at the time of experimentation, many aspects of your work seem self explicit or are daily routine, and you might not feel bothered to take notes. But will you be able to understand the exact design of your experiments, the concentrations of chemicals you used, or the aim of your experiments three years later when you write up your PhD thesis? Furthermore, good protocol notes are pivotal for trouble shooting. For example, an experiment which used to work a year ago can not be reproduced: were ingredients changed since then? Was there a trick you used to apply but have simply forgotten about? Good protocols will save you enormous time! Finally, your documentation and protocols are essential proof of your work which should withstand critical investigation by others. Good Laboratory Practice (GLP) in today's academic or industrial research laboratories describes a wide spectrum of working practices. Scientific research and investigation, by its nature, affects many medical, legal and commercial decisions. Because of the importance of their work, scientists have historically adopted a series of working practices aimed at making their work verifiable.

5.2. Guidelines for the laboratory protocol

You must keep a laboratory note book which is state-of-the-art at any stage of your experiments. Try to keep it understandable not only to you, but also to others. This way you make sure that you will be able to understand and capitalise on your own protocol notes in years to come. We recommend to consider the following:

a) NUMBER and DATE each page used. Use pages in order and do not leave any pages blank nor should any pages be removed. Use only blue or black permanent ink. Do not use correction fluid. Any errors must be ruled through with a single line so that the original text is still visible. An explanation of the reason for the correction must be made in the margin.

b) In case several experiments run in parallel, develop a system to keep them separate in your notes in a clear and understandable way.

c) Write down the AIM OF EACH EXPERIMENT before you start it. This focusses your work and will determine the way in which you document your results.

d) Enter details about your experimental objects. For example, in our module you should enter details about the GENOTYPE (wild type, mutant, transgene?) and the AGE or DEVELOPMENTAL STAGE (which embryonic, larval stage?) of your specimens.

e) Enter details about MATERIALS/CHEMICALS (e.g. fixatives, antibodies etc.). Note that you will carry out only one experiment, but you will be asked to document also the experiments of other groups in the course. Please, take down details (i.e. genotypes and antibodies) of ALL EXPERIMENTS carried out in the course. Record the model and serial numbers of any equipment used in the experiment (this information may be given to you when appropriate).

f) Enter SINGLE STEPS OF YOUR EXPERIMENTS into your protocol. If you use standard operating procedures, protocols or recipes detailed in external sources, you may refer to these in a precise manner (*see g*), but state any changes you have introduced in your actual experiment. Note SPECIAL OBSERVATIONS, PROBLEMS, TIPS, TRICKS, EXPLANATIONS or THOUGHTS which might help you in future.

g) Use clear REFERENCES TO EXTERNAL SOURCES: e.g. refer to pages in the script describing the method in a precise way (e.g. *'larvae were dissected and fixed as described in protocol 1 on page XX of the BL2332 manual, module 1'*). State the existence and location of any original data, objects (*e.g. slides with specimens*) or documentation not attached to the lab book.

h) What was the OUTCOME at intermediate stages or upon termination of the experiment? Which STATEMENTS can you deduce? Has the aim of the experiment (*see c*) been reached? If not, which action needs to be taken?

Please note that failure to complete the notebook will be construed as absence from the practical: as far as we are concerned, if you did not record the experiment, you didn't do it!!

5.3. How to file microscopic specimens

Immediately after you have embedded your specimens you MUST LABEL the respective slides with PENCIL on the FROSTED SLIDE EDGE; Please, write down the following items (*letters refer to accompanying Figure*):

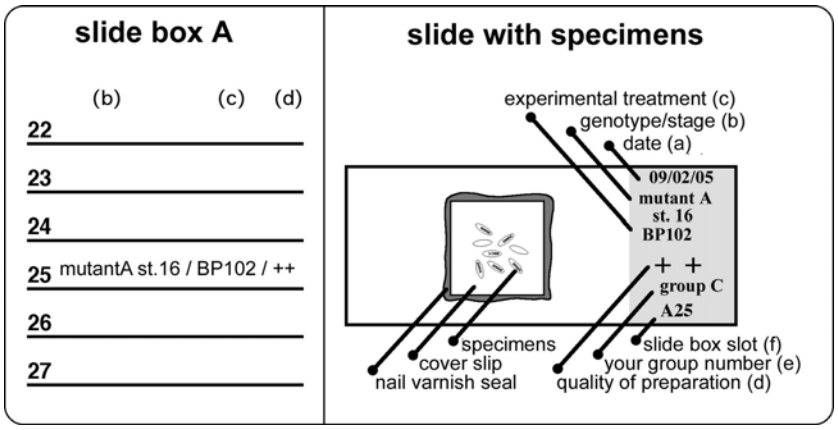


Figure 14. *How to file slides. On the right side a slide with embedded specimens (sealed with nail varnish) is shown; the frosted edge (grey) contains important information (a - f; see text). In this example, BP102 stainings of stage 16 embryos of the mutant strain A have been carried out by group C, they are of high quality and the slide belongs into slot 25 of slide box A. The respective position of the list in the lid of the slide box (left) contains information (b), (c) and (d) allowing for rapid browsing for appropriate preparations.*

a) The DATE at which you carried out the experiment. This information will link directly to the respective notes in your protocol book and will help you to recall the conditions and details of the respective experiment.

b) The DEVELOPMENTAL STAGE of the specimens ("*stage 16*" embryo or "*L3*" = 3rd instar larval stage) and its GENOTYPE (e.g. "*wt*" = wild type, *'mutant A*', or '*elav::comm*' = *elav-Gal4;UAS-comm*).

c) The EXPERIMENTAL TREATMENT (e.g. '*BP102*' = staining with BP102 antiserum); the short information of b) and c) provide a quick overview and reassure the identity of specimens.

d) Add a QUALITY JUDGEMENT upon first microscopic inspection after embedding and before you file the slide in a slide box:

" - " = no staining or too much background

" +/- " = weak but identifiable specific staining

" + " = in principle good staining, but preservation of tissue or orientation of specimens not optimal

" ++ " = all aspects of high quality

Please, reconfirm with the course assistants who may even suggest to discard slides, if the quality is insufficient. This way we make sure that during our documentation sessions no time is wasted on bad preparations in and that preparations of highest quality can be found efficiently.

