M.Sc. (IIIrd Semester) Examination, 2015
Zoology
Paper: LZT-301
(Developmental Biology and Immunology)
Model answer

Section: A

Answer No.1. (i) b (ii) c (iii) a (iv) a (v) a (vi) a (vii) a (viii) c (ix) b (x) c

Section: B

Answer No. 2.

Immune tolerance:

Immune tolerance or immunological tolerance describes a state of unresponsiveness of the immune system to substances or tissue that have the capacity to elicit an immune response.

Tolerance is classified into central tolerance or peripheral tolerance depending on where the state is originally induced—in the thymus and bone marrow (central) or in other tissues and lymph nodes (peripheral).

Central tolerance

Central tolerance refers to the tolerance established by deleting autoreactive lymphocyte clones before they develop into fully immunocompetent cells. It occurs during lymphocyte development in the thymus and bone marrow for T and B lymphocytes, respectively. In these tissues, maturing lymphocytes are exposed to self-antigens presented by medullary thymic epithelial cells and thymic dendritic cells, or bone marrow cells. Self-antigens are present due to endogenous expression, importation of antigen from peripheral sites via circulating blood, and in the case of thymic stromal cells, expression of proteins of other non-thymic tissues by the action of the some specific transcription factor.

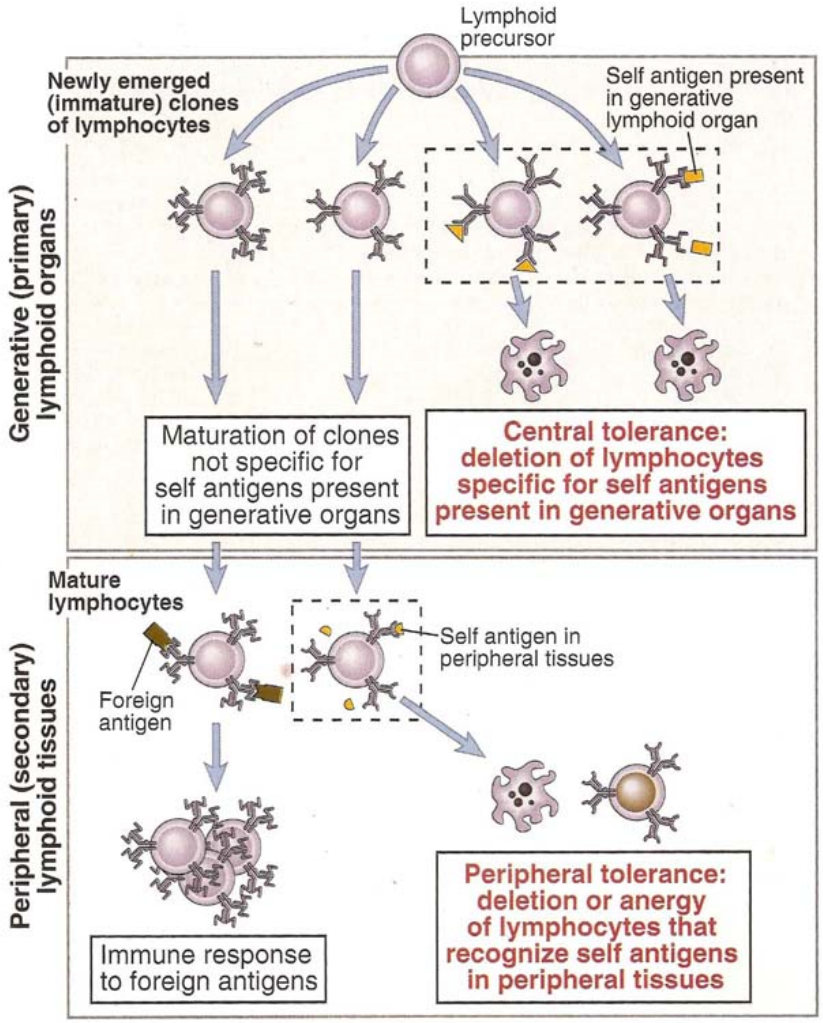
Those lymphocytes that have receptors that bind strongly to self-antigens are removed by induction of apoptosis of the autoreactive cells, or by induction of anergy, a state of non-activity. Weakly autoreactive B cells may also remain in a state of immunological ignorance where they simply do not respond to stimulation of their B cell receptor. Some weakly self-recognizing T cells are alternatively differentiated into natural regulatory T cells (nTreg cells), which act as sentinels in the periphery to calm down potential instances of T cell autoreactivity.

Peripheral tolerance

Peripheral tolerance develops after T and B cells mature and enter the peripheral tissues and lymph nodes. It is established by a number of partly overlapping mechanisms that mostly involve control at the level of T cells, especially CD4+ helper T cells, which orchestrate immune responses and give B cells the confirmatory signals they need in order to produce antibodies.

Several mechanisms are involved in induction and maintenance of tolerance, including clonal deletion, clonal anergy, receptor editing, receptor down-modulation and lymphocyte sequestration. The number of antigen presenting cells, the number and activity of regulatory T cells and regulatory B cells, the nature and amount of antigenic peptides generated and the presence of co-stimulatory signals in a particular tissue

are also important. Depending on the site and the level of antigen expression, different states of peripheral B-cell and T-cell tolerance can be reached.



Autoimmunity

Autoimmunity is the system of immune responses of an organism against its own cells and tissues. Any disease that results from such an aberrant immune response is termed an autoimmune disease.

Examples includes Celiac disease, diabetes mellitus type 1, Sarcoidosis, systemic lupus erythematosus (SLE), Sjögren's syndrome, Churg-Strauss Syndrome, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, Addison's Disease, rheumatoid arthritis (RA), Polymyositis (PM), and Dermatomyositis (DM). Autoimmune diseases are very often treated with steroids.

An immune response to "self" antigens defines a state of autoimmunity. Clinically, autoimmune states may be mild or symptom-free, but in other cases may result in severe and fatal afflictions.

Autoimmune responses may result from two general causes. In one case, antigens normally "hidden" from the immune system ("sequestered" antigens) may be released into the circulation and trigger a response by the immune system. In this case no state of peripheral tolerance need exist, and the immune system is capable of generating an effective response on exposure to the antigen. Examples of clinical or experimentally induced autoimmunity to such sequestered antigens include those directed against thyroglobulin, eye lens protein and spermatozoa antigens.

A different situation exists when the normally effective tolerant state of the immune system to "self" antigens is for some reason abrogated. The final result of such a breakdown of tolerance, however, is the same as in the case above: a damaging and potentially fatal autoimmune reaction.

