C8545 Developmental Biology

Lesson 2

Early Development of *Drosophila*

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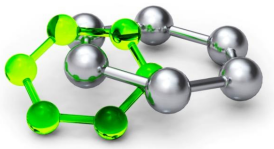
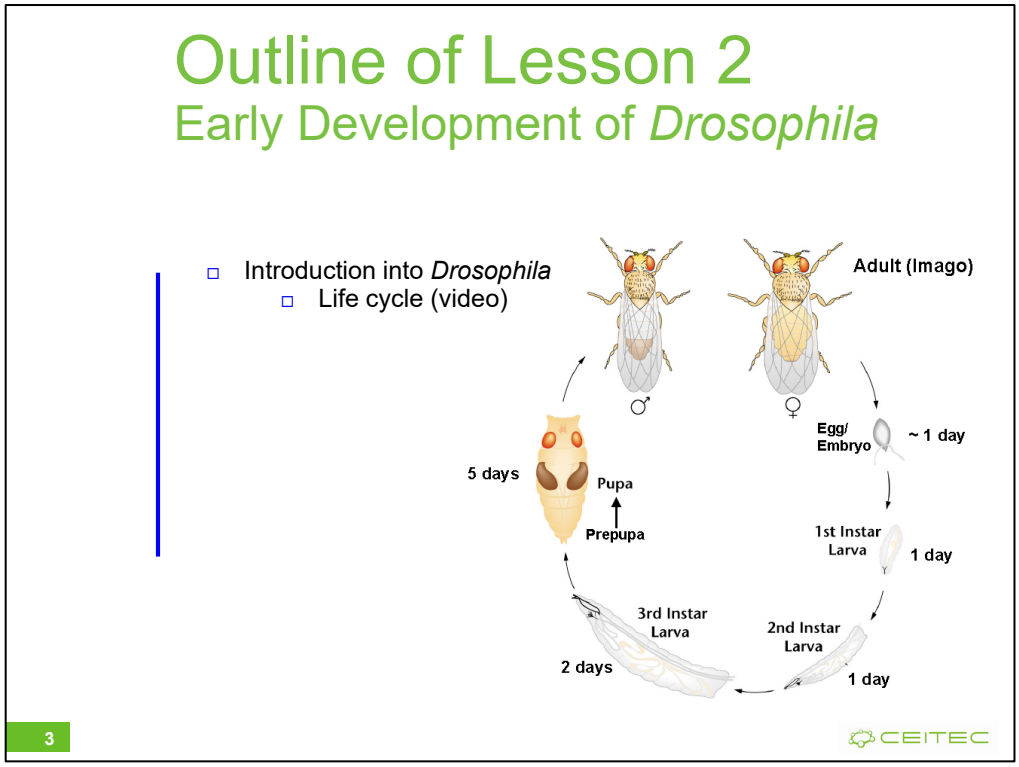


Outline of Lesson 2
Early Development of *Drosophila*

- Introduction into *Drosophila*
  - Life cycle (video)
  - Anatomy (video)
  - Mating (video)
- Oogenesis
  - molecular mechanisms of the early axis formation
- Early embryogenesis
- Gastrulation
  - Video of gastrulation in *Drosophila*
- Overview of metamorphosis
- Introduction into genetic and molecular mechanisms of the early embryogenesis in *Drosophila*



Drosophila is a *holometabolous* insect —that is, an insect that has a larval and a pupal stage prior to the adult stage. This is in contrast to *hemimetabolous* insects that develop via nymph stages.

Eggs hatch in 22–24 hours at 25˚ C. The larva that emerges looks like a tiny worm and is called the **first instar larva.**

It feeds on the substrate that the eggs were laid in and, after another 25 hours, molts into a larger wormlike form, the **second instar larva.** This feeds as well and, after about 24 hours, molts into the **third instar larva.** This is the largest of the larval forms.

It feeds, but it also starts to climb upward out of its food, so that it will be in a relatively clean and dry area to undergo pupation. The third instar molts into a **pupa** after 30 hours.

The pupa is stationary, and in its early stages is yellowish-white. As it develops, the pupa becomes progressively darker. During the pupal stage, the larva is **metamorphosing** into the adult fly, also called the **imago.**

The time span of the whole life cycle from laying the egg till the new imago is highly dependent on the external conditions, particularly temperature. At 28 ˚ C it

takes 7 days.

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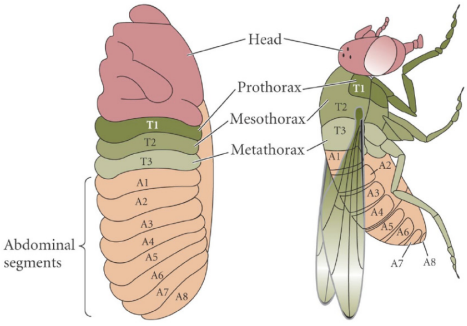

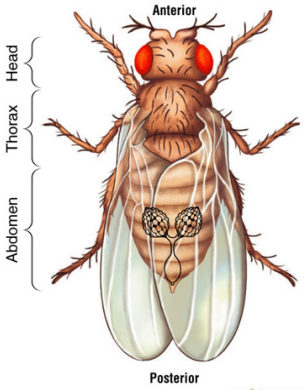

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Look for the tiny external **head,** three **thoracic segments**, eight **abdominal segments,** and a **telson** extending beyond the anus.

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5

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There is quite complicated **courtship behavior** of *Drosophilas*. The female must give an acceptance signal by slowing down, extruding her ovipositor, and spreading her wings, in order for mating to occur.

There is no known incidence of rape in *Drosophila*.

A female rejects a male by kicking with her hind legs, fending with her middle legs, flicking her wings, producing a rejection buzzing sound by fluttering her wings, or moving away rapidly.

If she has already mated, she also will extrude her genitalia to reject the male. A male courts anything that produces the right “taste” or “smell” (even other males if they are immature). He orients himself toward the female’s head, taps her with his forelegs, “tasting” her to make sure she is the right species, and then pursues her when she moves, extending and vibrating one wing producing a **courtship song.**

Though the “love song” of the *Drosophila is* species-specific, females do respond to the songs of other species as well. Females “hear” the song through their antennae; the **aristae** (feathery extensions of the antennae) augment the vibrations, and they are sensed by Johnston’s organ in the second segment of the antenna.

Later in the courtship, the male extends his proboscis to touch the female’s genitalia. If all the active courtship of the male has stimulated the female enough to accept the male, the two mate with the male positioned on top of the female.

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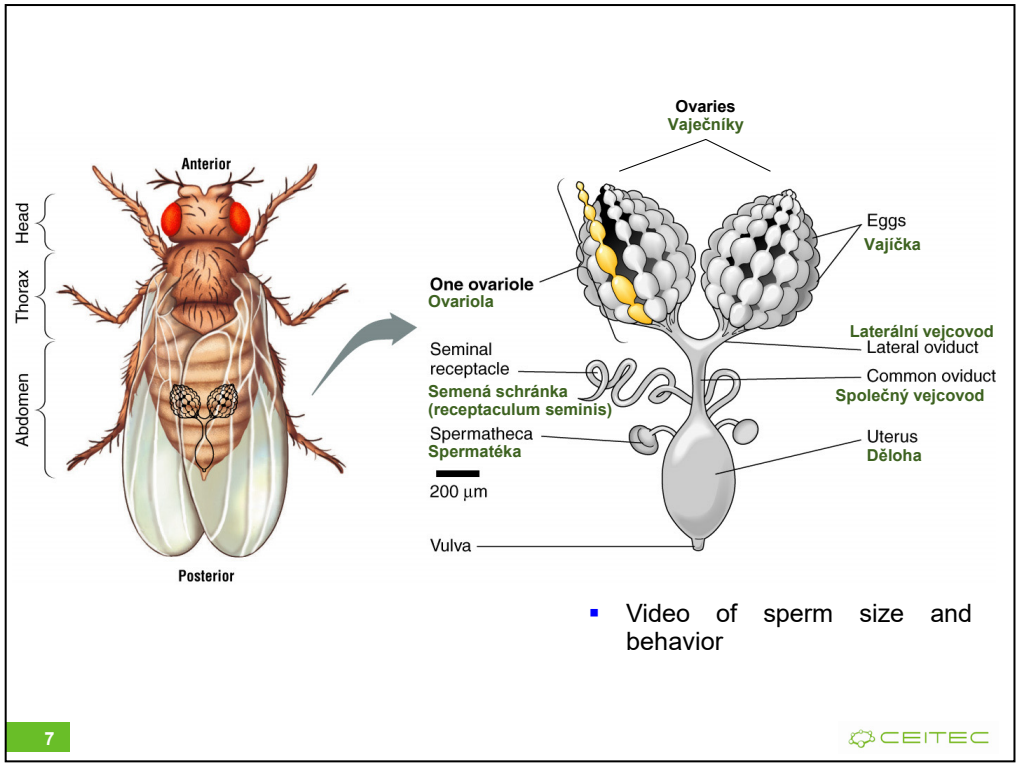


In *Drosophila*, the egg is being activated few minutes before fertilization. It resumes the meiosis and translation from mRNA stored in the egg gets started.

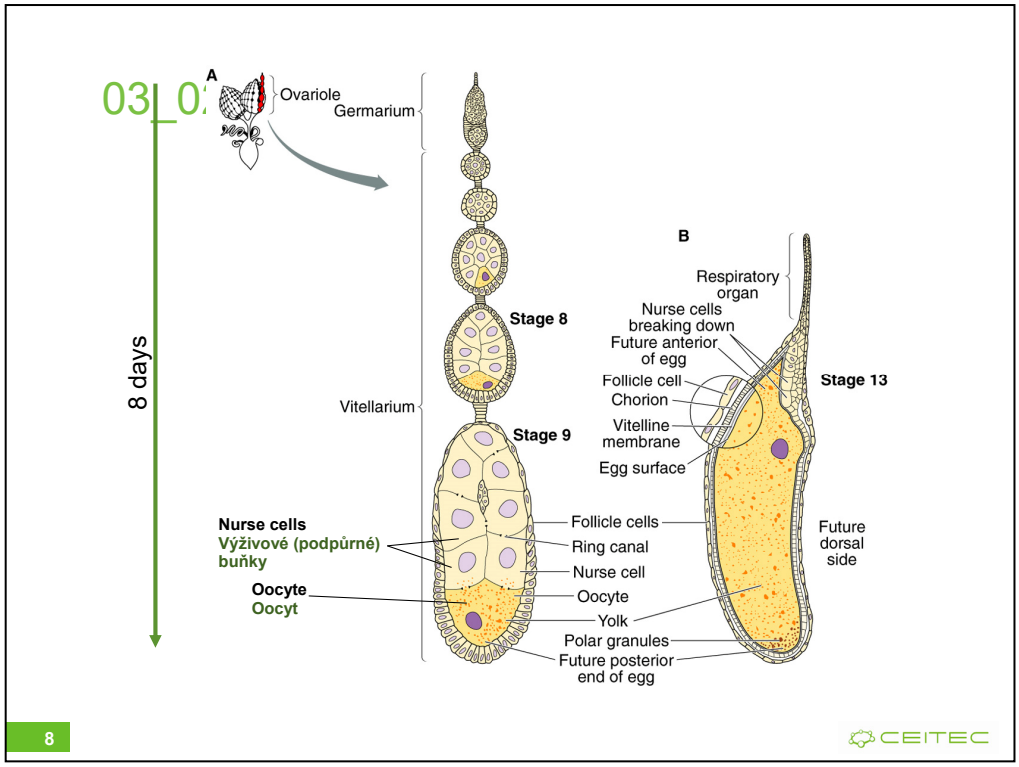
Sperm travel through the male penis into the female uterus and then swim into the female **seminal receptacle** and **spermathecae,** where they are stored for fertilization.

Genus *Drosophila* has extremely large sperm cells. *D. melanogaster* has sperm cells that are 1.76 mm long, about 300 times longer than human sperm (and about as long as the male fly producing them). But even these huge sperm are miniscule compared to the sperm of *D. bifurca*. These sperm are 58 mm long--over 20 times the length of the male fly.

