MANCHESTER 1824

The University of Manchester

BIOL20332
GENETICS RSM

BIOL20972
DEVELOPMENTAL BIOLOGY RSM

MODULE 2

17-27/02/2014

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Faculty of Life Sciences

MODULE 2: BASIC INFORMATION

MODULE 3: BASIC INFORMATION

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 sections 7 and 12 (see time table next page). LAB COATS are REQUIRED! Please, direct questions regarding this module to Andreas.Prokop@manchester.ac.uk.
Course assistants in 2013: Victoria Coyne, Jessica Doyle, Daniel Howard

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 shar.es/Yis4C)
- Roote+Prokop-SupplMat-3.ppt (available on shar.es/YcX2f)

1.5. Assessment

35% for module 3 that are split as follows:

- 20% for genetic problem solution. Online assessment
- 15% for submitted figure and legend. Marked by lecturer

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 AT shar.es/Yis4C)*
- ***FlyGenetics-IntroStudents.doc*** *(AVAILABLE AT shar.es/Yis4C)*

Monday, 1st week (17th of February) - section 7 of 2MUL

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 (18th of February) - section 7 of 2MUL

11.00-12.30 PC cluster2, bays C: self-study of "Roote+Prokop-SupplMat-3.ppt" (*AVAILABLE AT shar.es/Yis4C*)
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 (20th of February) - section 7 of 2MUL

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*
13.45-14.30 Survey and Genetic skills: *Solving a simple task handed out in the course*
14.30-16.00 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 (24th of February) - section 12 of 2MUL

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 (25th of February) - section 12 of 2MUL

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 27th of February**)*

Thursday, 2nd week (27th of February) - section 12 of 2MUL

11.00-12.30 *PC cluster 2, bays C:* Genetic skills assessment: *solve genetic crosses*
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 how genetic research in *Drosophila* has been and continues to be instrumental in generating fundamental understanding of biological processes.

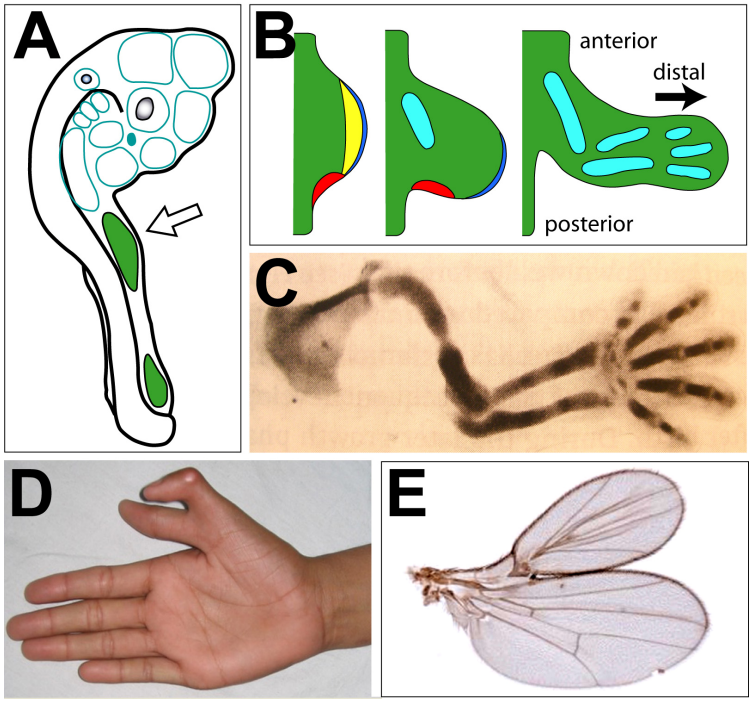


***Figure 1**. Patterning during limb formation. **A**) In the developing embryo, limb buds (green) are formed in stereotypic positions. **B**) Determinants in the apical ectodermal ridge (dark blue), the progress zone (yellow) or polarising region (red) pattern the developing limb bud along the anterior-posterior and proximal-distal axis, as illustrated by the cartilage structures (light blue), the precursors of future bones of arm and hand (see **C**). **D,E**) 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 patterning functions were first discovered and understood in flies where they cause comparable phenotypes, as illustrated by this wing duplication.*

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, cells have to know whether they contribute to the distal hand or proximal arm, to an anterior thumb or the posterior 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, and much of this understanding was pioneered by work on the *Drosophila* wing (Fig.1E).

Week 2: The **guided growth of axons** is a key developmental process that electrically wires the brain and 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) which can be up to a meter away in our body (Fig.2A,B). 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 for every neuron. For this, the growing tips of axons called **growth cones** (arrow heads in Fig.1A) make stereotypic step-by-step directional decisions along their path through interpreting chemical and physical properties of the surrounding tissues. For this, they use cocktails of guidance cues in form of diffusible or contact-mediated molecules which have been precisely arranged in space and time through the processes of pattern formation. To ensure that each axon will make the right growth decisions that will lead to functional neural circuits, patterning and cell differentiation processes in the nervous system have to ensure that each growth cone is equipped with its appropriate individual receptive machinery. Therefore, it is pivotal that patterning processes, during neurogenesis in the nervous system as well as in all tissues that axons grow through, are precisely coordinated. In this RSM module we will look at genetic mechanisms governing one particular choice event at the midline of the CNS, i.e. to either stay on the same body side (red, blue, light green in Fig.1) or to cross over to the other half (dark green in Fig.1).

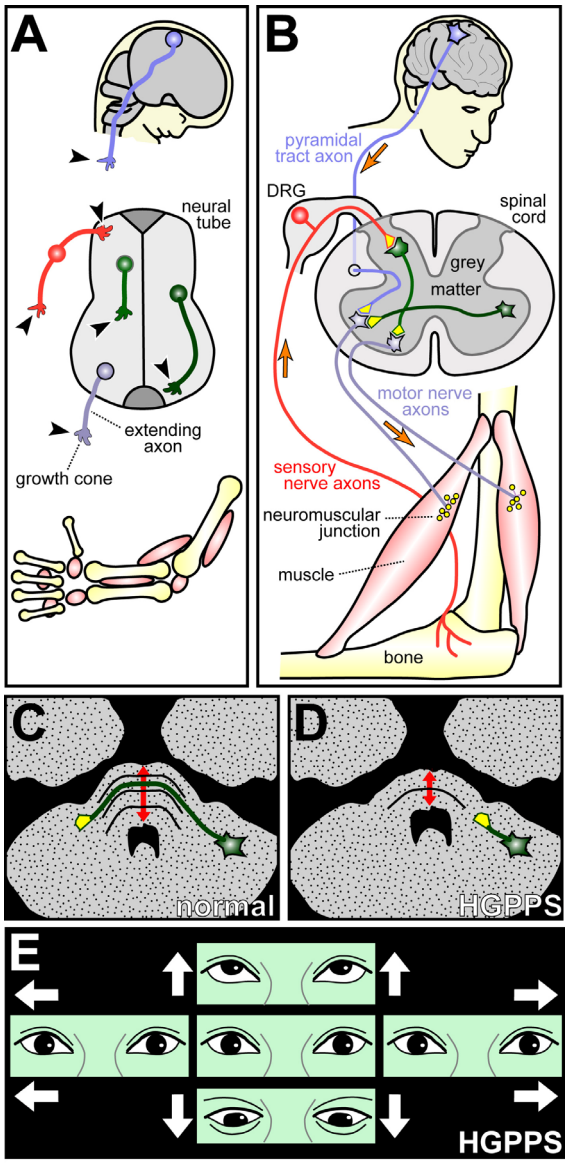


Figure 2. *Examples of inherited human diseases relevant to this Genetics RSM module:*
A) *In the developing nervous system, neuronal axons grow out along reproducible paths via growth cones at their tips (arrow heads).* ***B)*** *Stereotypic guidance of axonal growth ensures that sensory neurons (red), interneurons (green) and motorneurons (blue) arrange into correctly hard-wired circuits that conduct action potentials along slender axons over long distances (orange arrows) and pass them on at synapses to their appropriate partner cells (yellow triangles and circles).* ***C-D)*** *Mutations of the robo3 gene cause Human gaze palsy with progressive scoliosis (HGPPS; Jen et al., 2004, Science 304, 1509ff.), and HGPPS patients have a severe reduction of commissures (red double-arrows), suggesting that commissural neurons (green) fail to cross the CNS midline.* ***E)*** *HGPPS patients show a coordination deficit across the body axis, for example the inability to move eyes horizontally. Work in* Drosophila *was instrumental in paving the way to a molecular understanding of this disease (Fig.8).*

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 (Figs. 1D, 2C-E).

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 full genomic sequences were available, it has become

clear that many genes are evolutionary conserved and 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*, 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. In this context, you will get introduced to **transposable elements** and the manifold opportunities they offer as **transgenic tools**, as well as to **loss-of-function** analyses using gene **mutations** (see the "Rough guide" for details). We will discuss how research based on these genetic strategies can help to advance understanding even 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 be trained in the **design of genetic mating schemes**. You will learn how classical Mendelian rules are applied, and what genetic tools are available for carrying out genetics as well as experimental work in *Drosophila*, and thus appreciate the use of this model organisms in great depth. A Manual and a PowerPoint presentation are available on Blackboard as eLearning resources to prepare you for this part of the course.

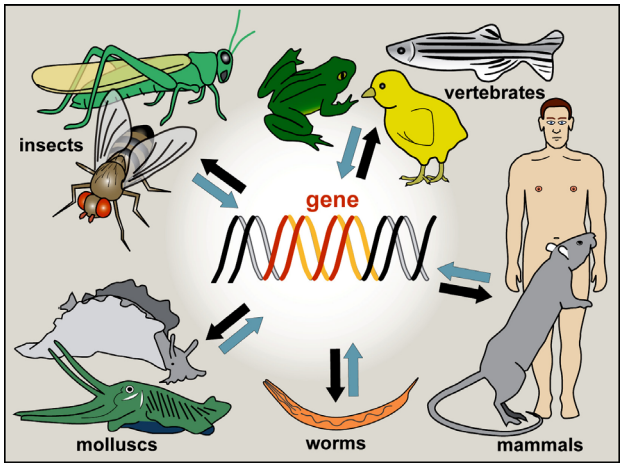


***Figure 3**. The use of model organisms: Many different model organisms are being used to study biological mechanisms and phenomena. Researchers have to weigh advantages versus disadvantages of each particular model organism to choose the most suitable one for as respective scientific problem. Since 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. Groups of two students will work together throughout the course. Experimental procedures to be carried out will be the same for everybody, but the genotypes and antibodies used will differ between groups. 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

OBJECTIVE: The aim of this experiment is to describe the specific spatial expression patterns of selected genes in imaginal discs of Drosophila *larvae, in order to illustrate developmental patterning mechanisms as well as the experimental use of transgenic enhancer trap lines or promoter constructs.*

3.1. Background on imaginal discs

As a holometabolous insect, *Drosophila* has a pupal stage in which the worm-like maggot metamorphoses into the elaborate shape of the adult fly. To accelerate the pupal stage, in which the animal is immobile and vulnerable, all adult tissues are "pre-assembled" in the larva and set aside as defined entities which are 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 imaginal discs can be clearly assigned to the future structures of the adult fly (Fig.5). In week 1 we will capitalise on the well described leg and wing discs to study genes that regulate their patterning.