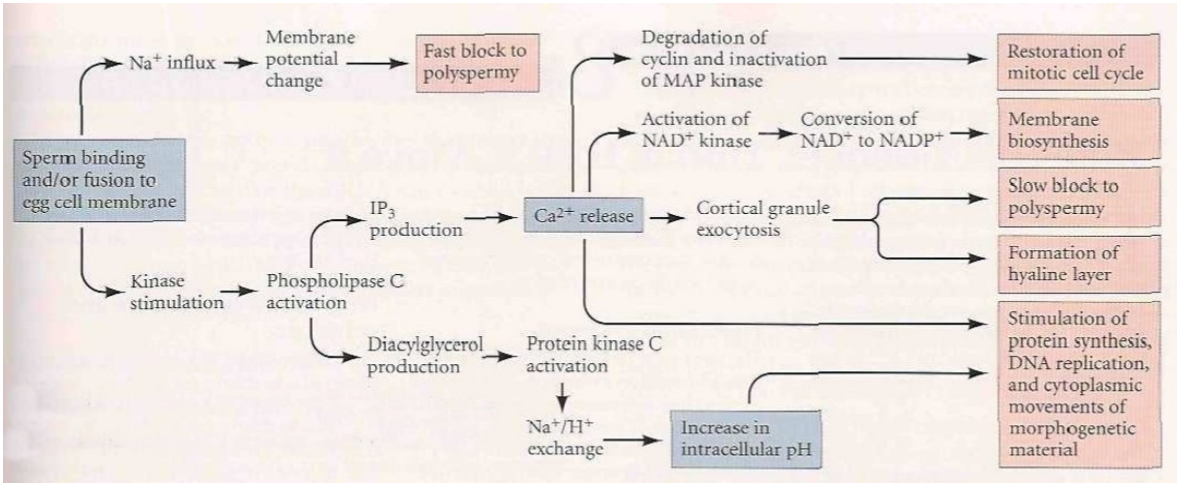
Breaking of the normal tolerant state of an organism's immune system may result from a variety of influences:

i) Decrease in Treg activity.

ii) Increase in TH activity (e.g., with adjuvants).

iii) Immune response to foreign antigens which happen to cross-react with "self"

Answer No. 3. Following is the possible mechanism for the activation of egg metabolism

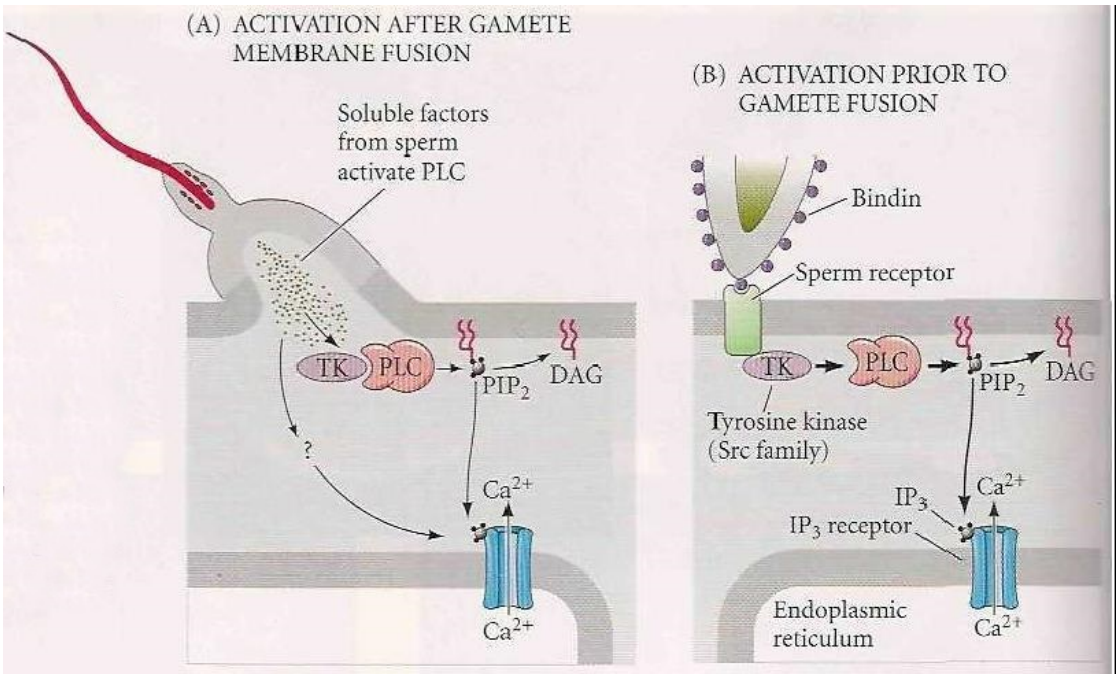


Release of intracellular calcium ions

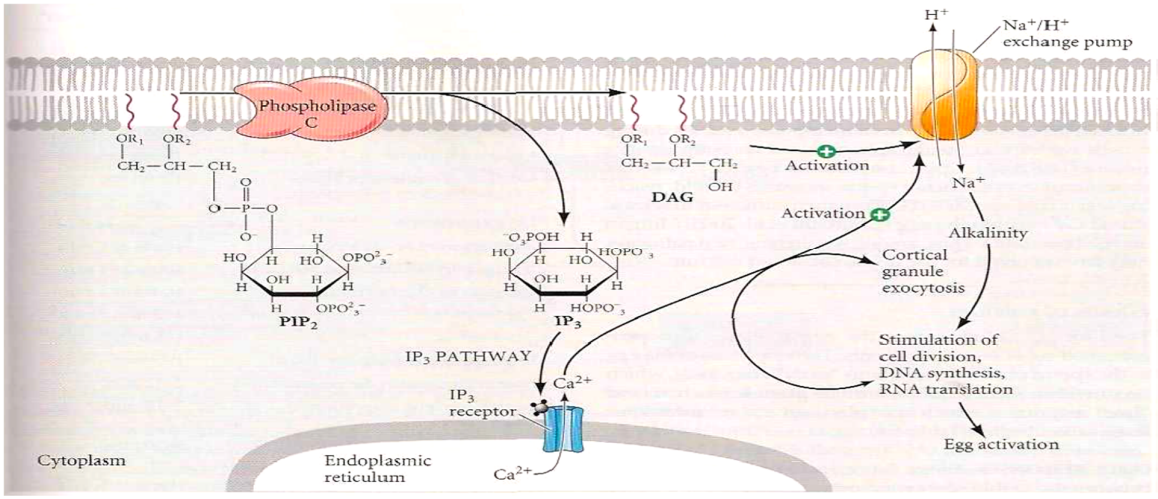
Calcium ions release the inhibitors from maternally stored messages, allowing these mRNAs to be translated; they also is the inhibition of nuclear division, thereby allowing cleavage to occur. However, the process of release of calcium ions varies between species.

First mechanism: This is first proposed by Jacques Loeb (1899, 1902). It states that a soluble factor from the sperm is introduced into the egg at the time cell fusion, and this substance activates the egg by changing the ionic composition of the cytoplasm. This mechanism probably works in mammals.

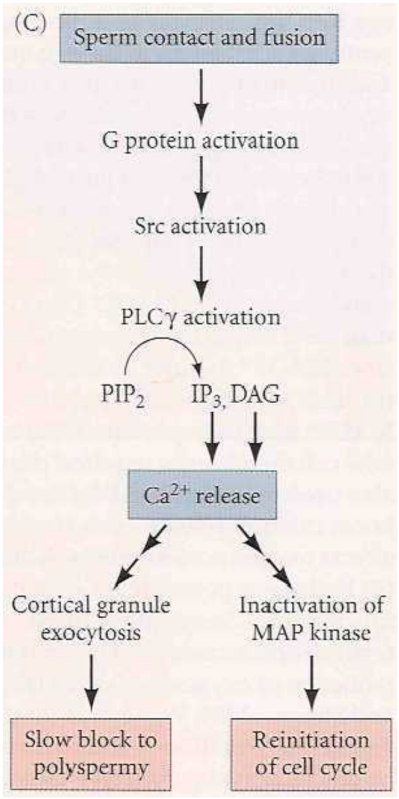
Second mechanism: This was proposed by Frank Lillie (1913), is that the sperm acts like a big hormone, binding to receptors on the egg cell surface and changing their conformation, thus initiating reactions within the cytoplasm that activate the egg. This is probably what happens in sea urchins.



