BIMA52 HT 2016 Examination Developmental Biology	2016-10-26
number	passed ☐ not passed ☐
time when left back:	Points: *(Erasmus grading:)*

You can write your answers either in English or in Swedish.
IMPORTANT: write your number on the bottom of each side.

Short questions: Answer these questions using one or two short sentences.

1a) Name at least 4 structures that are formed from the Müllerian duct! (2p)

Oviduct, uterus, cervix, upper portion of vagina, labia majora, clitoris

1b) Name at least four proteins that play a role in sperm-egg recognition. (2p)

Izumo, Juno, Bindin, EBR1, ZP2, ZP3

1c) Describe *C. elegans* as a model system. Which are its advantages and where are its limitations? (2p)

Easy to manipulate, short generation time; too simple organism, only 959 cells

1d) Which mechanisms determine the early A-P axis formation in *C. elegans* and name two proteins that play a role in it.

Sperm entry, PAR1, 2; PAR3, 6, aPKC

2a) What is the role of follicle cells during specification of the terminal regions (anterior and posterior end) in the Drosophila embryo? (2p)

Answer: Follicle cells produce the localized determinant Torsolike that activates the Trunk ligand of the torso receptor tyrosine kinase only in terminal regions of the egg.

2b) Where is the mesoderm located in the Drosophila embryo before gastrulation. Briefly outline the cellular mechanism (not molecular!) that leads to internalization of the mesoderm during gastrulation. (2p)

Answer: Mesoderm is located at the ventral side. Shape changes of individual cells drive invagination of the ventral epithelium.

2c) The hunchback gene induces development of anterior structures. Maternally produced hunchback mRNA is uniformly distributed in the zygote and thus also present in the posterior region. Briefly explain how the developing Drosophila embryo solves this problem. (2p)

Answer: In the posterior region of the embryo maternal hunchback is degraded by nanos. Hunchback is thus limited to anterior regions.

2d) Give an example of an organizer that is active after the Drosophila embryo has cellularized or in the larva. (2p)

Answer: Wingless/Engrailed segmental organizer, or Dpp-expressing anterior-posterior boundary of the wing imaginal disc, or Wingless expressing dorsal/ventral boundary of the wing imaginal disc.

3a) Briefly outline the signaling pathway that determines ventral identity in the amphibian gastrula. (2p)

Answer: TGFb pathway: Ligand induced dimerization of the serine-threonine kinase receptor leads to Smad/Co-smad phosphorylation and formation of an active Smad transcription factor.

3b) What happens in the cytoplasm of cells opposite the site of sperm entry in the amphibian zygote and how is this event related to the sperm entry? (2p)

Answer: b-catenin is stabilized. Components activating the canonical WNT pathway are transported by sperm induced cortical rotation.

3c) How can you distinguish “invagination” from “involution” based on the behavior of individual cells during these processes? (2p)

Answer: Invagination is the movement of a cell sheet driven by individual cell shape changes. Involution is a driven by migration of individual cells.

3d) Suggest an experiment showing that an induction process specifies mesoderm in the amphibian embryo. (2p)

Answer: Animal cap assay. Transplanting an animal cap onto an isolated vegetal half of an embryo will induce the animal cap to become mesoderm.

4a) What is the difference in developmental potency (what cells can become) between cells at the uncompacted eight-cell stage and the sixteen-cell stage? (2p)

Answer: Cells at the uncompacted eight cell stage are totipotent. At the 16 cell stage inner cells can form all embryonic tissues whereas outer cells form trophectoderm.

4b) The secreted signaling molecule hedgehog is produced as an inactive precursor. Mention two things that need to occur to produce the active hedgehog ligand. (2p)

Answer: Hedgehog needs to be cleaved by its own endopeptidase activity and modified by palmitoylation and addition of a cholesterol moiety.

4c) Mention an early sign of left right asymmetry in the mammalian or bird embryo and how this early asymmetry spreads to lateral tissues? (2p)

Answer: Left sided expression of hedgehog or nodal. Nodal diffuses laterally and induces its own expression in lateral tissues.