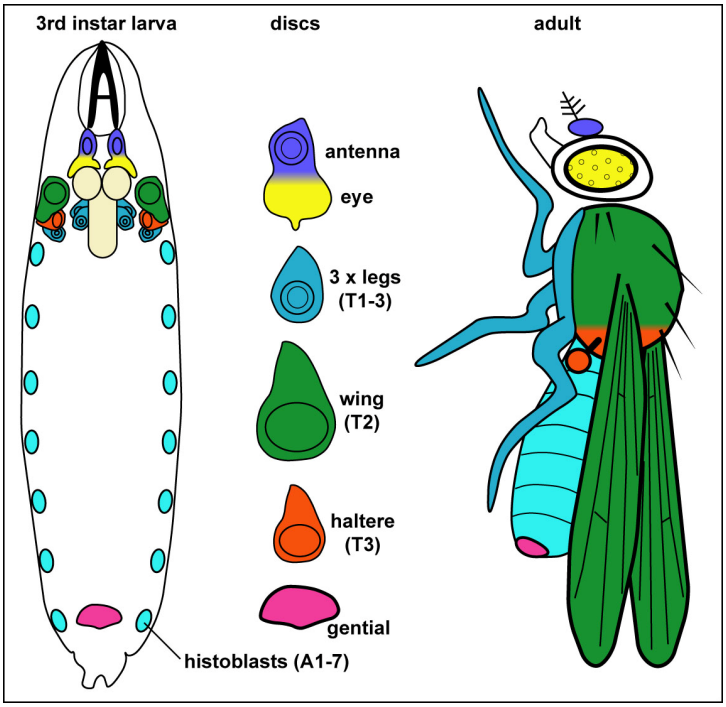


***Figure 4**. Position and prespective fate of the various* Drosophila *imaginal discs. The image shows the position of the various imaginal discs in the late larva (3rd instar larva, left) their shapes and relative sizes (middle) and, following a consistent colour coding, the part of the adult fly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

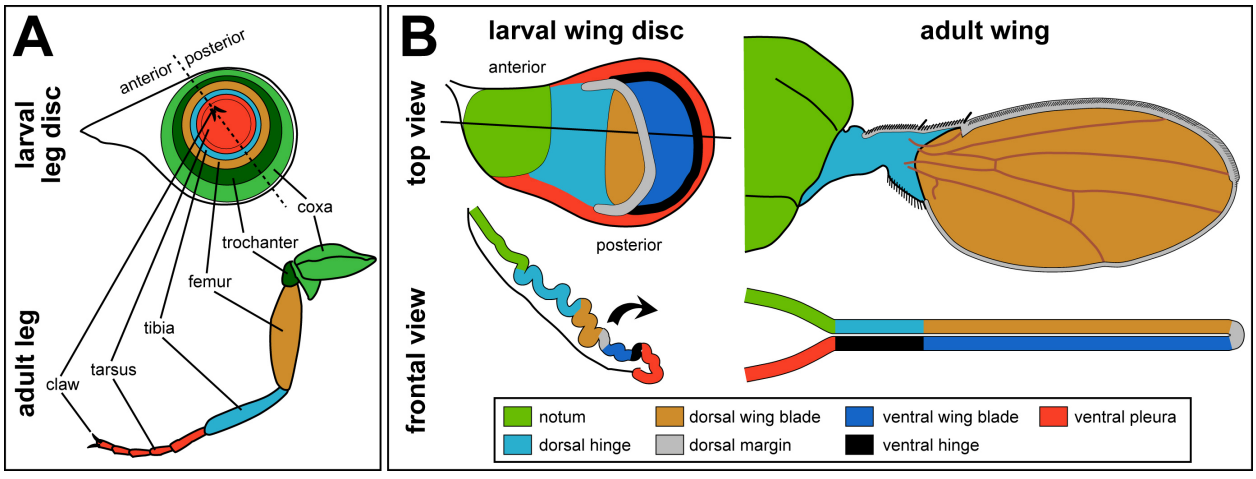


***Figure 5**. Fate maps of* Drosophila *imaginal discs. **A**) Correlation of leg disc areas to structures of the adult leg (named and colour coded). **B**) Top and frontal view of a larval wing disc and adult wing, both colour coded to illustrate the correlation between leg disc areas and wing structures.*

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, you will (1) dissect out imaginal leg and wing discs from *Drosophila* larvae, (2) stain them with X-Gal to make the spatial expression patterns mediated by the various P-element insertions visible, (3) embed these specimens on a slide, (4) use a compound microscope to analyse and document the stained imaginal discs aiming to map the localisation of stained areas as precisely as possible into a standardised scheme (Appendix G) and try to understand what prospective wing or leg structures the staining can be assigned to (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):

Group	larval genotype	staining	comments
	A-lacZ	*2hr 37ºC, then o.N. 26ºC*	
	B-lacZ	*2hr 37ºC*	
	C-lacZ	*2hr 26ºC*	
	D-lacZ	*2hr 37ºC (may be 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 figshare.com (http://shar.es/Yis4C).

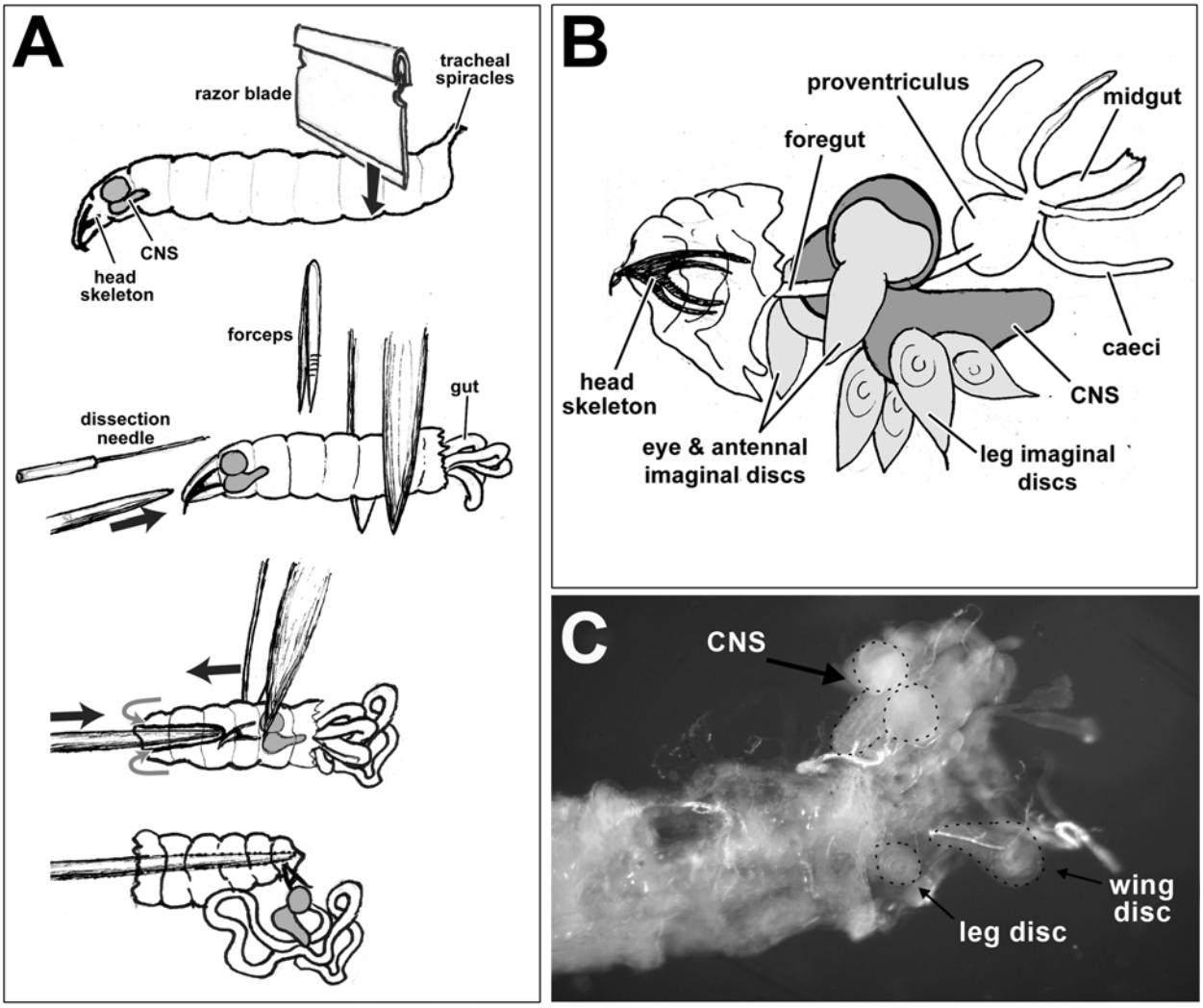


***Figure 6**. Dissection of late* Drosophila *larvae. **A)** The different steps of the inversion technique: larvae are cut close to their posterior tips with a blade (anterior tip can be identified by the dark head skeleton, posterior tip by the prominent spiracles), gently hold the larva with a forceps avoiding to squeeze its tissues, 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)** Photo of an inverted larval carcas with CNS, wing discs and the third pair of leg discs as seen under the microscope.*

Step-by-step procedure:

1) Fill a Sylgard dish 1/3 with 1X PBS
2) Select a few larvae with a forceps (use fat whitish larvae 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; choose the black side of the dissecting microscope's bottom plate and position the goose neck of the light source such that the light shines in a flat angle from the side at the larva (spotlight effect).
4) Hold larvae with the forceps to limit the wiggling movements of the posterior tip; avoid squashing internal organs by applying only gentle pressure.
5) 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*).
6) 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 inwards by moving the needle forwards between

the shanks of the forceps so that the larva is gradually turned inside-out (*grey curved arrows* in *Fig. 6A*). Make sure that the inversion is complete; otherwise the specimen will flip back into its natural outside-in orientation when stripping the carcas off the needle.

7) Upon completion of the body wall inversion, all internal organs are exposed (*Fig. 6A*). Whilst the carcass is still placed on the needle, carefully remove the biggest chunks of digestive tract and fat body. Make sure that you do not remove the CNS and imaginal discs which are connected to the anterior part of the body wall (*Fig. 6C*).
8) 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 at its very tip to obtain a narrow opening) to remove the PBS. But make sure that specimens do not fall dry!
9) Replace with 0.5-1ml of fixation solution (*1% glutaraldehyde in PBS; see Section 4.4.1 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 encodes the enzyme β-galactosidase and 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 concept of a reporter gene is that it "reports" the transcriptional activation of a gene regulatory element under experimental conditions. In our RSM module, we use P-element insertions which mediate *lacZ* expression resembling the patterns of endogenous genes. In order to detect the *lacZ* expression patterns in the larval discs, we will assay its enzymatic activity using X-Gal as substrate (*Fig. 7*). Alternatively, the presence of β-galactosidase can be visualised through immunohistochemistry using antibodies specifically directed against this enzyme (*see week 2 and Protocol 5*).