IP3: THE RELEASER OF CALCIUM IONS



Throughout the animal kingdom, inositol 1,4,5-trisphosphate (IP3) is the primary mechanism for releasing $Ca2^{+}$ from intracellular storage. The membrane phospholipid phosphatidyl inositol 4,5-bisphosphate (PIP_2,) is split by the enzyme phospholipase C (PLC) to yield two active compounds: IP_3 and diacylglycerol (DAG). IP_3 is able to release Ca^{2+} into the cytoplasm by opening the calcium channels of the endoplasmic reticulum. DAG activates protein kinase C, which in turn activates a protein that exchanges sodium ions for hydrogen ions, raising the pH of the egg.



The PLC activation results in the liberation of Ca^{2+} and the alkalinization of the egg, and both of the compounds this activation creates—IP_3 and DAG— are involved in the initiation of development.

In sea urchin eggs, IP_3 is formed initially at the site of sperm entry and can be detected within seconds of the eggs being fertilized.

Binding of Ca^{2+} to these receptors releases more $Ca2^{+}$, which binds to more receptors, and so on. The resulting wave of calcium release is propagated throughout the cell, starting at the point of sperm entry.

PHOSPHOLIPASE C, THE GENERATOR OF IP3

The active PLC in echinoderms is a member of the 7 (gamma) family of PLCs.

KINASES: A LINK BETWEEN SPERM AND PLCy

G proteins are found in the cortex of the egg. G proteins activate activate PLC. Indeed, more than one pathway and more than one way are involved in activating Ca^{2+} release.

Answer No. 4.

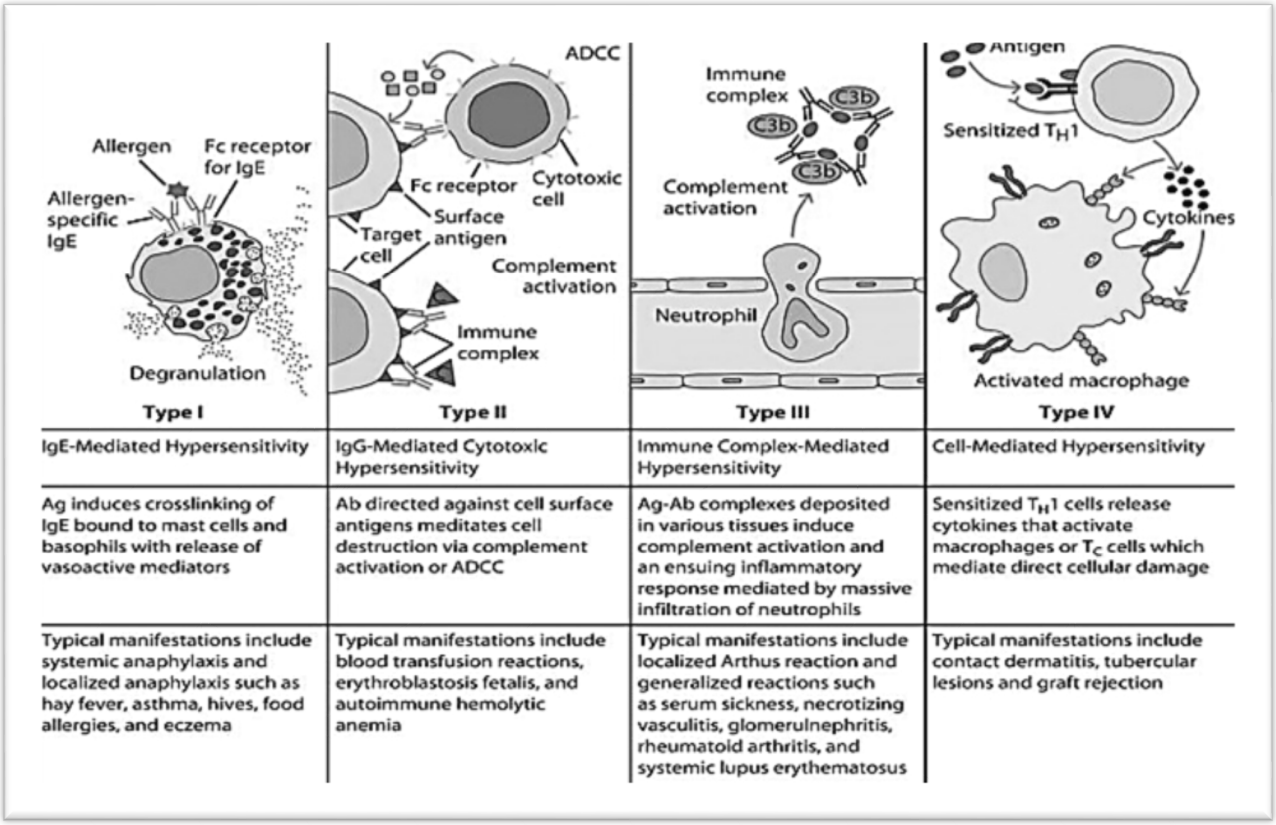
Hypersensitivity:

Hypersensitivity describes an abnormal or pathologic immune reaction that is caused by an immune response to repeated exposure to an antigen.

Hypersensitivity refers to excessive, undesirable (damaging, discomfort-producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host.

Hypersensitivity diseases have been grouped into four major categories based upon their underlying causes. These groups are:

1.) Immediate (type I) hypersensitivity
2.) Antibody-mediated (type II) hypersensitivity
3.) Immune complex-mediated (type III) hypersensitivity
4.) Cell-mediated (type IV) hypersensitivity



Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

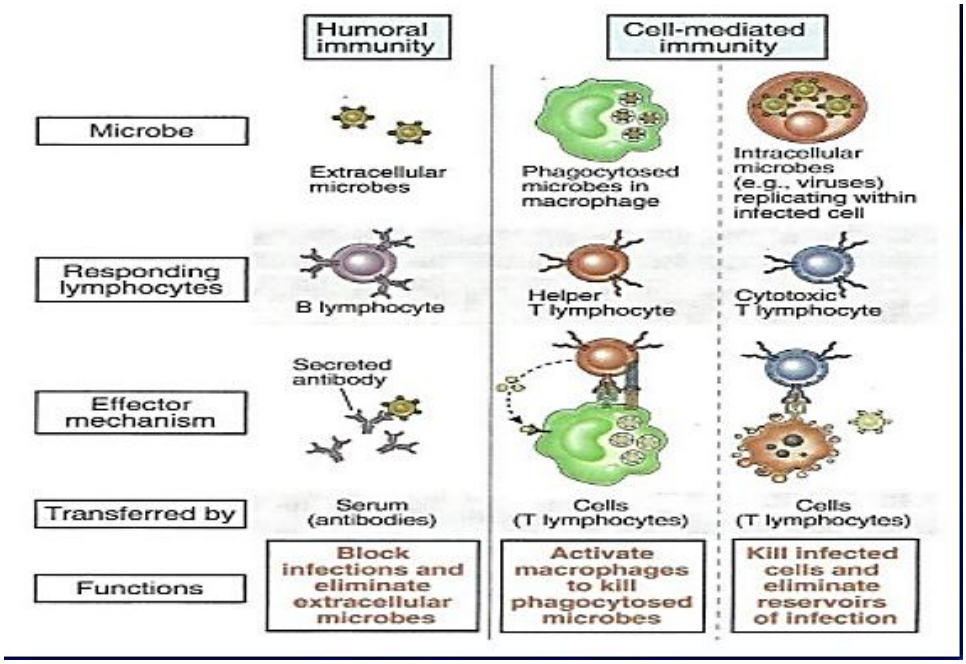
Answer No. 5.

