MANCHESTER 1824

The University of Manchester

BIOL20332
GENETICS RSM

BIOL20972
DEVELOPMENTAL BIOLOGY RSM

MODULE on FLY GENETICS

18-20 Feb 2020

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Faculty of Biology, Medicine & Health

MODULE 2: BASIC INFORMATION

1. GENERAL INFORMATION

1.1. Location & Contacts

This part of the RSM is organised and taught by Dr. Andreas Prokop and consists of 1 unit lasting 3 days. This module will be taught in the teaching labs indicated in the time table next page. LAB COATS are REQUIRED! Please, direct questions regarding this module to Andreas.Prokop@manchester.ac.uk.
Course assistants in 2018: Liliana Correia, Lorenzo Gallicchio, Alex Dyson

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) .. have been introduced to basic fly genetics
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. Assessment

- 18% for submitted figure and legend, to be handed in on last course day
- 7% laboratory protocol

1.5. Time table of the module

Monday 17 February (location: MUL2, section tba)

11.00-12.00 Instructions: *Immuno-histochemistry & Fixation of embryos*

12.00-13.00 Lunch

13.00-15.00 Practical work: *Dechorionation (15'), heptane/FA fixation (30'), devitellination and transfer to PBT (15'), buffer time: 15'*

Instructions: how to keep a laboratory protocol

15.00-15.15 *Adding 1st antibody*

15.15-16.15 Genetic skills: *Intro to fly genetics (incl. marker exercise)*

16.15-16.45 Genetic skills: *Designing genetic mating schemes (voluntary)*

Tuesday 18 February (location: MUL2, section tba)

11.00-11.30 Practical work: *Adding 2nd antibody*

11.30-12.00 Transferrable skills session: *How to organise a figure for scientific publication (**assessed task - TO BE HANDED IN ON FRIDAY 01 MARCH**)*

12.00-13.00 Lunch

13.00-13.15 Practical work: *Adding ABC-solution*

13.15-13.30 Instructions: *DAB staining and filing of specimens*

13.30-15.00 Practical work: *Carry out DAB staining, embedding, filing*

15.00-16.00 Practical work: larval crawling assays and climbing assays

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Thursday 20 February (location: MUL2, section 4/5)

11:00-12.00 Practical work: *analysis and documentation of preparations*

12.00-13.00 Lunch

13.00-14.30 Practical work: *analysis and documentation of preparations*

15.00-16.30 *Final discussion (lecture theatre: tba)*

- results of 2nd week
- taking midline guidance as an example to explain how the various techniques experienced on this whole module are being used to study biological problems
- 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 orchestrated 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. Here we will focus on the **guided growth of axons** as 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 slender, often meter-long cellular processes of neurons that form the core of nerves and nerve tracts. They are the cables that conduct electrical messages in form of action potential and pass them on at synaptic contacts with appropriate target cells (other neurons, muscles or gland cells) which can be up to a meter away in our body (Fig.1A,B). This organisation is the cellular basis of coordinated behaviour and requires that axons wire up correctly. The precision of this wiring process is achieved during development through the guided growth of axons along paths which are 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 paths. They interpret chemical and physical properties of the surrounding tissues, in form of diffusible or contact-mediated molecules which have been precisely arranged in space and time through the processes of pattern formation. To sense these cues, each growth cone has to be equipped with its appropriate individual receptive machinery, and this is a function of their birth history. Therefore, it is pivotal that patterning processes, during neurogenesis in

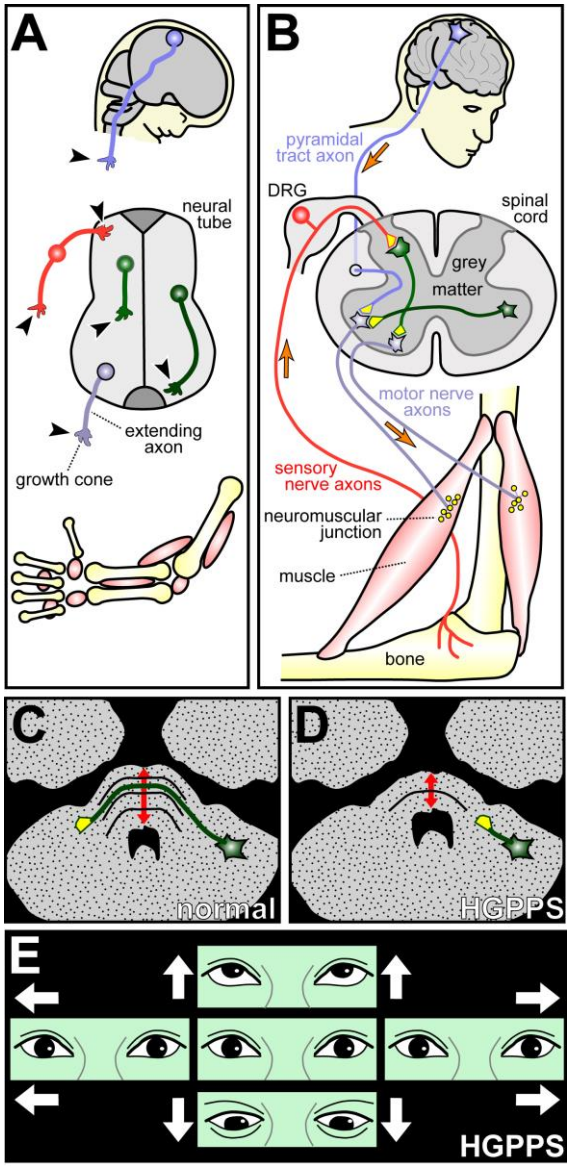

Figure 1. *Examples of inherited human diseases relevant to this Genetics RSM module:*
A*) In the developing nervous system, neuronal axons grow out along reproducible paths via growth cones at their tips (arrow heads).* ***B****) Stereotypic guidance of axonal growth ensures that sensory neurons (red), interneurons (green) and motorneurons (blue) arrange into correctly hard-wired circuits that conduct action potentials along cable-like axons over long distances (orange arrows) and pass them on at synapses to their appropriate target 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.3).*

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 where growing axons either stay on the same body side (red, blue, light green in Fig.1) or cross over to the other half (dark green in Fig.1).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).