4d) Mammalian eggs are isolecithal whereas bird eggs are telolecithal (these terms refer to the distribution of the yolk). Outline briefly which adaptations this difference necessitates during embryonic development in the respective animal classes. (2p)

Answer: Mammals: formation of a placenta. Rotational cleavage. Formation of a yolk sac. Birds: Meroblastic discoidal cleavage. Epiboly of the ectoderm.

5a) What becomes of the part of the ectoderm that will not form the nervous system? (2p)

Epidermis

5b) How is white and grey matter distributed in the spinal cord? Why is this so? (1+1p)

A drawing of the spinal cord in cross section is helpful.
Grey matter closest to the central canal, white matter outside of this. Grey matter contains the cellbodies of the cells formed close to the lumen of the neural tube. White matter comes from the (myelinated) processes/nerve fibers from for instance the cells in the grey matter.

5c) When an axon of a neuron in the peripheral nervous system is injured, it may grow back and re-create a connection with its original target. What is the navigating part of such a growing axon called? Give a name of an *intracellular* protein that is important for the growth of the navigating part? (1+1p)

Growth cone.
Actin or tubulin, or some other protein that is clearly in the intracellular compartment of the growth cone.

5d) The formation of eyes starts with a single eye-field. Name a molecule that is important for splitting up the single eye-field into two developing eyes! (2p)

Sonic hedgehog
Pax 6 (has to be downregulated)

6a) Tetra-amelia is a human syndrome that is characterized by the absence of four limbs. Explain briefly how a loss-of-function mutation of the *Wnt3* gene can cause this syndrome! (2p)

Answer: During normal limb development, Wnt3 is an important signaling intermediate in the positive feedback between mesodermal Fgf10 and ectodermal Fgf8 signals. The absence of Wnt3 prevents the induction of Fgf8 in the AER and consequently the outgrowth of the limb

6b) Name two transcription factors and their site of origin that regulate dorsoventral patterning of the limb bud! (2p)

Answer: En2 in the ventral limb bud epidermis. Lmx1 in the dorsal mesenchyme.

6c) The ability of sectioned planarians to regenerate missing head and tail structures is prevented, when the animals are exposed to high-energy irradiation. However, when a single neuroblast from an untreated planarian is grafted into the irradiated animal, regeneration is rescued. Briefly explain what the experiment tells about the role of neuroblasts in regeneration? (2 p)

Answer: Neuroblasts are pluripotent stem cells that when activated can proliferate and give rise to all cell types of the planarian.

6d) Two distinct heart fields give rise to the heart in birds and mammals. What evolutionary advantage do you see in the invention of a secondary heart field? (2 p)
Answer: The secondary heart field gives rise to an extra ventricle (right ventricle) that connects to the pulmonary circulation and allows the body to more efficiently supplied with oxygen-rich blood.

7a) Which signaling centers help determine the dorsal pancreas anlage? (2p)

Notochord (FGF2), dorsal aorta, mesodermal mesenchyme (FGF10)

7b) Which structures arise from the dermamyotome?

Dermis of the back, skeletal muscles

7c) What is the role of notch signaling in somite formation?

Notch serves as the molecular clock signal during somitogenesis, critical for border formation of new somites.

7d) Describe retinoic acid's teratogenicity if taken during pregnancy. Which are the medical indications? Which can be the phenotypes for the born child? (2p)

13-cis-retinoic acid against acne. Abortion, congenital malformations: cleft palate, absent/defective ears and jaws, CNS defects.

In the essay section, describe more in detail. But: stay focused such that we understand what you mean! Use the back side if you need more space.

E1. a) Your task is to inactivate ZP2 the mouse completely such that the inactivation is inheritable. Explain all steps, from the moment on where you have a plasmid with the cloned ZP2 gene at hand, up to stage where your newly-designed mouse is born. DRAWINGS!
b) Later, you decide to generate a mutant mouse (again where the mutation is inheritable) which will reveal polyspermy. Describe two proteins that could serve as potential targets, and explain briefly which approach you would like to utilize with the two proteins to achieve the goal.