Humoral and Cell mediated immunity:

The immune system distinguishes two groups of foreign substances. One group consists of antigens that are freely circulating in the body. These include molecules, viruses, and foreign cells. A second group consists of self cells that display aberrant MHC proteins. Aberrant MHC proteins can originate from antigens that have been engulfed and broken down (exogenous antigens) or from virus-infected and tumor cells that are actively synthesizing foreign proteins (endogenous antigens). Depending on the kind of foreign invasion, two different immune responses occur:

Humoral response (or antibody-mediated response): These response involves B cells that recognize antigens or pathogens that are circulating in the lymph or blood ("humor" is a medieval term for body fluid). The response follows this chain of events:

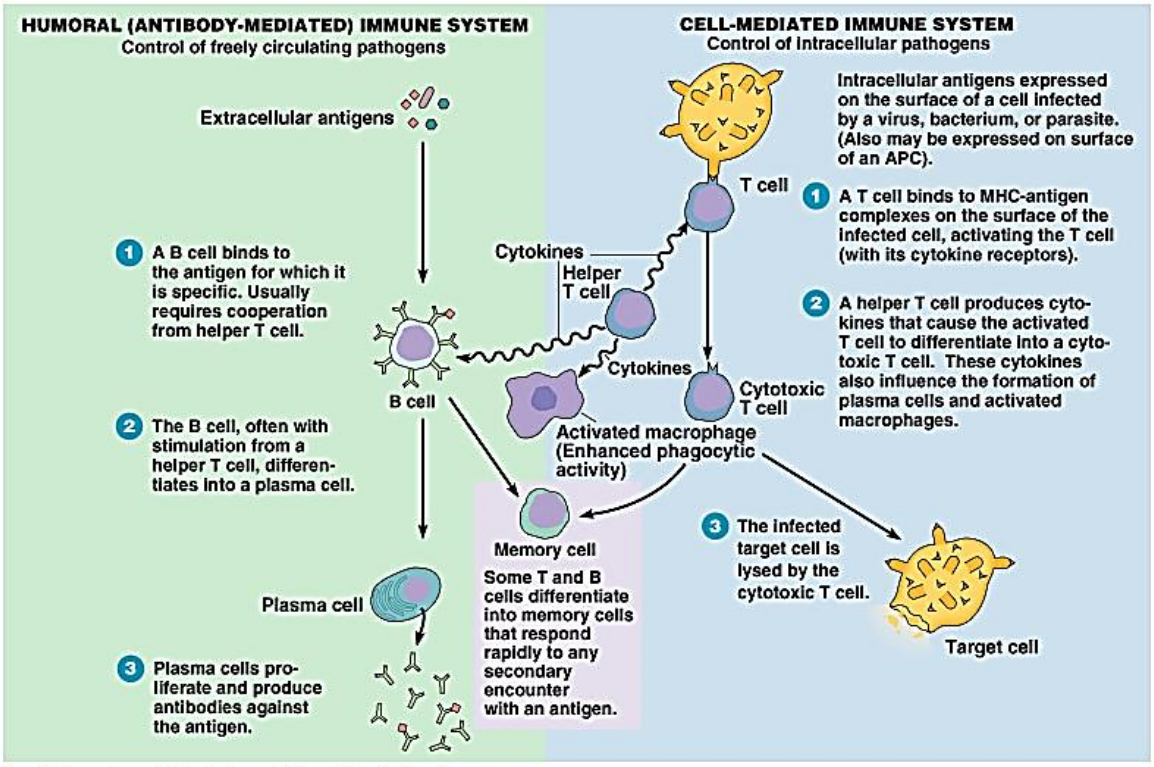
1. Antigens bind to B cells.
2. Interleukins or helper T cells costimulate B cells. In most cases, both an antigen and a costimulator are required to activate a B cell and initiate B cell proliferation.
3. B cells proliferate and produce plasma cells. The plasma cells bear antibodies with the identical antigen specificity as the antigen receptors of the activated B cells. The antibodies are released and circulate through the body, binding to antigens.
4. B cells produce memory cells. Memory cells provide future immunity.



Components of specific immune system

Cell-mediated response: These response involves mostly T cells and responds to any cell that displays aberrant MHC markers, including cells invaded by pathogens, tumor cells, or transplanted cells. The following chain of events describes this immune response:

1. Self cells or APCs displaying foreign antigens bind to T cells.
2. Interleukins (secreted by APCs or helper T cells) costimulate activation of T cells.
3. If MHC-I and endogenous antigens are displayed on the plasma membrane, T cells proliferate, producing cytotoxic T cells. Cytotoxic T cells destroy cells displaying the antigens.
4. If MHC-II and exogenous antigens are displayed on the plasma membrane, T cells proliferate, producing helper T cells. Helper T cells release interleukins (and other cytokines), which stimulate B cells to produce antibodies that bind to the antigens and stimulate nonspecific agents (NK and macrophages) to destroy the antigens.



General characteristics and importance:

Humoral Immunity Or Antibody Mediated Immune System (AMIS):