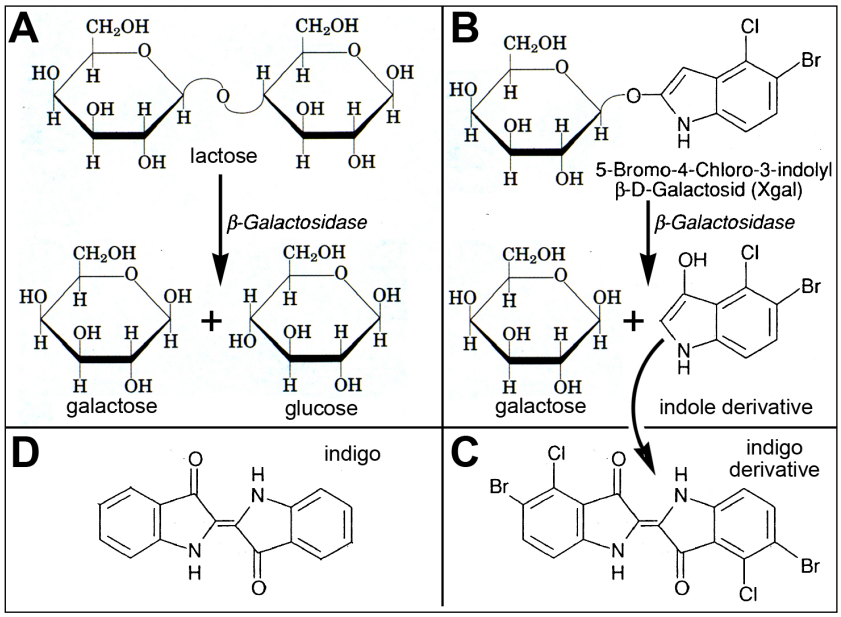


***Figure 7**. The mechanism of X-Gal staining. **A**) In* E. coli*, β-galactosidase cleaves lactose into galactose and glucose. **B**) In a similar manner, it can cleave X-Gal into galactose and an indole derivative. (**C**) Upon oxidation (curved arrow) this indole derivative dimerises into an indigo derivative with conjugated double-bonds that absorbs light efficiently. **D**) Indigo in comparison.*

3.4.2. SOLUTIONS

- X-GAL SOLUTION: 5% X-Gal (*5-bromo-4-chloro-3-indolyl-ß-d-galactoside; Fig. 7B*) in N,N-dimethyl formamide.
- INCUBATION BUFFER: 3 mM $K_4FeII(CN)_6$, 3 mM $K_3FeIII(CN)_6$ (*kept as 30 mM stock solutions, respectively*) and 1 mM $MgCl_2$ (*kept as 10 mM stock solution*), all dissolved in PBS (*0.2 M, 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

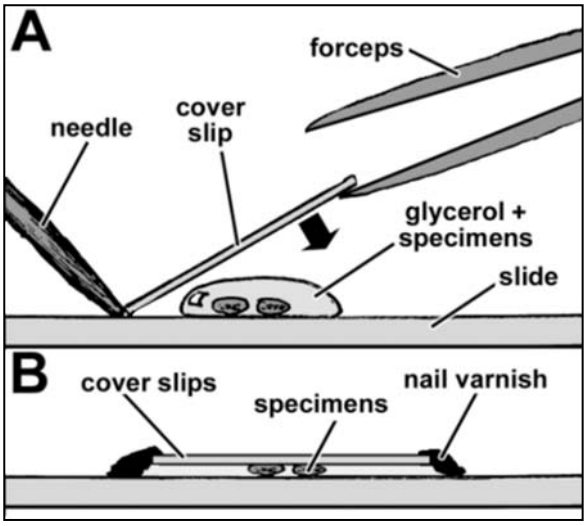
1) Following fixation in 1% glutaraldehyde (*end of Protocol 1*), wash larval specimens once in incubation buffer.
2) Make sure that the staining solution has cooled down to room temperature equal to the temperature of the specimens (this prevents precipitation of X-Gal crystals), then replace the incubation buffer with staining solution. The X-Gal component in the staining solution serves as substrate for β-galactosidase, whereas $K_4FeII(CN)_6$ and $K_3FeIII(CN)_6$ act as oxidants for the released indole derivative (*Fig. 7B,C*).

The following will be performed by course assistants:

3) Incubate at room temperature or at 37°C for several hours or over night (see table in 3.2.).
4) Once staining can be seen, shake the microfuge tube and tip its content straight into a glass well. Hold the microfuge tube against light to check whether specimens remained in the tube. If so, fill it up once more with staining solution and repeat the above process.
5) When satisfactory staining can be seen, stop staining reaction by removing staining solution and adding 70% glycerol.

3.5. Embedding of larval discs (Protocol 3)

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



1) Using a forceps, stained specimens are transferred singly into a big drop of 70% glycerol on a slide.
2) Place the slide under a dissection microscope (*white side of the base plate facing up*)
3) Using needle and forceps, remove wing and leg discs from the stained carcasses and gather them on one side of the drop.
4) Note that wing discs tend to come off the carcasses and float loosely in the glass vials. Check carefully!
5) 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 one shank of the forceps into this drop.
6) When a couple of leg and wing discs have been collected in this drop, use a forceps to place a cover slip with one side next to the glycerol drop (*use needle to keep it in position; A in Fig.*), then slowly lower the whole cover slip (*arrow*). Using this procedure you prevent inclusion of air bubbles.
7) Seal all fringes of the cover slip with nail varnish (*B in Fig.*).
8) Make sure that your slides are properly labelled and filed according to the instructions given under 'General guidelines'.

4. EXPERIMENTAL WORK OF WEEK 2

OBJECTIVE: The aim of this experiment is to describe nervous system defects of selected Drosophila *mutant embryos, in order to illustrate molecular mechanisms that regulate axonal CNS midline crossing as well as the experimental use of genetic loss-of-function analysis.*

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 common principle is that axons of certain classes of neurons cross the midline of the nervous system, whereas axons of other sub-groups of neurons never cross but stay ipsilateral (on the same side of the body where their soma lies; Fig.8). Mechanisms underlying this simple choice are shared between vertebrates and *Drosophila*. They have been discovered first in *Drosophila* through the use of genetic screens (explained in the "Rough guide). Here, you will get to know some of these genes and experience how their functions can be studied.

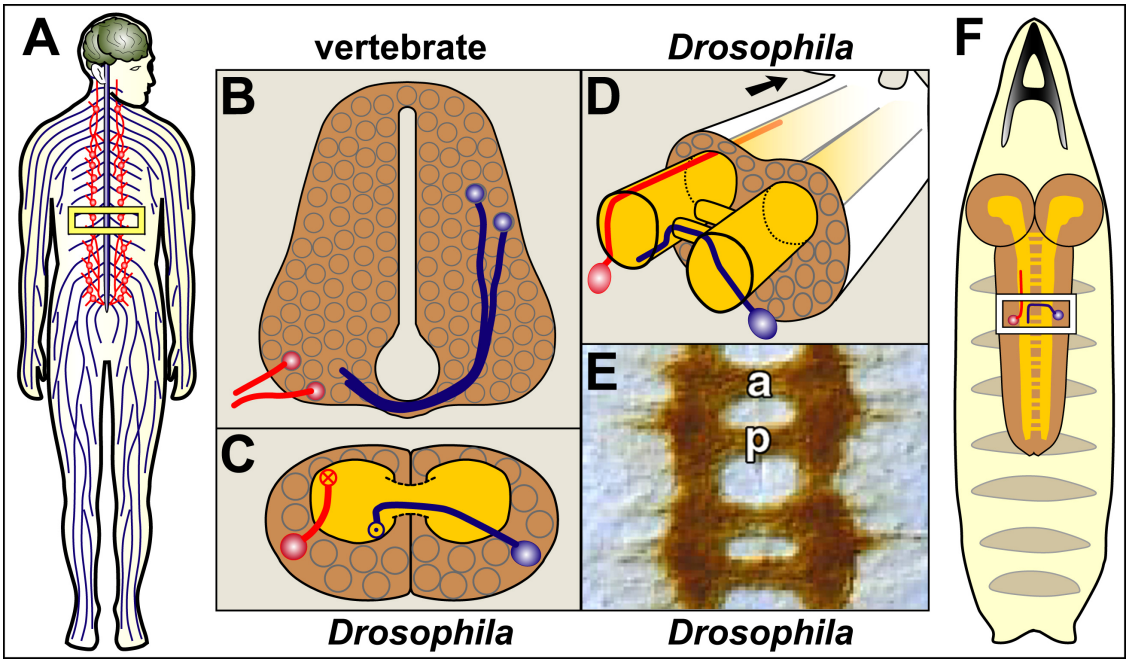


***Figure 8**. Axon crossing at the CNS midline. **A)** Schematic view of the human nervous system with brain (green), spinal cord, nerves and autonomous nervous system. **B**) Cross section through the developing vertebrate spinal cord (corresponding to boxed area in A) with blue axons crossing the midline. **C-E)** Different views of the developing ventral nerve cord of* Drosophila *(corresponding to boxed area in F); axons crossing the midline are shown in blue, ipsilateral axons in red; the specimen in E is stained with BP102 antibody (brown) which highlights the axonal area called neuropile (orange in C, D, F; a, anterior; p, posterior commissure). **F**) Top view of a* Drosophila *larva.*

4.2. Overview over experiments of the second week

You will study the functions of genes involved in the guidance of neuronal growth at the midline of the embryonic *Drosophila* CNS. For this, you will perform antibody staining (immunohistochemistry) to analyse the phenotypes of mutant embryos in which the 'pathfinding or guidance genes' A, B and C are dysfunctional. As readout, you will use antibodies which mark preferentially the neuropile (*BP102, α-Fas2; Figs. 8 and 9*). Complementary to this, you will use antibody staining of wildtype embryos to analyse the natural expression of proteins A and C, or to detect ß-Gal-expression of the C-lacZ enhancer trap line (mimicking the anti-C pattern). Like in week 1, gene names will not be disclosed until the final discussion.

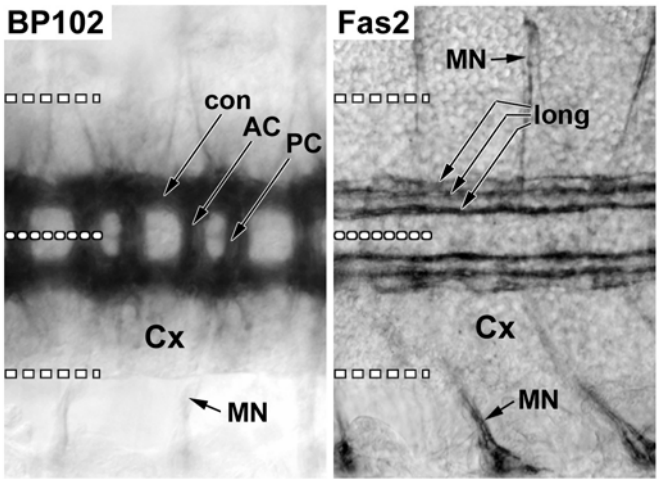


***Figure 9**. Staining patterns of BP102 and Fas2 in the embryonic CNS (compare Fig.8, D-F). Horizontal views of CNSs (dashed lines, lateral borders; dotted line, midline) stained for BP102 (labelling the synaptic neuropile) or Fas2 (labelling a subset of 3 longitudinal axon bundles within the connectives of the neuropile, as well as all motornerves). AC, anterior commissure; con, connective; Cx, cortex; long, longitudinal axon bundles; MN, motornerve; PC, posterior commissure.*

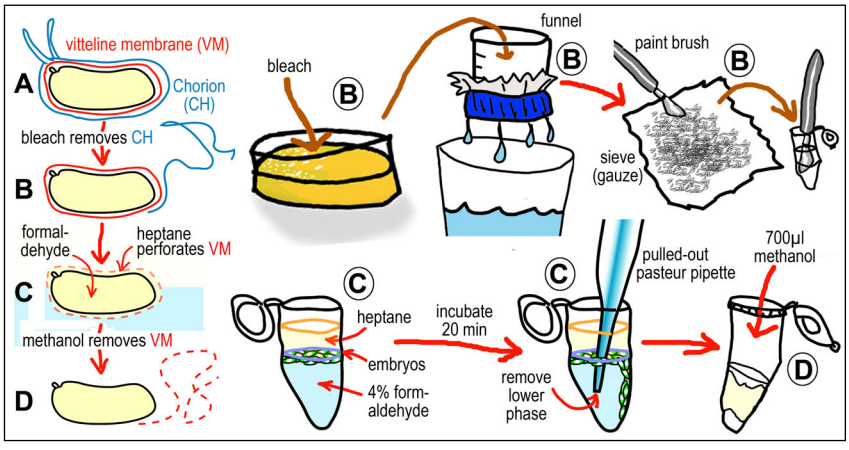
Table of experiments (week 2):

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 of maggot and fly and is a protective sheet impenetrable to any aqueous solutions. 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; see **A** in image[1]) which can be removed through straightforward chemical procedures:

1) Hand-pick all flies and bigger dirt particles off the agar plates.
2) To remove the chorion, fill the petri dishes with 50% household bleach for 1 min.; under the dissection micro-scope you can observe that embryos float to the surface and shed their chorion (dorsal appendages no longer visible; **B** in image).
3) Pass embryos together with the bleach through a funnel with sieve (see image); use water to wash any remaining embryos off the plate



[1] images modified from original drawings by Christoph Rickert (Mainz)

into the funnel.

