INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

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IBO Challenge 2020

A Substitute for The 31st IBO 2020 Nagasaki, JAPAN

Clypeaster japonicus

Saccharina japonica

Glirulus japonicus



Leucothea japonica

Branchiostoma japonicum

Mauremys japonica

Hyla japonica

Anguilla japonica

Narke Japonica

Eutrema japonicum

Practical Exam I

Animal Physiology

signature

2020.8.11.

Cryptomeria japonica

Corbicula japonica

Ulnaria japonica

Hydroglyphus japonicus

Luehdorfia japonica

Halichondria japonica

Nihonhimea japonica

Perkeomeris japonica

Aspergillus japonicus

Fibrocapsa japonica

Meyersia japonica

Alveopora japonica

Omphalotus japonicus

Mimobdella japonica

Ephebe japonica

Coturnix japonica

Oxycomanthus japonicus

Panulirus japonicus

Notholca japonica

Scolopendra subspinipes japonica

Perophora japonica

Lychaete japonica

Delisea japonica

Prasiola japonica

Lotus japonicus



Osmunda japonica

Conocephalum japonicum



Nipponia nippon

Instructions of this exam

1. To see the Photoset No.1 and No.2, you have to connect to the server with information below.

   Find the URLs of your country's server at:

   https://bit.ly/IBO2020file

   or refer to the URL list at the end of this exam file/booklet.

   Then, enter the server using the following username and password.

   Username: ibo2020

   Password: ibo2020apnagasaki

   If you have a connection problem, try the following alternative servers:

   Competitors in Asia, Oceania, or America: 18.181.44.86/index.html

   Competitors in Europe: 18.194.137.48/index.html

   or 3.126.91.168/index.html

   You can see photos in each file when you click it. Click a selected photo to see its enlarged image. Use the Back button on the browser or "previous page" button to return to the previous page. If there is any trouble (for example, it freezes, you can't see pictures anymore, and so on), press the reload button on the browser.

2. Write your signature on each page of the answer sheet.

To understand functions of various tissues and organs in adult animals, it is important to study how they are created. This practical exam involves two tasks aimed at understanding cellular and molecular mechanisms regulating animal development. Answer the five questions in Part 1 and the two questions in Part 2.

Part 1

Photo set No.1 on the server has photographs of sections of a 2-day-old chicken embryo (Figure 1). Observe the sections and answer the following questions.