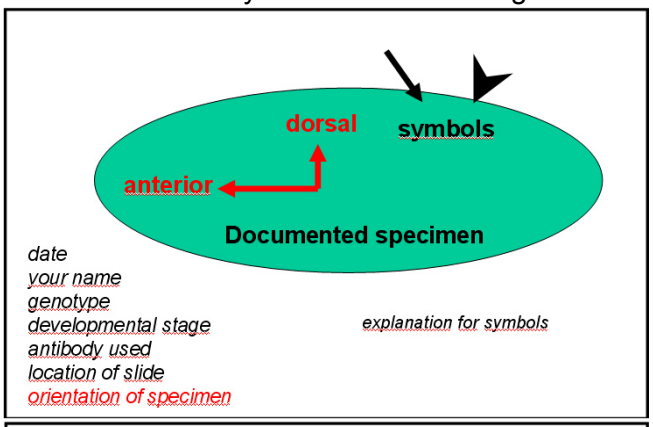
e) Take down YOUR GROUP NUMBER. This way other groups in the course can consult you about specific aspects of your experiment or reassure the specimens' identity.

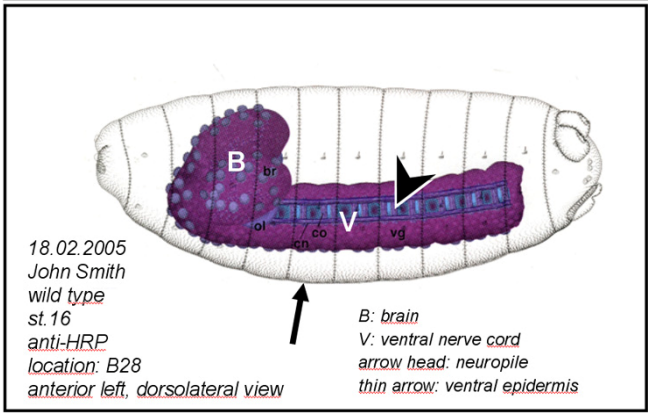
f) Once the slide has been inspected and all information noted on the frosted slide edge, bring it to the common slide box, choose the next available slot and write the number of this SLIDE BOX SLOT onto the slide (e.g. '*B25*' = slide box B, slot 25). *Vice versa*, insert some information about the slide into the list in the lid of the slide box: insert the GENOTYPE, EXPERIMENTAL TREATMENT, DEVELOPMENTAL STAGE and QUALITY JUDGEMENT into this list (see accompanying figure). This way, browsing through the slide box list enables you to find the kind of specimens you intend to document, respectively. Subsequently, preparations can be brought back to their appropriate location to be used by others. Remember to insert the slide box slot position on your documentation sheet to be able to find

the documented specimen efficiently. Questions about your documentation may arise and you might want to reinspect the exact specimen you documented.

5.4. Documentation of results

A crucial part of any laboratory work is the analysis and documentation of data. In this module of the RSM you will carry out microscopic analyses and draw your results. You might think that drawing is old fashioned. However, on the contrary, it is an excellent means to sharpen your observation skills and will demonstrate to the course assistants the degree to which you have understood the features to be seen in the microscope. NEVER DOCUMENT BEFORE YOU HAVE SEEN A FEATURE REPEATEDLY IN DIFFERENT SPECIMENS AND DRAW THE MOST REPRESENTATIVE EXAMPLE. DRAW WHAT YOU SEE - NOT WHAT YOU WANT TO SEE. To facilitate your documentation we will provide you with prepared sheets in which schemes of embryos or tissues are given. ASK QUESTIONS DURING YOUR ANALYSIS: Which clues allow you to assign an immunohistochemical staining to a certain tissue or part of a tissue? Is there a difference between individual specimens, i.e. might there be a subpopulation of mutant embryos in your preparation? If so, define the difference and draw this observation. It is essential that you insert written information on your documentation sheets, as indicated in the figure on the left. The following information should be given:





- DATE
- YOUR NAME
- GENOTYPE
- DEVELOPMENTAL STAGE
- EXPERIMENTAL TREATMENT
- STORAGE LOCATION of the respective slide (i.e. slide box slot)
- ORIENTATION of documented specimen (is it a horizontal or lateral view? where is anterior? where is dorsal?).

Make sure that you will be able at that later stage to understand what you drew. Very helpful is the use of PRECISELY DEFINED SYMBOLS pointing at specific features of the drawing. Such symbols will also facilitate the writing of figure legends, AS WILL BE ONE OF YOUR ASSESSED TASKS!!!

6. COMPOSING A FIGURE AND FIGURE LEGEND FOR SCIENTIFIC PUBLICATIONS

Examples of figures and figure legends are given throughout this manual. However, have a look at scientific publications and look at the figure design and their descriptions. You will find good and bad examples, but ask yourself why you would make this distinction. Please, find below a number of rules or suggestions you can follow when composing a figure and its legend.

1) Think of a statement you want to make with a figure and choose the images supporting this statement.

2) Formulate a title for your figure (e.g. 'Mutations affecting the embryonic neuropile in *Drosophila*').

3) Arrange images in a logical sequence and in right orientation (anterior left or up, dorsal up or right)

4) Label single images with capital letters, and refer to these letters when explaining your images

5) Explain what is to be seen (e.g. which species, which tissue, which stage, which staining); to this end, ...

6) ....group statements common to all or several images, instead of repeating them throughout the legend (e.g. 'All specimens are stained with anti-X', or 'A-C show embryos at stage 16, D-G late larval CNSs'); this strategy saves space and facilitates readability.

6) ....make brief statements about the specific aspect of each chosen image [e.g. 'The neuropile of wild type embryos is composed of commissures and connectives (A), whereas commissures are missing in mutant X (B), and connectives are absent in mutant Y (C)'].

7) Make extensive use of symbols or abbreviations in the figure to guide the reader unequivocally and efficiently through your images [e.g. 'The neuropile of wild type embryos is composed of commissures (white arrow heads in A) and connectives (white arrows in A), whereas commissures are missing in mutant X (black arrow heads in B) and connectives are absent in mutant Y (black arrows in C)']; use symbols consistently throughout your figure.