During **oviposition,** the eggs emerge from the female's **ovipositor** posterior end first**.** The female oviposits preferentially on a moist food surface in a humid atmosphere. If the air is too dry, the female may feed, but she won't oviposit.

Fertilization is internal, and sperm are stored within the female's body in a **seminal receptacle** and the paired **spermathecae.**

The females reach their maximal fertility a the age of 4-7 days. In this time period they lay 50-70 eggs per day.



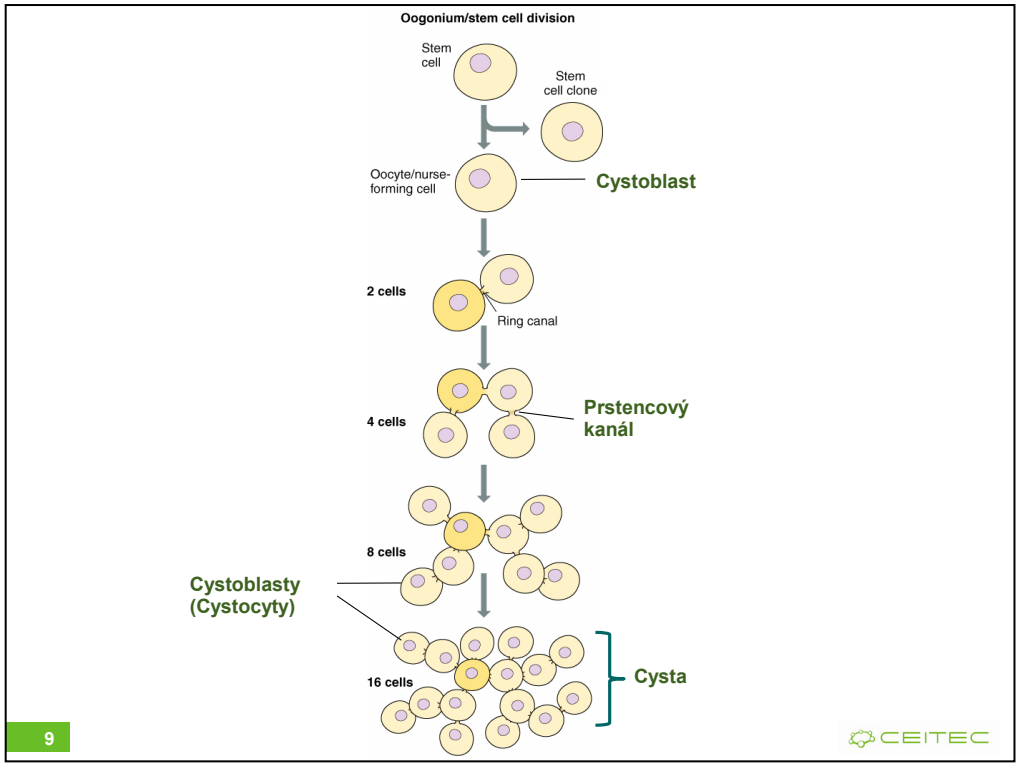
In spite of *Drosophila* and human beings diverged more than 600 milon years ago, the major developmental principles are conserved. The genetic power of studies on *Drosophila* allowed identification of their molecular mechanisms.

One of these conserved mechanisms is that the egg cell (oocyte) is polarized **before fertilization**. The diversification is demonstrated by differential distribution of *maternal components*. These maternal components are produced by the oocyte itself, but the majority of proteins, mRNAs and organelles of the egg cell are produced in the adjacent *nurse cells*.

Many other cell types contribute to the egg formation.
•Eggs are formed in ***ovaries***, paired female sex organ, connected to oviducts.
•Each ovary is composed of several ***ovarioles***. In each ovariolus, eggs are being formed in a developmental sequence.
•The egg formation goes through defined developmental stages. The duration of the entire cycle takes approximately 8 days, however, it is dependent on temperature.
•In the final developmental stage, the egg is enclosed by the layer of somatic cells, called *follicle cells*.
•Follicle cells produce membraneous structure *chorion*, that surrounds oocyte.
•Yolk is produced in fat body, the organ with function partially resembling liver in mammals.

At the anterior end of the egg there are two small filaments, extensions of the

chorion, extend from the dorsal surface. These are **respiratory filaments** and serve for gas exchange, as their name implies. Eggs are laid half-buried in rotten fruit or the medium in culture jars, and the filaments protrude into the air.



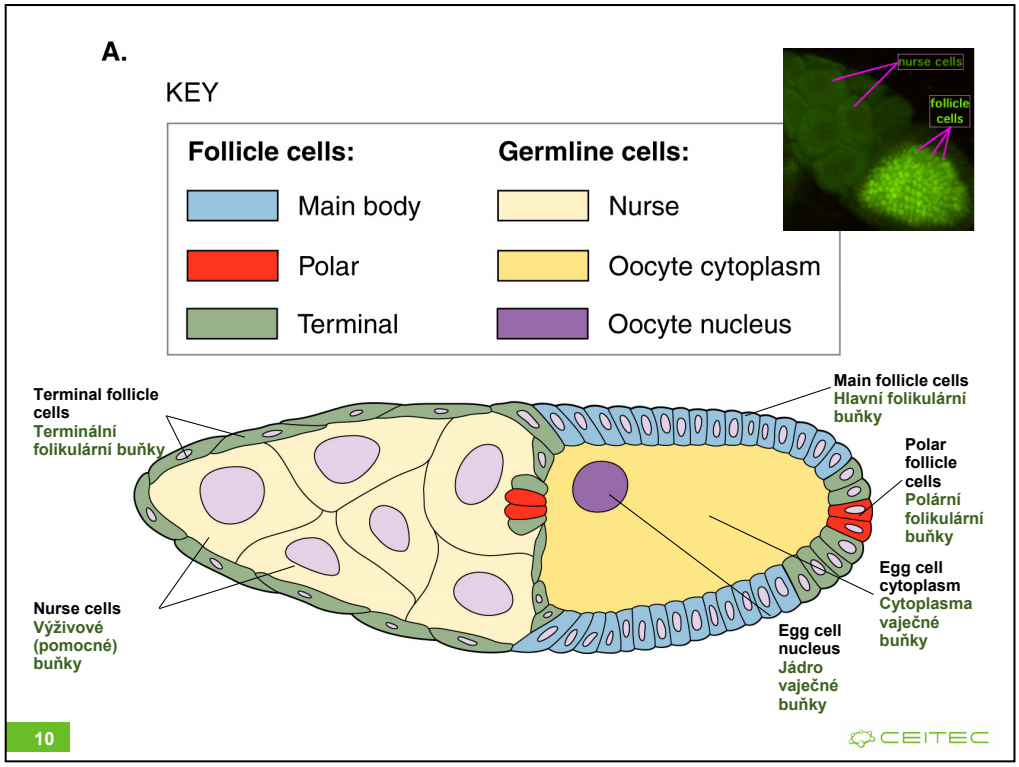
The oocyte formation starts in the tip of each ovariolus by the mitosis of egg stem cell, the oogonium (see the Lesson 01).

Series of subsequent mitotic divisions leads to the formation of 16 cells, connected via cytoplasmic bridges. Two of these 16 cells are connected via 4 bridges, the remaining via two or three.

One of these two cells undergoes meiosis and forms egg cell. The remaining ones undergo polyploidization (mitosis without cytokinesis, forming 2n x 256 chromosomes) and enlarge. These cells form nurse cells and pump mRNA, proteins and organelles via the cytoplasmic channels into the egg cell.

Almost half of volume of the egg cell is created by yolk that is composed of

phosphorylated protein *vitellogenin*.



The oocyte is assymetric, with anterior, posterior, dorsal and ventral poles distinguishable.

There are three major follicle cell types:
1. Main body cells (blue)
2. Terminal follicle cells (green)
3. Polar follicle cells (red)

Most of the yolk is located centrally, with cytoplasm placed peripherally close to the plasma membrane.

Thus, the oogenesis leads via mitosis, meiosis, stockpilling and diversification to the formation of ***pre-polarized egg cell.***

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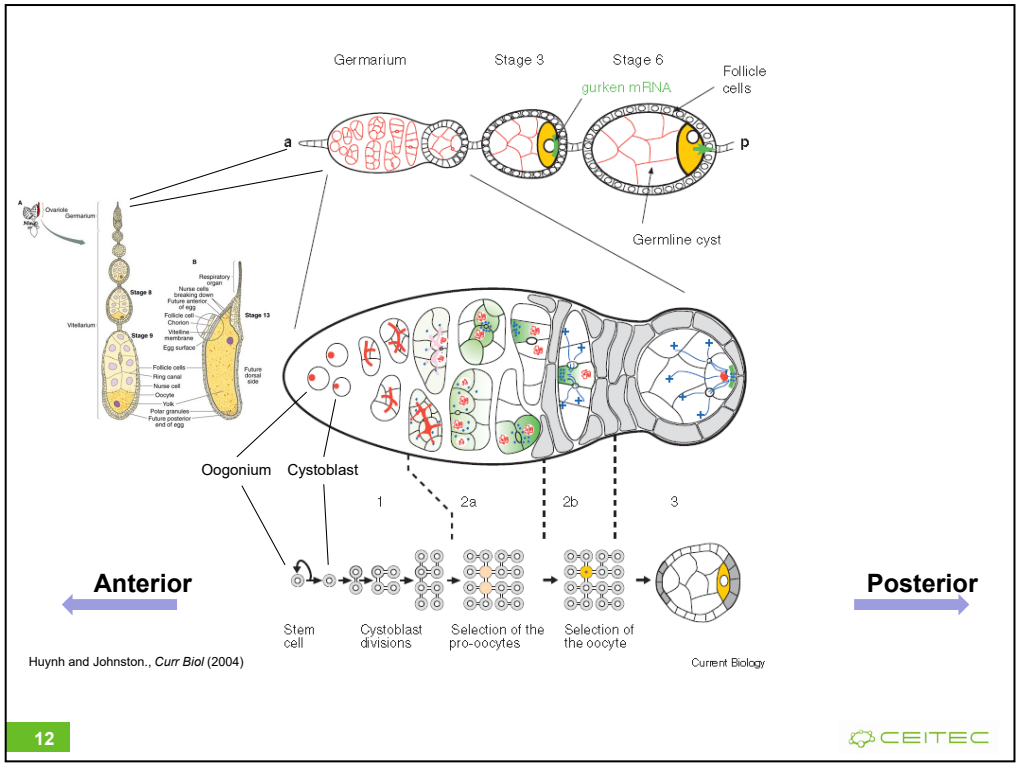
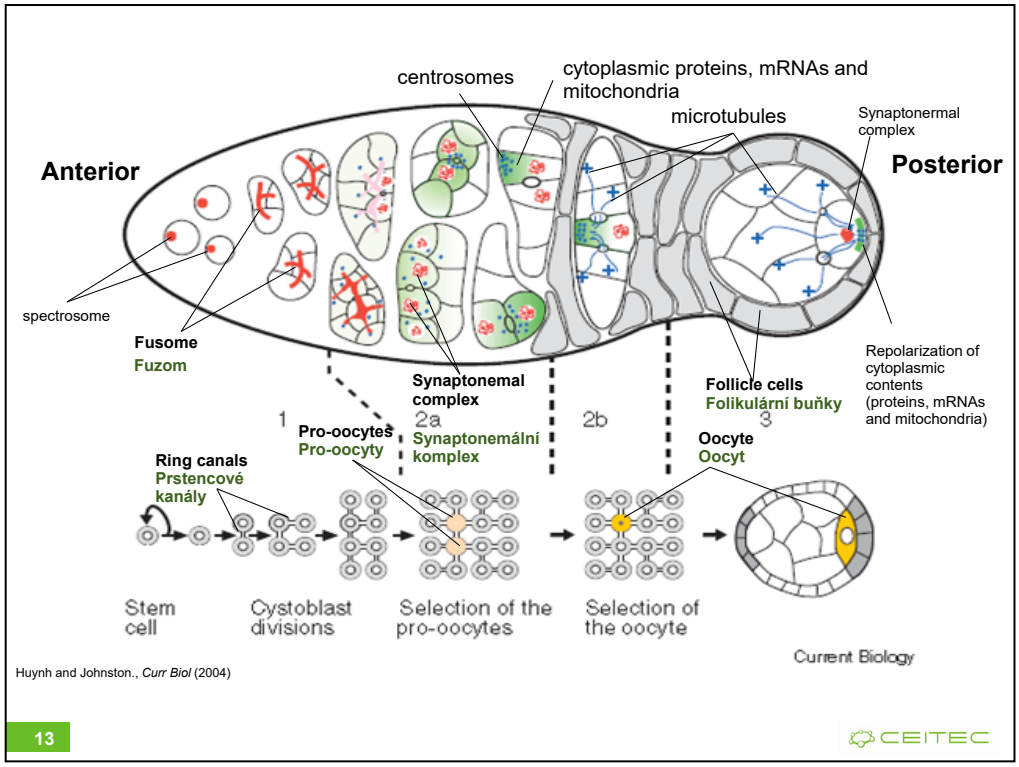


The polarization of the oocyte in *Drosophila* take place very early in the oogenesis, before then polarized localization of mRNA takes place (see later).