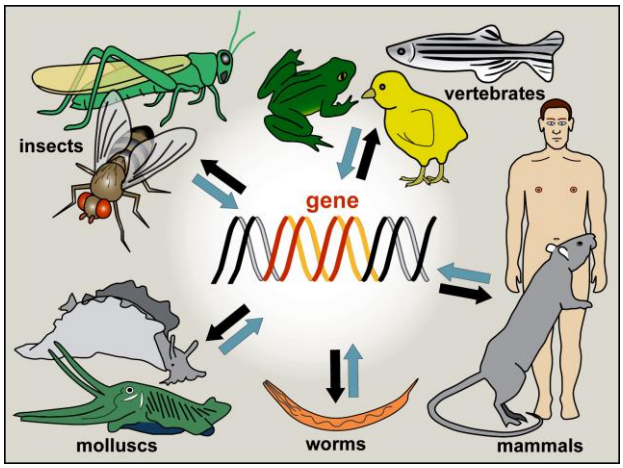

***Figure 2**. Using model organisms:* *Different model organisms are being used in biology. Researchers have to choose the most suitable organisms for their scientific questions, considering their particular pros and cons. Since genes are well conserved across the animal kingdom, insights obtained in simple model organisms tend to be translatable into the biology of higher organisms and humans, speeding up investigation and improving understanding of fundamental biological mechanisms and processes - often relevant for studying disease.*

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

The use of model organisms is common practice in biomedical 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.2). 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)[1]. Therefore, it is a trend in contemporary biomedical science to view model organisms like *Drosophila* as tools or “test tubes” for human genetics. Further advantages of using fly are explained in detail in the accompanying document "FlyGenetics-IntroStudents.doc" (available on shar.es/Yis4C) or read the recent advocacy article[2,3]. During this RSM module, you will experience two important aspects of work with flies:

- You will learn **how *Drosophila* genetic tools can be employed to decipher principal mechanisms** underpinning biological processes. To illustrate this, pattern formation of fly wings and axonal growth guidance will be used as examples. You will learn about analyses using **classcial and genomically engineered gene mutations**, as well as about **transposable elements** and the manifold opportunities they offer as **transgenic tools**. We will discuss how research using these tools can unravel mechanisms and processes of fundamental biology and advance work on inherited human diseases.

You will learn how **applied *Drosophila* genetics** is used to achieve the above outcome. To understand and appreciate this strategy, you will be trained in ***Drosophila* genetic mating scheme design**, which will introduce you to the ways in which the rules of classical Mendelian genetics are applied to capitalise on mutations and transgenic tools. This is **immediately relevant for state-of-the-art research in fly laboratories** and introduces you to **principles of work with all genetic model organisms including mice**. This training is supported by a fly genetics manual and a PowerPoint presentation available as eLearning resources (see page ii).

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[1] see droso4schools.wordpress.com/why-fly & www.flyfacility.ls.manchester.ac.uk/forthepublic

[2] Prokop, A. (2016). Fruit flies in biological research. *Biol Sci Rev* **28,** 10-14 -- tinyurl.com/ybvpoqmw

[3] Prokop, A. (2018). Why funding fruit fly research is important for the biomedical sciences. *Open Access Govern* **20,** 198-201 -- tinyurl.com/y7b25jpm

EXPERIMENTAL PART

Students will work in groups of two throughout the course. Experimental procedures will be the same for everybody, but the genotypes and antibodies used will differ between groups. Stained preparations will be filed in a common slide box accessible to all students (*please, read the respective general guidelines in Section 4 to assure optimal use of preparations*). All students will be expected to have analysed and documented the outcome of all experiments across the course. Results will be discussed at the end of the week and the documentation will form an essential part of the assessment.

3. EXPERIMENTAL WORK

OBJECTIVE: To understand how molecular mechanisms of axonal guidance can be studied via genetic loss-of-function analysis combined with immunohistochemistry. For this, you will study nervous system defects of selected Drosophila *mutant embryos.*

3.1. 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.3*). Mechanisms underlying this simple choice are shared between vertebrates and *Drosophila*. They have been discovered first in *Drosophila* through the use of genetic screens (*Fig.2 of "FlyGenetics-IntroStudents.doc")*. Here, you will get to know some of these genes and experience how their functions can be studied.

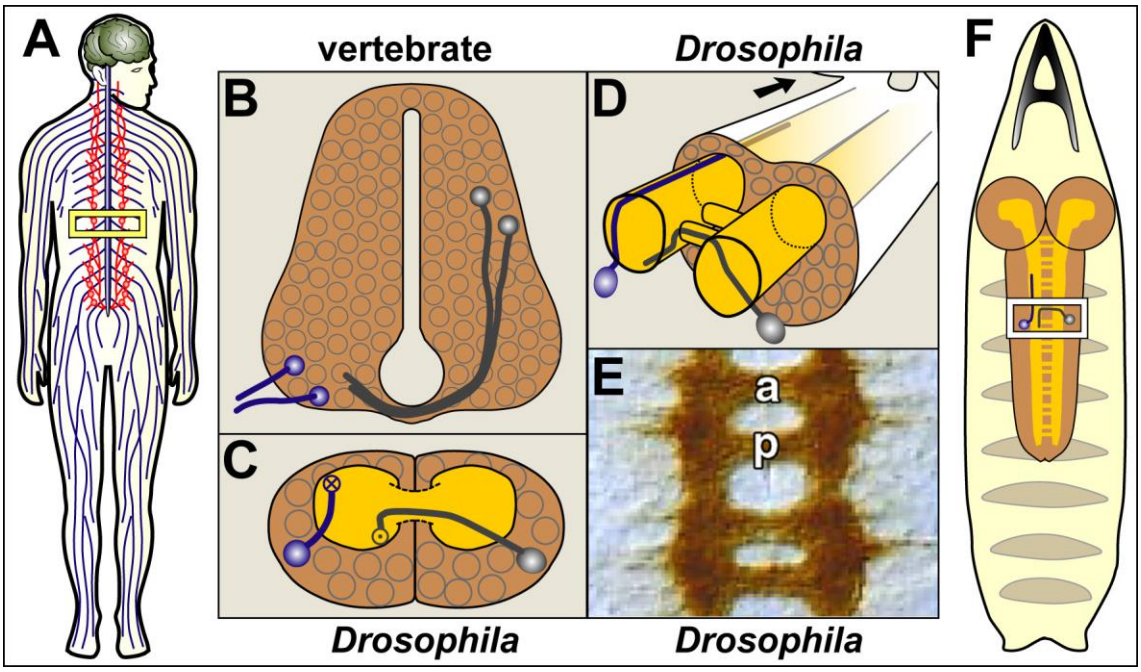


Figure 3. *Axon crossing at the CNS midline*. ***A)*** *Schematic view of the human nervous system with brain (green), spinal cord and nerves (blue), and the autonomous nervous system (red).* ***B)*** *Cross section through the developing vertebrate spinal cord (corresponding to boxed area in A) with grey commissural axons crossing the midline and blue motoraxons projecting ipsilaterally.* ***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 grey, ipsilateral axons in blue; 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.*

3.2. Overview of experiments

You will study the functions of genes involved in axonal growth guidance 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. 3 and 4*). 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.