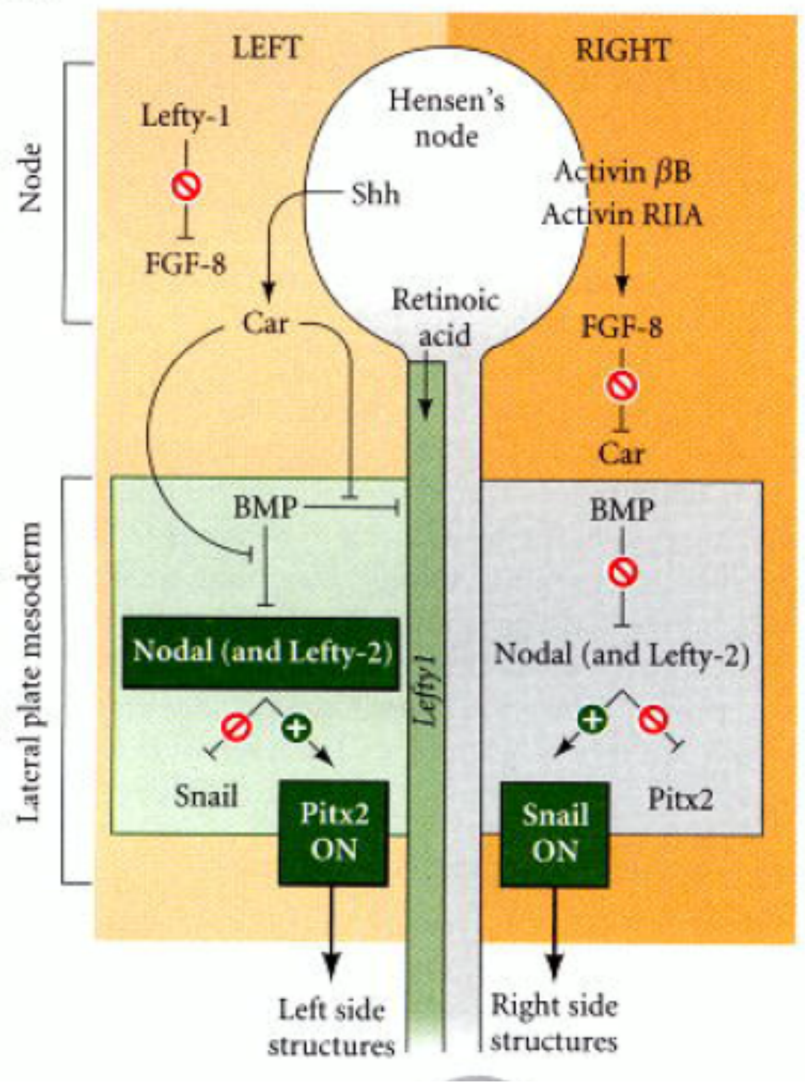
1. It consists of B-lymphocytes which produce the antibodies that circulate in the body fluids.
2. It defends the body against viruses and bacteria.
3. It does not respond to transplants.
4. It does not provide immunity against cancer.

Cell Mediated Immunity Or Cell Mediated Immune System (CMIS):

1. It consists of T-lymphocytes which produce normally 4 types of T-cells.
2. It defends the body against all pathogens including fungi and protozoa.
3. It reacts against transplants.
4. It provides immunity against cancer.

Answer No. 6. (a) Left-right axis formation in chick

The vertebrate body is not symmetrical. Rather, it has distinct right and left sides. The heart and spleen, for instance, are generally on the left side of the body, while the liver is usually on the right side. The distinction between the right and left sides of vertebrates is regulated by **two major proteins:** the paracrine factor **Nodal** and the transcription factor **Pitx2**. However, the mechanism by which *nodal* gene expression is activated in the left side of the body differs among vertebrate classes.



As the primitive streak reaches its maximum length, transcription of the *sonic hedgehog* gene ceases on the right side of the embryo, due to the expression on this side of activin and its receptor. Activin signaling blocks the expression of *sonic hedgehog* and activates the expression of *fgf8*. FGF8 prevents the transcription of the *caronte* gene. In the absence of Caronte, bone morphogenetic proteins (BMPs) are able to block the expression of *nodal* and *lefty-2*. This activates the *snail* gene (*cSnR*) that is characteristic of the right side of avian embryonic organs. On the left side of the body, the Lefty-1 protein blocks the expression of *fgf8*, while Sonic hedgehog activates *caronte*. Caronte is a paracrine factor that prevents BMPs from repressing the *nodal* and *lefty-2* genes, and also inhibits BMPs from blocking the expression of *lefty-1* on the ventral midline structures. Nodal and Lefty-2 activate *pitx2* and repress *snail* (*cSnR*). Lefty-1 in the ventral midline prevents the Caronte signal from passing to the right side of the embryo. Experimentally induced expression of either *nodal* or *pitx2* on the right side of the chick is able to reverse the asymmetry or cause randomization of the asymmetry on the right or left sides. The course to either left- or right-sidedness can be interfered with at any point along the pathway. If activin is blocked by the experimental

addition of follistatin to the embryo, the asymmetry of *sonic hedgehog* expression vanishes, and the heart has an equal chance of looping either way. Conversely, when activin soaked beads are placed to the left side of Hensen's node, the *sonic hedgehog* gene (usually expressed only on the left side) is repressed. This, in turn, suppresses the transcription of *nodal*. In this situation, too, the heart tube forms randomly, having an equal probability of being left or right. A similar condition can be produced by implanting cells secreting Sonic hedgehog into the right side of the node. In these cases, *nodal* is induced symmetrically in the lateral plate mesoderm, and the heart has a 50% chance of having a left-handed tube.

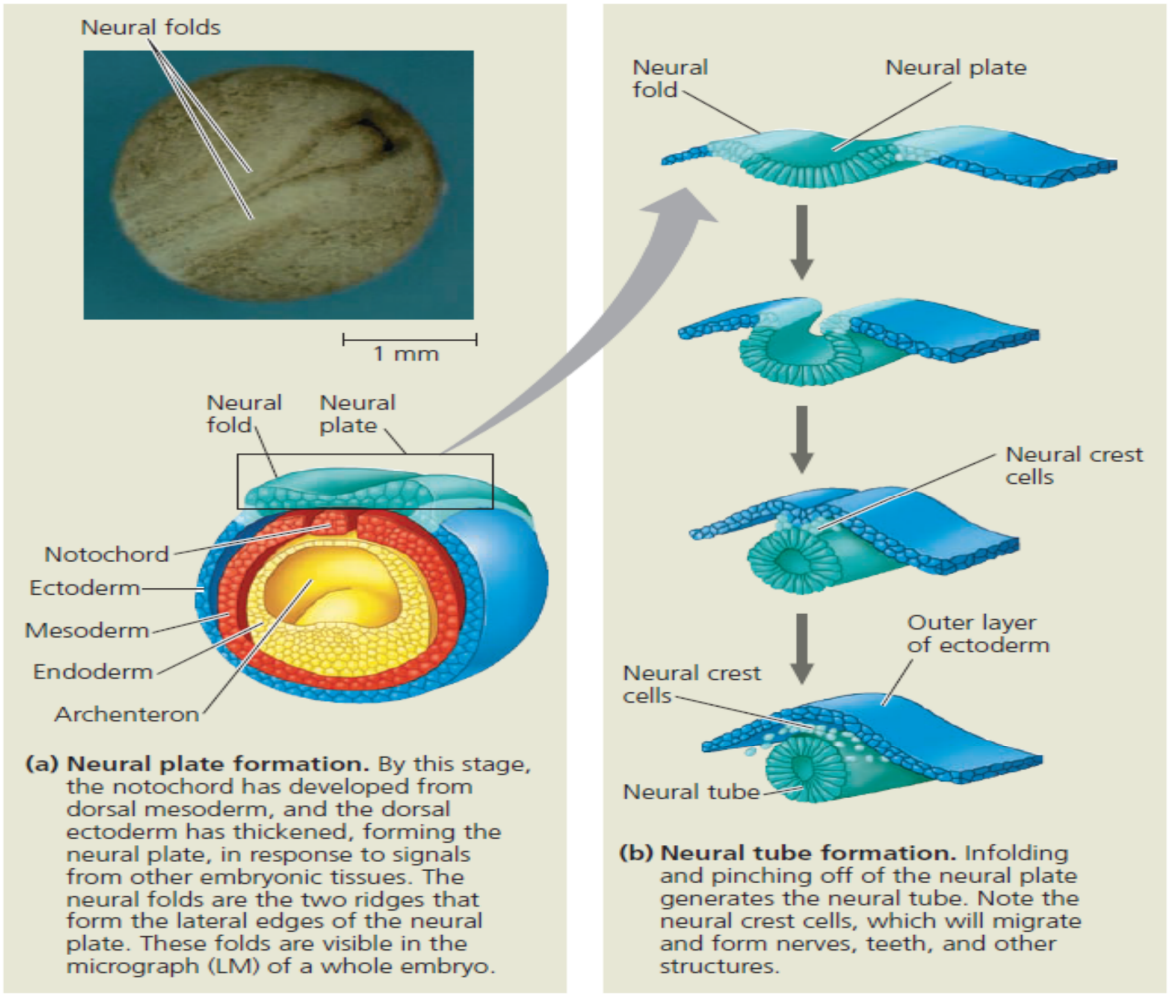
(b) Specifying the left-right axis in amphibia