The *Drosophila* ovary is composed of 16–20 ovarioles, each of them progressively produces mature egg chambers. New egg chambers are generated at the anterior of the ovariole in a region called the germarium, which has been divided into four regions according to the developmental stage of the cyst (see the figure above and the previous slides).

Oogenesis begins in region 1, when a germline stem cell divides asymmetrically to produce a posterior **cystoblast**, and a new germline stem cell, which remains attached to the neighboring somatic cells at the anterior pole of germarium.

The cystoblast then undergoes precisely four rounds of mitosis with incomplete cytokinesis to form a cyst of 16 germline cells, which are interconnected by stable cytoplasmic bridges called ‘ring canals’. During these divisions, a cytoplasmic structure called the **fusome** anchors one pole of each mitotic spindle and, therefore, ensures that cells follow an invariant pattern of division (see also later, slide # 14).



The germarium is divided into four morphological regions along the anterior–posterior axis.

The germline stem cells reside at the anterior tip of the germarium (left) and divide to produce *cystoblasts*, which divide four more times in region 1 to produce 16 cell *germline cysts* that are connected by ring canals.

The stem cells (oogonia) and cystoblasts contain a *spectrosome* (red circles), the cell organelle that is rich in cytoskeletal proteins such as actin, α- and β-spectrin, the adducin-like Hts protein, and ankyrin (see later, slide # 15). Spectrosome further develops into a branched structure called the **fusome**, which orients each division of the cyst. Fusome is a cytoplasmic organell rich in membrane skeleton proteins (see also later).

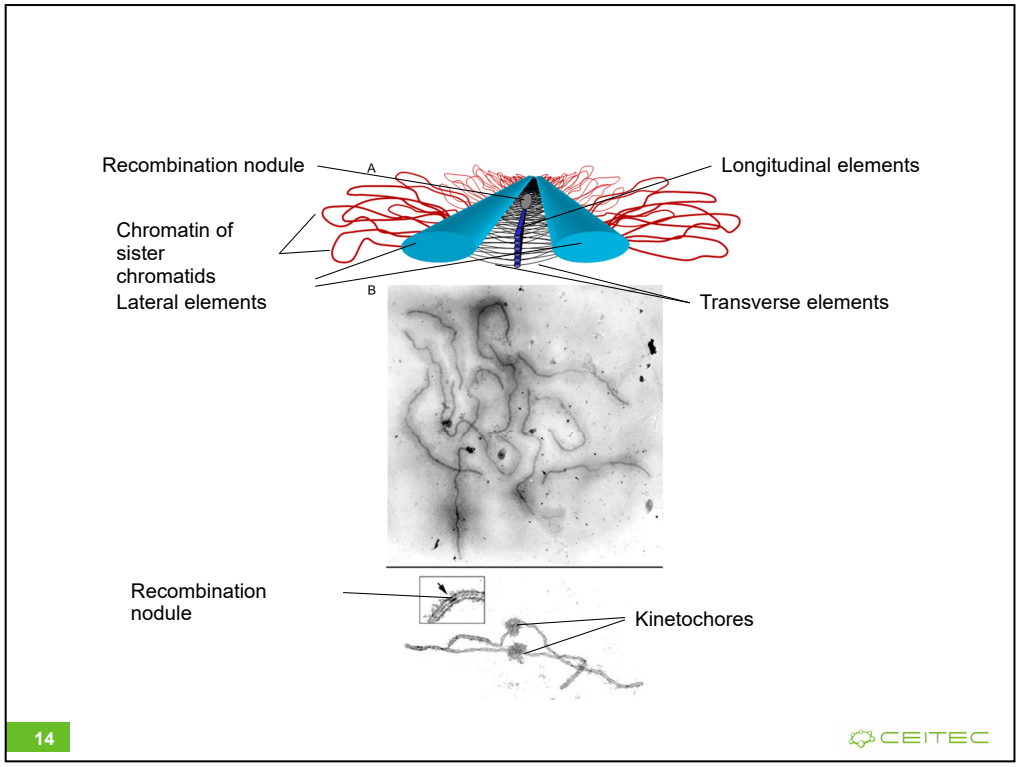
In early region 2a, the *synaptonemal complex* (red lines) forms along the chromosomes of the two cells with four ring canals (pro-oocytes, yellow) as they enter meiosis. The synaptonemal complex then appears transiently in the two cells with three ring canals, before becoming restricted to the pro-oocytes in late region 2a.

By region 2b, the oocyte has been selected, and is the only cell to remain in meiosis.

In region 2a, cytoplasmic proteins, mRNAs and mitochondria (green), and the centrosomes (blue circles) progressively accumulate at the anterior pole of the oocyte.

In region 2b, the minus-ends of the microtubules are focused in the oocyte, and the plus-ends extend through the ring canals into the nurse cells.

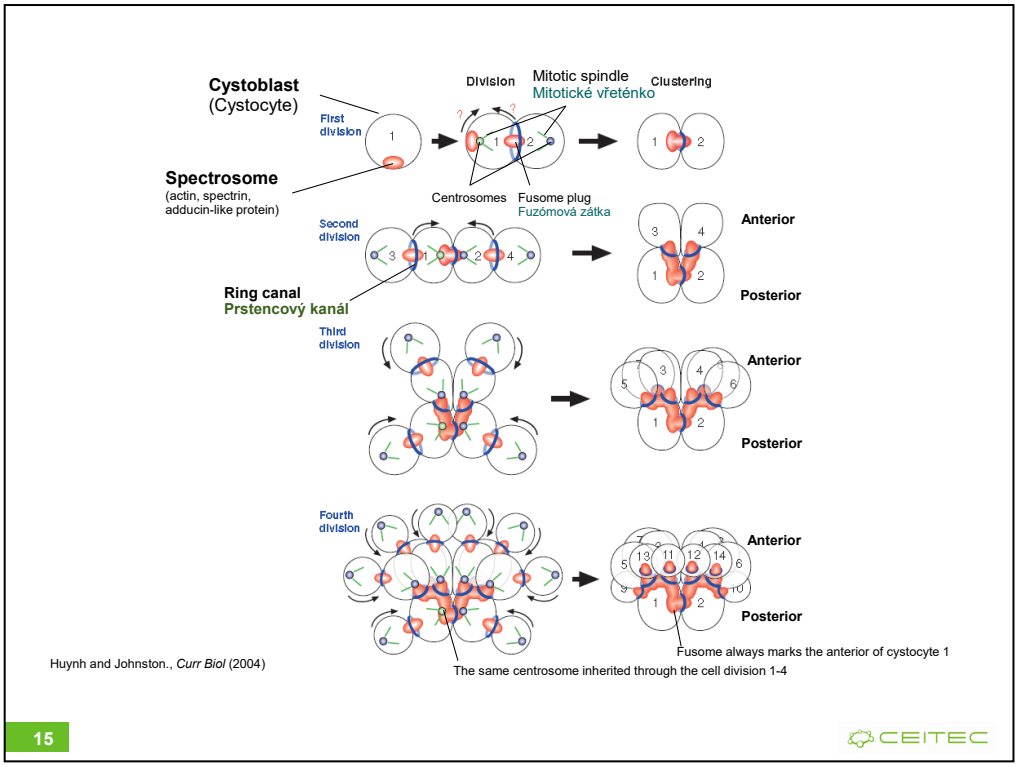
The follicle cells (gray) also start to migrate and surround the germline cells. As the cyst moves down to region 3, the oocyte adheres strongly to the posterior follicle cells and repolarizes along its anterior– posterior axis, with the microtubule minus-ends and specific cytoplasmic components now localized at the posterior cortex (Huynh and Johnston., *Curr Biol,* 2004).



The Synaptonemal Complex. (A) Model of the SC. Lateral elements (light blue rods) of homologous chromosomes align and synapse together via a meshwork of transverse filaments (black lines) and longitudinal filaments (dark blue rods). The longitudinal filaments are collectively referred to as the “central element” of the SC. Ellipsoidal structures called recombination nodules (gray ellipsoid) are constructed on the central region of the SC. As their name implies, recombination nodules are believed to be involved in facilitating meiotic recombination (crossing over). The chromatin (red loops) of each homologue is attached to its corresponding lateral element. Because there are two “sister chromatids” in each homologue, two loops are shown extending laterally from each point along a lateral element.

(B) Top: Set of tomato SCs. Chromatin “sheaths” are visible around each SC. Bottom: Two tomato SCs. The chromatin has been stripped from the SCs, allowing the details of the SC to be observed. Each SC has a kinetochore (“ball-like” structure) at its centromere. Recombination nodules, ellipsoidal structures found on the central regions of SCs, mark the sites of crossover events (see inset).

Resource: Wikipedia, http://en.wikipedia.org/wiki/Synaptonemal_complex.



The question remains, what is the mechanism of the oocyte selection. Two main models have been proposed to explain how the oocyte is selected.

First model is based on the symmetrical behavior of the two pro-oocytes until mid-late region 2a, and proposes that there is a competition between the two pro-oocytes to become the oocyte. The 'winning' cell would become the oocyte, while the 'losing' cell would revert to the nurse cell fate.

A second model suggests that the choice of the oocyte is biased by the **establishment of some asymmetry as early as the first cystoblast division**, which is maintained until the overt differentiation of the oocyte. The role played by the fusome and the analysis of its formation provides the strongest evidence in support of this model.

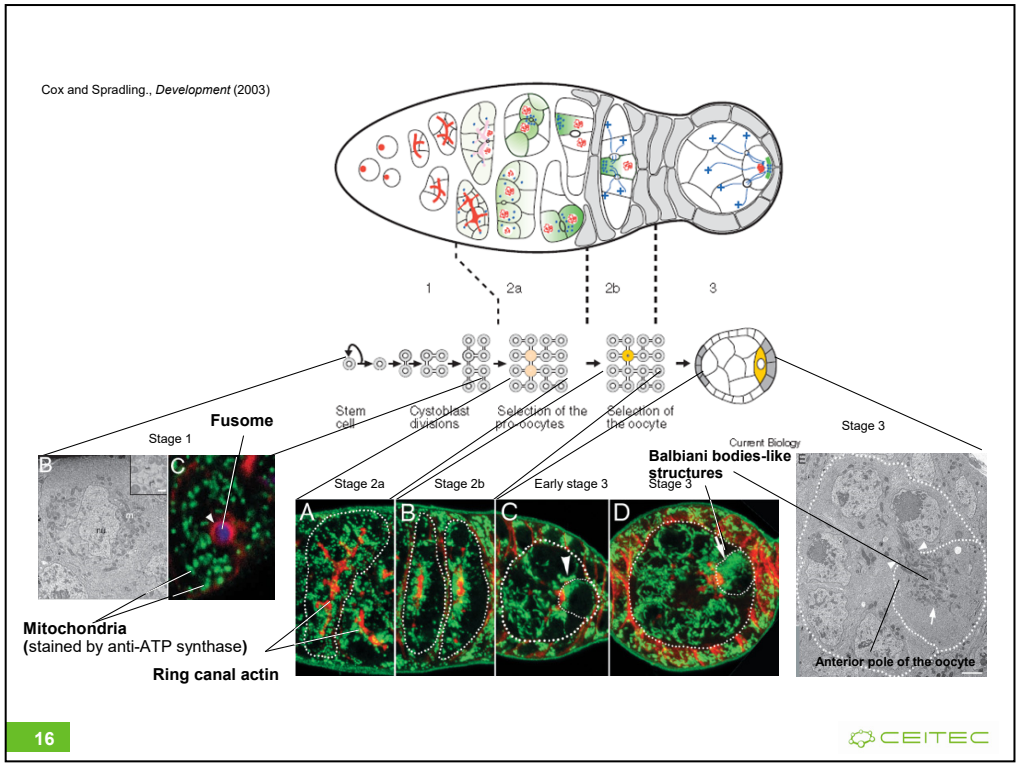
The fusome arises from a spherical structure called **the spectrosome** in the germline stem cell, which is made of small membranous vesicles kept together by components of the sub-membraneous cytoskeleton, such as *α spectrin, β-spectrin*, and *Hts* (an adducin-like protein).