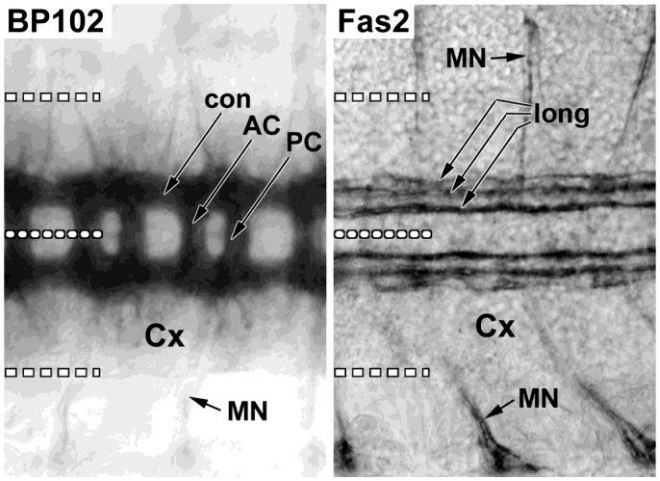


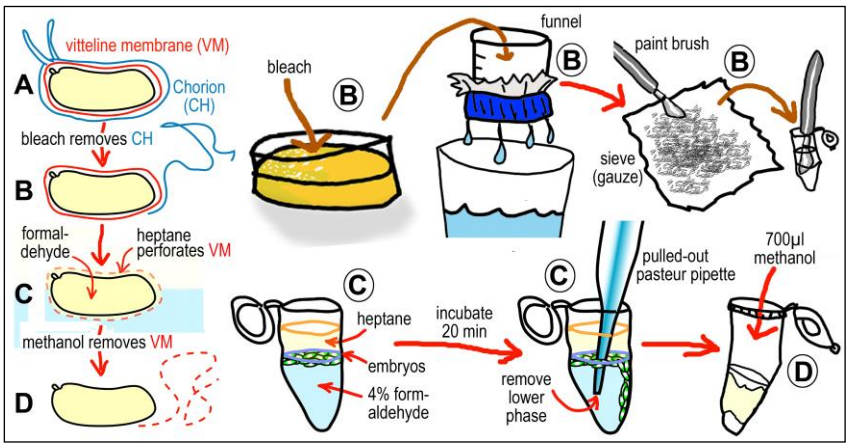
***Figure 4**. Staining patterns of BP102 and Fas2 in the embryonic CNS (compare Fig.3, D-F). Horizontal views of CNSs (dashed lines, lateral borders; dotted line, midline) stained for BP102 labelling the synaptic neuropile, or Fas2 labelling motornerves (MN) a subset of 3 longitudinal axon bundles within the connectives of the neuropile (long). Further abbreviations: AC, anterior commissure; con, connective; Cx, cortex; PC, posterior commissure.*

Experimental table - to facilitate your note keeping as will be explained on the course

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

3.3. Fixation of embryos (Protocol 1)

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 gut is constricted into three portions but also cuticle starts being secreted. The cuticle forms the exoskeleton of maggots and flies and is a protective sheet impenetrable to aqueous solutions. Therefore, standard procedures allow only embryos up to an age of about 13hr (*and slightly older*) to be fixed and analysed as whole mount

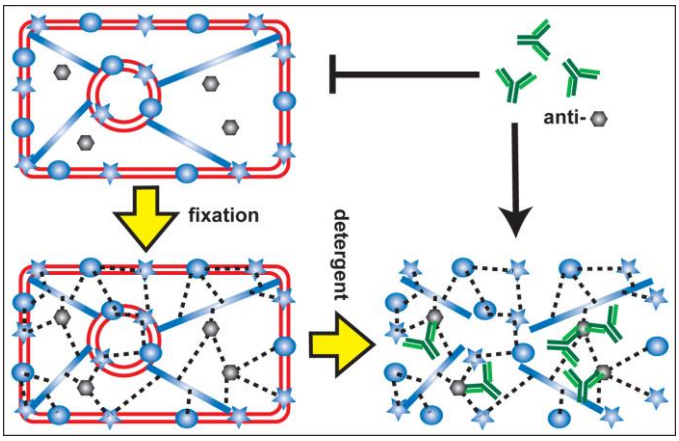


preparations. The only obstacles are two extra-embryonic membranes (*vitelline membrane and chorion; see **A** in image*[4]) which can be removed through straightforward chemical procedures:

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 microscope 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 into the funnel.
4) Wash embryos thoroughly 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 vitelline membrane, remove all liquid (*including heptane*) and fill the tube with methanol.
10) Wash once more with methanol (whole tube volume), then remove and fill tube with PBT. Note that in PBT the embryos take some time to sink to the bottom.

3.4. Immunohistochemistry (Protocol 2)

3.4.1. BACKGROUND

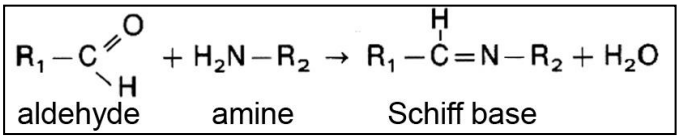


Immunohistochemistry provides the possibility to visualise proteins in their natural position in tissues or organisms. Antibodies can be produced by repeatedly injecting proteins or peptides with specific epitopes into animals, such as rabbits, goats, horses, donkeys, sheep, rats, guinea pigs, mice. 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*). Aldehyde fixation is based on a chemical reaction (*Schiff*

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

condensation; see image on the right) 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.



To visualise where antibodies are localising in tissues (*or in Western blots*) they can be associated with specific detection agents (*image next page*). Such agents can be:

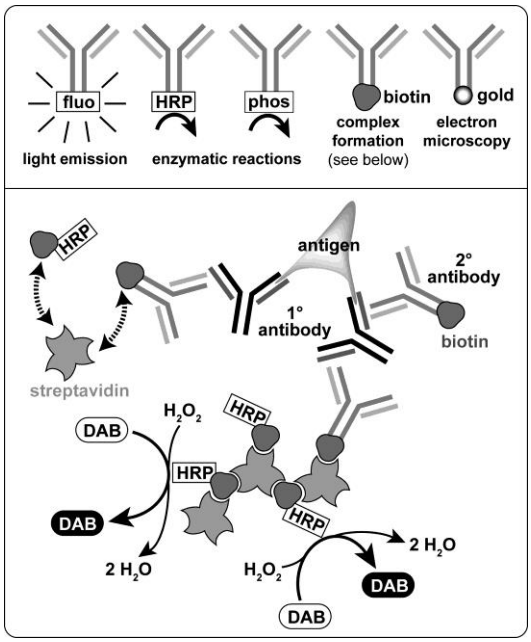
(a) fluorescent markers (*fluo in upper panel*) which can be detected directly in fluorescent microscopes

(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 visualise 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 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.