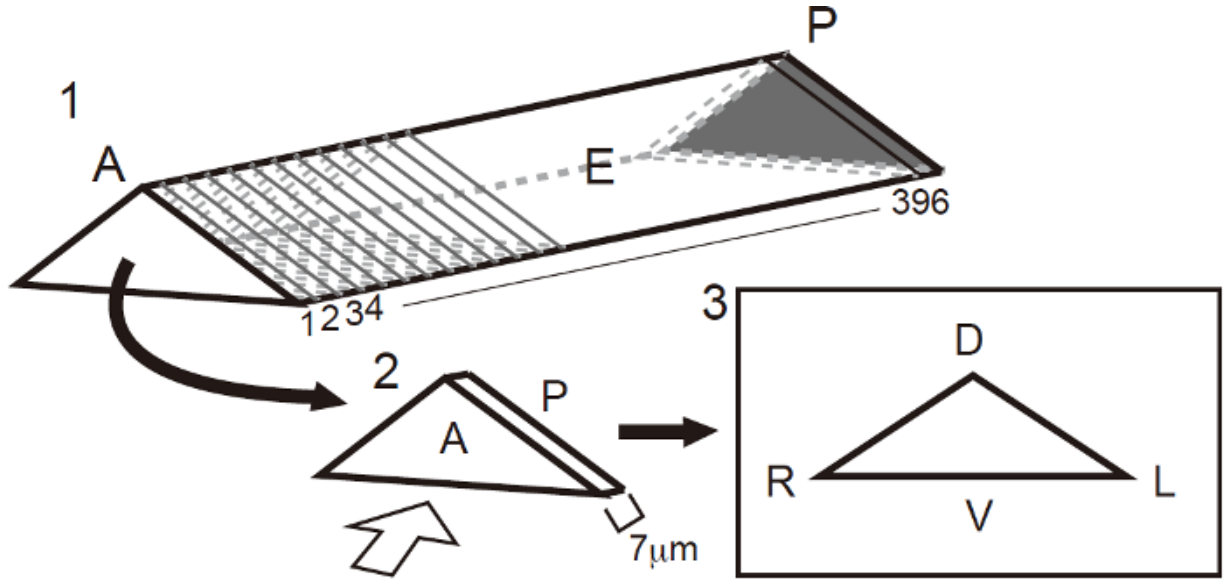


Figure 1

1: A chicken embryo (E) was cut into 7-μm sections from anterior (A) to posterior (P). Here, the chick embryo is represented by a triangular prism.

2: A photo of each section (#1-396) was taken from the front side (A).

3: In each photo, dorsal (D), ventral (V), right (R) and left (L) sides of the embryo are shown as indicated.

Q1. You can find several sections that resemble the diagram in Figure 2A. Choose one of them and draw it. Your drawing should show structural characteristics of embryonic tissues as detailed as you see it in Figure 2B (e.g. cell membranes and nuclei). Draw your answer as large as possible in the frame of the answer sheet. No labeling of the embryonic structures in your drawing is required. [20 marks]

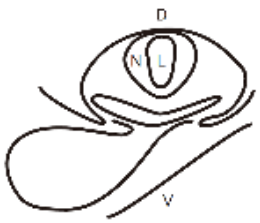


Figure 2A: Schematic diagram of a section of the chick embryo

N: the neural tube

L: lumen of the neural tube

D: Dorsal side of the embryo

V: Ventral side of the embryo



Figure 2B: Example answer for a sketch of chicken embryo.

Q2. The neural tube (N in Figure 2A) extends anterior-posteriorly. Examine all sections on the slide. Reconstitute and draw the shape of the entire neural tube from the most anterior to the most posterior end, as if it is viewed from the dorsal side of the embryo. Pay attention to projections and change of the diameter of the neural tube. Draw with the anterior at the top of the frame, and posterior at the bottom of the frame, as large as possible. Note that the embryo may have a slightly curved shape. [15 marks]

Q3. When an external electric field is applied to a cell, structure of cell membrane changes and small holes are formed temporarily, allowing macromolecules to enter the cell. Plasmid DNA for expressing GFP, green fluorescent protein, in cells was injected into the lumen of the neural tube of a chick embryo (L in Figure 2A). Electrodes were placed on the left and right sides of the embryo, and an electric field of direct current was applied. Using this method, GFP can be expressed in neural tube cells. In Figure 3, an embryo was sectioned and sections were viewed from the front side. GFP protein signals were observed. Consider how the positive and negative electrodes are aligned, and fill in the blanks A-D in the following sentence. [4 marks]

DNA contains A which are B, and therefore, plasmid DNA moves towards the C electrode. The D electrode is placed on the right side of the embryo.

choices of A:

1. phosphate groups
2. bases
3. pentose sugars
4. hydroxyl groups

choices of B:

5. negatively charged
6. positively charged
7. neutral

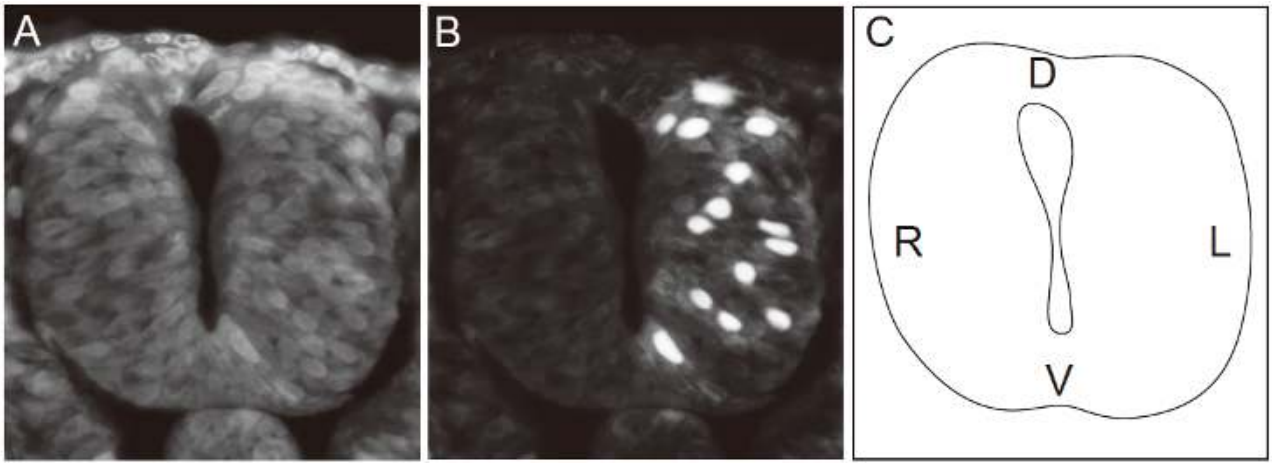
choices of C and D:

8. negative

9. positive

10. neutral

Figure 3: Neural tube with overexpression of GFP protein.



A: Nuclear DNA staining in a section of the neural tube. B: Bright white GFP-positive nuclei were detected. C: A schematic drawing of the neural tube in A and B showing dorsal (D), ventral (V), right (R) and left (L) sides.

Q4. The neural tube is regionalized along the dorsoventral axis, as shown in Figure 4. Different types of neurons are generated in each region. An experiment was conducted to examine how a secreted factor, Wnt3a, is involved in the regionalization of the neural tube. Wnt3a is the ligand of the Wnt signaling pathway (Figure 5) and its mRNA was detected only in the D1 region.

Wnt3a gene was overexpressed in the neural tube of the chicken embryo using the same method and position of the electrodes described in Q3. The embryos were sectioned as described in Q3 and localization of three proteins, P1, P2 and P3, was examined (Figure 6).

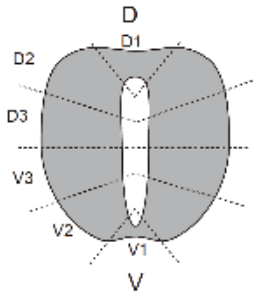


Figure 4: Schematic diagram of neural tube differentiation

The neural tube is divided into several dorsoventral regions, and different neurons develop in each region.