During the first incomplete division, the spectrosome (red) of the cystoblast interacts with one of the centrosomes (green and blue spheres) to anchor one pole of the mitotic spindle (green lines).

A fusome plug (red) forms in the arrested furrow or ring canal (blue). The spectrosome (or 'original' fusome) and the fusome plug come together to fuse. The direction of these movements is not known.

The same mechanism is repeated for the second, third and fourth division: first, one pole of each mitotic spindle is anchored by the fusome and a new fusome plug forms in each ring canal. Then the ring canals move centripetally for the fusome plugs to fuse with the central fusome (black curved arrows).

This behavior has several crucial consequences: cystocyte 1 has more fusome than the other cystocytes; the same centrosome (green sphere) could be inherited by cystocyte 1 from the first division through the fourth division; and the fusome always marks the anterior of cystocyte 1, after the clustering of the ring canals.



Fusome ensures directed localization of mRNAs, proteins or even entire organelles during oocyte diffrentiation.

Mitochondria associate with a fusome and form structures similar to **Balbiani bodies** in vertebrates, ensuring thus allocation of maternally-inherited mitochondria. Balbiani bodies are „clouds“ of mitochondria, ER, Golgi and other organelles.

In the germline stem cells and in the cystoblasts and growing cysts (Stage 1), the mitochondria are uniformly distributed (Stage 1, Figure B, C, left-hand panels).

A transmission electron micrograph showing the nucleus (nu) and mitochondria (m) of a region 1 cystocyte. The inset shows a cross-section of a ring canal containing a fusome plug surrounded by mitochondria. (C) Another region 1 cystocyte sectioned facing a ring canal (arrowhead): mitochondria (anti-ATP synthase) are green, the fusome (1B1 antibody) is blue and ring canal actin (rhodamine phalloidin) is red.

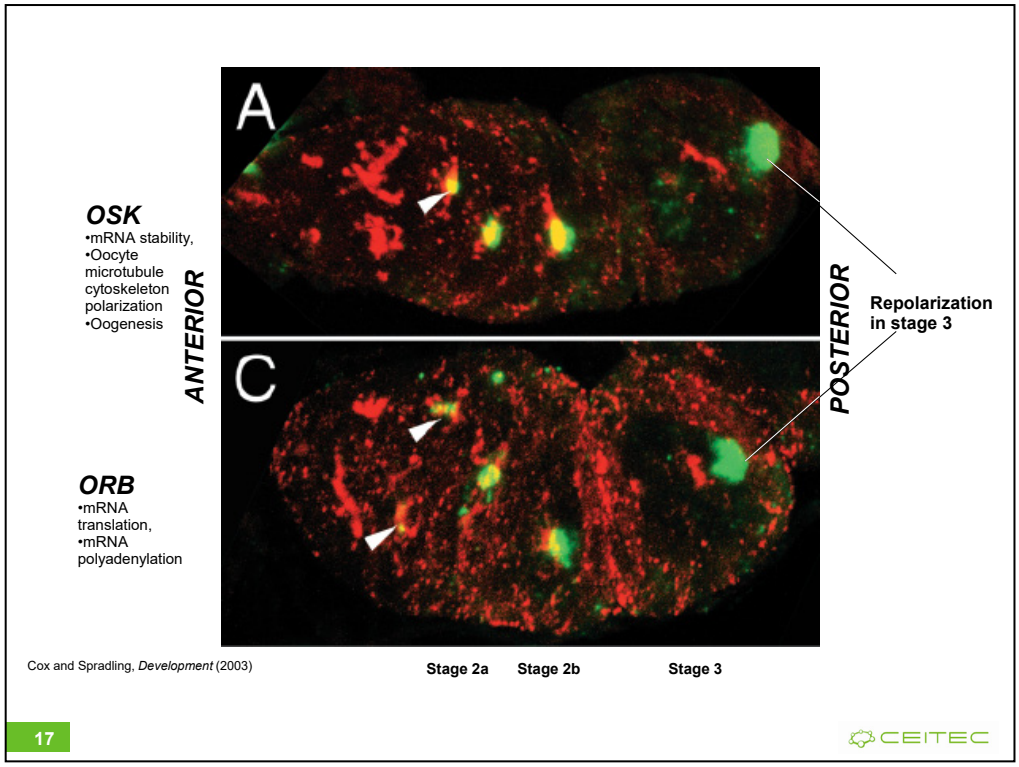
However, as the oocyte development proceeds, the mitochondria associate with the newly formed fusome (Stage 2a, Figure A) and they move toward the center of the fusome (yellow, Stage 2b, Figure B).

In the early stage 3 (Figure C), the clouds of mitochondria (arrowhead) accumulate near the ring canals that connect to the oocyte (small broken circle).

In region 3 follicles and young budded egg chambers, a Balbiani body containing many aggregated mitochondria (arrow, Figure D)) is visible in the anterior of the oocyte (small broken circle).

Figure E. Electron micrograph of a region 3 follicle (large outline) reveals mitochondria entering the oocyte (small outline) via a ring canal (arrowheads) to form the Balbiani body (arrow).

According to Cox and Spradling, Development, 2003.



As mentioned previously, not only organelles, but also mRNAs and proteins get “sorted” by the fusome.

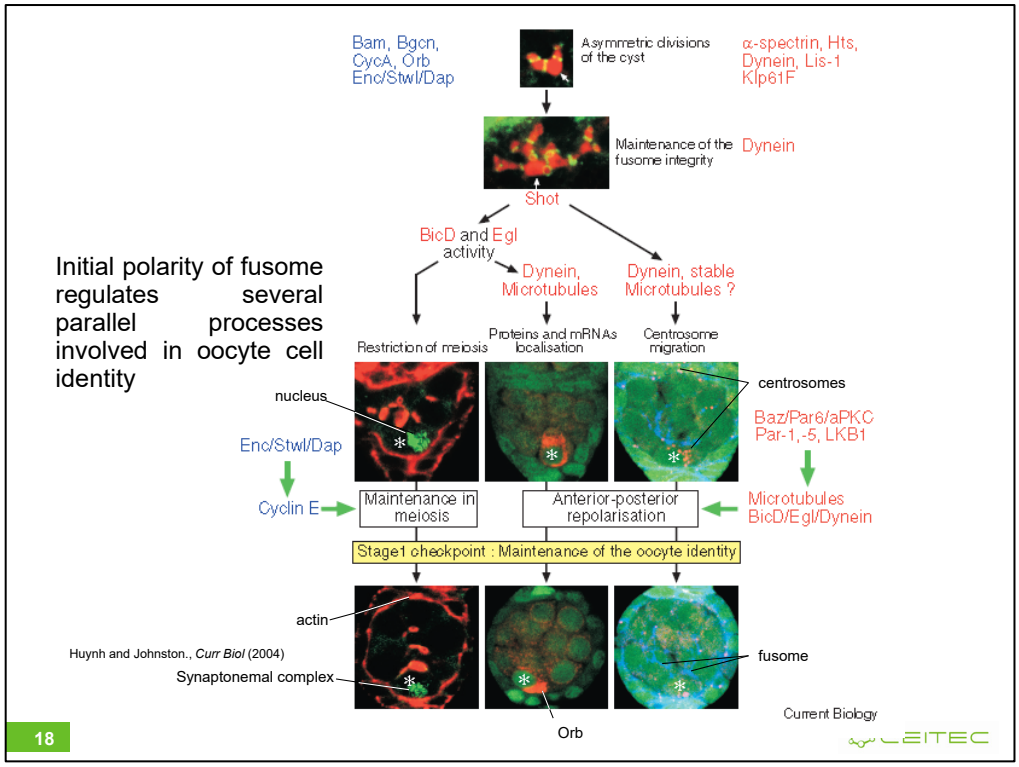
Several mRNAs and proteins were shown to localize into pro-oocytes that develop into oocytes, among them, *OSKAR (OSK)* mRNA.

OSKAR encodes a protein of unknown molecular mechanisms. It was shown to be involved in regulation of mRNA stability, oocyte microtubule cytoskeleton polarization, oogenesis, visual learning, visual behavior and long-term memory, germ cell development, posterior abdomen determination etc.. Similarly, another gene *ORB* encodes a protein involved in protein-protein interactions that regulates oocyte anterior/posterior axis specification and is involved in regulation of mRNA translation, mRNA polyadenylation etc. (according to www.flybase.org).

The fusome very probably plays an important role in the directed localization of those RNAs and proteins, as could be seen on the figure showing co-localization of *OSK* (upper panel, green signal) and *ORB* (lower panel, green signal) with fusome (red signal).

(A) In situ hybridization reveals that *OSK* mRNA (green) associates with the central fusome (red) within the future oocyte beginning in region 2 (arrowhead). The RNA moves to the posterior of the oocyte during stage 3 (rightmost follicle).

(C) *ORB RNA (green),* like *OSK RNA,* associates with the centre of the fusome (red) beginning early in region 2 (arrowheads), and then moves to the oocyte posterior in stage 3 (rightmost follicle).



The early steps in the determination and polarisation of the oocyte.

The early differentiation of the oocyte is a multistep process. Genes involved at each step are indicated though the list is not exhaustive. Regulators of cytoplasmic differentiation are shown in red, whereas regulators of the cell cycle are shown in blue.

The top panel shows a 4-cell cyst (α-spectrin, in red, marks the fusome and anilin, in green, marks the ring canals). One cell has more fusome than the other cells (white arrow).

The panel below shows a 16-cell cyst after the last division with one cell having more fusome (white arrow).

There are **three different pathways to restrict oocyte identity to one cell** (asterisk).

The left panels show the actin in red and the synaptonemal complex in green.

In the middle panels, nuclear GFP is shown in green, and Orb (an oocyte-specific cytoplasmic protein) is labelled in red.

On the right panels, γ-tubulin marks the centrosomes in red, α-spectrin marks the fusome in blue, and nuclear GFP is shown in green. Orb and the centrosomes are clearly seen migrating from the anterior of the oocyte to the posterior, revealing the repolarisation of the oocyte.

Although it was originally thought that the oocyte was specified by the transport of determinants along a single polarised microtubule cytoskeleton, recent results have uncovered a more complex reality, in which **multiple processes function in parallel to restrict different aspects of oocyte identity to one cell**.