3.4.2. 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 pre-incubation step, shake/rotate for ~20 min (*not longer than an hour*)
- DAB staining solution: 0.01% H_20_2 and 0.05% DAB in PBT

3.4.3. STAINING PROTOCOL FOR IMMUNOHISTOCHEMICAL PROCEDURES

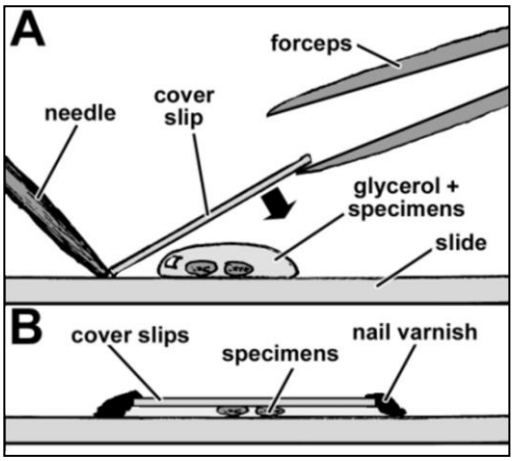
1) Remove methanol and fill the tube with PBT for 1hr to dissolve cell membranes and thoroughly rinse from fixative and methanol. If the PBT appears murky and embryos do not sink to the bottom, carefully remove PBT and fill the tube once more with methanol and share vigorously. Then repeat the PBT step.
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 2 times with PBT (*filling up the tube each time*) 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 (*filling up the tube each time*) 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 times with PBT (*filling up the tube each time*) 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 the 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 fill the glass well half with PBT.
14) Through slow rotating movements concentrate embryos at the bottom of the well, and use a 1000 µl laboratory pipette (*blue tip; the very tip may be cut with a razor blade to widen it opening*) to transfer embryos into a fresh centrifuge tube.
15) Rinse at least one more time with PBT (*filling up the tube*) and dispose waste liquid into the original 15ml Flacon tube.
16) Remove PBT into the 15 ml Falcon tube and add 1ml of 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 80 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 the 80 µl drop with embryos to a microscope slide (*with frosted edge*) 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.

3.5. Embedding of larval discs (Protocol 3)

Required materials: 1 forceps, 1 dissection needle, stained specimens, slides with frosted edges, pencil, 22mm x 22mm cover slips, 90% glycerol

1) After the 80 µl drop with embryos was transferred to the slide, place the slide under a dissection microscope (*white side of the base plate facing up*).
2) 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.
3) Seal all fringes of the cover slip with nail varnish (*B in Fig.*).



Make sure that your slides are properly labelled and filed according to the instructions given under 'General guidelines'.

GENERAL GUIDELINES

4. DOCUMENTATION OF EXPERIMENTS AND FILING OF DATA

4.1. Why bother? A short introduction

This RSM aims to reflect scientific laboratory work as much as possible and equip you with transferable skills, in particular in areas of experimental planning, data analysis, data/specimen storage and note keeping. This will become relevant for many of you for a number of reasons. 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 in interpreting 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 on this course to interpret your specimens and data independently, 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 respective areas in the wing disc; you should apply logic and precise thinking rather than “guesstimation”. 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 efficiently and

reliably. This requires discipline, care and feasible strategies, all of which can be trained on this course. Notably, your documentation and protocols will be essential proof of your work and must be good enough to withstand critical or even legal investigation by others.

4.2. 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, since this is a good test demonstrating how likely it is that you will understand your own notes in years to come. Consider the following, which will also be discussed repeatedly during the course:

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) For each day, give a BRIEF OVERVIEW of the day's task, so that the reader can make sense of detail that follows; for example "*Preparing stage 16 embryos of different genotypes for antibody stainings*"

e) Note down SINGLE STEPS OF YOUR EXPERIMENTS. If you use standard protocols you may not have to explain each step but rather refer to the protocol in a precise manner; for example "*embryos were released from their chorion and vitelline membrane and then fixed as described in protocol 1 of the BL20332 RSM manual 2018, module 1"*.

f) However, note down INDIVIDUAL PARAMETERS & DEVIATIONS FROM THE PROTOCOL. 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 with relevant concentrations*) and potentially also equipment used. Also keep written track of where you are at any stage of the protocol by noting down the TIMES WHEN YOU CARRIED OUT THE INDIVIDUAL STEPS.

g) Note down the intermediate and final OUTCOMES of your experiments: Did the staining work? Note down how many slides with specimens you generated, what their quality was and where exactly they were stored (*see Section 4.3*). 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*)?

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 note"*).

4.3. 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. 10). 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. 10, 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, make sure also others can work. Therefore, NEVER HAVE MORE THAN TWO SLIDES AT YOUR PLACE and always RETURN SLIDES INTO THE CORRECT BOX AND SLOT.

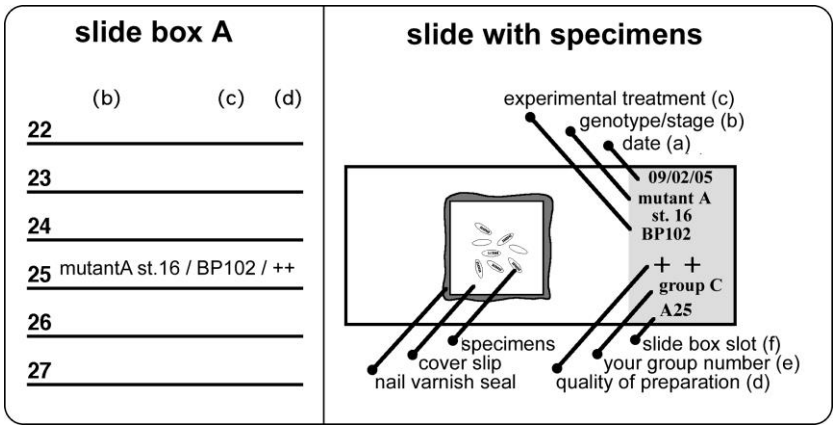


Figure 10. *How to file slides. A slide with embedded specimens (sealed with nail varnish) is shown on the right; the frosted edge (grey) contains important information (see text). In this example, BP102 staining of stage 16 embryos of the mutant strain A has been carried out by group C, they are of 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.*

4.4. 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 you saw under the microscope [5].