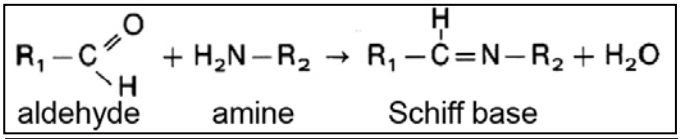
4) Wash embryos thorough-ly with water to remove all residues of bleach.
5) Detach the sieve and, using a small paint brush, transfer the embryos from the sieve into a microfuge tube containing 500µl of heptane and 500µl of a 4% formaldehyde solution in PBS. Embryos float at the interface of hydrophobic 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 (**C** in image).
7) Use a pulled-out Pasteur pipette to remove most of the lower phase.
8) To remove the vitelline membrane from the embryos (**D** in image), add 700µl of methanol, close the tube immediately and shake rigorously for one minute (ideally using a vortex); the vast majority of embryos should turn white (alcoholic denaturation) and sink to the bottom of the tube; 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, then add PBT. Note that in PBT the embryos take some time to sink to the bottom.

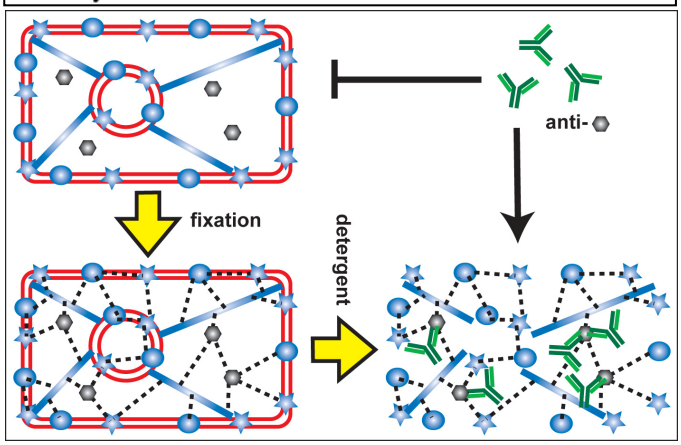
4.4. Immunohistochemistry (Protocol 5)

4.4.1. BACKGROUND

Immunohistochemistry provides the possibility to visualise proteins in their natural position in tissues or organisms. Antibodies can be produced by repeatedly injecting proteins or peptides with specific epitopes into animals, such as rabbits, goats, horses, donkeys, sheep, rats, guinea pigs, mice. After a few weeks, their natural immune response usually provides amplified amounts of 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*).

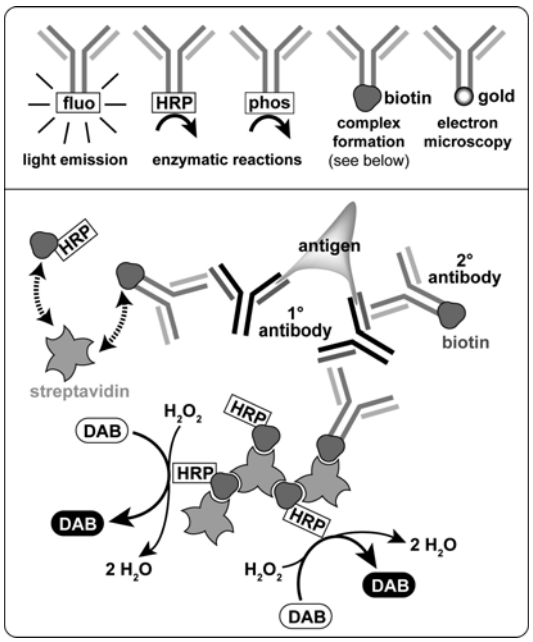
Here, we will use antibodies raised against *Drosophila* proteins or against β-Gal from *E. coli*, which will be used as readout for the analysis of mutant phenotypes or to detect expression patterns of endogenous or reporter genes. For this, *Drosophila* embryos will be fixed with aldehyde (*usually formaldehyde; see Protocol 4*). Aldehyde fixation is based on a chemical reaction (Schiff condensation; *see upper image*) which cross-links amino groups (*black dashed lines in lower image*) to preserve tissues in natural shapes and proteins in their typical sub-cellular positions. Subsequent treatment with detergent strips the cell membranes of their lipid components (*red double lines*) and makes intracellular proteins accessible to antibodies. Ideally, antibodies will localise to all cells and tissues where where their target epitopes/proteins are expressed.





In order to visualise the specifically localised 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 forms complexes with avidin, (d) gold particles which are impenetrable for electrons and are therefore visible as sharp black dots under the electron microscope. In order to improve visibility and sensitivity of antibody stainings amplification steps can be used. For this, 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*). This has two experimental advantages: Firstly, primary antibodies do no longer have to be labelled themselves using time consuming biochemical procedures, but can they can be detected using secondary antibodies linked to any label of choice, providing a versatile detection system that can be flexibly adapted to different experimental needs. For this, a range of secondary antibodies is usually kept in laboratories, ready for use at any time. Secondly, a single primary antibody is usually recognised by several secondary antibodies at a time, i.e. the signal of antibody stainings is usually enhanced and the detection sensitivity increased when employing secondary antibodies.



Here we will use the ABC-detection system (*strept**a**vidin-**b**iotin-**c**omplex; 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 will assemble around or be trapped at biotin-coupled secondary antibodies. If the added free biotin is itself linked to a detection agent, this will cause a significant accumulation of this agent at an antigenic site. Here we will use biotin coupled to the enzyme HRP, which is a peroxidase derived from horseradish plants. Its natural task is to perform a redox reaction that catabolises H_20_2, thus protecting cells from this reactive oxygen species. Accordingly, the staining procedure will involve that you add H_20_2 as the electron acceptor and diaminobenzidine (*DAB*) as the electron donor for the HRP reaction. Oxidised DAB produces a conjugated double bondage system which absorbs light and produces a brownish stain.

4.4.2. SOLUTIONS & MATERIALS

- Pulled out Pasteur pipette, laboratory pipettes, table centrifuge, razor blade, disposal bags for incineration
- PBT: PBS containing 0.1-0.3% of the detergent Triton-X
- Primary antibodies diluted in PBT: anti-A (*1:5; mouse*), anti-C (*1:5; mouse*), anti-Fas2 (*1:10; mouse*), anti-BP102 (*1:10; mouse*), anti-ßGal (*1:1000; rabbit*)
- Biotin-coupled secondary antibodies diluted in PBT: anti-mouse and anti-rabbit (*both 1:400; donkey*)
- Streptavidin-biotin-complex (ABC) solution: 1:100 solution A (avidin) and 1:100 solution B (HRP-coupled biotin) in PBT; as preincubation step, shake/rotate for ~20 min (not longer than an hour)
- DAB staining solution: 0.02% H_20_2 and 0.1% DAB in PBT

4.4.3. STAINING PROTOCOL FOR IMMUNOHISTOCHEMICAL PROCEDURES

1) Remove methanol and add PBT for 1hr to dissolve cell membranes and thoroughly rinse from fixative and methanol.
2) Use a pulled-out Pasteur pipette to remove as much PBT as possible without loosing specimens and add the antibody solution assigned to you (*will be handed out*).
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 solution of secondary biotin-coupled anti-mouse or anti-rabbit antibody (*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) Rinse 2 times with PBT over a period of 10 min.
8) Remove PBT and add the ready prepared ABC solution (*will be provided*); keep agitating for 1hr at room temperature.
9) Rinse 2-3 times with PBT over a period of 10-15 min; in parallel prepare your dissecting scope (light from top, white side of bottom plate up)
10) Remove PBT and add DAB staining solution (*handed out in a 15ml Falcon tubes*). You must wear gloves to avoid skin contact! DAB-contaminated liquids, tips and tubes will be collected in yellow bags for incineration!
11) Close centrifuge tube, shake it, then quickly open and pour its content into a glass well; take up some liquid from the glass well to wash out embryos that got stuck in the tube.
12) Observe the progressing staining reaction under the dissection microscope - stop on time (usually within 1-5 minutes) to avoid excessive background staining - course assistants will be around to help you make the right decision
13) Stop the reaction by removing the DAB staining solution with a pulled-out Pasteur pipette into the original 15ml Falcon tube and replace with PBT.
14) Rinse at least one more time with PBT and dispose waste liquid into the original 15ml Flacon tube.
15) Through slow rotating movements concentrate embryos at the bottom of the well, and use a 1000 µl laboratory pipette (*blue tip*) to transfer embryos into a fresh centrifuge tube.
16) Remove PBT into the 15 ml Falcon tube and fill the centrifuge tube up with 90% glycerol.
17) Centrifuge at 3000 rpm for 2 minutes; embryos will settle on one side of the tube, easy to take up with a pipette.
18) 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 whilst sucking in embryos.
19) Transfer to a microscope slides (100 µl per slide) and embed as explained in Protocol 3 (section 3.5.).
20) DAB-litter 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

This RSM aims to reflect scientific laboratory work in areas of data analysis, data/specimen storage and note keeping. First, in your future scientific life (*e.g. during your final year or PhD project*) you will routinely carry out experiments of which no one knows the results. Hence, you will have to invest considerable time to interpret your data and extract sensible

information/statements, determine the statistical significance of your observations, and design and carry out control experiments to further validate your findings. To illustrate what this means, we will encourage you to interpret your specimens and data independently, to speculate, discuss and hypothesise. For example, you will use the basic information provided in this manual to develop criteria that you can use to assign specific X-Gal staining under the microscope to its respective area in the wing disc; you should apply logic and precise thinking rather than "guestimation". At the end of each week, we will use your results to discuss the experimental outcome.

Second, it is a frequent requirement in scientific life to revisit data or specimens that were generated years ago. In such a situation you must be able to find and access relevant materials, understand your notes, and reproduce experimental procedures or conclusions. It is pivotal that you take good notes about your experiments and file your specimens and data in efficient and reliable ways. This requires discipline, care and feasible strategies. Also note that your documentation and protocols are essential proof of your work which should withstand critical investigation by others.

5.2. Guidelines for the laboratory protocol

You must keep a laboratory note book which is up-to-date at any stage of the experiment. An experiment that has not been properly recorded has not been done!! However, consider that in a real laboratory situation you will not have the time to write long texts and explanations. Instead, try to keep your notes concise but nevertheless precise and understandable. Try whether others can understand them - as a good test demonstrating how likely it is that you will understand your own notes in years to come. Consider the following:

a) NUMBER and DATE each page used. Use permanent ink and no correction fluid. Any errors must be crossed out with a single line so that the original text is still visible. The correction must be justified in words.