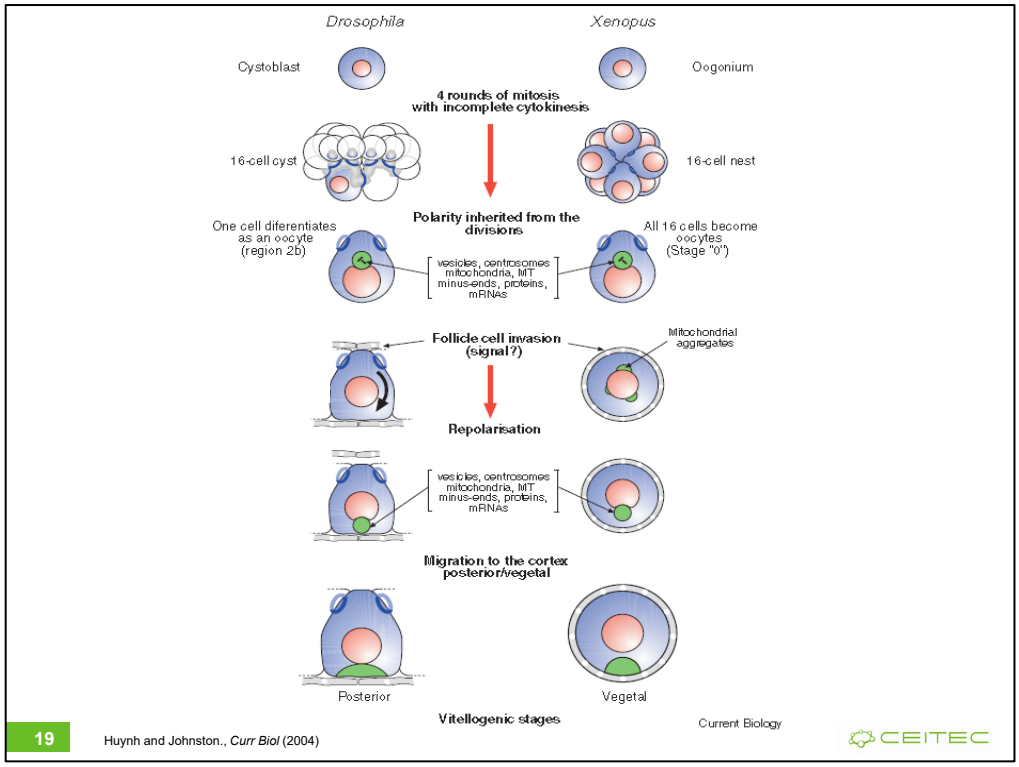
However, **all of these processes probably depend on the initial polarity of the fusome**, which may act in three distinct ways to select the oocyte:

First, the fusome organizes a polarised network of dynamic microtubules that direct the localisation of oocyte-specific proteins and mRNAs into one cell, presumably through the Dynein-dependent transport of cargoes that are linked to the motor through BicD, Egl and the Dynein light chain.

Second, the fusome also nucleates stable microtubules that are associated with Shot, and the centrioles may migrate along these in a process that could also involve Dynein. Dynein is a group of proteins that act as molecular motors. They have the ATPase activity and connect via their light and heavy chains the cargo vesicle with the microtubules and transport the cargoes along the microtubules towards its minus end that is usually oriented towards the cell center. In comparison, the kinesins are motor proteins that move toward the microtubules' plus end.

Finally, the fusome appears to regulate a Dynein light chain and microtubule-independent activity of the BicD/Egl complex that controls entry into meiosis.

Although the molecular mechanisms are only emerging, these results also suggest an exciting link between oocyte differentiation, vesicular trafficking and mRNA transport.



The mechanisms of the oocyte polarization and early axis formatioon are conserved in *Drosophila* and vertebrates. The figure above shows similarities between the early steps of *Drosophila* and *Xenopus* oogenesis.

In both *Drosophila* and *Xenopus*, the oocyte inherits an anterior–posterior axis of symmetry from the cyst divisions.

Specific cytoplasmic components (green) accumulate in a depression above the nucleus (red). The oocyte then polarizes along this axis when the somatic follicle cells surround it. It is suggested that a signal coming from the follicle cells may trigger this polarization.

This polarization is clearly seen in *Drosophila* with the translocation of specific cytoplasmic proteins, mRNAs and the centrosomes to the posterior of the oocyte (black arrow). The situation is less clear in *Xenopus*, as the cell rounds up and seems to lose any polarity. However, we propose that the same components that were located above the nucleus after the cyst division, are now part of the Balbiani body on the vegetal side (green sphere).

At the following stage, these components migrate to the posterior/ vegetal cortex of

the oocyte. This has been clearly demonstrated in *Xenopus*, and is here hypothesized for *Drosophila* . Both oocytes then enter the vitellogenic stages (Huynh and Johnston., *Curr Biol* , 2004).

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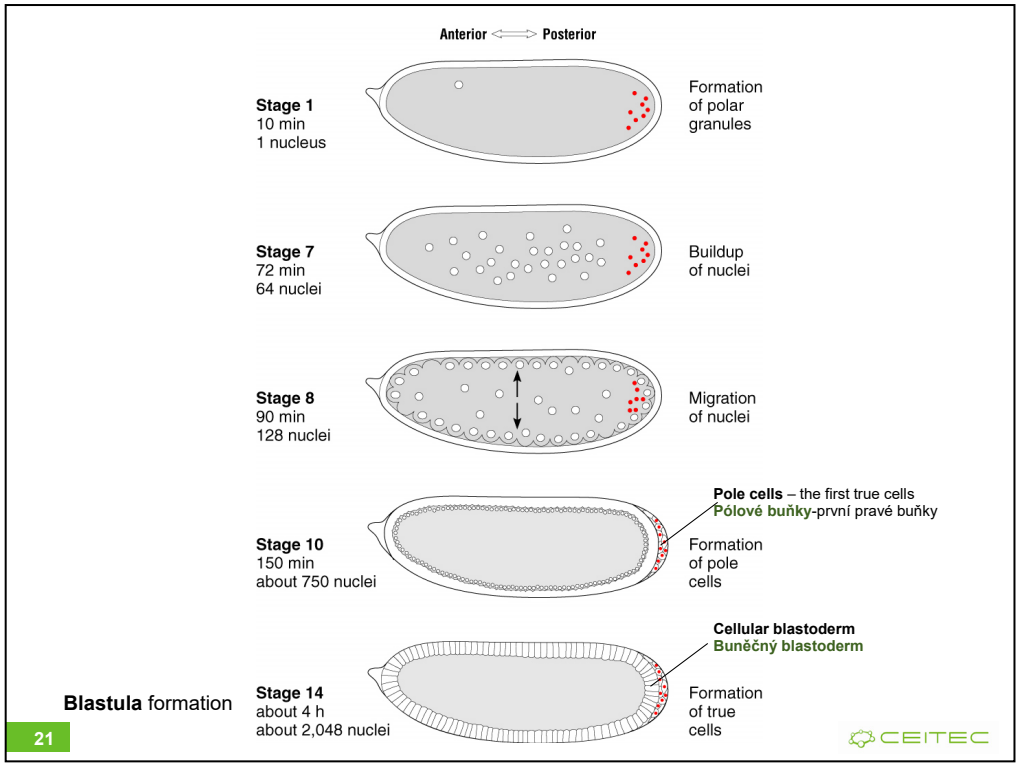


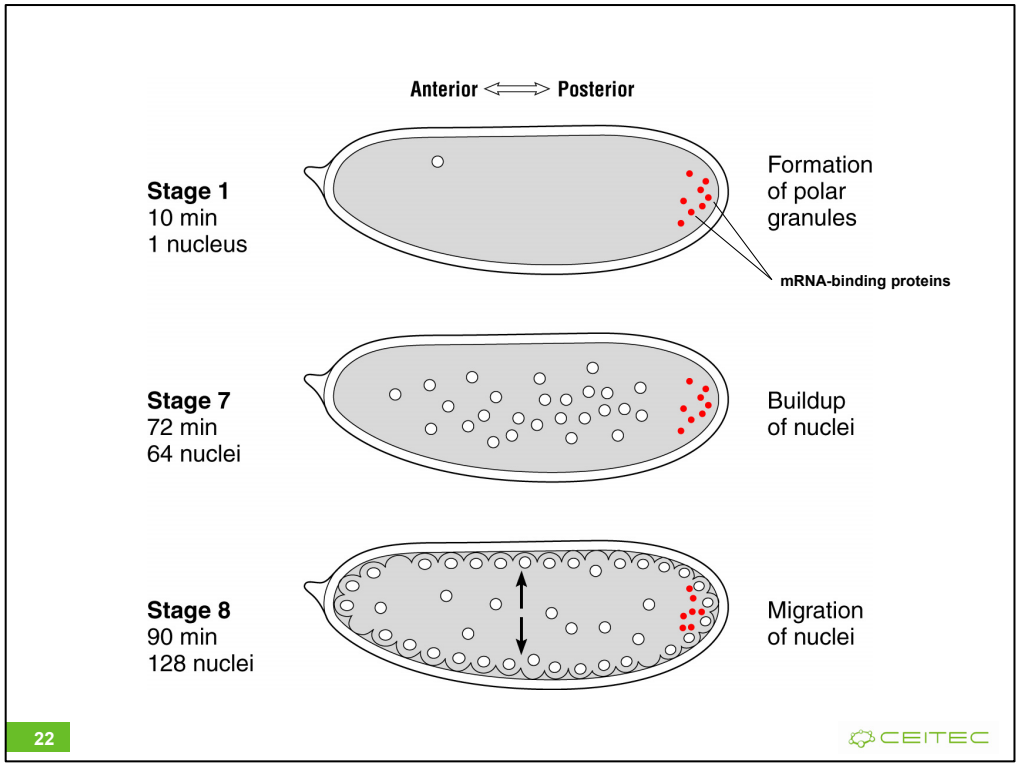
Fertilization occurs when the sperm enters the oocyte via *micropyle*, an opening located in the chorion. Meiosis is completed, syngamy takes place and a series of cell divisions starts.

The first divisions occur in cycles (9 min. each) and the embryo is at the stage of *syncytium*. The stages numbering refers to the number of cell division cycles.

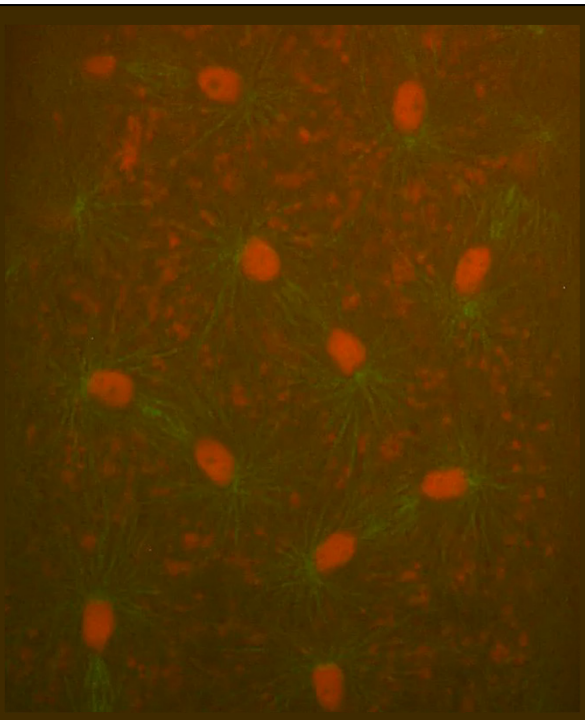
Nuclei migrate to the perifery (superficial cortical cytoplasmic layer).

Polar granules are located at the posterior ("tale") and of the embryo and they become a part of the first true cells*, the* ***pole cells***.