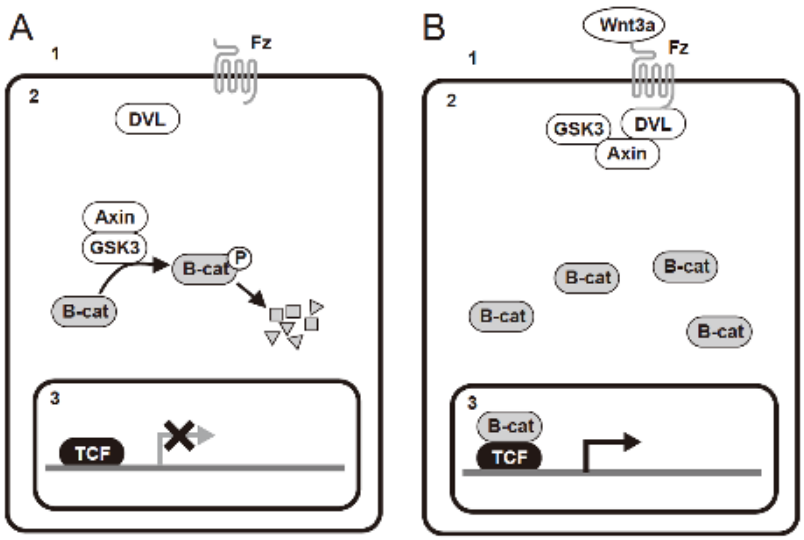


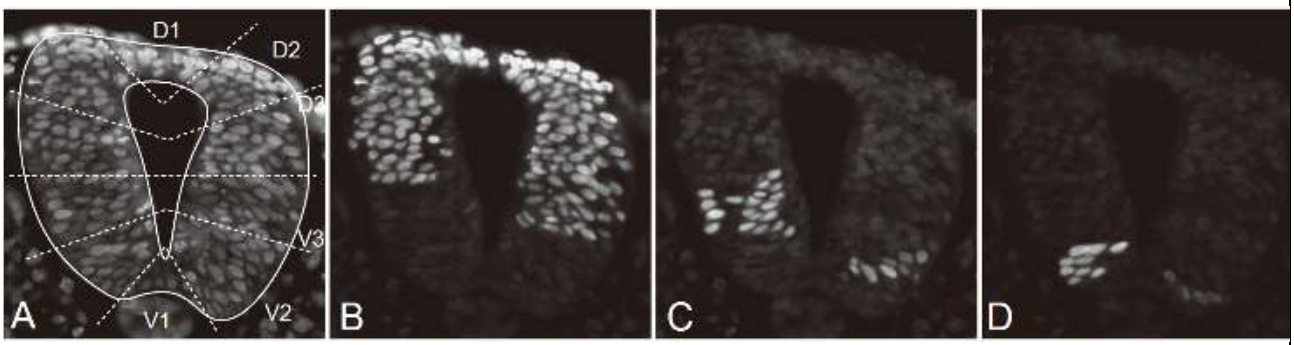
Figure 5: Schematic diagrams of Wnt signaling pathway

A: In the absence of the Wnt ligand, the complex of GSK3 and Axin phosphorylates B-cat and degrades phosphorylated B-cat. In the nucleus, TCF binds to the regulatory region of the Wnt t signal target gene and inhibits its transcriptional activity.

B: In the presence of the Wnt ligand, the Wnt ligand binds to the receptor Fz, and upon stimulation, intracellular DVL binds to the intracellular domain of Fz and inhibits GSK3 function. As a result, B-cat is not degraded and translocates to the nucleus. In the nucleus, B-cat / TCF complex binds to the regulatory region of the target gene of the Wnt signal and activates its transcription.

1: extracellular space, 2: cytoplasm, 3: nucleus

Figure 6: Expression of various proteins in the neural tube when *Wnt3*a was overexpressed.



A: Nuclear DNA staining of a section of neural tube with region names. Localization of P1 (panel B), P2 (panel C) and P3 (panel D) protein shown in the same section of A. Top of the pictures is the dorsal side.

4-1. Based on data from these experiments, identify the localization of the following proteins in the neural tube of a **NORMAL** developing chicken embryo. Choose the best choice of localization. [4 marks]

proteins:

4-1-1: B-cat in nuclei

4-1-2: TCF in nuclei

choices of localization:

a. Present with similar expression levels throughout the neural tube

b. Present throughout the neural tube, but stronger on the ventral side and weaker on the dorsal side

c. Present throughout the neural tube, but stronger on the dorsal side and weaker on the ventral side

d. Localize only in D1.

e. Localize only in V1.

4-2. P2 protein regulates differentiation of neurons in V3 region of the **NORMAL** developing neural tube. Which of the following is the most probable mechanism of P2 to regulate neural differentiation? Please answer based on localization of P2 protein in cells of V3 region in Figure 6 C? [2 marks]

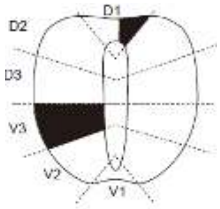
a. P2 acts as a transcription factor and represses the expression of another gene.

b. P2 is secreted extracellularly and activates a signal in another cell.

c. P2 acts in the cytoplasm and transmits the signal received by the expressing cell.

d. As a transmembrane receptor, P2 binds extracellular proteins.