[5] Chabrier, R., Janke, C. (2017). The comeback of hand drawing in modern life sciences. *Nature Reviews Molecular Cell Biology* **19,** 137 -- *dx.doi.org/10.1038/nrm.2017.126*

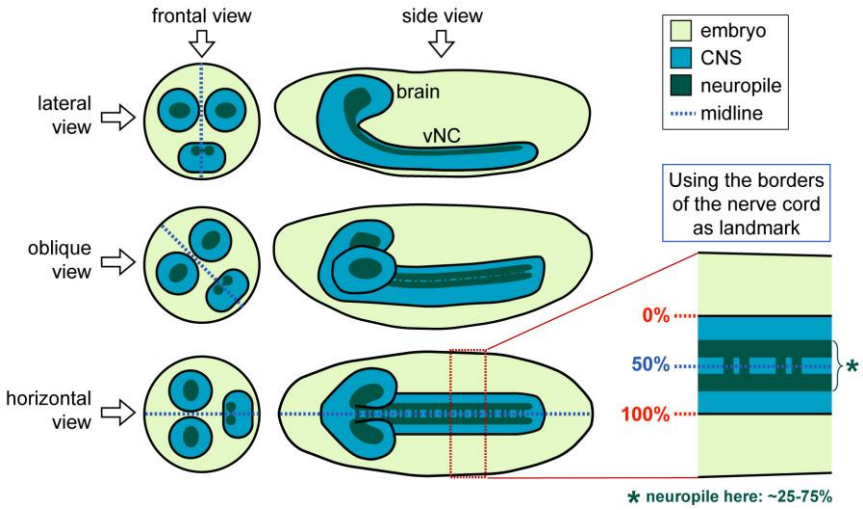
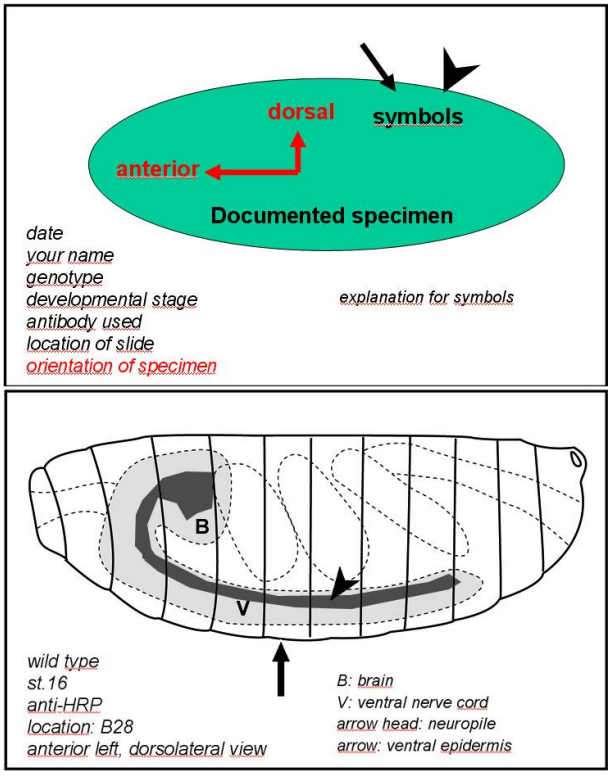


Figure. 10-2 *How to identify embryo orientation and map staining position. You will see embryos in side view: embryos are in horizontal orientation if the vNC is on the midline (blue stippled line), it is in lateral orientation when the vNC is lying furthest away from the midline and the brain hemispheres are lying one over the other. To map the actual staining in the vNC, use the percentage system indicated bottom right.*

Before you start your analysis, it is important that you ADJUST YOUR MICROSCOPE, including the width and dioptre 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 you may use sheets displaying 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 a 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.