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) Before starting, be clear about your experiment from its design through to its documentation. Write down the AIM OF EACH EXPERIMENT before you start. This is a good way to make sure you really understand the rationale. If you are unclear or disagree, re-discuss with your supervisor. Consider that you want to invest your time ONLY in sensible experiments!

d) Enter experimental details. For example, in this module you must note down the GENOTYPE (wild type, mutant, transgene?), the DEVELOPMENTAL STAGE of specimens (embryonic, larval), the MATERIALS/CHEMICALS used (e.g. fixatives, antibodies etc.). Please, take down details, such as genotypes and antibodies, of ALL EXPERIMENTS carried out on this course, since you are expected to document them all. Usually, you would also take down some notes about important EQUIPMENT used, but this is not relevant here.

e) Note down SINGLE STEPS OF YOUR EXPERIMENTS. If you use protocols or recipes which are detailed in external sources, you may refer to them in a precise manner (*see f*), but state any changes you have introduced in your actual experiment and keep written track of where you are at any stage of the protocol.

f) SPECIAL OBSERVATIONS, PROBLEMS, TIPS, TRICKS, EXPLANATIONS or THOUGHTS that cross your mind might help you in future, and you might want to write them down. However, make sure you separate them out from the actual experimental details (e.g. box them in as "side remark").

g) Use clear REFERENCES TO EXTERNAL SOURCES: e.g. refer to pages in the script (*'larvae were dissected and fixed as described in protocol 1 of the BL20332 RSM manual 2014, module 2'*). Note down the existence and location of any used materials, such as slot references for specimen slides used for documentation.

h) Note down the intermediate and final OUTCOMEs of your experiment. Which STATEMENTS can you deduce? Has the aim of the experiment been reached (*see c*)? Is

further work required (e.g. repeat of experiment using different parameters, control or validation experiments)?

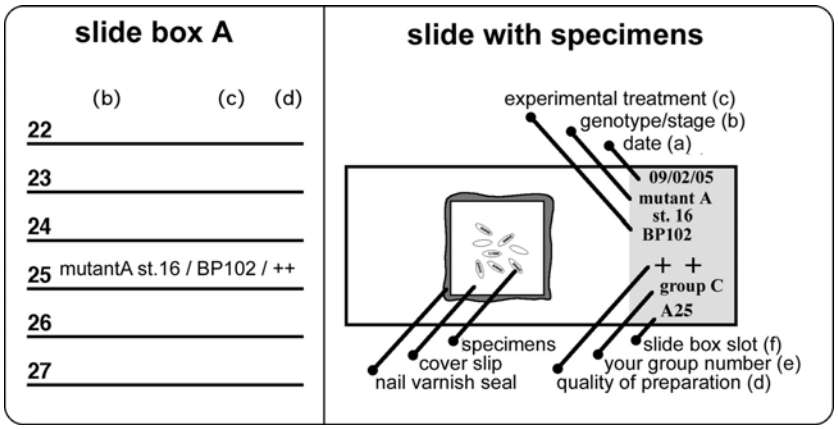


Figure 14. *How to file slides. A slide with embedded specimens (sealed with nail varnish) is shown on the right; the frosted edge (grey) contains important information (see text). In this example, BP102 staining of stage 16 embryos of the mutant strain A has been carried out by group C, they are of high quality and the slide belongs into slot 25 of slide box A. The lid of the slide box is shown on the left; it lists information (b), (c) and (d) from the slide, allowing all students to browse efficiently for appropriate preparations.*

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*):

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 (*e.g. "stage 16" embryo or "L3" = 3rd instar larval stage*) and their GENOTYPE (*e.g. "wt" = wild type, "mutant A", or "C-lacZ"*).

c) The EXPERIMENTAL TREATMENT (*e.g. "BP102" = stained with anti-BP102*); information under b) and c) provides a quick overview and reassure the identity of specimens.

d) A QUALITY JUDGEMENT (*given by course assistants before you file the slide*):

"-" = no staining or too much background (*usually discard right away*)

"+/-" = weak but identifiable specific staining

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

"++" = all aspects of high quality

This rating will make sure that, during the documentation sessions, preparations of highest quality are used most and no time is wasted on bad preparations.

e) YOUR GROUP NUMBER; this allows other groups to consult with 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 and appropriate slot and write the SLOT NUMBER onto the slide (“f” in Fig. 14). This ensures that slides will be returned to their place of origin and can be reused by others.

g) Insert key information (*genotype/stage, experimental treatment, quality of specimen*) into the appropriate space of the LIST IN SLIDE BOX LID (Fig. 14, left). This facilitates quick browsing for suitable specimens.

h) Remember to write down the SLIDE BOX SLOT POSITION IN YOUR LABORATORY NOTE BOOK, to be able to find and revisit your own specimens.

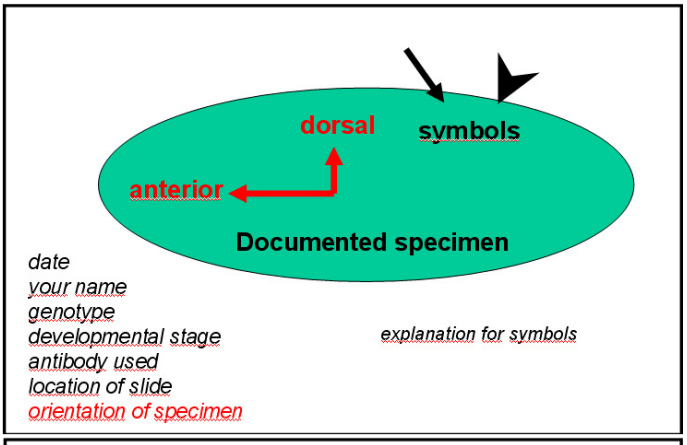
i) When documenting, NEVER HAVE MORE THAN TWO SLIDES AT YOUR PLACE and make sure you RETURN SLIDES INTO THE CORRECT BOX AND SLOT.

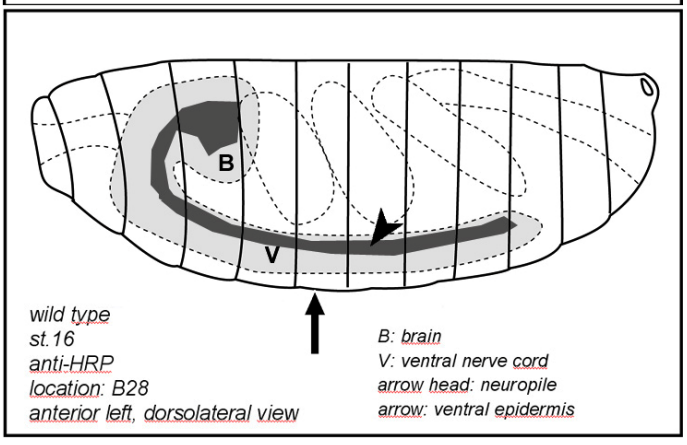
5.4. Documentation of results

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 under the microscope.

Before you start your analysis, it is important that you SET YOUR MICROSCOPE PROPERLY, including the width and diopter of the eye pieces, the condenser, the contrast, and the intensity of the light source (Appendix F). Only with the right settings will you be able to extract maximal information. An experienced microscopist will never rest his/her hands but constantly move the focal plane to achieve a three-dimensional impression, or use changes in contrast to see the actual staining (low contrast) and map it into the surrounding tissue (usually visible with higher contrast).

Then start looking at the specimens. During the analysis it is important that you ASK QUESTIONS: Which criteria allow you to assign an observed staining to a certain structure within a tissue? Are there reproducible classes of embryos which display reproducible differences? These might be differences in developmental stage or mutant versus wildtype embryos. What are your arguments to distinguish between these possibilities? Importantly, NEVER DOCUMENT BEFORE YOU HAVE SEEN A FEATURE REPEATEDLY IN DIFFERENT SPECIMENS, and draw only representative examples. DRAW WHAT YOU SEE - NOT WHAT YOU WANT TO SEE. To facilitate your documentation we will provide you with sheets with schematic outlines of embryos or imaginal discs (Appendix G). It is essential that you provide written information with your documentation, 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, which will be ONE OF YOUR ASSESSED TASKS.

6. COMPOSING A FIGURE AND FIGURE LEGEND FOR SCIENTIFIC PUBLICATIONS

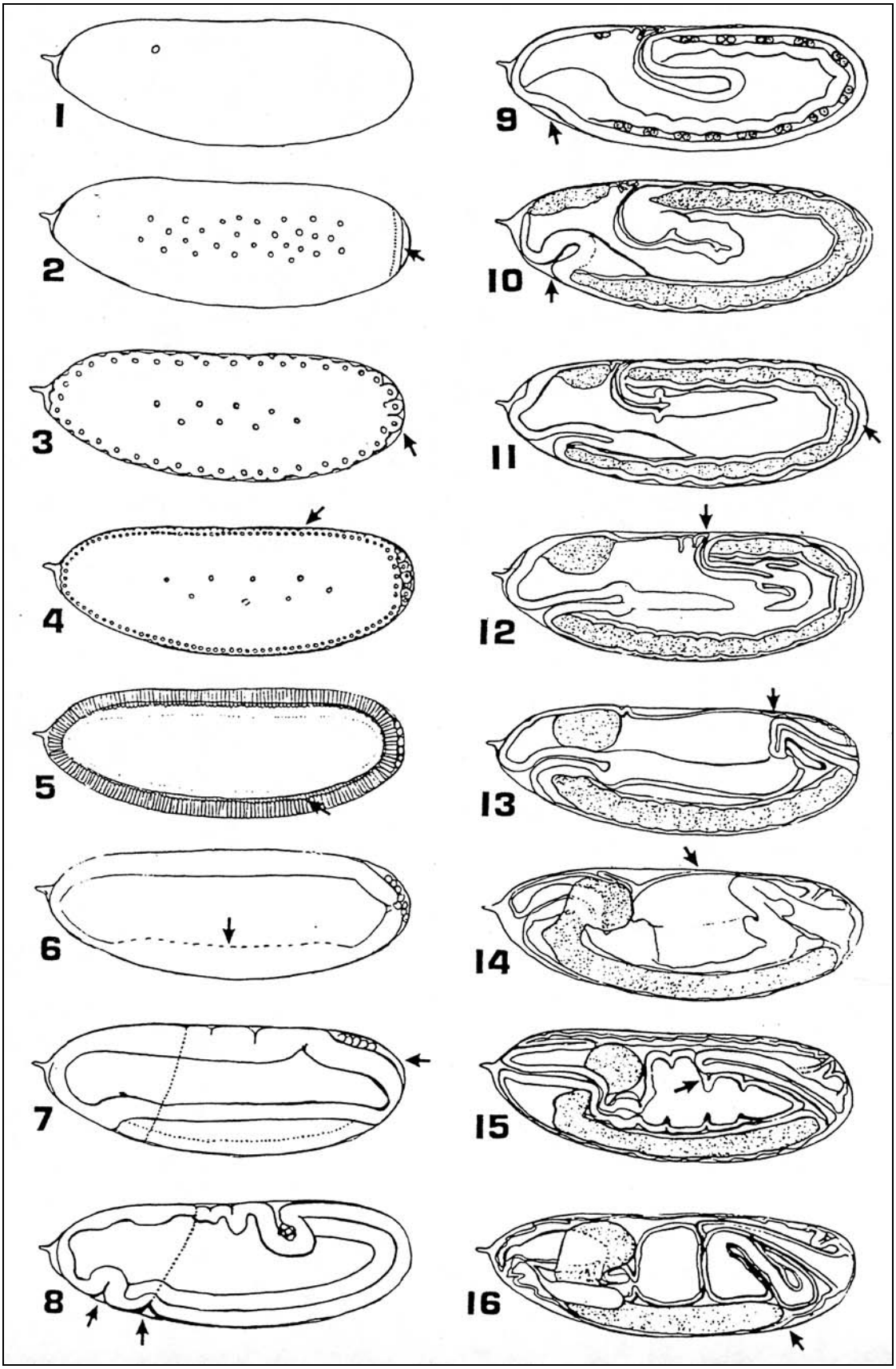
Examples of figures and figure legends are given throughout this manual. However, have a look at scientific publications and look at the figure design and their descriptions. You will find good

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: www.sdbonline.org/fly/aimain/2stages.htm

B) Genetic crosses: Training tasks for over the weekend

When solving these tasks, revisit the manual and presentation for help. If this does not solve the problem, please, come forward with specific questions.