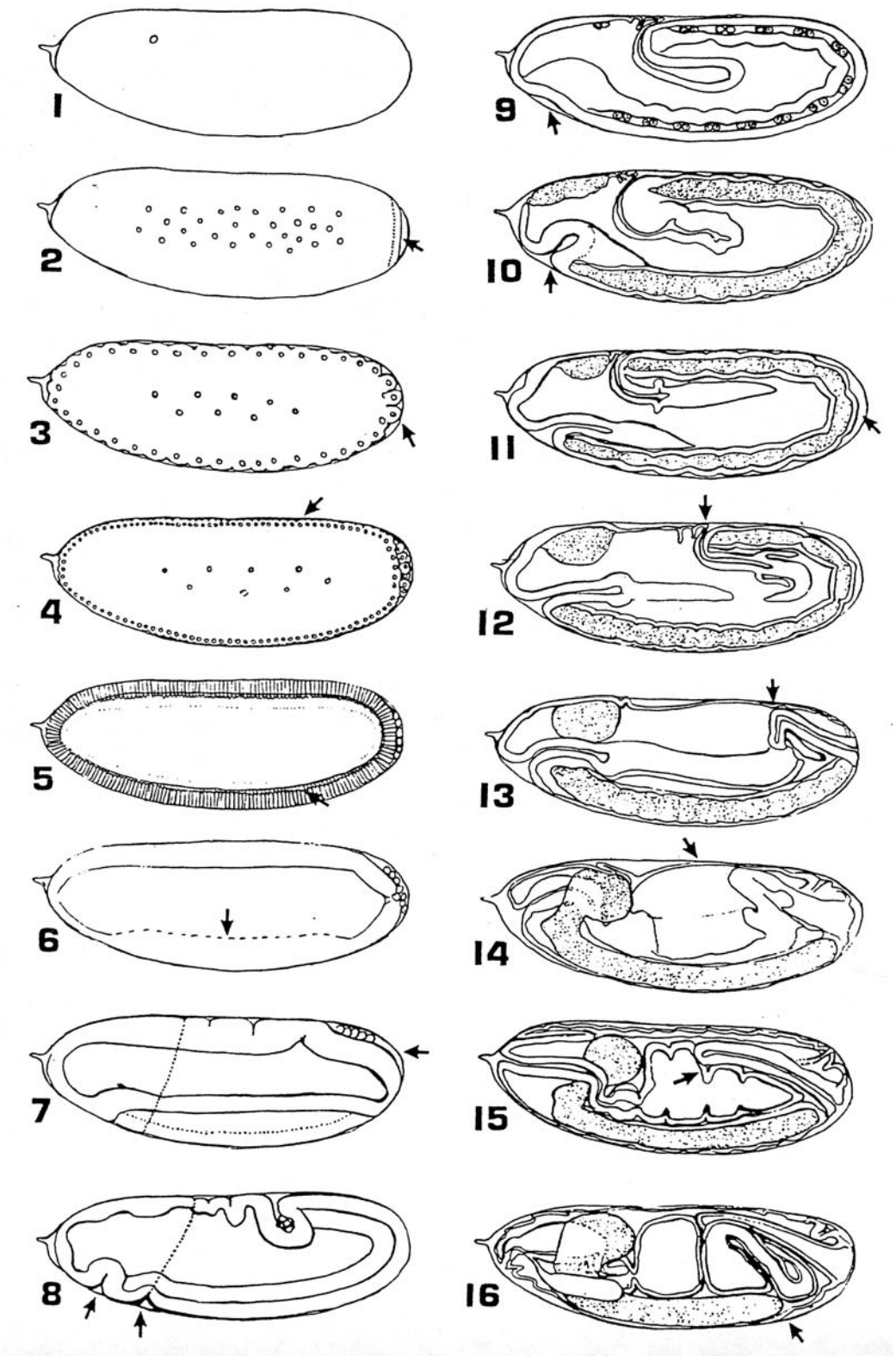
8) You may indicate further information directly within each image, especially if it concerns features differing from image to image (e.g. indicate abbreviated genotype in the top right corner of each image and the respective antibody staining in the bottom right corner). This allows a specialist reader to grasp the content of a figure without having to read through the complex figure legend.

9) All used abbreviations must be explained in the legend.

10) A figure must show a scale bar, if biological material is shown.

APPENDIX

A) Embryonic stages of Drosophila



For more detailed information see:

http://www.flybase.org/allied-data/lk/interactive-fly/atlas/00contents.htm

B) Genetic crosses: Training tasks for over the weekend

Task 1: You have a stock carrying the recessive mutation *m1* over a standard CyO balancer (stock 1). For experimental reasons you want to bring *m1* over a GFP-expressing CyO balancer which, unfortunately, harbours no further dominant genetic marker that would easily distinguish it from the normal CyO balancer chromosome. Currently, you keep the GFP-expressing CyO balancer in a fly stock carrying the recessive mutation *m2* (stock 2), and you can use this stock as a source for the desired balancer. Design a safe strategy by which you can bring the *m1* mutation over this GFP-expressing CyO balancer. You may use stock 3 as a further aid.

stock 1	stock 2	stock 3
$\frac{+}{+}$; $\frac{m1}{CyO}$; $\frac{+}{+}$	$\frac{+}{+}$; $\frac{m2}{CyO,ry+,GFP}$; $\frac{ry^-}{ry^-}$	$\frac{+}{+}$; $\frac{If}{CyO}$; $\frac{+}{+}$

Tip: Be aware that *m1* and *m2* are recessive mutations. How do you make sure that you can follow these chromosomes safely throughout the cross without risking to mix them up?

Task 2: The *M48-Gal4* P-element insertion stock (stock 3) shows Gal4 expression in a subset of commissural neurons in the CNS, the axons of which can be visualised with the help of a *UAS-lacZ* insertion stock (stock 4) and X-Gal staining. You would like to test, whether the *commissureless* mutation (stock 1) affects the axonal pattern of the *M48-Gal4*-positive neurons in homozygosis. To be able to select the *commissureless* mutant animals, the mutation should be kept over a GFP-expressing TM3 balancer, the presence of which can be easily spotted under a fluorescent microscope (stock 2).

a) Which is the genotype of the embryos you would want to analyse?

b) What are the genotypes of the parents of the embryos in (a)?

b) Design crosses to generate the parental strains in (b) as established maintainable fly strains, using the following stocks as source:

stock 1	stock 2
$\frac{+}{+}$; $\frac{+}{+}$; $\frac{comm}{TM6B, Hu}$	$\frac{w^-}{w^-}$; $\frac{If}{CyO}$; $\frac{TM3, Ser, GFP}{Sb}$
stock 3	**stock 4**
$\frac{w^-}{w^-}$; $\frac{M48\text{-}Gal4, w^+}{M48\text{-}Gal4, w^+}$; $\frac{+}{+}$	$\frac{w^-}{w^-}$; $\frac{UAS\text{-}lacZ, w^+}{UAS\text{-}lacZ, w^+}$; $\frac{+}{+}$

Tip: The w^+ on the P-elements gives orange eyes, the endogenous *white* locus on the first chromosome gives red eyes.