Answer:

for a): the student should start:
1) with drawing a map of a possible plasmid with all elements that are necessary for successful integration: a) targeting sequences, b) neo®, c)TK.
2) integration into ES cells ("129" line resulting in brown fur) should be discussed, including selection: a) Neomycin, b) Ganciclovir.
3) injection of "129" stably-transfected ES cells into "129" blastocysts
4) insertion of blastocysts into C57BL/6 mice (has black fur)
5) breeding for ZP 2 homozygote
6 points

for b): the student should discuss the target proteins first and the molecular targets:
- ZP2
- ovastacin
- Juno

ZP 2 is crucial for sperm binding, but gets cleaved after successful fertilization. This step also allows to prevent polyspermy. If the cleavage site of ZP2 is altered, then ovastacin can no longer cleave ZP2, hence the egg remains susceptible for further sperm binding, and polyspermy can occur.
Ovastacin cleaves ZP2. If absent or inactive, ZP2 remains uncleaved and polyspermy can occur, as well.
Juno gets cleaved off after fertilization, and freely-swimming Juno can saturate Izumo receptors of other sperms, thereby preventing polyspermy. If the GPI anchor of Juno is altered such that cleavage cannot occur, then polyspermy is possible because there are no freely-swimming Juno proteins around that can saturate the Izumo receptors of the other sperms.

For all 3 proteins, a conventional gene targeting scheme (as discussed above) with the altered proteins will allow polyspermy.
4 points

E2. Describe the mechanism establishing and patterning the ventral side in the Drosophila embryo/egg. Subdivide your description into events before and after fertilization. (10p)

Answer:
Before fertilization:
Gurken associated with the oocyte nucleus signals to adjacent non-terminal follicle cells to induce dorsal fate and inhibition of pipe. Ventral follicle cells express pipe, which sulfates components of the vitelline envelope.

After fertilization:
A protease cascade consisting of gastrulation defective, snake and easter activates the spätzle ligand in the vitelline space in regions where the vitellin membrane has been sulfated. Spätzle activates the Toll receptor, which transduces the signal through pelle and tube to the Dorsal/cactus complex. As a result Dorsal enters the nucleus and activated the transcription of target genes.
Dorsal forms a ventral to dorsal gradient of nuclear localization. High nuclear concentrations of dorsal activate the expression of ventral genes twist and snail. Medium dorsal concentrations activate lateral genes such as rhomboid. Low concentrations of dorsal allow dorsal genes such as decapentaplegic and zerknüllt to be expressed.

E3. Describe the mechanism that establishes positional information along the anterior-posterior axis in the ectoderm of the amphibian embryo during gastrulation. Suggest an experiment that shows that regional identities have been established in the dorsal ectoderm after gastrulation. (10p)

Answer: During gastrulation mesodermal cells of the Spemann organizer involute and migrate anteriorly along the inside of the dorsal ectoderm. This establishes a ventral-to-dorsal gradient of BMP signaling and a posterior-to-anterior gradient of WNT signaling in the mesoderm. Anterior dorsal cells in the mesoderm express the WNT inhibitors Frzb and Dickkopf and the BMP inhibitors chordin, noggin and follistatin. These inhibitory signals from mesodermal cells are received by overlaying ectodermal cells and interpreted to establish positional information. Establishment of posterior identities is supported by retinoic acid and fibroblast growth factor signals.

E4. Compare the role of extraembryonic tissues during positioning and extension of the anterior-posterior axis in chicken and mouse. (10p)

Answer: The early chicken embryo is discoidal and consists of two layers: an epiblast and an underlying extraembryonic hypoblast. The posterior marginal zone from which the anterior posterior axis emerges is positioned by TGFb signals from cells underlying the epiblast intersecting with cells in the area opaca in which WNT signaling is active.
Elongation of the primitive streak from the posterior marginal zone is inhibited by Cerberus secreted from the primary hypoblast. As the secondary hypoblast underlying the posterior marginal zone extends anteriorly, Cerberus inhibition is relieved and nodal from the epiblast drives extension of the primitive streak.