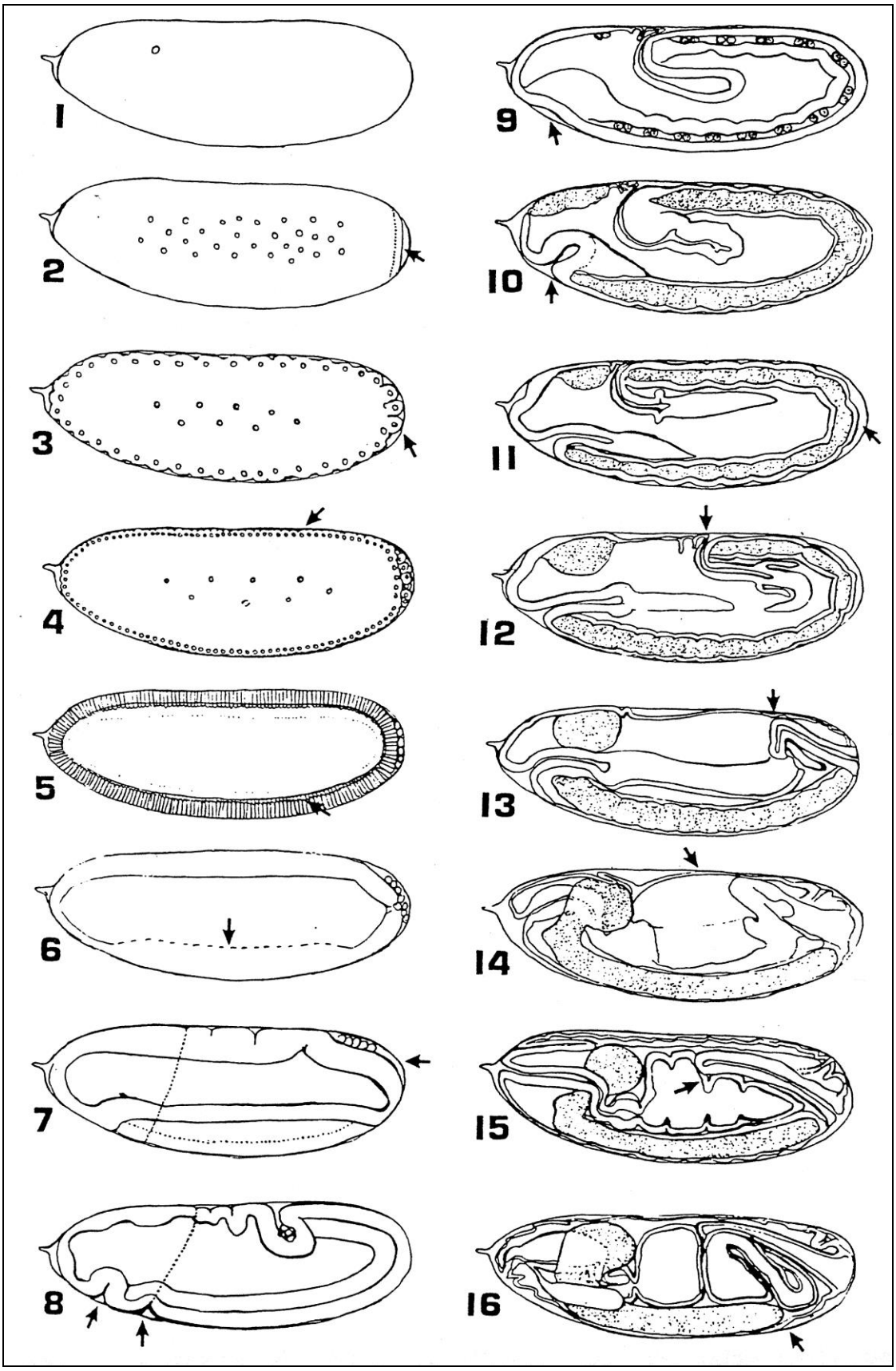
5. COMPOSING A FIGURE AND FIGURE LEGEND FOR SCIENTIFIC PUBLICATIONS

Examples of figures and figure legends are given throughout this manual. However, also have a look at scientific publications and look at their figure designs and legends. You will find good and bad examples, but think about the criteria that have determined your judgement. Please, find below a number of rules or suggestions you can follow when composing a figure and its legend. This information can also be downloaded as a PowerPoint presentation in which you will find images illustrating each statement (*"05 - Figure legend.ppt" - available on shar.es/Yis4C*).

1) Think of a statement you want to make with a figure and choose the images to support it.
2) Formulate a legend title (*e.g. 'Mutations affecting the embryonic neuropile in Drosophila'*).
3) Arrange images in a logic sequence and in right orientation (*anterior left/up, dorsal up/right*).
4) Label single images with capital letters, and refer to these letters in text and legend.
5) Explain what is to be seen (*e.g. which species, tissue, stage, staining*); to this end, ..
   a) .. group statements common to all/some images, instead of repeating them for each image separately (*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 reading.
   b) .. 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.
6) 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.
7) All used abbreviations must be explained in the legend.
8) A figure must show a scale bar if biological material is displayed.

APPENDIX

A) Embryonic stages of *Drosophila*



For more detailed information see: www.sdbonline.org/fly/aimain/2stages.htm

B) 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:

- Prokop, A. (2013). A rough guide to *Drosophila* mating schemes. *figshare.com*, dx.doi.org/10.6084/m9.figshare.106631 -- LINK

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
- Layman's guide to synapses

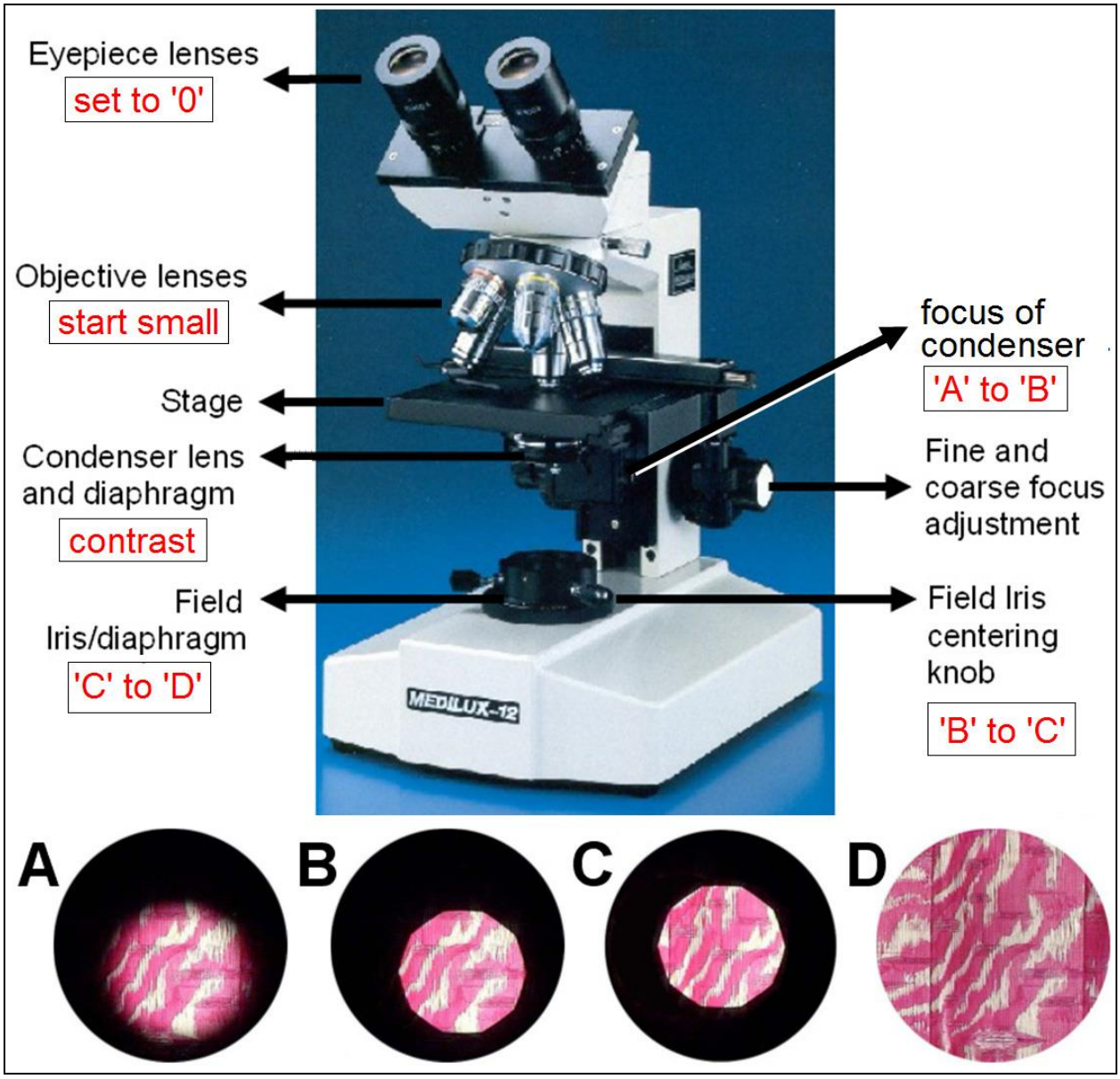
DSHB, a good resource for monoclonal antibodies:

- dshb.biology.uiowa.edu

A good read on *Drosophila* history and background:

- droso4schools.wordpress.com/why-fly/#books
- www.flyfacility.manchester.ac.uk/forthepublic/outreachresources/#History

C) 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.