C) Genetic crosses: Training tasks for 2nd Monday

Task 1: You have a stock carrying the recessive mutation *m1* over a standard CyO balancer (stock 1). For experimental reasons you want to bring *m1* over a GFP-expressing CyO balancer which, unfortunately, harbours no further dominant genetic marker that would easily distinguish it from the normal CyO balancer chromosome. Currently, you keep the GFP-expressing CyO balancer in a fly stock carrying the recessive mutation *m2* (stock 2), and you can use this stock as a source for the desired balancer. Design a safe strategy by which you can bring the *m1* mutation over this GFP-expressing CyO balancer. You may use stock 3 as a further aid.

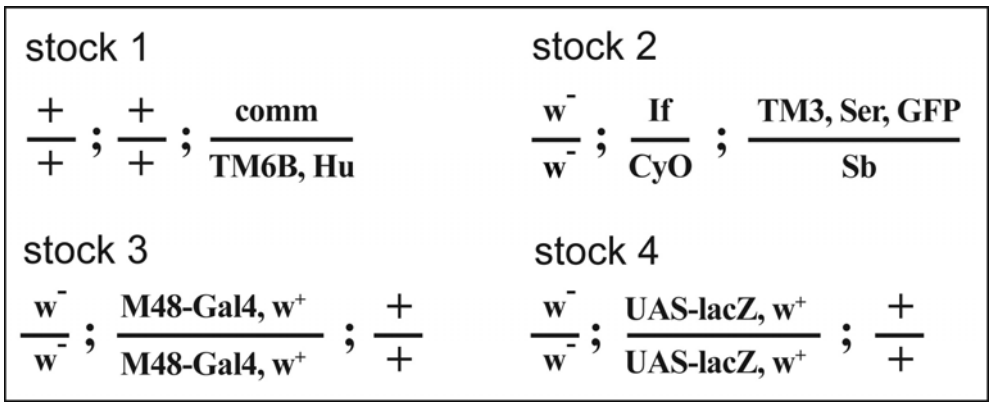
Tip: Be aware that *m1* and *m2* are recessive mutations. How do you make sure that you can follow these chromosomes safely throughout the cross without risking to mix them up?

Task 2: The *M48-Gal4* P-element insertion stock (stock 3) shows Gal4 expression in a subset of commissural neurons in the CNS, the axons of which can be visualised with the help of a *UAS-lacZ* insertion stock (stock 4) and X-Gal staining. You would like to test, whether the *commissureless* mutation (stock 1) affects the axonal pattern of the *M48-Gal4*-positive neurons in homozygosis. To be able to select the *commissureless* mutant animals, the mutation should be kept over a GFP-expressing TM3 balancer, the presence of which can be easily spotted under a fluorescent microscope (stock 2).

a) Which is the genotype of the embryos you would want to analyse?

b) What are the genotypes of the parents of the embryos in (a)?

b) Design crosses to generate the parental strains in (b) as established maintainable fly strains, using the following stocks as source:



Tip: The w^+ on the P-elements gives orange eyes, the endogenous *white* locus on the first chromosome gives red eyes.

D) Genetic crosses: Training tasks for 2nd Tuesday

1) You want to carry out experiments with a P-element insertion P(lacZ,w+) on the third chromosome (stock 2) in combination with a dominant mutation, likewise on the third chromosome (stock 1). You need to recombine both onto the same chromosome. Design a suitable crossing scheme. You may make use of stock 3.

stock 1	stock 2	stock 3
$\frac{+}{+}$; $\frac{+}{+}$; $\frac{M}{TM3, Sb}$	$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{lacZ,w^+}{lacZ,w^+}$	$\frac{w^-}{w^-}$; $\frac{If}{CyO}$; $\frac{Sb}{TM6B, Hu}$

Tip 1: Remember that dominant markers show a phenotype in heterozygosis. Let's say, mutation *M* causes gaps in wing veins.

Tip 2: The w+ on the P-element produces an orange rather than red eye colour.

2) You have identified a novel 2nd chromosomal mutation called ***shot*** which, when in homozygosis, correlates with an exciting brain phenotype. You want to proof that the brain phenotype is indeed caused by loss of *shot* function. To this end you perform a gene rescue experiment in embryos. This experiment involves that you express the cloned *shot* gene in the nervous system of *shot* homozygous mutant embryos, with the aim of recovering normal brain morphology.

- For this you have generated a ***P{UAS-shot,w+}*** transgenic line (stock 1) where the P-element is inserted on the third chromosome; unfortunately the insertion turns out to be lethal in homozygosis.
- You hold a suitable transgenic fly stock carrying the viable ***P{sca-Gal4,w+}*** insertion on the second chromosome (stock 2); this Gal4 line targets expression throughout the nervous system.

- stock 1: ***w/w; +; P{UAS-shot+,w+}/TM3,Sb*** (orange eyes; shorten to $\mathbf{P^U w^+}$)
- stock 2: ***w/w; P{sca-Gal4,w+}/P{sca-Gal4,w+};+/+*** (orange eyes; shorten to $\mathbf{P^G w^+}$)
- stock 3: ***+/+; shot/CyO; Sb/TM6B,Hu***
- stock 4: ***w/w; If/CyO,S; CxD/TM3,Ser*** (dominant S on CyO,S causes rough eyes; dominant D on CxD causes lack of alulae from wing hinges)

Design the genetic crosses required for this task, using the above stocks. To make this task easier, please, address first the following questions:

a) Write down the genotype of the embryos in which you can assess rescue of the *shot* mutant phenotype.

b) To obtain these embryos, you will have to establish two independent parental stocks that can be kept in the laboratory for future purposes. Please, write down the genotypes of these two parental stocks.

c) Design the crossing strategies to obtain these two parental fly lines using the above stocks.