4-3. In the following experiments (4-3-1, 2, and 3), wild-type or mutated components of the Wnt signaling pathway (see Figure 5) were overexpressed or inhibited in the neural tube. Indicate the expression region of the specified protein using Figure 6 as reference. The schematic diagram of the neural tube on the answer sheet shows the dorsal side at the top. Indicate the protein expression regions by painting a schematic diagram of the neural tube with a pencil. [12 marks]

	Example answer 2: Protein P1 is expressed in D1 and V3 on the left and right sides of the neural tube, respectively. Note that this schematic figure of a section of the neural tube is viewed from the front (see Figure 3C for its orientation).

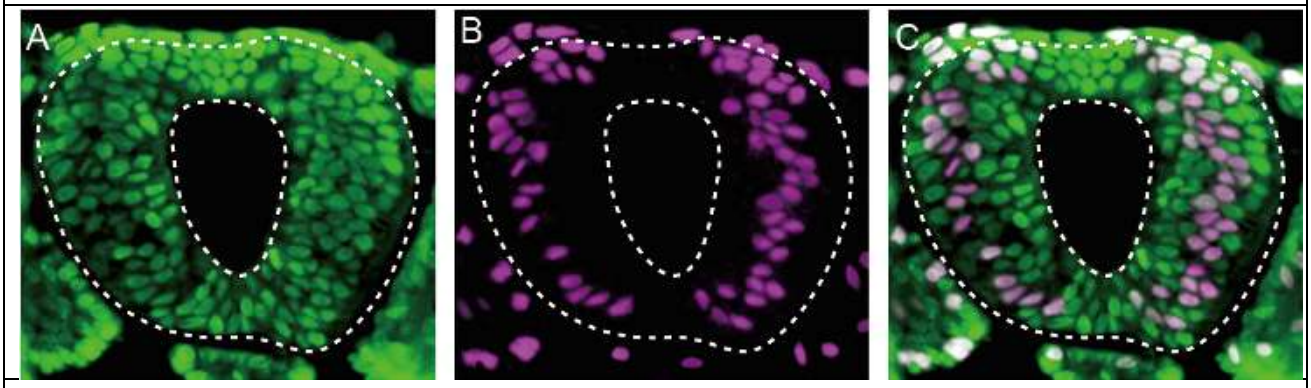
4-3-1. What is the expression region of protein P1, when the gene encoding a mutant B-cat protein (with an amino acid mutation that makes it non-phosphorylatable by GSK3) in the neural tube with the method and position of the electrodes described in Q3?

4-3-2. What is the expression region of P2, when the translation of both DVL and GSK3 is on both sides of the neural tube?

4-3-3. What is the expression region of P3, when the gene encoding the TCF protein is overexpressed in the neural tube with the method and position of the electrodes described in Q3?

Q5. After *Wnt3a* was overexpressed in the neural tube as described in Q4, 5-bromo-2'-deoxyuridine (BrdU), a thymidine analogue, was added to culture for 4 hours. The embryos were then sectioned, BrdU was detected with anti-BrdU antibody and nuclear DNA was stained (Figure 7).

Figure 7: Localization of BrdU-positive cells in the neural tube with overexpression of *Wnt3a*



A: photograph of a section showing nuclear DNA.
B: the same section in A showing the location of BrdU. C. merging of A and B photos.
The dashed white line indicates the neural tube.