stage 16
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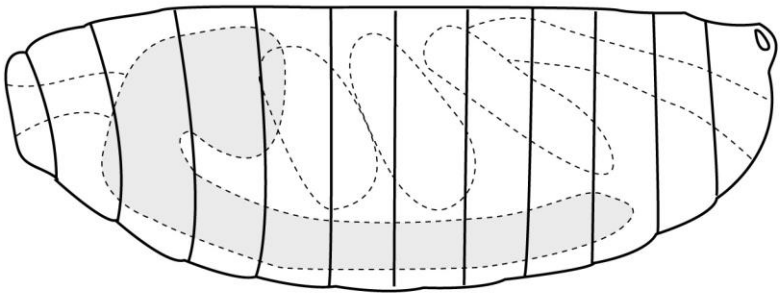

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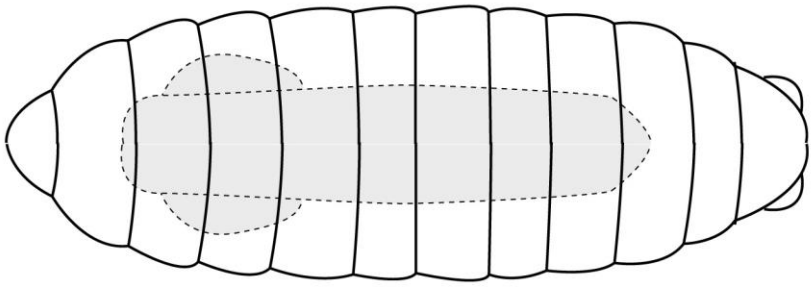

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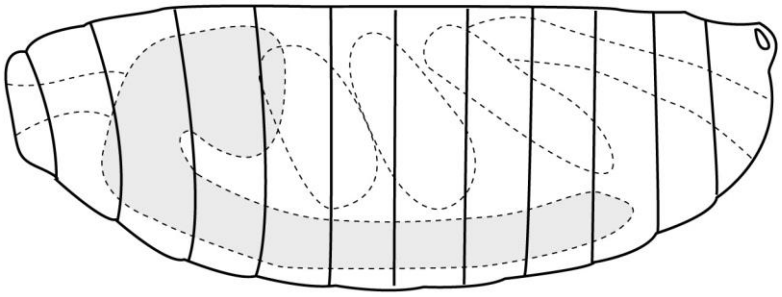

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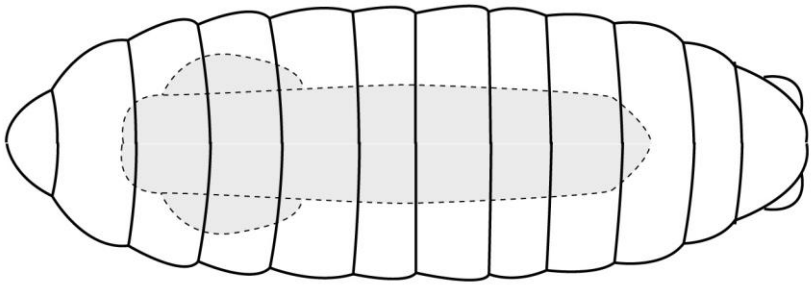

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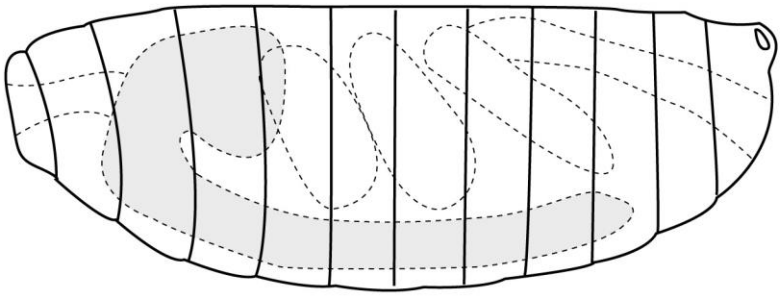

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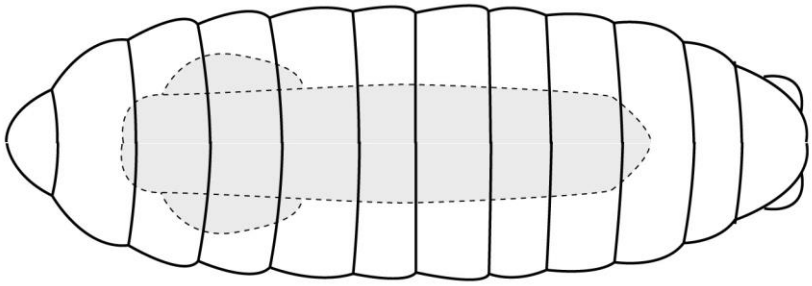

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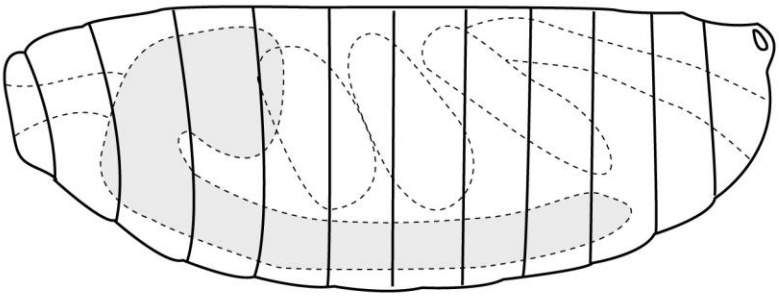

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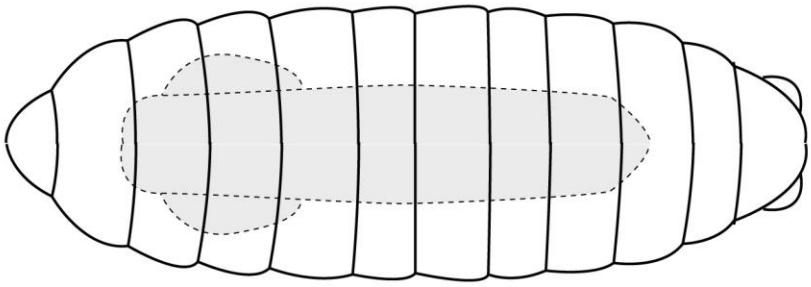

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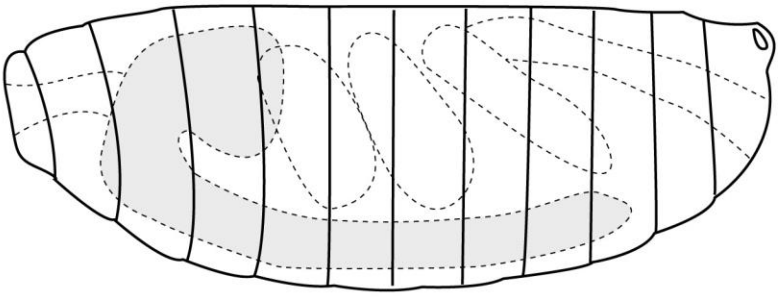