E) Additional resources

Information about the theoretical aspects of pattern formation

http://www.eb.mpg.de/dept4/meinhardt/home.html

http://www.cmp.caltech.edu/~mcc/Patterns/index.html

General data bases for *Drosophila*:
http://www.flybase.org
http://flybase.bio.indiana.edu/allied-data/lk/interactive-fly/aimain/1aahome.htm

An important stock centre for *Drosophila* fly strains:
http://flystocks.bio.indiana.edu/

A manual to *Drosophila* genetics: Greenspan, R. J. (1997). "Fly pushing: The theory and practice of *Drosophila* genetics." Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York

Information about *Drosophila*-specific experimental procedures: Sullivan, W., Ashburner, M., and Hawley, R. S. (2000). "*Drosophila* Protocols." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Information about the embryonic development of *Drosophila* (especially embryonic stages):
Campos-Ortega, J. A., and Hartenstein, V. (1997). "The embryonic development of *Drosophila melanogaster*." Springer Verlag, Berlin
http://www.flymove.de
http://www.flybase.org/allied-data/lk/interactive-fly/atlas/00contents.htm

Information about the Gal4/UAS system: Duffy, J. B. (2002). GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. *Genesis* 34**,** 1-15

Information about the *Drosophila* nervous system:
http://www.prokop.co.uk
http://flybrain.neurobio.arizona.edu

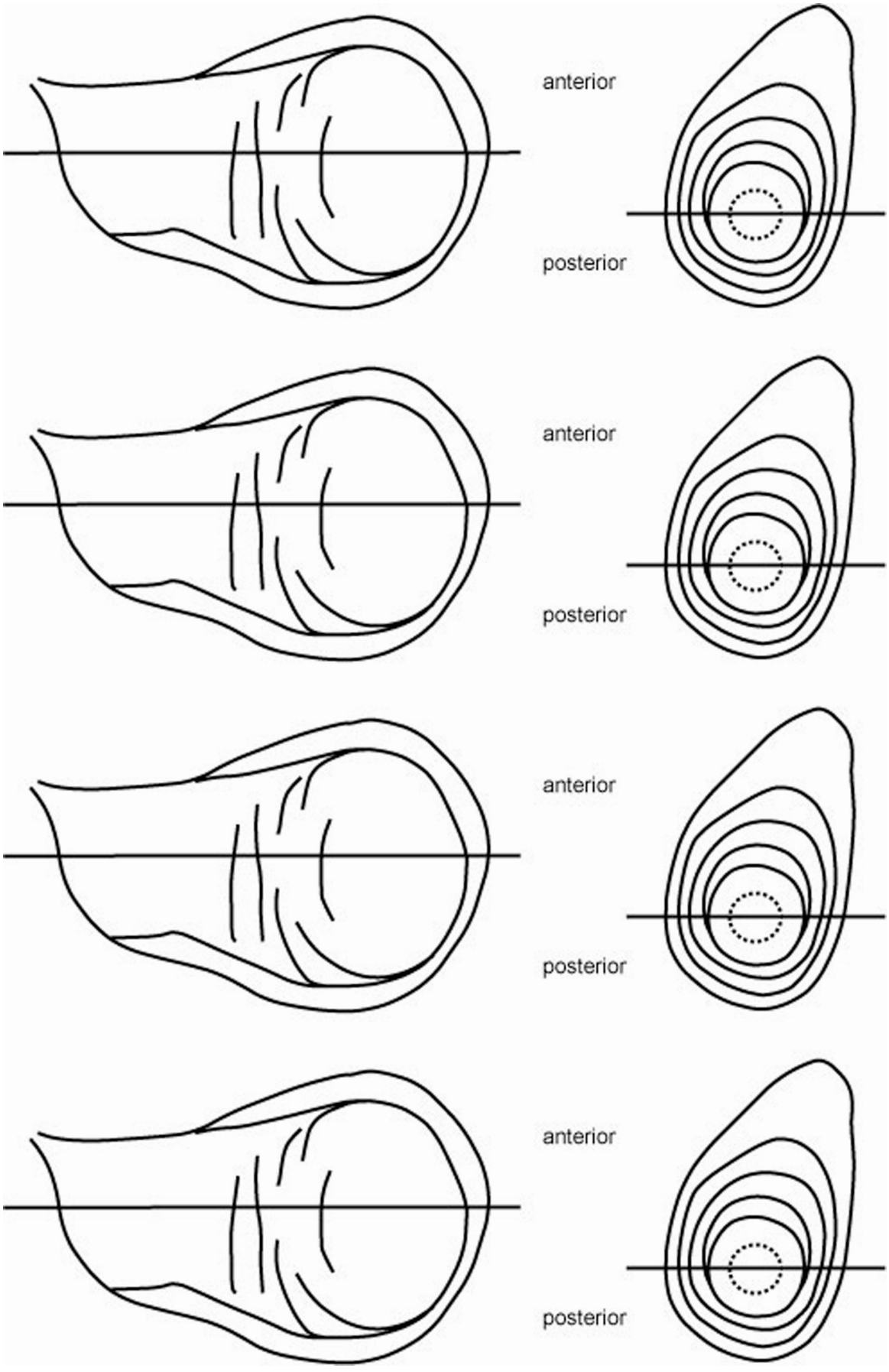
Medline, a general data base for scientific literature:
http://www.ncbi.nlm.nih.gov