Left-right axis appears to be initiated at fertilization through the Vg1 protein. In a still unknown fashion, this protein activates a Nodal protein solely on the left side of the body. The Nodal protein activates expression of Pitx2, which is critical in distinguishing left-sidedness from right sidedness in the heart and gut tubes. In all vertebrates studied so far, the crucial event in left-right axis formation is the expression of a ***nodal*** gene in the lateral plate mesoderm on the *left* side of the embryo. In *Xenopus*, this gene is *Xnr-1* (*Xenopus* nodal-related-1). If the expression of this gene is also permitted to occur on the right-hand side, the position of the heart (which is normally on the left side) and the coiling of the gut are randomized. The pathway by which the Xnr-1 protein instructs the heart and gut to fold properly is also unknown, but one of the key genes activated by Xnr-1 appears to be ***pitx2***. Since this gene is activated by Xnr-1, it is normally expressed only on the left side of the embryo. However, if the Pitx2 protein is injected into the right side, too, the placement of the heart and the coiling of the gut are randomized. Pitx2 persists on the left side of the embryo as the heart and gut develop, controlling their respective positions. Pitx2 may be at the "heart of the heart".

Answer No. 7. Dorsal-ventral patterning

The neural tube patterns along the dorsal-ventral axis to establish defined compartments of neural progenitor cells that lead to distinct classes of neurons. This patterning occurs early in development and results from the activity of several secreted signaling molecules. Sonic hedgehog (Shh) is a key player in patterning the ventral axis, while bone morphogenic proteins (BMPs) and Wnt family members play an important role in patterning the dorsal axis. Other factors shown to provide positional information to the neural progenitor cells include fibroblast growth factors (FGFs) and retinoic acid. Retinoic acid is required ventrally along with Shh to induce Pax6 and Olig2 during differentiation of motor neurons. Three main ventral cell types are established during early neural tube development: the *floor plate cells*, which form at the ventral midline during the neural fold stage; as well as the more dorsally located *motor neurons* and *interneurons*. These cell types are specified by the secretion of the Shh from the notochord (located ventrally to the neural tube), and later from the floor plate cells. Shh acts as a morphogen, meaning that it acts in a concentration-dependent manner to specify cell types as it moves further from its source. The following is a proposed mechanism for how Shh patterns the ventral neural tube: A gradient of Shh that controls the expression of a group of homeodomain (HD) and basic Helix-Loop-Helix (bHLH) transcription factors is created. These transcription factors are grouped into two protein classes based on how Shh affects them. Class

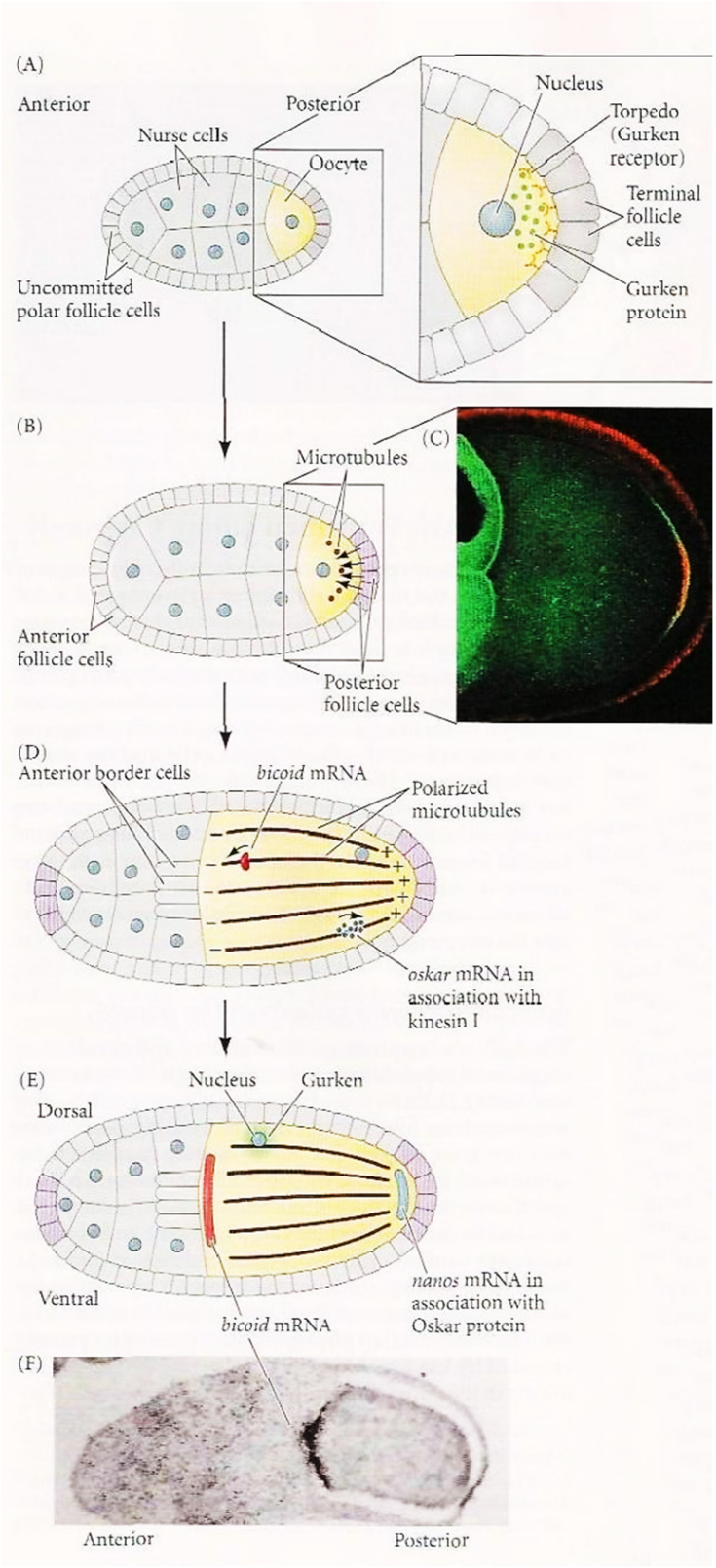
I is inhibited by Shh, whereas Class II is activated by Shh. These two classes of proteins then cross-regulate each other to create more-defined boundaries of expression. The different combinations of expression of these transcription factors along the dorsal-ventral axis of the neural tube are responsible for creating the identity of the neuronal progenitor cells. Five molecularly distinct groups of ventral neurons form from these neuronal progenitor cells in vitro. Studies have shown that neural progenitors can evoke different responses based on the length of exposure to Shh, with a longer exposure time resulting in more ventral cell types. At the dorsal end of the neural tube, BMPs are responsible for neuronal patterning. BMP is initially secreted from the overlying ectoderm. A secondary signaling center is then established in the roof plate, the dorsal most structure of the neural tube. BMP from the dorsal end of the neural tube seems to act in the same concentration-dependent manner as Shh in the ventral end. This was shown using zebrafish mutants that had varying amounts of BMP signaling activity. Researchers observed changes in dorsal-ventral patterning, for example zebrafish deficient in certain BMPs showed a loss of dorsal sensory neurons and an expansion of interneurons.