5-1: Where is BrdU detected, concerning their cell cycle stage? Choose all possible options. [2 marks]

a. in all cells in the neural tube
b. in cells that were in G1 phase during culture with BrdU
c. in cells that were in M phase during culture with BrdU
d. in cells that were in S phase during culture with BrdU
e. in cells that were in G2 phase during culture with BrdU
f. in cells that were in S phase at the onset of culture with BrdU, and entered G2 phase during four-hour culture with BrdU.
g. in cells that were in G1 phase at the onset of culture with BrdU, and entered S phase during four-hour culture with BrdU.
h. in cells that were in M phase at the onset of culture with BrdU, and entered G1 phase during four-hour culture with BrdU.
i. in cells that were in G2 phase at the onset of culture with BrdU, and entered M phase during four-hour culture with BrdU.

5-2: From the previous experiment, you hypothesized that higher levels of Wnt3a protein promote cell division in the neural tube. Using Photos in Figure 7, you decide to design an experiment to test this hypothesis. In this experiment, you will determine numerical values from counting the number of cells in two of three groups listed below, each in two different selected regions of the neural tube. [15 marks]

Value Options:

a. Total number of cells

b. Number of BrdU-positive cells

c. Number of BrdU-negative cells

The answer should be written down in the following way:

- ◊ In A1 and A2: enclose in a box the part of the neural tube which you have used for counting.
- ◊ A3 and A4: write down the cell number you have obtained through counting (choose two values from Value Options, i.e. choose only two-rows to fill out in both columns.).
- ◊ A5: write down the formula you have used to obtain final numerical value for comparison.
- ◊ A6 and A7: Write down final numerical values in the two regions, based on which your conclusion will be drawn.

Part 2

Q1. The development of the ascidian embryo has been studied for more than a century. The lineage of the embryonic cells is invariant, which means that the number and position of the cells are the same between individuals. Figure 8 is a schematic diagram of the 32-cell stage embryo and shows the cells' names. Researchers identified various transcription factors expressed in specific cells at the 32-cell stage. Embryos in photo set No.2 are 32-cell stage embryos stained to show mRNA for gene *W* in bluish-black color. Several individuals are shown from various viewing angles.