Task 1: You have a stock carrying the recessive, homozygous lethal 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, homozygous lethal 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 1: 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?

Tip 2: Does the ry marker have to be considered during this cross?

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 (*comm*; stock 1) in homozygosis affects the axonal pattern of the *M48-Gal4*-positive neurons. To be able to select the *comm* mutant animals, the mutation should be kept over a GFP-expressing TM3 balancer (stock 2), the presence of which can be easily spotted under a fluorescent microscope.

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

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

c) Design a mating scheme to generate the parental fly stocks (b) as stable 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 1: The w^+ on the P-elements gives orange eyes in *white* mutant background, the endogenous *white* locus on the first chromosome gives red eyes.

Tip 2: Only one copy of Gal4 and one copy of the UAS-construct are required to perform your experiment in *comm* mutant embryos

C) Genetic crosses: Training tasks for 2nd Monday

When solving these tasks, revisit the manual and presentation for help. If this does not solve the problem, please, come forward with specific questions.

Task 3: You keep a fly stock that carries a homozygous lethal, recessive *gcm* mutant allele and is wild type for the *white* locus on its first chromosome. However, for a recombination experiment with a P-element line you need a *white* mutant background. Design a strategy by which you can combine the recessive non-lethal white mutation with the *gcm* mutation.

mutant stock 1	balancer stock
$\frac{+}{+}$; $\frac{gcm}{CyO}$; $\frac{+}{+}$	$\frac{w^-}{w^-}$; $\frac{If}{CyO}$; $\frac{+}{+}$

Task 4: You want to recombine the homozygous viable P-element insertion *P{lacZ,w}*^{RRK} with the recessive, homozygous lethal *repo* mutation. Both are on the third chromosome but kept in two separate fly stocks.

a) Design a scheme using recombination in which you bring both genes onto the same chromosome, stabilised over a balancer chromosome.
b) How do you check for the presence of mutation and P-element?

mutant stock	P-element line	balancer stock
$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{repo}{TM6B, Hu}$	$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{P\text{-}(lac\text{-}w^+)^{RRK}}{P\text{-}(lac\text{-}w^+)^{RRK}}$	$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{TM3, Ser}{CxD}$

Tip 1: *CxD* bears the dominant *Dichaete* marker, which is visible as loss of the alula (a part of the proximal wing); it is only a partial balancer.
Tip 2: Recombination simply occurs during meiosis in the germline of female flies. Selecting the chromosomes in which recombination has occurred is the actual challenge in this question.
Tip 3: The w^+ marker of *P-(lac-w*$^+$*)*^{RRK} causes orange eyes in *white* mutant background.

D) Genetic crosses: Training tasks for 2nd Tuesday

When solving these tasks, revisit the manual and presentation for help. If this does not solve the problem, please, come forward with specific questions.

Task 5: 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, homozygous lethal 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: The dominant mutation *M* shows a phenotype in heterozygosis consisting in gaps in wing veins.

Tip 2: The w^+ on the P-element produces an orange eye colour in *white* mutant background.

Task 6: 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 *P{sca-Gal4,w+}* insertion on the second chromosome (stock 2); this Gal4 line targets expression to the nervous system.

- stock 1: ***w/w; +; P{UAS-shot+,w+}/TM3,Sb*** *(orange eyes; shorten to* ***PUw+****)*
- stock 2: ***w/w; P{sca-Gal4,w+}/P{sca-Gal4,w+};+/+*** *(orange eyes; shorten to* ***PGw+****)*
- stock 3: ***+/+; shot/CyO; Sb/TM6B,Hu***
- stock 4: ***w/w; If/CyO,S; CxD/TM3,Ser*** (*S*: rough eyes; *D*: lack of alulae from wing hinges)

Design the genetic crosses required for this task, using the above stocks. To make this task easier, answer 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

General data bases for *Drosophila*:

- www.flybase.org
- flybase.bio.indiana.edu/allied-data/lk/interactive-fly/aimain/1aahome.htm

An important stock centre for *Drosophila* fly strains:

- 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* and embryonic stages):

- Campos-Ortega, J. A., and Hartenstein, V. (1997). "The embryonic development of *Drosophila melanogaster*." Springer Verlag, Berlin
- Lawrence, P. (1992). "The making of a fly: the genetics of animal design." Blackwell Science, Oxford
- www.flymove.de
- 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:

- www.prokop.co.uk/Research/Drosi-Info/Drosi-info.html
- flybrain.neurobio.arizona.edu

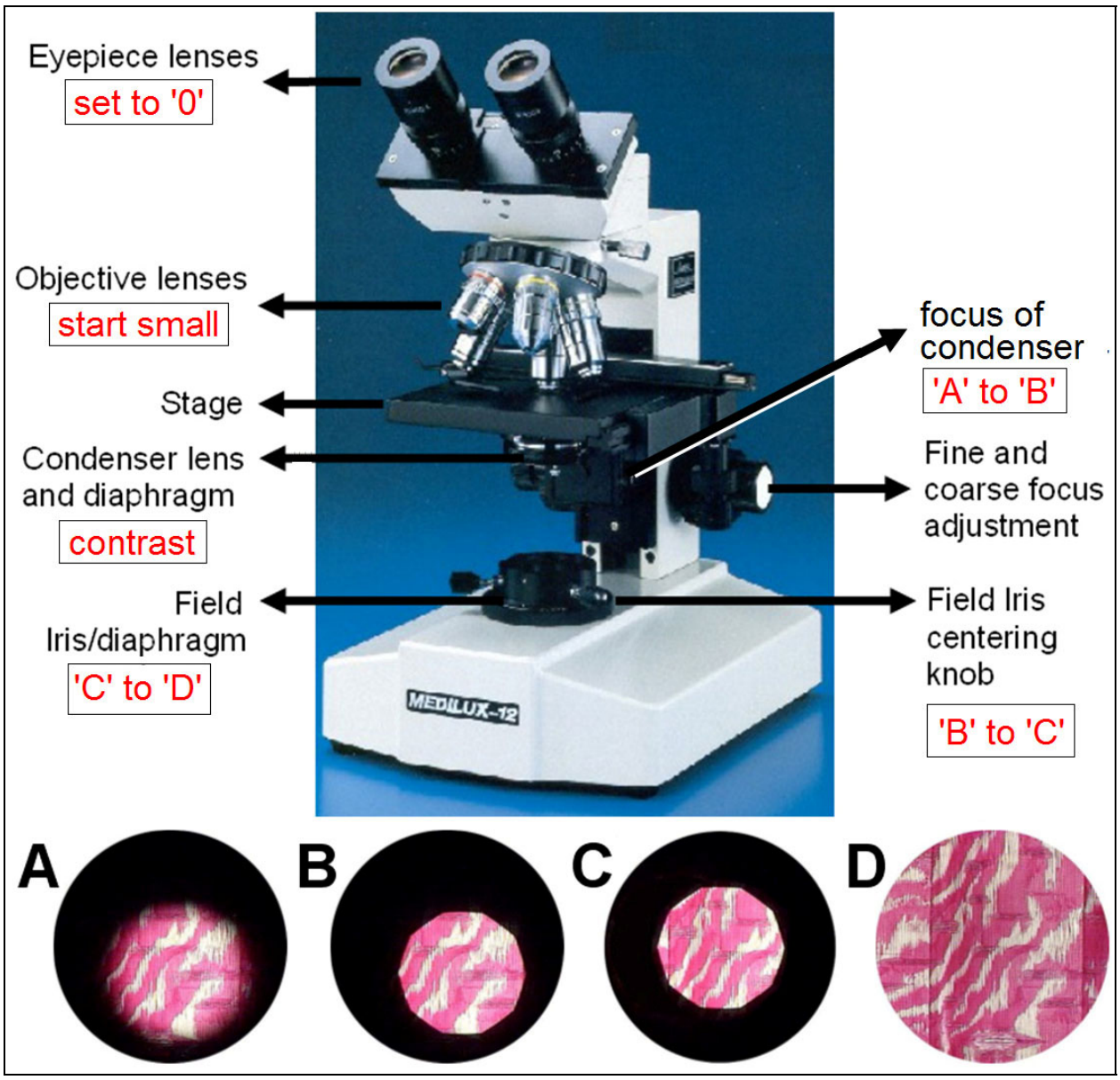
DSHB, a good resource for monoclonal antibodies:

- dshb.biology.uiowa.edu

A good read on *Drosophila* history:

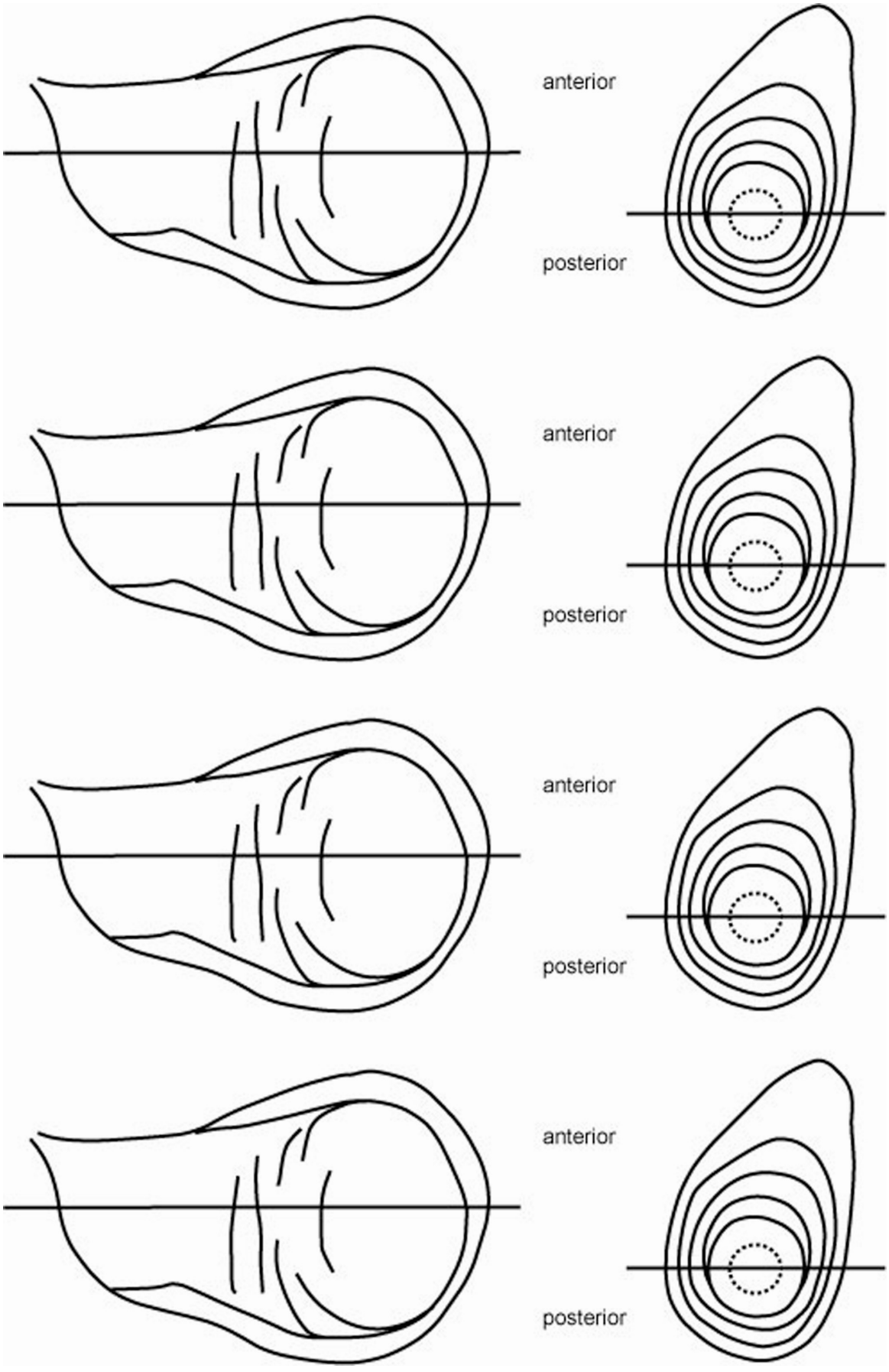
- Kohler, R. E. (1994). Lords of the fly. *Drosophila* genetics and the experimental life (Chicago, London, The University of Chicago Press)
- Weiner, J. (1999). Time, Love, Memory : A Great Biologist and His Quest for the Origins of Behavior (New York, Alfred A. Knopf)
- Martinez Arias, A. (2008). *Drosophila melanogaster* and the development of biology in the 20th century. *In* "*Drosophila*. Methods and Protocols" (C. Dahmann, Ed.), Vol. 420, pp. 1-25. Humana Press - LINK
- Ashburner, M. (1993). Epilogue. *In* "The development of *Drosophila melanogaster*" (M. Bate and A. Martínez Arias, Eds.), Vol. 2, pp. 1493-1506. CSH Laboratory Press, Cold Spring Harbor
- Green, M. M. (2010). 2010: A century of *Drosophila* genetics through the prism of the *white* gene. *Genetics* **184,** 3-7 - LINK
- Keller, E. F. (1996). *Drosophila* embryos as transitional objects: the work of Donald Poulson and Christiane Nusslein-Volhard. *Hist Stud Phys Biol Sci* **26,** 313-46 - LINK