Answer No. 8. Anterior-posterior polarity in the oocyte of Drosophila

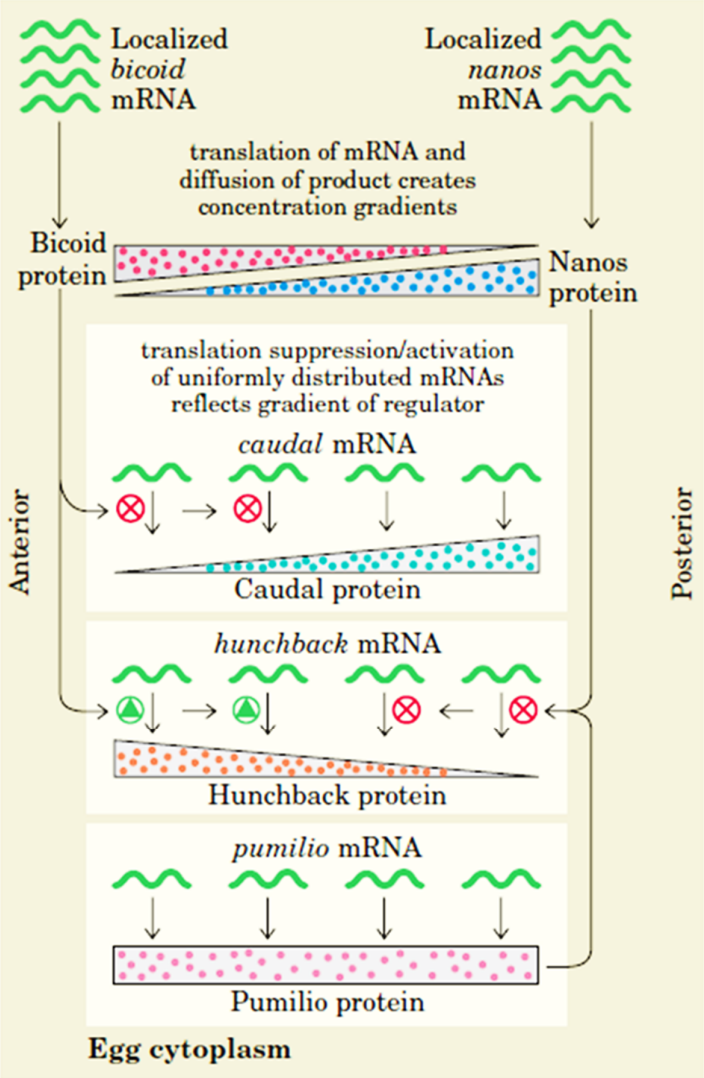
The follicular epithelium surrounding the developing oocyte is initially uniform with respect to cell fate, but this uniformity is broken by two signals organized by the oocyte nucleus. These

signals involve the same gene, *gurken*. The *gurken* message appears to be synthesized in the nurse cells, transported specifically to the oocyte nucleus where it is positioned between the nucleus and the cell membrane and is translated into Gurken protein. At this time the oocyte nucleus is very near the posterior tip of the egg chamber, and the Gurken signal is received by the follicle cells at that position through a receptor protein encoded by the *torpedo* gene. This signal results in the "posteriorization" of these follicle cells. The posterior follicle cells send a signal back into the oocyte. The identity of this signal is not yet known, but it recruits the par-1 protein to the posterior edge of the oocyte cytoplasm. Par-1 protein organizes microtubules specifically with their minus (cap) at the anterior and plus (growing) ends at the posterior ends of the oocyte. The orientation of the microtubules is critical, because different microtubule motor proteins will transport their mRNA or protein cargoes in different directions. The motor protein kinesin is plus end directed cargo protein while Dynein is a "minus-directed" motor protein.



The *oskar* mRNA is transported by kinesin along the microtubules to the posterior end of the oocyte. At posterior cortex, the *oskar* mRNA produces the Oskar protein. Oskar protein recruits more par-1 protein, thereby stabilizing the microtubule orientation and allowing more material to be recruited to the posterior pole of the

oocyte. The posterior pole will thereby have its own distinctive cytoplasm, called **pole plasm**, which contains the determinants for producing the abdomen and the germ cells. This cytoskeletal rearrangement in the oocyte is accompanied by an increase in oocyte volume, owing to transfer of cytoplasmic components from the nurse cells. These components include maternal messengers such as the *bicoid* and *nanos* mRNAs, which are carried by motor proteins along the microtubules to the anterior and posterior ends of the oocyte, respectively. Mainly the products of the bicoid and nanos genes define anterior-posterior axis in Drosophila. The *bicoid* gene product is a major anterior morphogen, and the *nanos* gene product is a major posterior morphogen. The mRNA from the *bicoid* gene is synthesized by nurse cells and deposited in the unfertilized egg near its anterior pole. According to Nusslein-Volhard, *bicoid* mRNA is translated soon after fertilization, and the Bicoid protein diffuses through the cell to create, by the seventh nuclear division, a concentration gradient radiating out from the anterior pole. The Bicoid protein activates the expression of several segmentation genes and at the same time inactivates certain mRNAs. The amounts of Bicoid protein in various parts of the embryo affect the subsequent expression of other genes in a threshold-dependent manner. Genes are transcriptionally activated or translationally repressed only where the Bicoid protein concentration exceeds the threshold. Lack of Bicoid protein results in development of an embryo with two abdomens but neither head nor thorax. The *nanos* gene has an analogous role, but its mRNA is deposited at the posterior end of the egg and the anterior-posterior protein gradient peaks at the posterior pole. The Nanos protein is a translational repressor. In addition to the *bicoid* and *nanos* mRNAs, which are deposited in the egg asymmetrically, several other maternal mRNAs are deposited uniformly throughout the egg cytoplasm. Three of these mRNAs encode the Pumilio, Hunchback, and Caudal proteins, all affected by *nanos* and *bicoid*. Caudal and Pumilio are involved in development of the posterior end of the fly. Caudal is a transcription activator; Pumilio is a translational repressor. Hunchback protein plays an important role in development