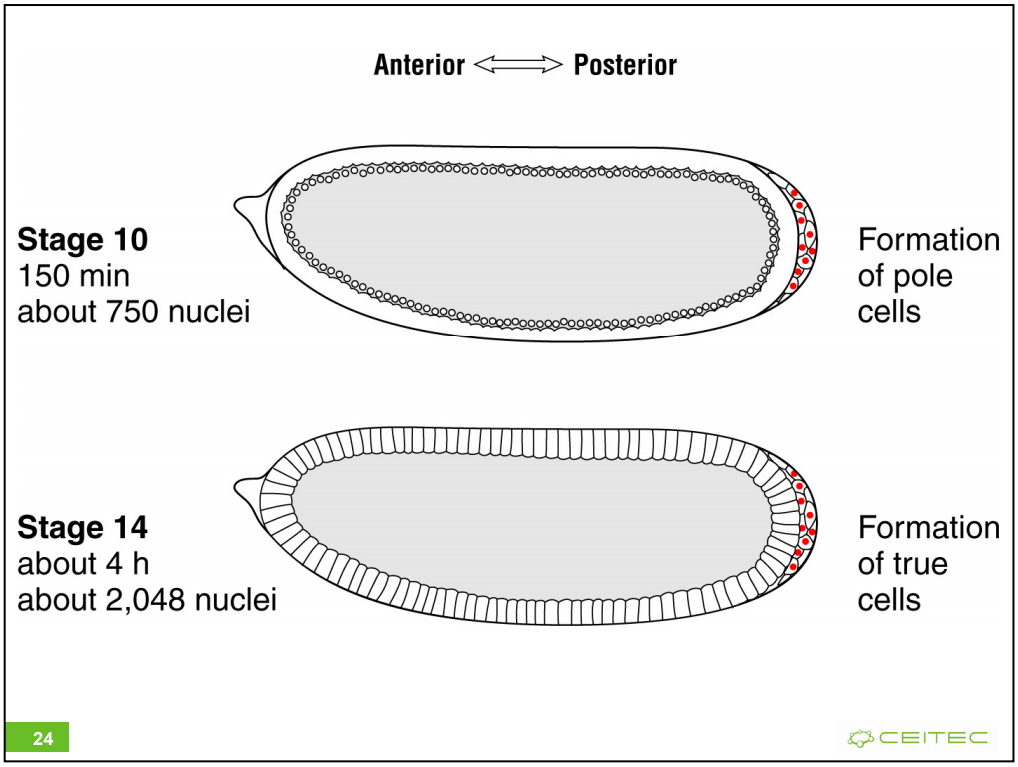
Via invagination of the plasma membrane at the perifery of the embryo, the cellularization takes place leading to the formation of ***cellular blastoderm***.



Polar granules consist of a tightly interwoven network of differentially localized RNA-binding proteins, which in turn localize specific mRNA species for differential storage, asymmetric segregation (as needed for asymmetric cell division), differential splicing and/or translational control. The germline granules appear to be ancestral and universally conserved in the germlines of all metazoan phyla (Wikipedia).



Synchronized nuclear divisions in the early *Drosophila* embryo


Anterior ⟺ Posterior
Stage 10
150 min
about 750 nuclei
Formation of pole cells
Stage 14
about 4 h
about 2,048 nuclei
Formation of true cells
24
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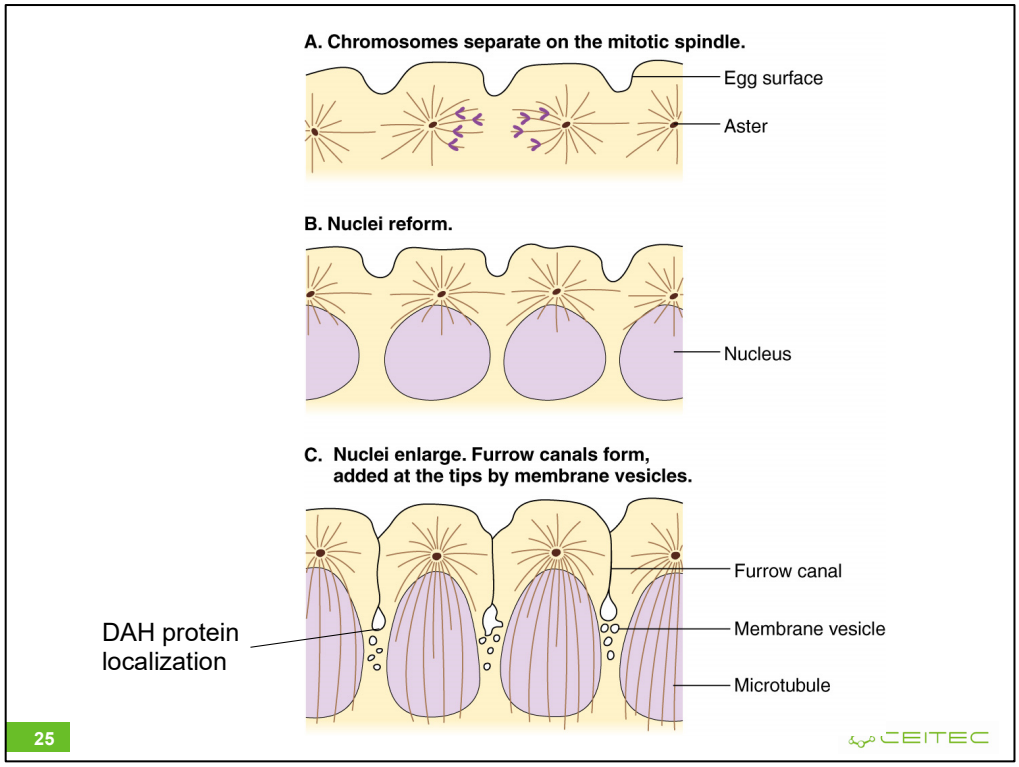
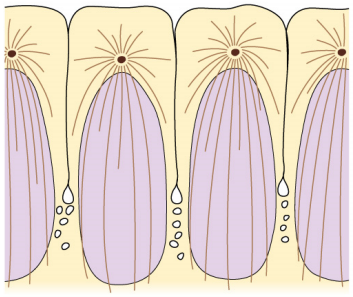


During cellularization of the blastoderm, the furrows appear at the surface of the egg. The microtubules of the mitotic spindle align perpendicularly to the surface and the furrow further invaginates along the expanding actin microtubules.

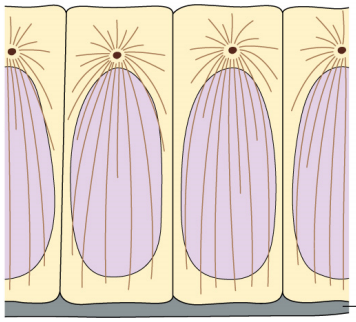
DAH protein is located in the front of the cleavage furrow and is critical for the progression of the cleavage.

discontinuous actin hexagon (*dah*) is a maternal-effect gene essential for the formation of cortical furrows during *Drosophila* embryogenesis, and DAH protein colocalizes with actin in these furrows. The DAH phosphorylation peaks during cellularization, a stage at which DAH function is critical. A kinase activity is coimmunoprecipitated with the DAH complex and hyperphosphorylates DAH in vitro (Zhang et al., *Molecular Biology of the Cell*, 2000).

D. Nuclei continue to grow and the furrow canals progress.



E. Cellularization is completed and yolk membrane is in place.



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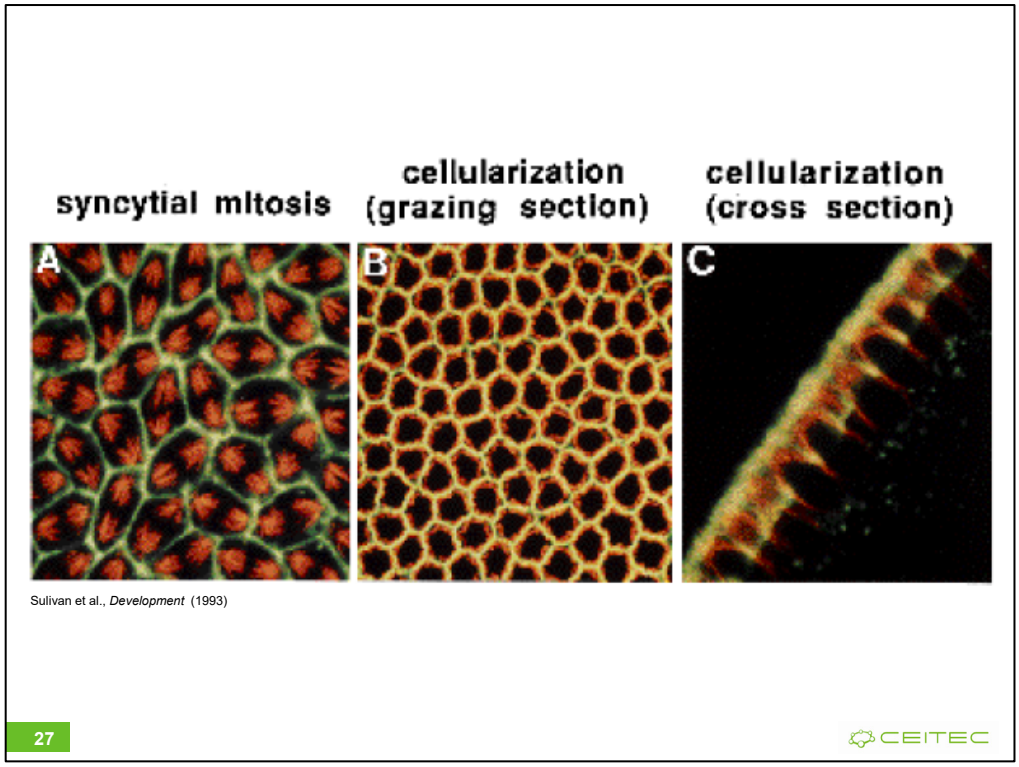
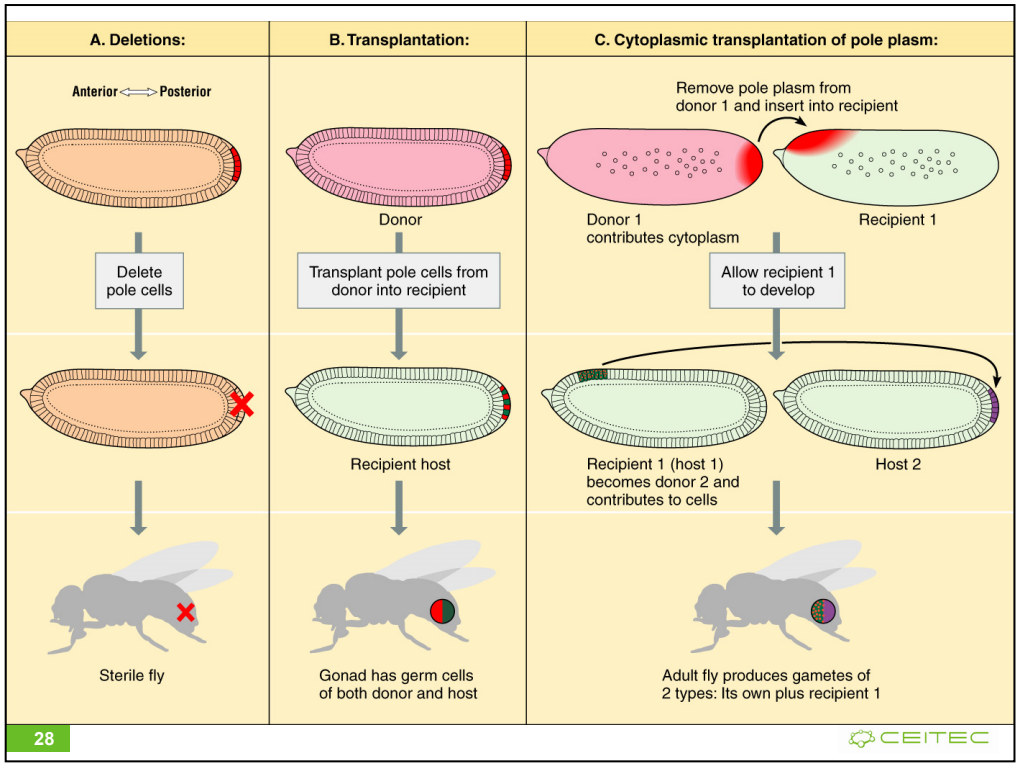


Confocal images of formaldehyde-fixed embryos derived from wild-type that have been stained for both actin (yellow/green) and tubulin (red).



Problem of the cell differentiation in the developing embryo.

During syncitium stage, the communication between nuclei is unimpeded because there are no cell membranes. The nuclei become undifferentiated and they can adopt different developmental fates, as shown by nuclei transplantation experiments.