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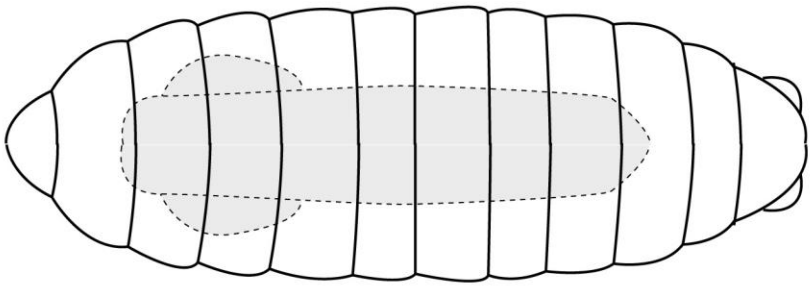

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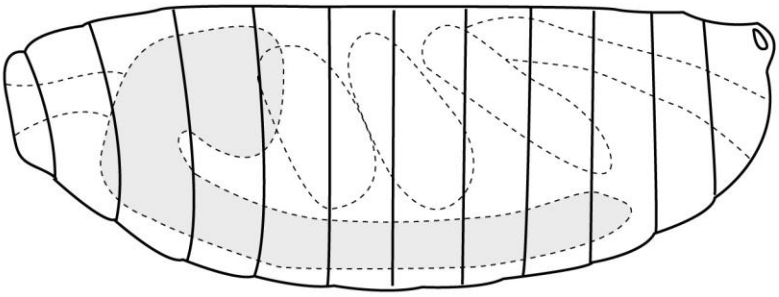

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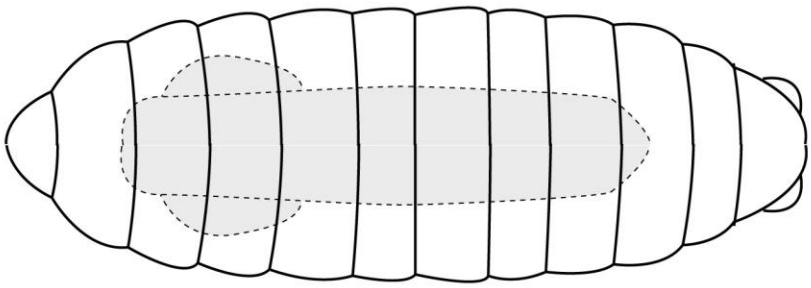

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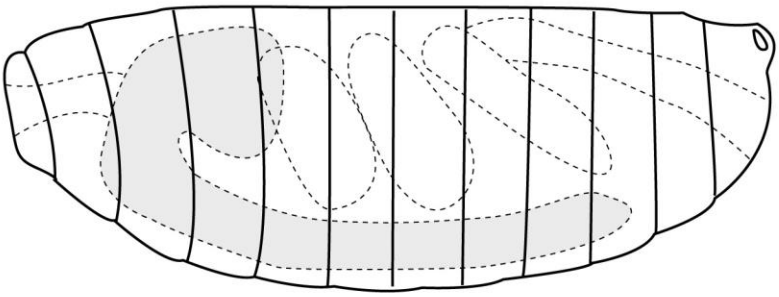

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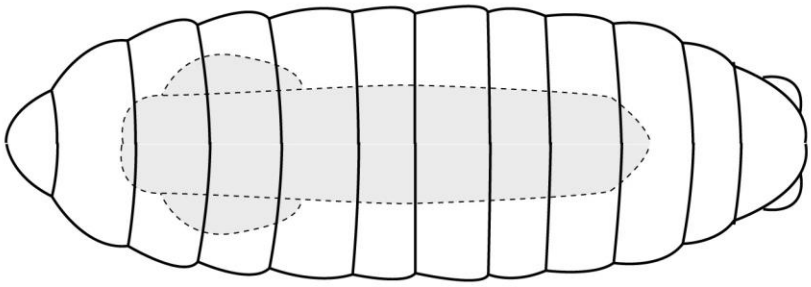

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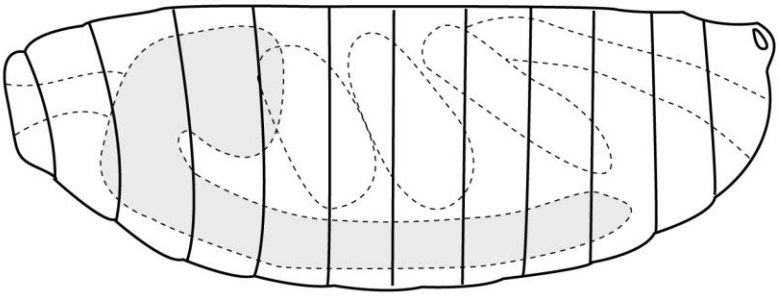

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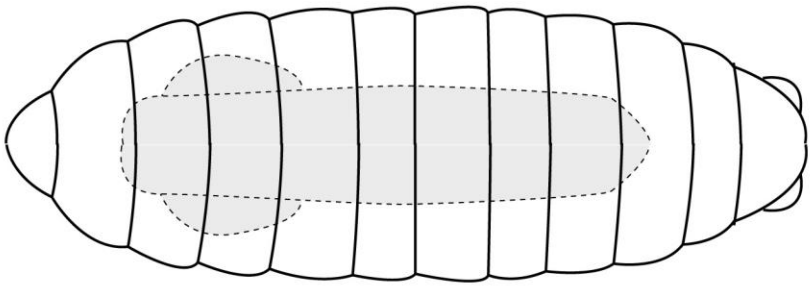

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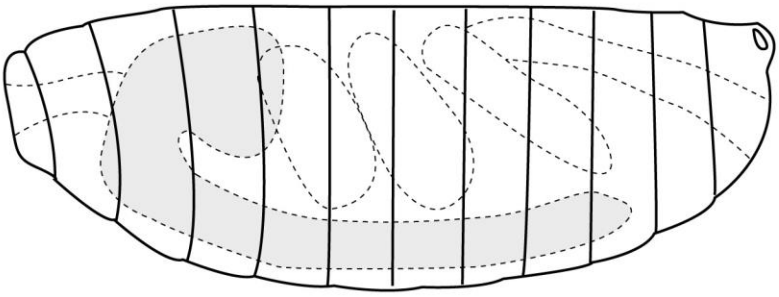

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