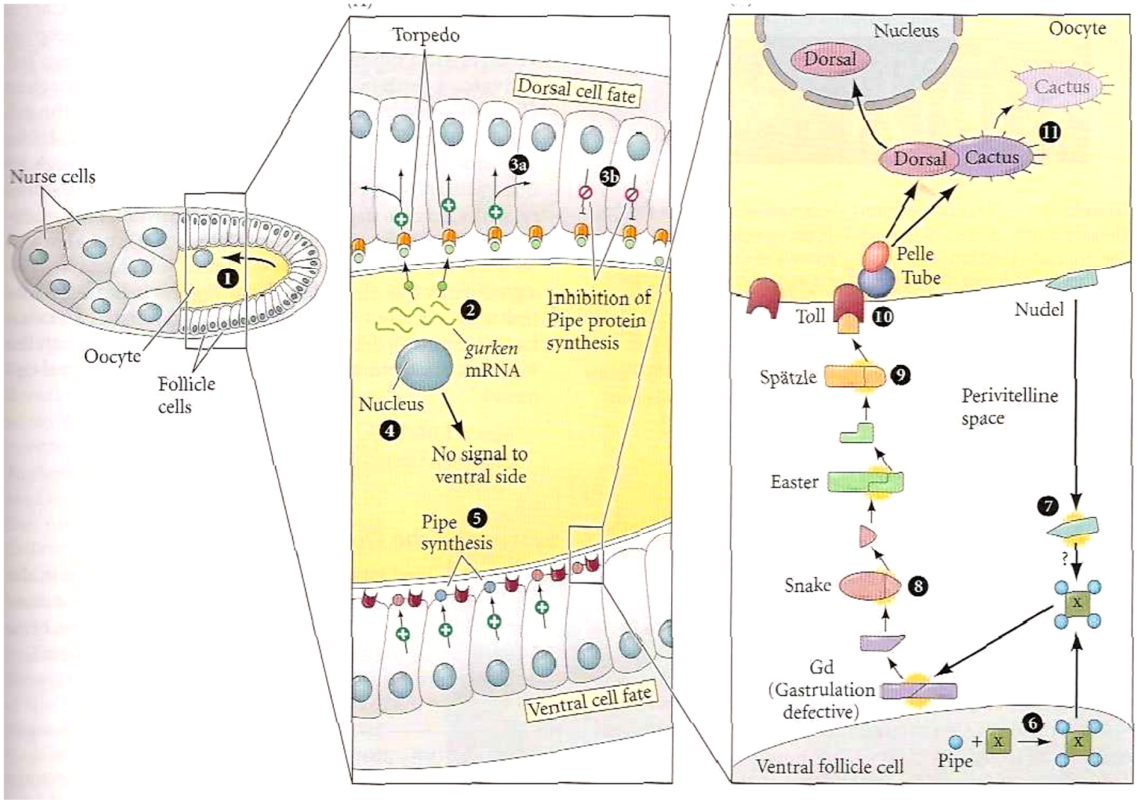


of the anterior end and is also a transcriptional regulator of a variety of genes, in some cases a positive regulator, in other cases negative. Bicoid suppresses translation of *caudal* at the anterior end and also acts as a transcription activator of *hunchback* in the cellular blastoderm. Because *hunchback* is expressed both from maternal mRNAs and from genes in the developing egg, it is considered both a maternal and a segmentation gene. The result of the activities of Bicoid is an increased concentration of Hunchback at the anterior end of the egg. The Nanos and Pumilio proteins act as translational repressors of *hunchback*, suppressing synthesis of its protein near the posterior end of the egg. Pumilio does not function in the absence of the Nanos protein, and the gradient of Nanos expression confines the activity of both proteins to the posterior region. Translational repression of the *hunchback* gene leads to degradation of *hunchback* mRNA near the posterior end. However, lack of Bicoid in the posterior leads to expression of *caudal.* In this way, the Hunchback and Caudal proteins become asymmetrically distributed in the egg.

DORSAL-VENTRAL POLARITY IN THE *DROSOPHILA* OOCYTE

Dorsal-ventral polarity in Drosophila is initiated by the dorsalized follicle cells specified by the Gurken-Torpedo signal. With the increase in oocyte volume, the oocyte nucleus moves to an anterior dorsal position. Here the *gurken* message becomes localized in a crescent between the oocyte nucleus and the oocyte cell membrane, and its protein product forms an anterior-posterior gradient along the dorsal surface of the oocyte. Since it can diffuse only a short distance, Gurken protein reaches only those follicle cells closest to the oocyte nucleus, and it signals those cells to become the more columnar dorsal follicle cells. This establishes the dorsal- ventral polarity in the follicle cell layer that surrounds the growing oocyte. Maternal deficiencies of either the *gurken* or the *torpedo* gene cause ventralization of the embryo. However, *gurken* is active only in the oocyte, whereas *torpedo* is active only in the somatic follicle cells. The Gurken-Torpedo signal initiates a cascade of gene activities that create the dorsal-ventral axis of the embryo. The activated Torpedo receptor protein inhibits the expression of the *pipe* gene, which results formation of Pipe protein only in the *ventral* follicle cells. In some as yet unknown way (probably involving sulfation), Pipe activates the Nudel protein, which is secreted to the cell membrane of the neighboring ventral embryonic cells. A few hours later, activated Nudel initiates the activation of three serine proteases that are secreted into the perivitelline fluid. These proteases are the products of the ***gastrulation*** *defective (gd), snake (snk),* and *easter (en)* genes. Like most extracellular proteases, these molecules are secreted in an inactive form and are subsequently activated by peptide cleavage. In a complex cascade of events, activated Nudel activates the Gastrulation-defective protease. The Gd protease cleaves the Snake protein, activating the Snake protease, which in turn cleaves the Easter protein. This cleavage activates the Easter protease, which then cleaves the Spatzle protein. It is obviously important that the cleavage of these three proteases be limited to the most ventral portion of the embryo. This is accomplished by the secretion of a protease inhibitor from the follicle cells of the ovary. This inhibitor of Easter and Snake is found throughout the perivitelline space surrounding the embryo. Indeed, this protein is very similar to the mammal ian protease inhibitors that limit blood clotting protease cascades to the area of injury. In this way, the proteolytic cleavage of Easter and Spatzle is strictly limited to the area around the most ventral embryonic cells. The cleaved Spatzle protein is now able to bind to its receptor in

the oocyte cell membrane, the product of the *toll* gene. Toll protein is a maternal product that is evenly distributed throughout the cell membrane of the egg, but it becomes activated only by binding the Spatzle protein, which is produced only on the ventral side of the egg. Therefore, the Toll receptors on the ventral side of the egg are transducing a signal into the egg, while the Toll receptors on the dorsal side of the egg are not. This localized activation establishes the dorsal-ventral polarity of the oocyte.



1. Oocyte nucleus travels to anterior dorsal side of oocyte where it localizes *gurken* mRNA.
2. gurken messages are translated. Gurken is received by Torpedo proteins during mid-oogenesis.
3. Torpedo signal causes follicle cells to differentiate to a dorsal morphology. Synthesis of Pipe is inhibited in dorsal follicle cells.
4. Gurken does not diffuse to ventral side.
5. Ventral follicle cells synthesize Pipe.
6. In ventral follicle cells, Pipe completes the modification of an unknown factor (x).
7. Nudel and factor (x) interact to split the Gastrulation-deficient (Gd) protein.
8. Activated Gd splits the Snake protein, and activated Snake cleaves the Easter protein.
9. Activated Easter splits Spatzle; activated Spatzle binds to Toll receptor protein.
10. Toll activation activates Tube and Pelle, which phosphorylate the Cactus protein. Cactus is degraded, releasing it from Dorsal.
11. Dorsal protein enters the nucleus and ventralizes the cell.

Resources: ***Different internet websites and textbooks***