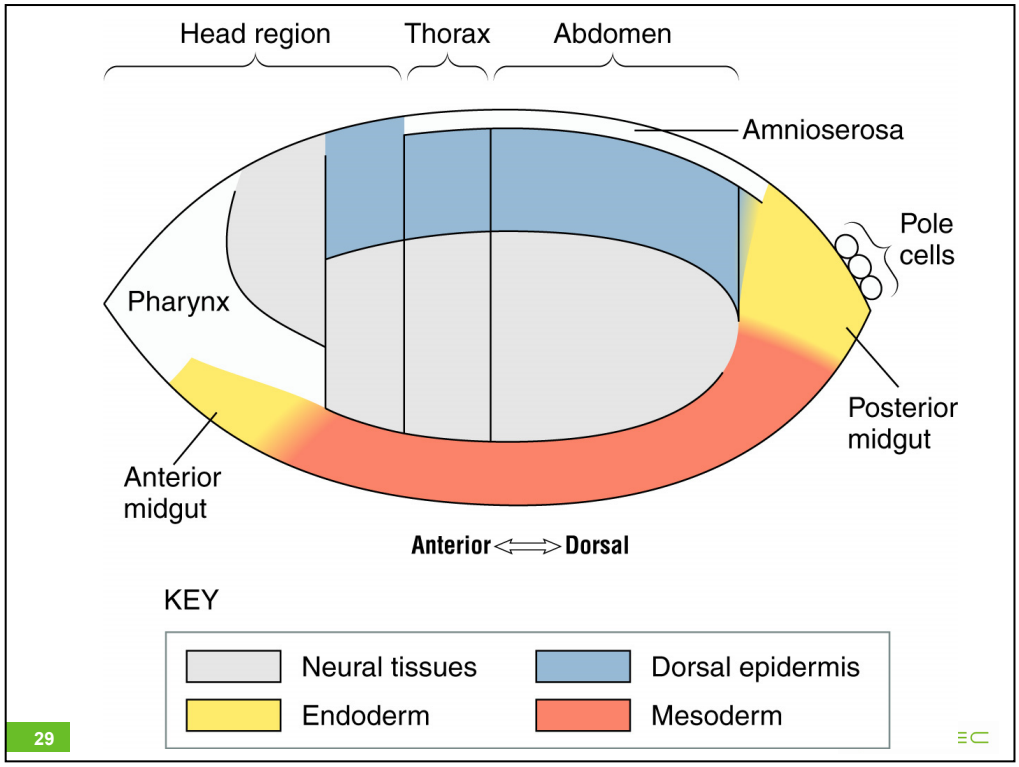
However, after cellularization the cells become predetermined and they cannot be transplanted to whatever part of the embryo.

The polar cells will develop into germ cells. When they are ablated, the adult is sterile (Fig. A).

When they are transplanted to the posterior pole, they will develop into the germ cells. That could be proven via genetic marker (allele) that could be observed in the progeny of the host adult.

However, even before cellularization, there is partial predetermination of the cytoplasm, as could be demonstrated by the transplantation of the posterior pole cytoplasm to the anterior region of the host 1 embryo (Fig. C). That leads to the differentiation of pole cells in that position. These cells can be then transplanted to the pole cells of the another host (host 2) leading thus to the transition of the genetic information of the host 1 to the host 2.

The above example provides evidence that there is a cytoplasmic/nuclear interaction that affects the future developmental fate of the cells, probably via regulation of the gene expression.



The cells from all peripheral regions of the cellular blastoderm were transplated and their developmental fate was determined.

The cells of the syncitium stage are ***totipotent***. However, after cellularization, the cells became predtermined.

The predetrmination of the developmental fate a the level of cells is expressed in the so called *fate map*. At the level of cytoplasm, this is called a map of *developmental potential*.

Originally, all cells of the syncitium are totipotent. However, the interaction of totipotent nuclei with different cytolpasm after cellularization leads to their differentiation, i.e. their *developmental fate is predetermined.*

Detemination is a dynamic process with different levels of restriction of the future development.

The ***amnioserosa*** is an epithelium that derives from about 200 cells at the dorsal midline of the blastoderm embryo. It is required for proper germ band extension and dorsal closure.

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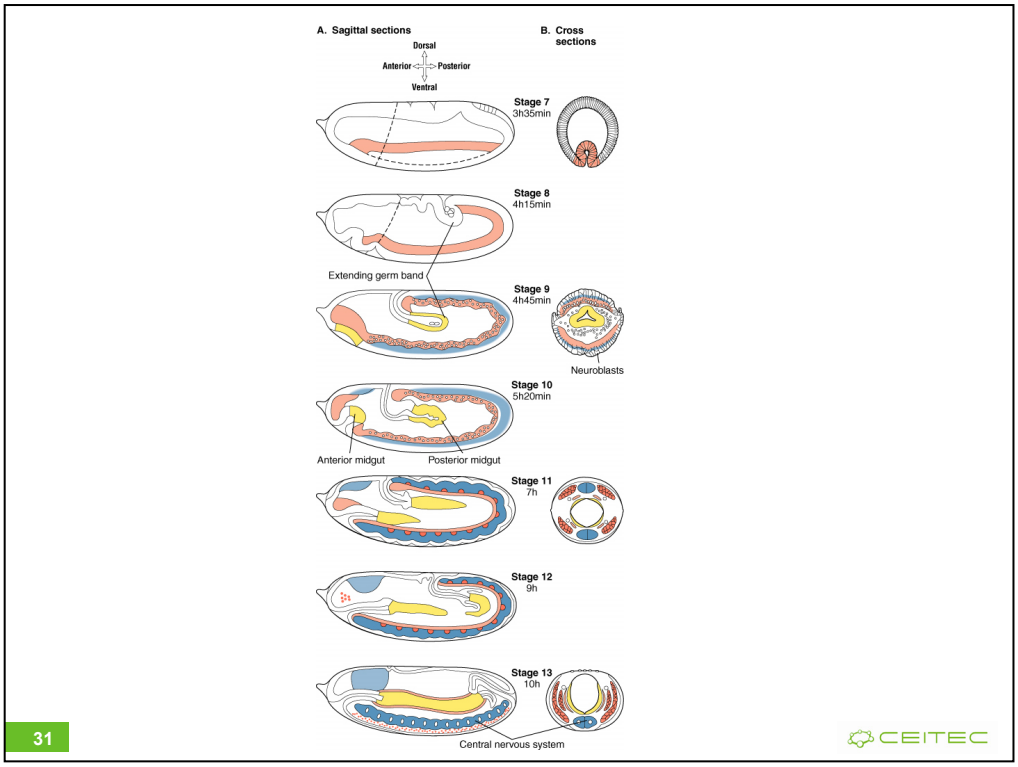
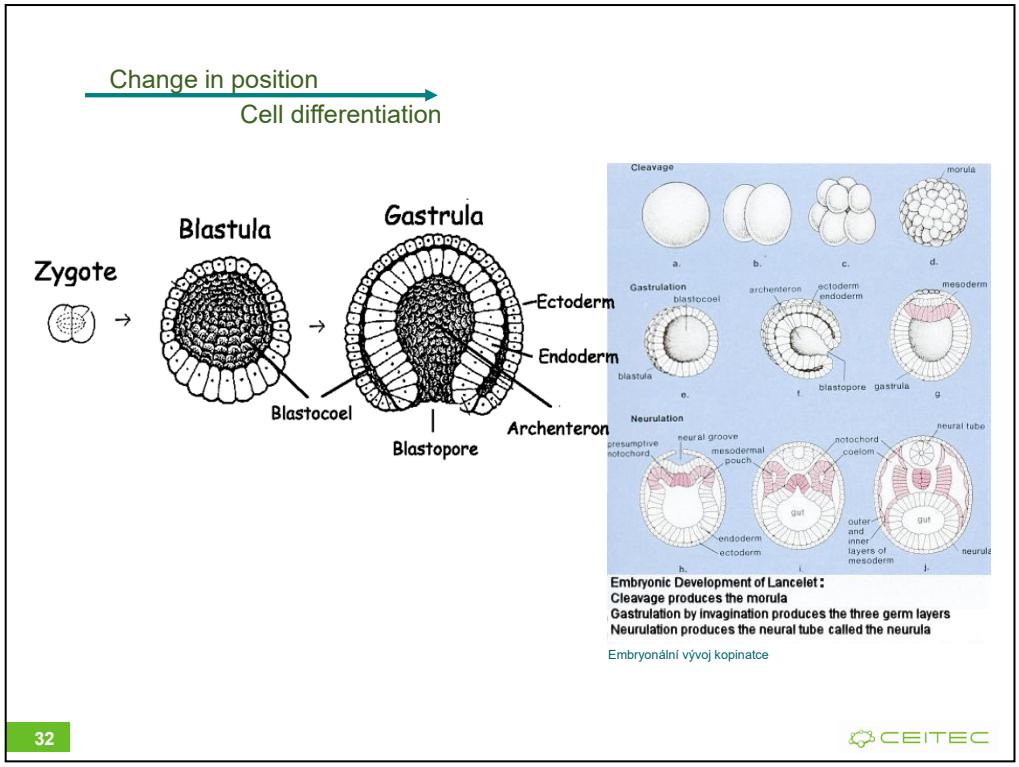


The differentiated cells of the blastoderm undergo series of cell and tissue movements. The details are specific in individual animals and never occur in plants.



During gastrulation, the partially predetermined cells of blastula undergo **specific process of movement**, leading into **change in their position** and is **accompanied with further differentiation**, i.e. further **restriction of the developmental potential**.

The process of gastrulation leads into formation of three distinct cell types:

Ectoderm is the cell layer on the surface.

Endoderm is the internal cell layer, precursor of some or all of digestive system cells.

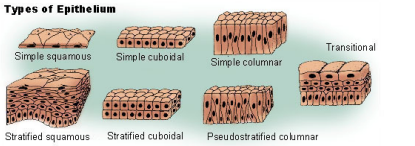
Mesoderm are the cells between ectoderm and endoderm and develops into most of the internal organs.

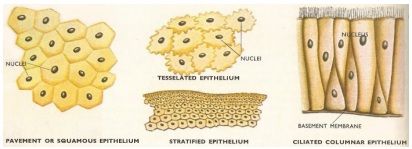
Cells from individual layers are restricted in their developmental fate. That is a result of process of differentiation that occurs concomitantly to gastrulation. However, the process of developmental differentiation is distinct from the anatomical position of those developing cells.