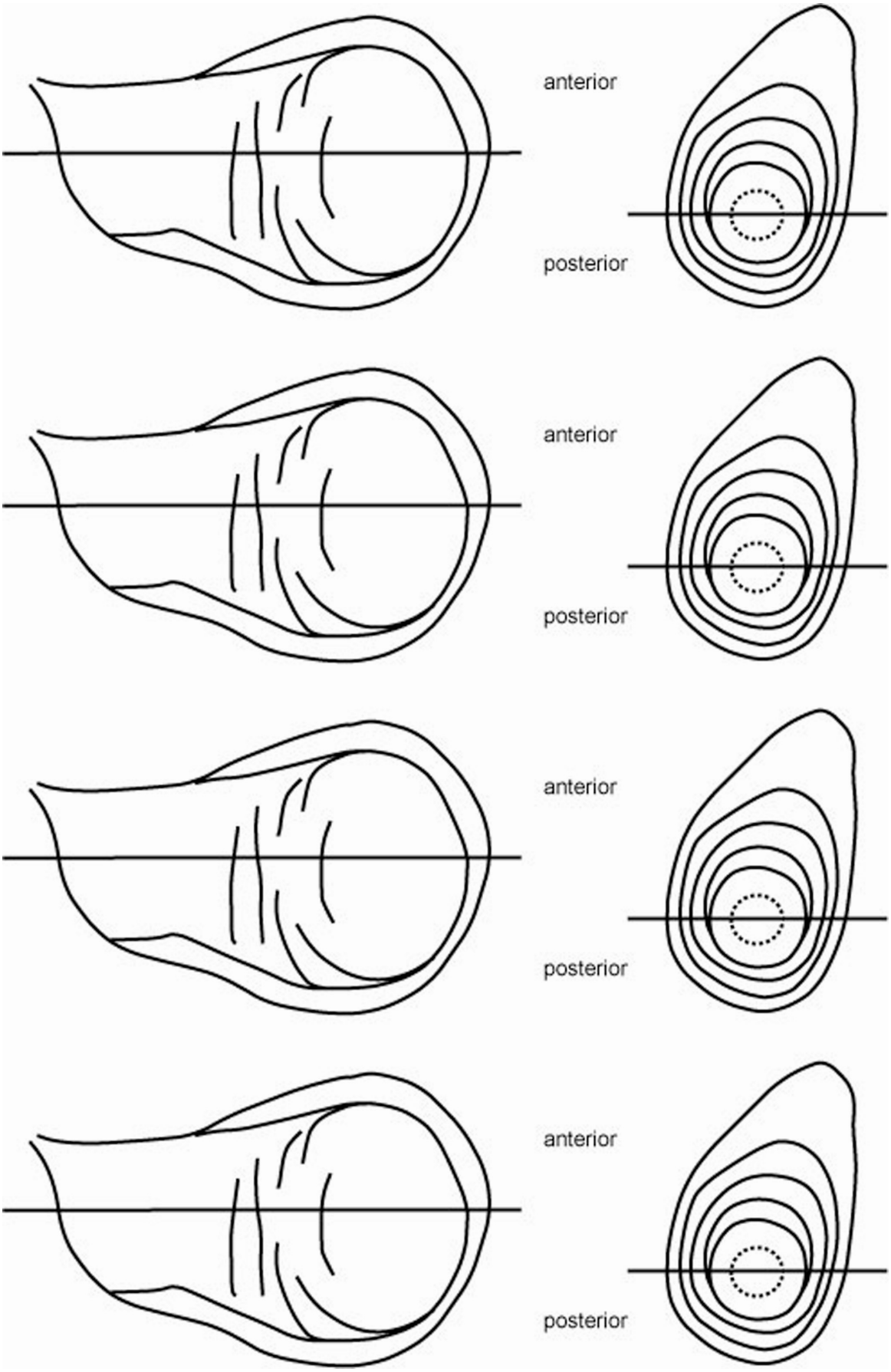
DSHB, a good resource for monoclonal antibodies:

http://www.uiowa.edu/~dshbwww

A good read on *Drosophila* history:
Kohler, R. E. (1994). Lords of the fly. *Drosophila* genetics and the experimental life (Chicago, London, The University of Chicago Press)
Weiner, J. (1999). Time, Love, Memory : A Great Biologist and His Quest for the Origins of Behavior (New York, Alfred A. Knopf)

F) Drawing templates

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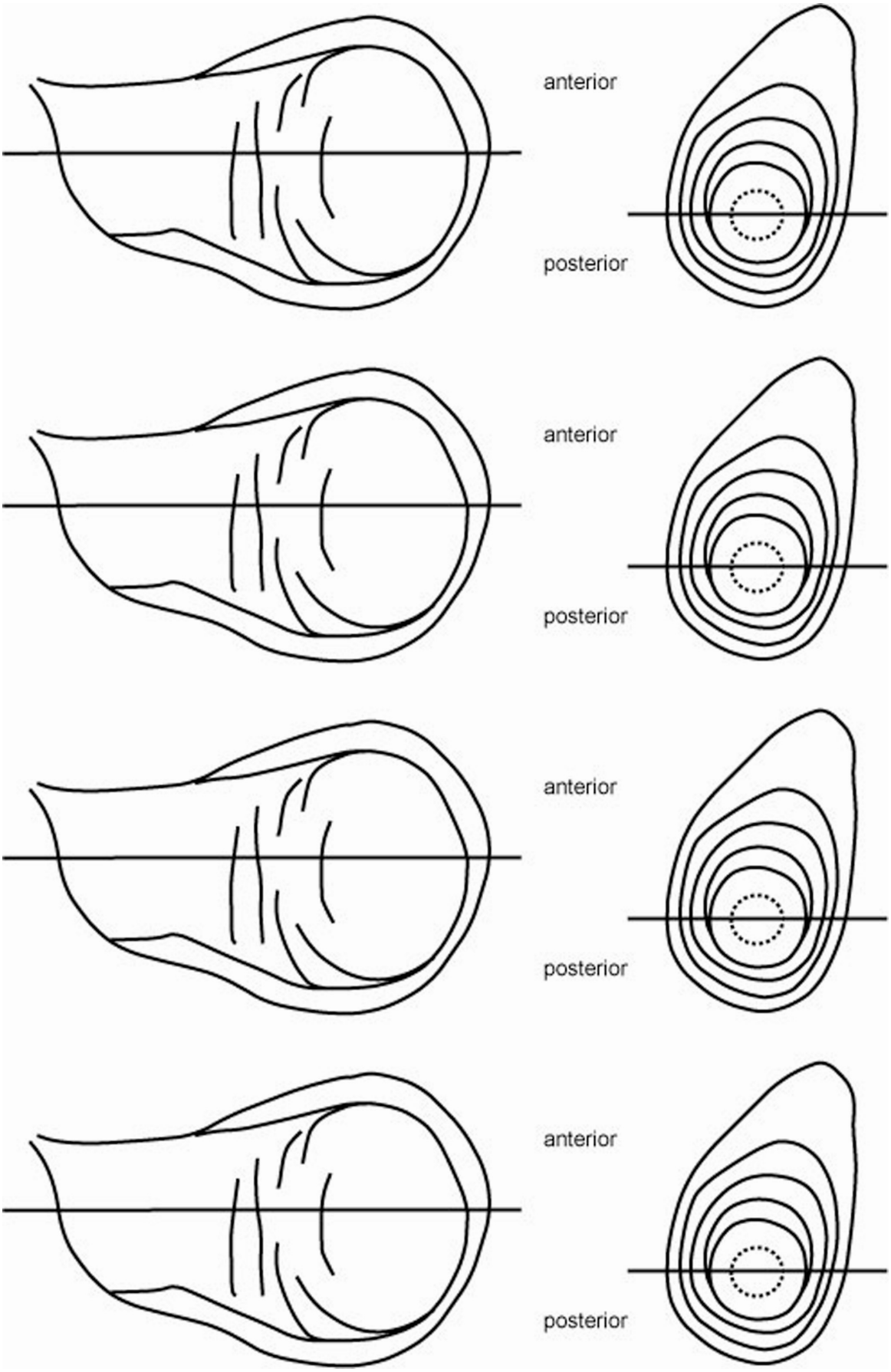
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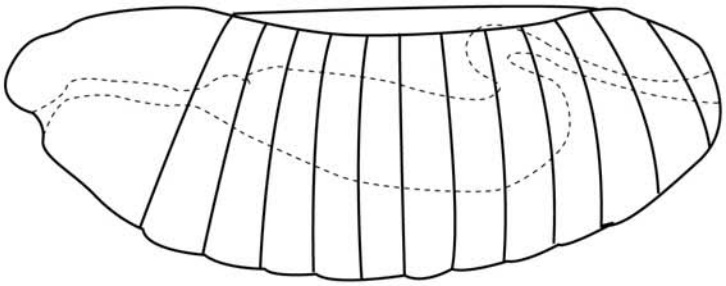