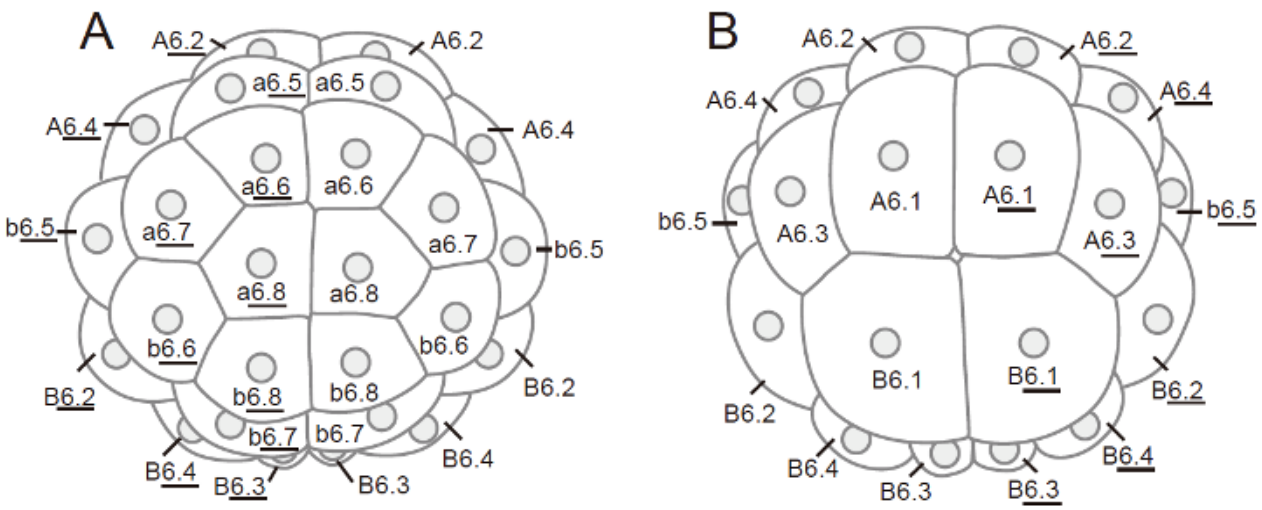


Figure 8: Schematic diagram of the ascidian embryo at the 32-cell stage.
A: Animal pole (top) view, B: Vegetal pole (bottom) view.
Names of the cells are shown in the diagram (ex. A6.1, b6.5). Names of cells in the animal hemisphere begin with a or b. Names of cells in the vegetal hemisphere begin with A and B. Anterior cells begin with A, a, and posterior cells begin with B, b. Cells that contribute to the right side of the bilaterally symmetrical larvae are distinguished by underlining. Cells in the periphery are shown in both diagrams. Gray circles are nuclei.

Photo set No.2
Images of each embryo are placed in separate lines on the server. The focal plane is arranged from the front to the back.
mRNA of gene *W* was stained blueish black. In ascidian embryos, mRNA is detected primarily in the nucleus.

1-1. Observe the images in photo set No. 2 and identify cells that are stained. Use the information shown in Figure 8 to identify the cells. Circle the name of stained cells on the answer sheet. [15 marks]

Q2. In cells, whose names are enclosed by boxes in Figure 9, mRNA of the gene that encodes secreted factor X is detected at the 32-cell stage.

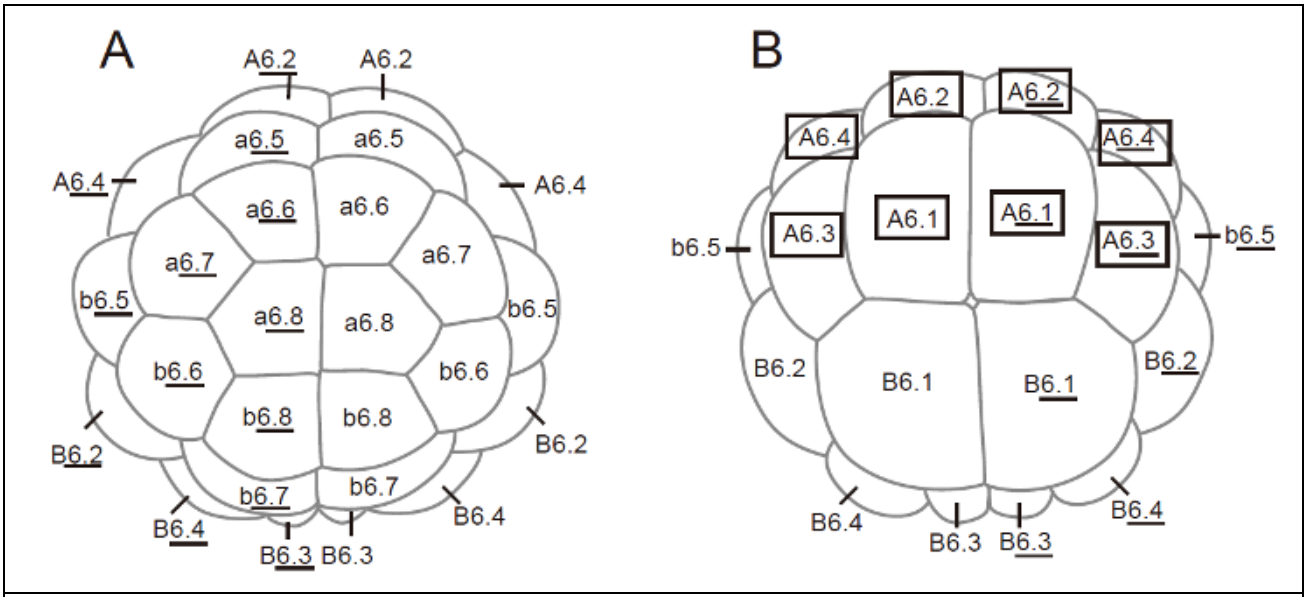


Figure 9: Expression of a gene that encodes a secreted factor X at the 32-cell stage. The names of the cells that express this factor is enclosed in a box. A: Animal pole view, B: Vegetal pole view

At the 64-cell stage, the daughter cells of the A6.2 cells express gene *Y* that is required for mesodermal differentiation. The following experiments were carried out to examine how the expression of this gene Y is regulated.