The early mouse embryo is also a bilaminar germ disc that is however formed into a cup. The inside of the cup is lined by the epiblast, the outside by the extraembryonic primitive endoderm corresponding to the chicken hypoblast. At the open end of the cup lies the extraembryonic ectoderm (EEE). Feedback signaling between the EEE and the epiblast establish nodal signaling in the epiblast. At the distal end of the cup the distal visceral endoderm (DVE) expresses BMP and WNT inhibitors. As the DVE translocates to the side of the cup to form the anterior visceral endoderm (AVE) its inhibitors make nodal signaling in the epiblast asymmetric with stronger nodal activity opposite the AVE. This positions the primitive streak. Nodal drives extension of the primitive streak towards the distal/anterior region of the cup.

E5. The developing spinal cord will with time go through a differentiation process and get a dorsal side related to sensory functions, as well as a ventral side related to motor functions. How is this differentiation obtained? (10p)

Obtained by patterning via gradients from the ventral as well as the dorsal side.

Ventrally, Sonic hedgehog from notochord, affects the so called floor plate, where Shh production is started.

Dorsally, TGF-beta family members (e.g. BMP4) from ectoderm, affects the so called roof plate, where production of same sort of factors is started.

Factors from the floor and roof plate then distribute throughout the developing spinal cord in a gradient from floor to roof, and roof to floor, respectively. The exact proportions of such factors available for the cells in the spinal cord will then decide which transcription factors will be active in these cells. The exact profile of transcription factors will decide the phenotype of the cell. Cells close to the floor plate will see much Shh but only little TGF-beta family factors. This will promote a motor neuron phenotype of these cells. Etcetera.

Essay 6) Knockout of the Sonic hedgehog (Shh) gene in mice leads to the formation of reduced limb structures with only one digit. What digit identity is formed in the mutant? How does Shh normally specify digit identity? Why does the absence of Shh lead to a shortened limb?
If in addition to Shh the Gli3 gene is inactivated, limb formation is rescued and extra fingers form. Explain! (10 p)

Answer: Only the most anterior digit 1 forms in the Shh null mutant. The timing and concentration of Shh normally determines digit identity. Cells of digits 5, 4 and partially of 3 must express Shh. Some cells of digit 3 and all cells of digit 2 do not express Shh, but are exposed to Shh protein due to paracrine signaling. Digit 1 is independent Shh.
In the absence of Shh, the AER is not maintained, leading to reduced outgrowth of the limb. Shh in the ZPA indirectly stimulates Fgf8 in the AER through inducing the BMP antagonist Gremlin and preventing BMP from inhibiting Fgf8.
In the developing limb, Shh prevents the cleavage of Gli3 to the truncated Gli3R, so that Gli3R can no longer inhibit the transcription of Shh target genes. Inactivation of Gli3 leads to polydactyly, and the phenotype is maintained when in addition to Gli3 Shh is inactivated.

Essay 7) Kidney development depends on continued communication between the epithelium and mesenchyme. Describe the sequence of morphological events resulting in kidney formation and the signaling molecules (including tissue source) involved in these processes. (10p)

Step1:
Metanephric mesenchyme (MM) is specified by expression of Hox11, WT, Foxc1 and 2 expression in anterior region inhibits expansion of MM

Step2:
Induction of ureteric bud (B) outgrowth by the mesenchyme (GDNF), ret receptor expression makes epithelium competent, GDNF is only expressed in posterior portion of MM

Step3:
Fgf2 and Bmp7 from UB stabilize MM and proliferation, this signals rescues MM cells from apoptosis

Step4:
Wnt signal from UB induces MM to aggregate and start mesenchymal to epithelial transition

Step5:
Wnt signaling in the MM completes transition to epithelium in autocrine fashion.

Step6:
Induction of branching and controlling of branching morphogenesis by GDNF, TGFb1 and BMP4

Step7:
FGF7 and FGF20 from UB induces stem cell competency in MM (production of appropriate number of nephrons).

Step 8:
Insertion of ureter into the bladder. Cells from urogenital sinus wrap around ureter to for smooth muscle cell layer, cells secrete BMP4 which induce cell maturation