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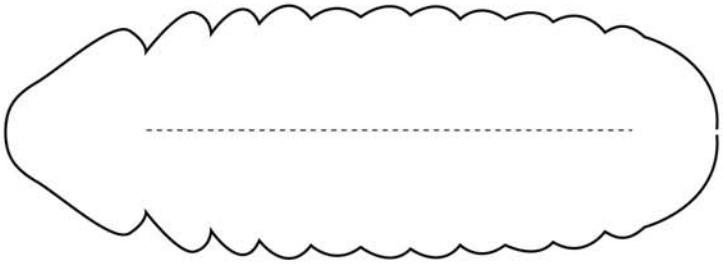

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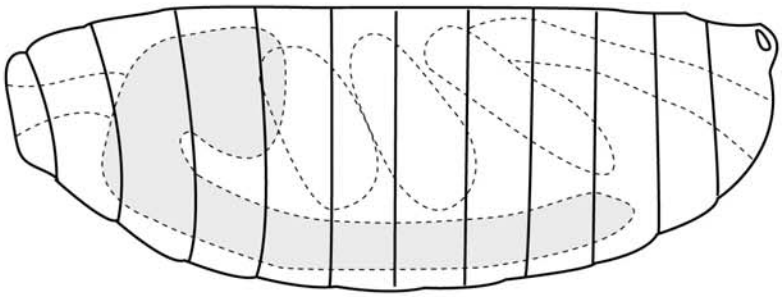

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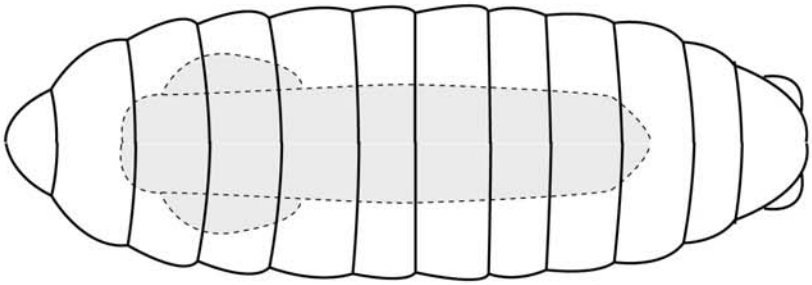

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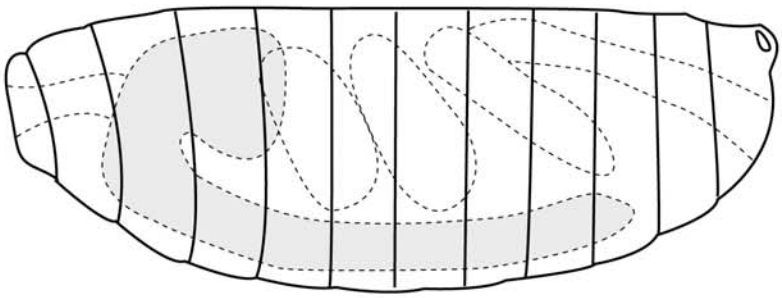

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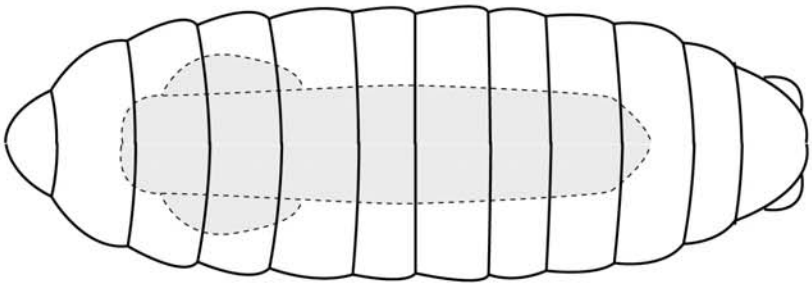

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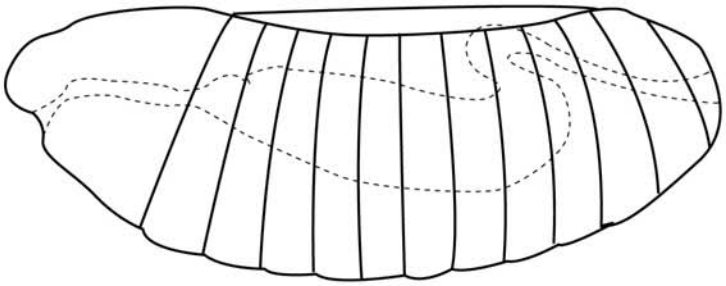

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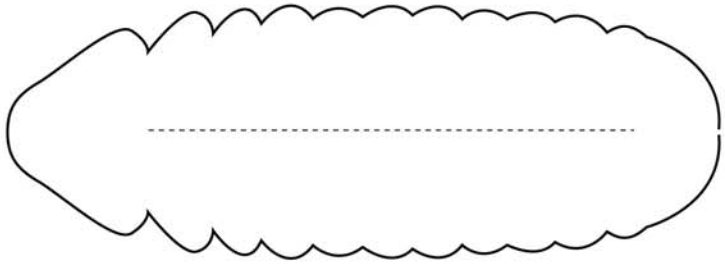

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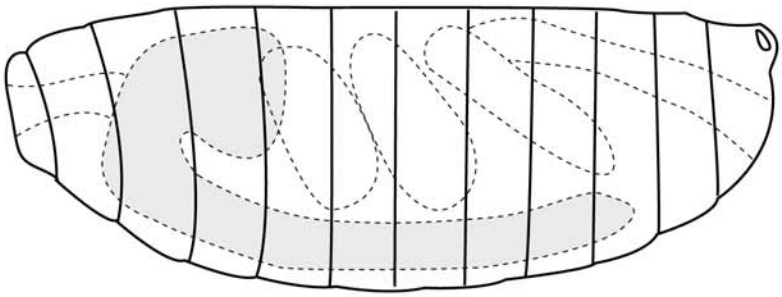