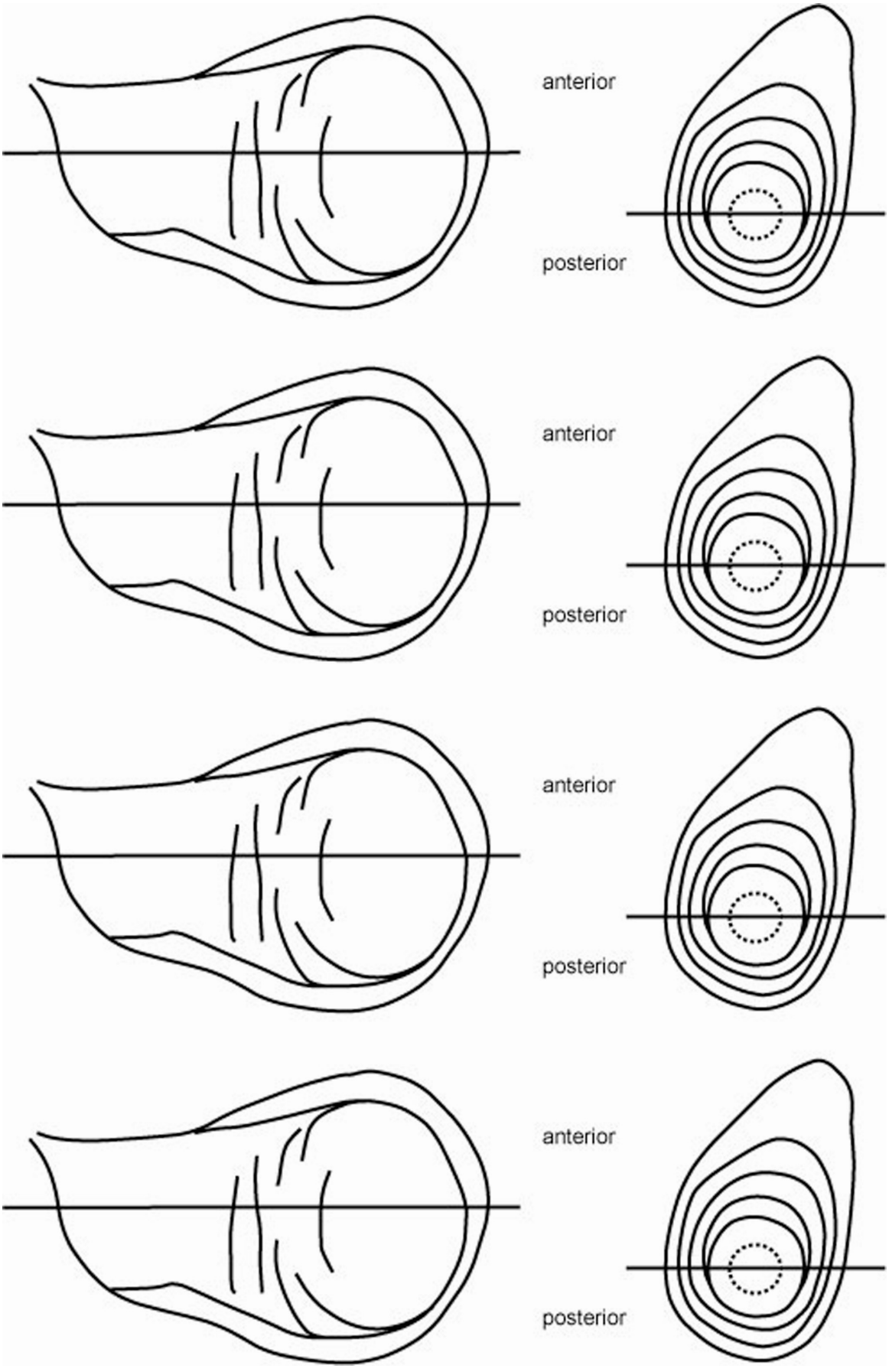
F) Microscope settings



Top: A typical course microscope; arrows point at various parts that require proper setting (as indicated by boxed red text, respectively). **Bottom:** The step-wise procedure required to koehler the microscope: first close the field iris diaphragm, then perform step A-B with the condenser, B-C with the field iris centering knob, C-D with with the field iris diaphragm.

G) Drawing templates

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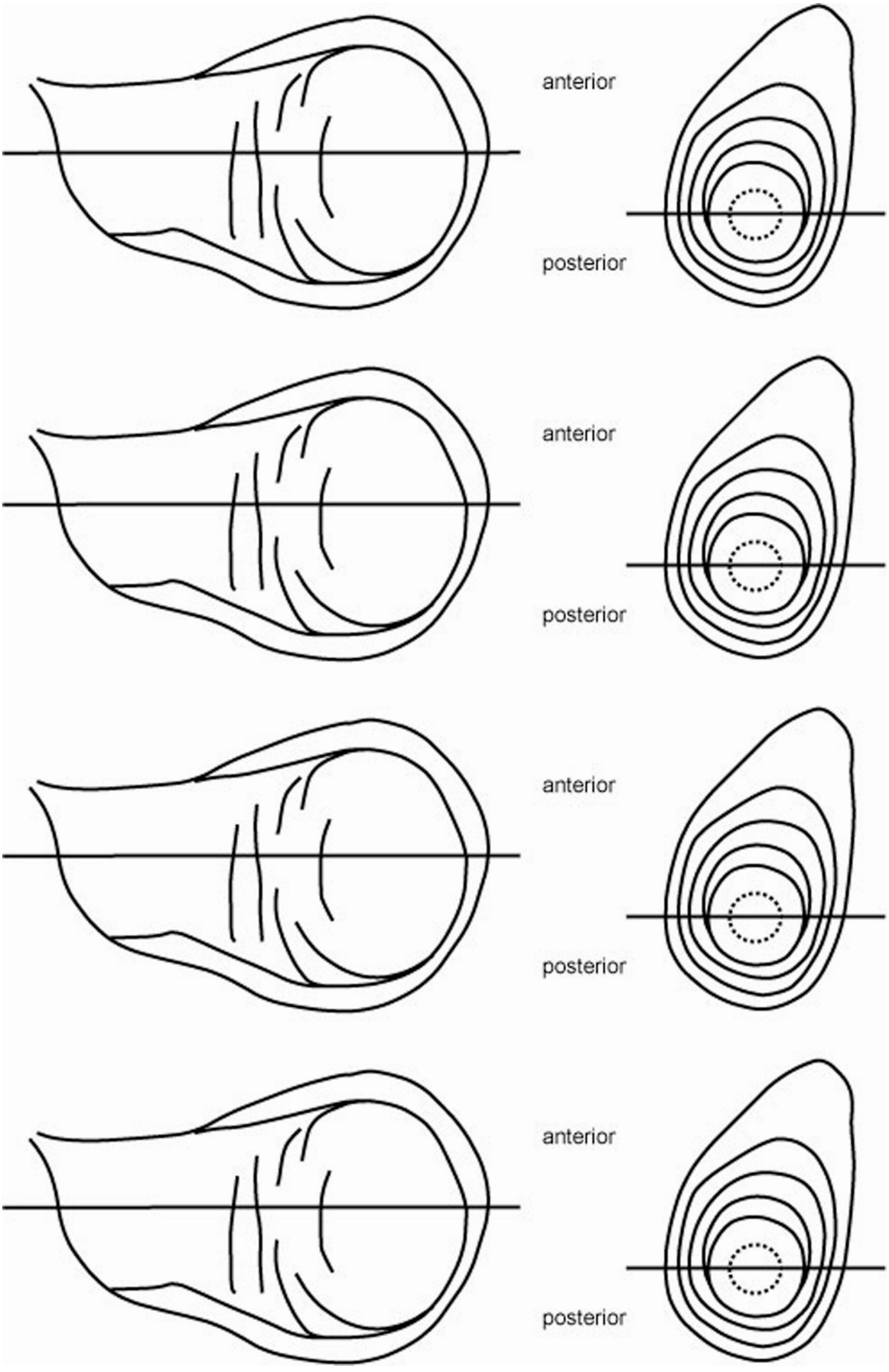
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stage 16
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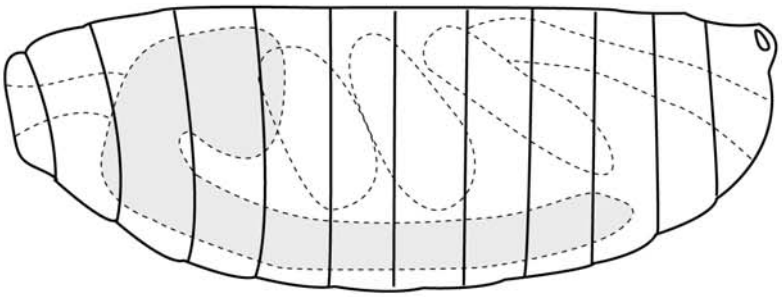

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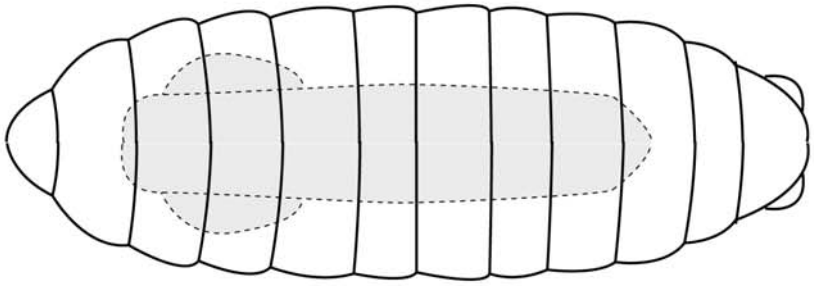