Experiment 1: The daughter cells of A6.2 cells did not express gene *Y* when the embryo was treated with an inhibitor of the receptor for secreted factor X.

Experiment 2: The daughter cells of A6.2 did not express gene *Y* when A6.2 cells were isolated from the embryo and cultured in isolation.

Experiment 3: Four A6.2 cells were collected from four 32-cell stage embryos and cultured together. The cells were in contact with each other during culture. Daughter cells of A6.2s did not express gene *Y*. The results were the same when the number of A6.2 cells that were cultured in contact was increased.

Experiment 4: The daughter cells of A6.2 expressed gene *Y* when the expression of gene *X* was suppressed in either A6.1, A6.1, A6.3, or A6.4 cells.

Experiment 5: The daughter cells of A6.2 did not express gene Y when the expression of gene *X* was suppressed in both A6.1 and A6.4 cells. The results were the same when the expression of gene *X* was suppressed in both A6.1 and A6.4 cells.

Experiment 6: The daughter cells of A6.2 expressed gene *Y* when the expression of gene *X* was suppressed in both A6.1 and A6.3 cells. The results were the same when the expression of gene *X* was suppressed in both A6.1 and A6.3 cells.

2-1. Using the results from experiment 1 to 6, choose the smallest possible combination of cells that must secrete factor X for daughter cells of A6.2 cells to express gene *Y*. It may happen that not all columns of A1-A5 on the Answer Sheet are required to be filled in. [10 marks]

Answer example:
There are two possible combinations.
Factor X secreted from a6.6 alone can induce Y in A6.2.
OR
Factor X secreted from two cells [b6.6 AND b6.6] can induce Y in A6.2.

A1	A2	A3	A4	A5
a6.6	b6.6 b6.6			

2-2. Choose all possibilities from the following that explains the results of experiment 2 and 3. [4 marks]
a. X is not transcribed in A6.2 cells.
b. X is transcribed but not translated in A6.2 cells.
c. X is translated but not secreted in A6.2 cells.
d. X is secreted, but the receptor for X is not expressed in A6.2 cells.

2-3. Gene *Z* that encodes a transcription factor is expressed specifically in A6.2 cell. Inhibition of translation of Z abolished the expression of gene *Y* in the daughter cells of A6.2.
You would like to test the hypothesis that Z and X are independently regulated factors that cooperate to regulate gene *Y* expression. Design the three experiments to prove this hypothesis in addition to experiments 1 to 6 and predict the results of each experiment if the hypothesis is correct.
Select cells to be manipulated in A1. Write the choice of experimental manipulation for that cell in A2. Write the name of the cell to be analyzed in A3. Write the possible result when the hypothesis is correct in A4. In each experiment, choose only one of the options for A1, A2, A3, and A4, respectively. [15 marks]

choices of cells to be manipulated/observed:
a. A6.2
b. A6.1, A6.1, A6.3, A6.4

choices of the experimental manipulation:

a. overexpress gene X

b. suppress translation of X

c. overexpress gene Z

d. suppress translation of Z

e. overexpress gene Y

f. suppress translation of Y

choices of the results:

a. Expression of gene X was decreased

b. Gene X expression was elevated

c. Gene X expression remained unchanged

d. Expression of gene Y was decreased

e. Gene Y expression was increased

f. Expression of gene Y was unchanged

g. Gene Z expression was reduced

h. Gene Z expression was increased

i. Gene Z expression remained unchanged

End of Practical Exam 1.

URL List

#	Participants	ID	URL for Practical Exam 1
1	Iran	11	13.127.152.58/index.html
2	Hungary	12	18.159.45.191/index.html
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6	Kazakhstan	17	13.127.152.58/index.html
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