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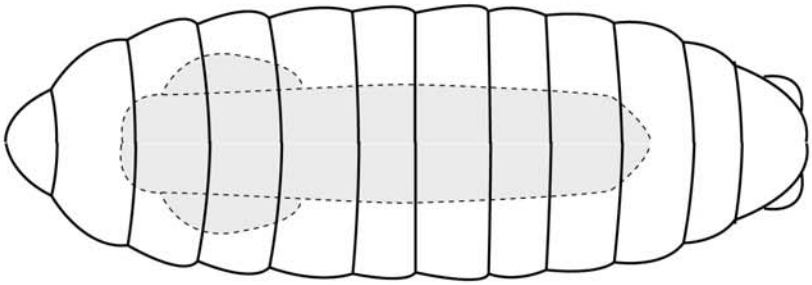

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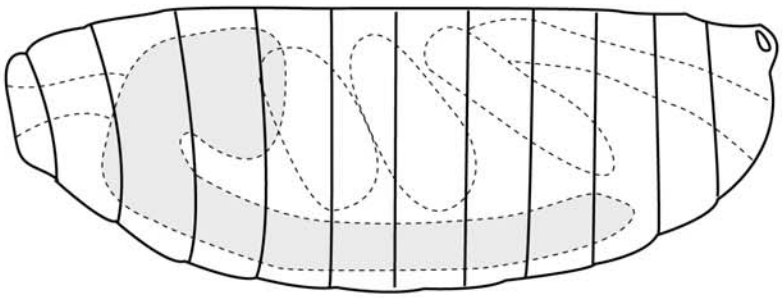

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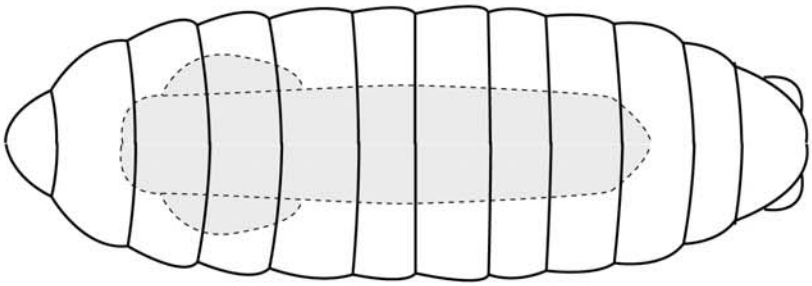

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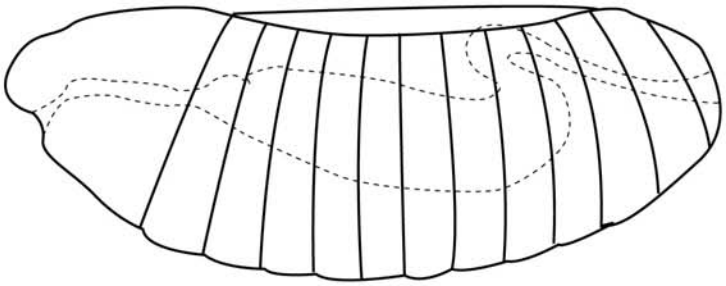

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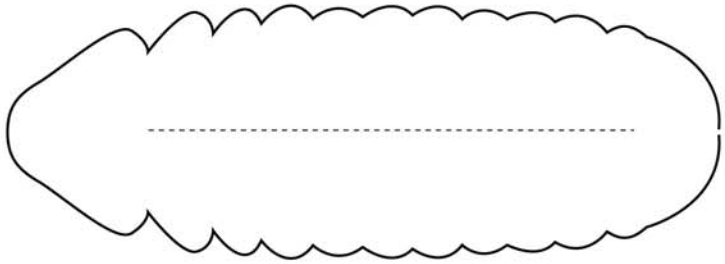

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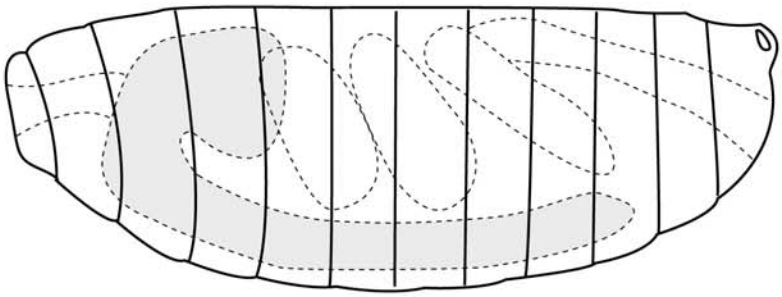

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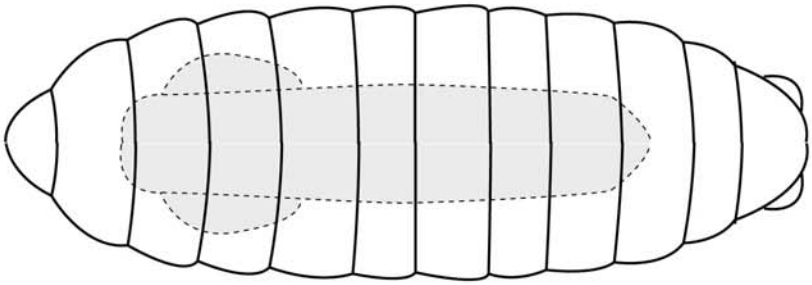

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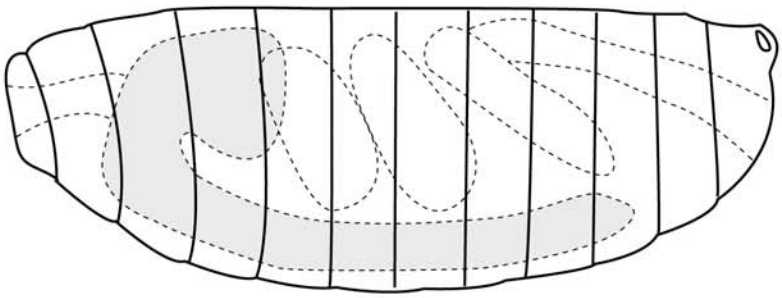

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