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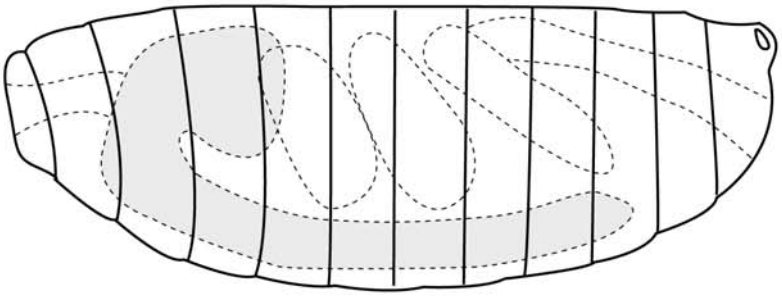

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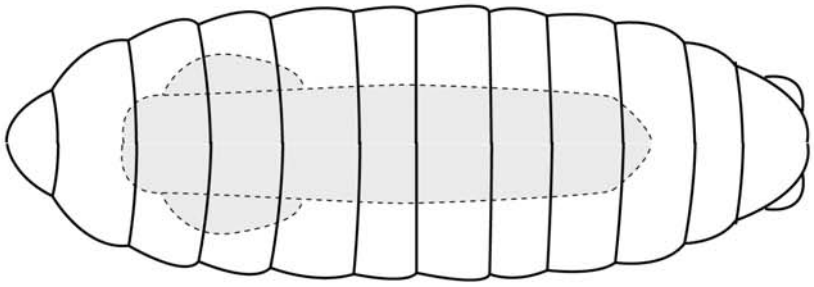

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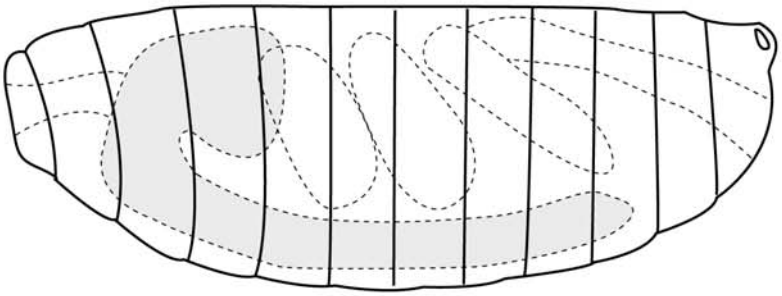

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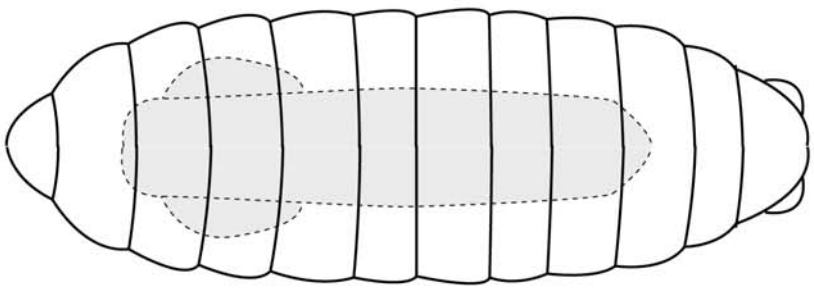

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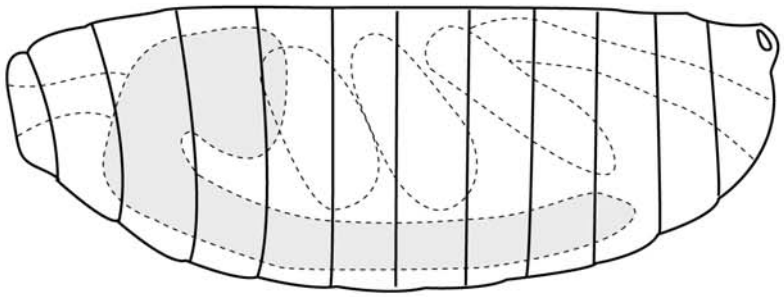

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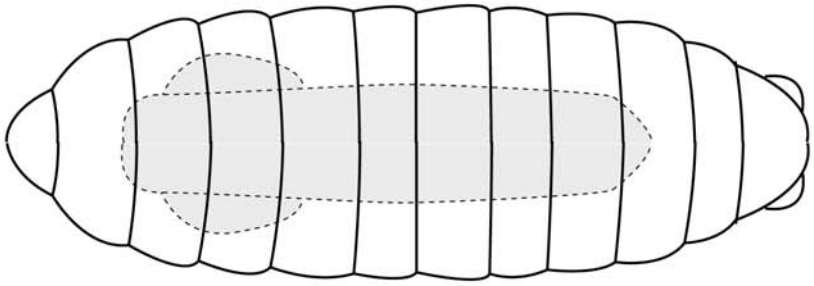

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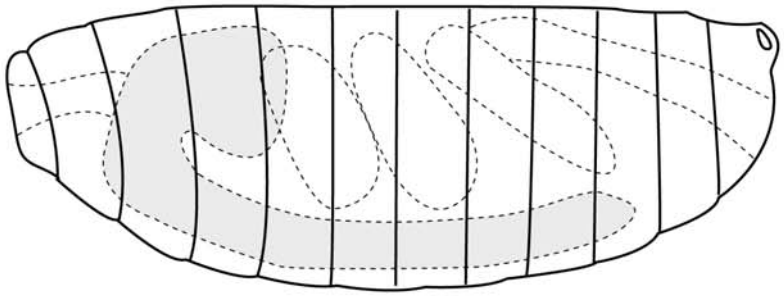

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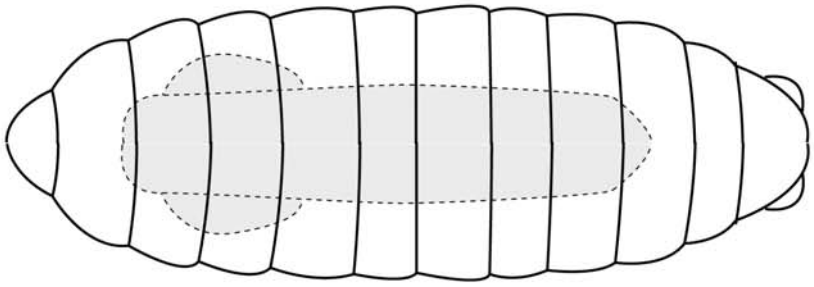

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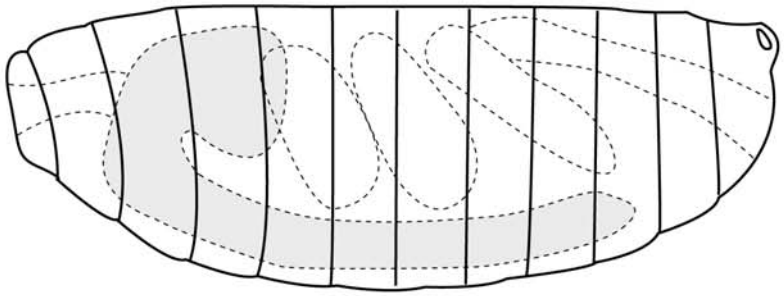

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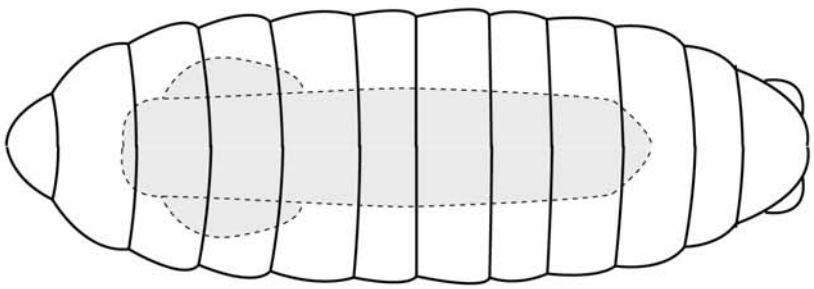

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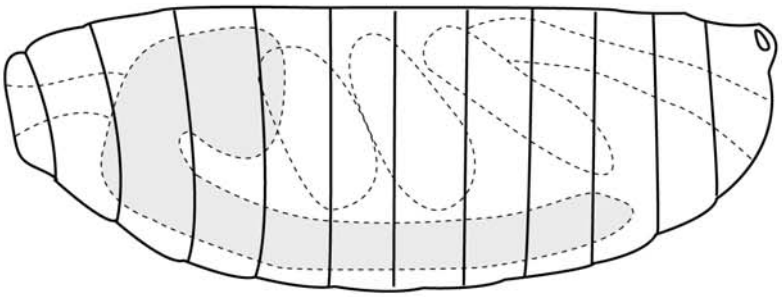

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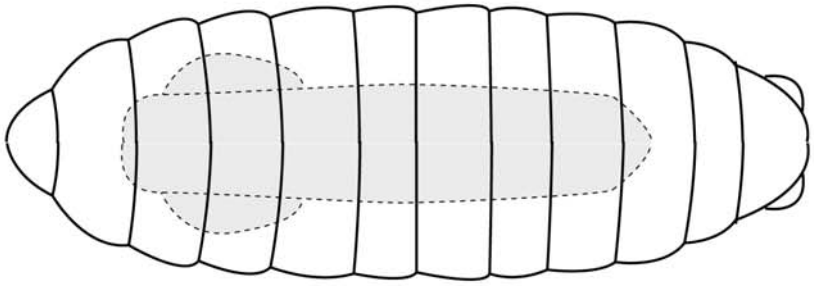

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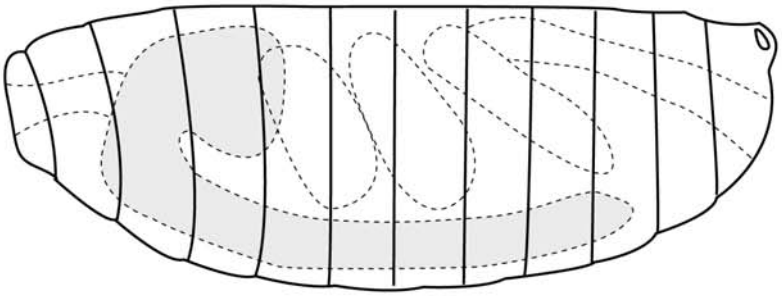

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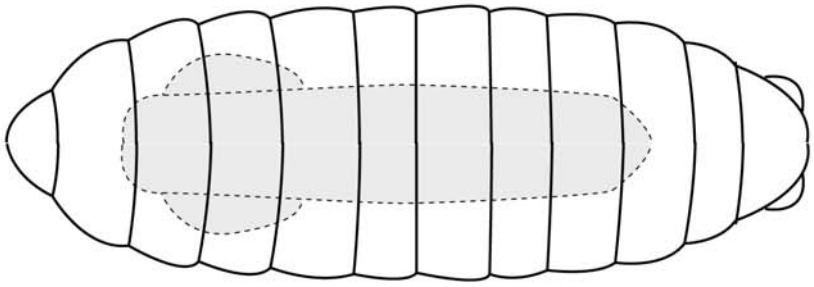

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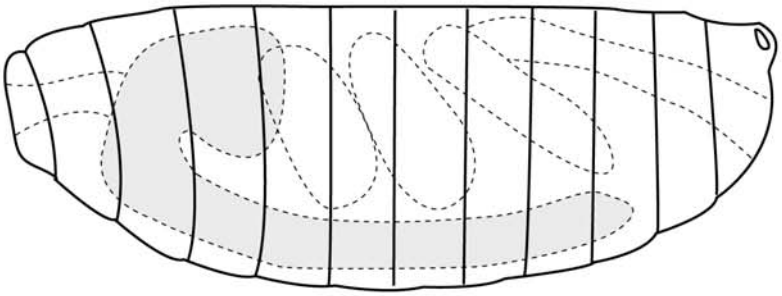

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