The source of the images:

http://images.google.cz/imgres?imgurl=http://chsweb.lr.k12.nj.us/mstanley/outlines/animals/antax/image51.gif&imgrefurl=http://smabiology.blogspot.com/2009_04_01_archive.html&usg=__BHUbg4eGIv11b1dpfydYrQkrV_Y=&h=413&w=927&sz=15&hl=cs&start=6&um=1&tbnid=9-EP7fbGTepCIM:&tbnh=65&tbnw=147&prev=/images%3Fq%3Dectoderm%26hl%3Dcs%26client%3Dfirefox-a%26rls%3Dorg.mozilla:cs:official%26sa%3DX%26um%3D1

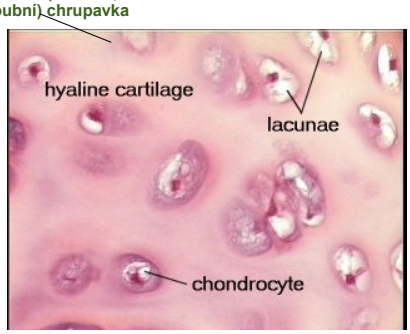
Epithelium
Epitel





Mesenchyme
Mesenchym

Hyalinní (sklovitá, kloubní) chrupavka



33

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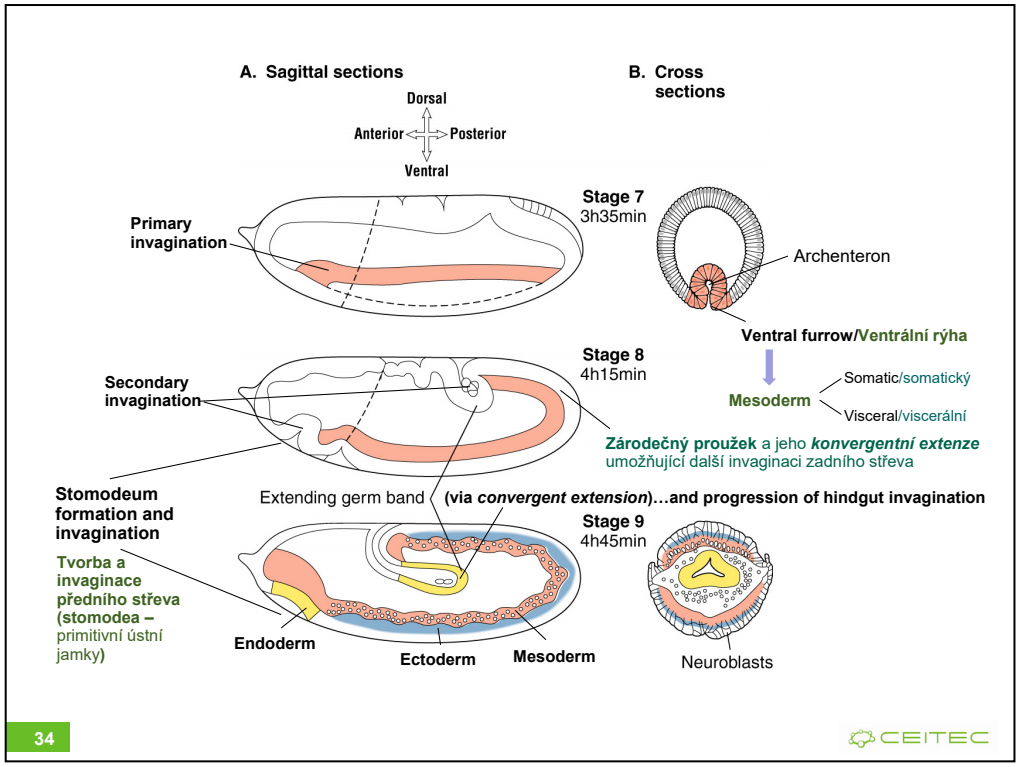
There is principal difference between tissue type, as based on the architecture and cell type (epithelium, mesenchyme), while germ layers differ in their embryonic origin (i.e. ectoderm, mesoderm and entoderm).

Epithelium is the tissue type, where the cells are tightly packed to each other, connected with cell junctions (e.g. skin epidermis).

Mesenschyme is a tissue, where the cells are not densely packed and there is a lot of extracellular matrix in the extracellular space (e.g. cartilage).

Ectoderm might form neurons, epithelium and mesenchyme, mesoderm and endoderm form epithelia and mesenchyme.

Source of the images: Wikipedia (http://en.wikipedia.org/wiki/Epithelium) and The Internet Encyclopedia of Science (http://www.daviddarling.info/encyclopedia/ETEmain.html).



Gastrulation in *Drosophila*. The time from the end of cleavage is indicated.

In *Drosophila*, the gastrulation starts by the formation of *ventral furrow*. The internal tubular structure is also called a*rchenteron*.

In *Drosophila*, the ventral cells invaginate (the band of width of about 18 cells) and the cells of the ventral furrow differentiate into motile mesenchymal layer, the future *mesoderm*.

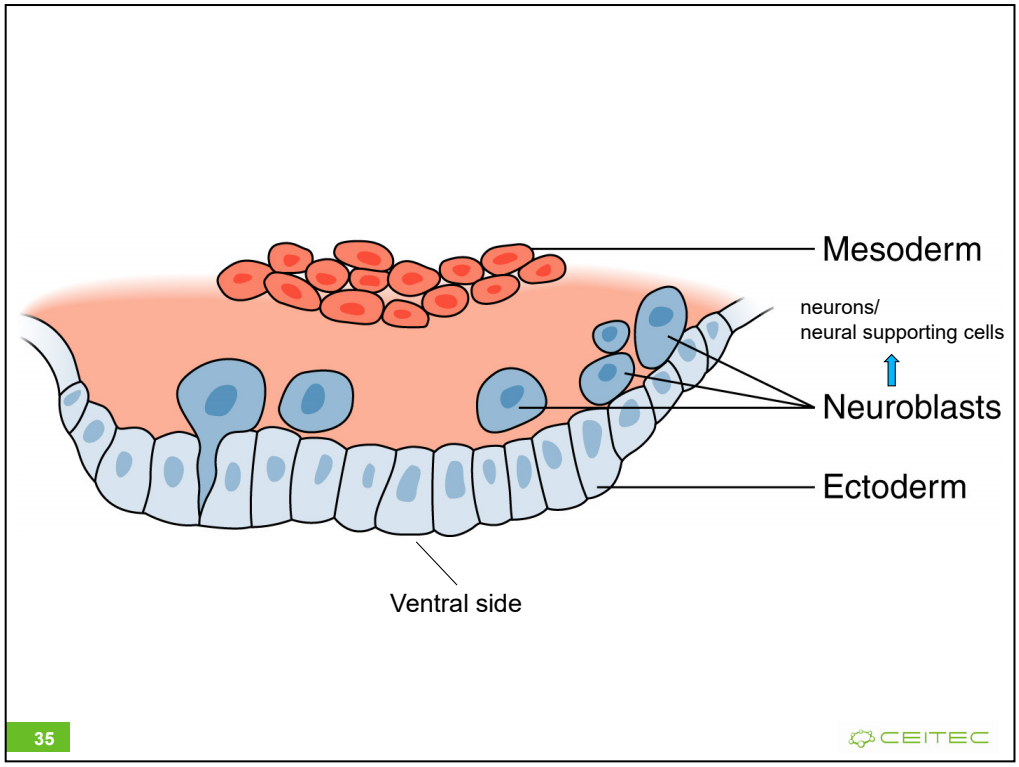
The surface layer of mesoderm is designated as a *somatic mesoderm* and develops into muscles. The internal mesoderm cell layers, what is called *visceral mesoderm*, gives rise to lipid body and the muscles of the gut.

There is a conventional color coding used: **ectoderm** is **blue**, **endoderm yellow** and **mesoderm red**.

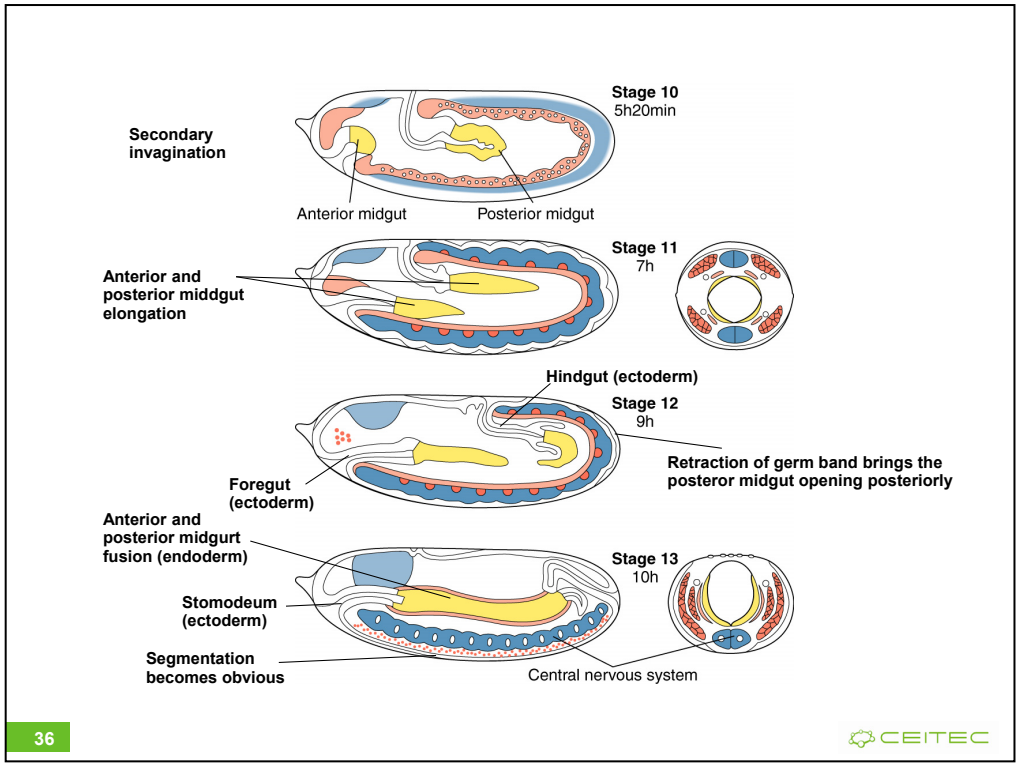
Cells at the surface of the ventral portion of the embryo undergo *convergent extension*. That is due to shuffling of the cells that leads to extension of the posterior portion of embryo to the position of the “scorpion attacking posture”. This process is called germ band extension and is peculiar to insects; however, that process is only

temporal and the extended germ band retracts (see the figures, stage 8-13).

Midgut is of endodermal origin, however, the stomodeum, foregut and hindgut are of ectodermal origin (see later and the animation of gastrulation).



Some of the ventral ectodermal cells ingress into the yolky space towards the mesoderm layer. These cells will divide and will become neurons and neural supporting cells.



After the first invagination allows formation of prospective mesoderm, the second invagination occurs. Both posterior and antherior cells invaginate, leading to formation of pockets that elongate and fuse, forming thus a long internal tube of endoderm that will become the *midgut*.

Cells on the midgut surface invaginate, too and allow formation of *foregut* and *hindgut*.

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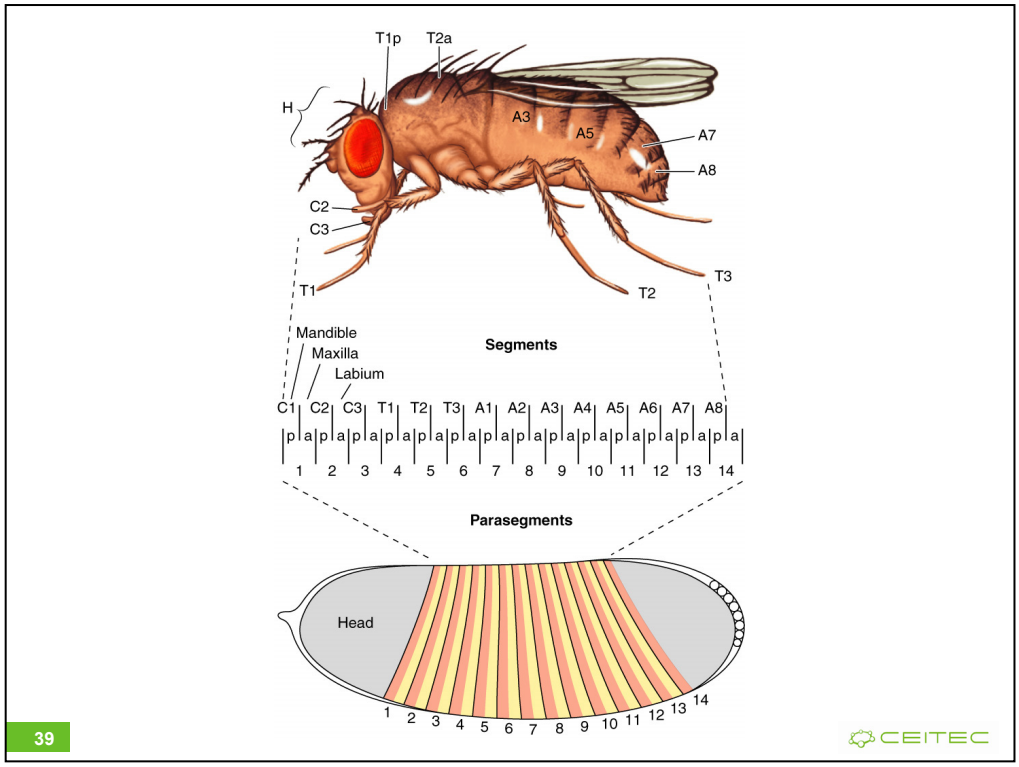
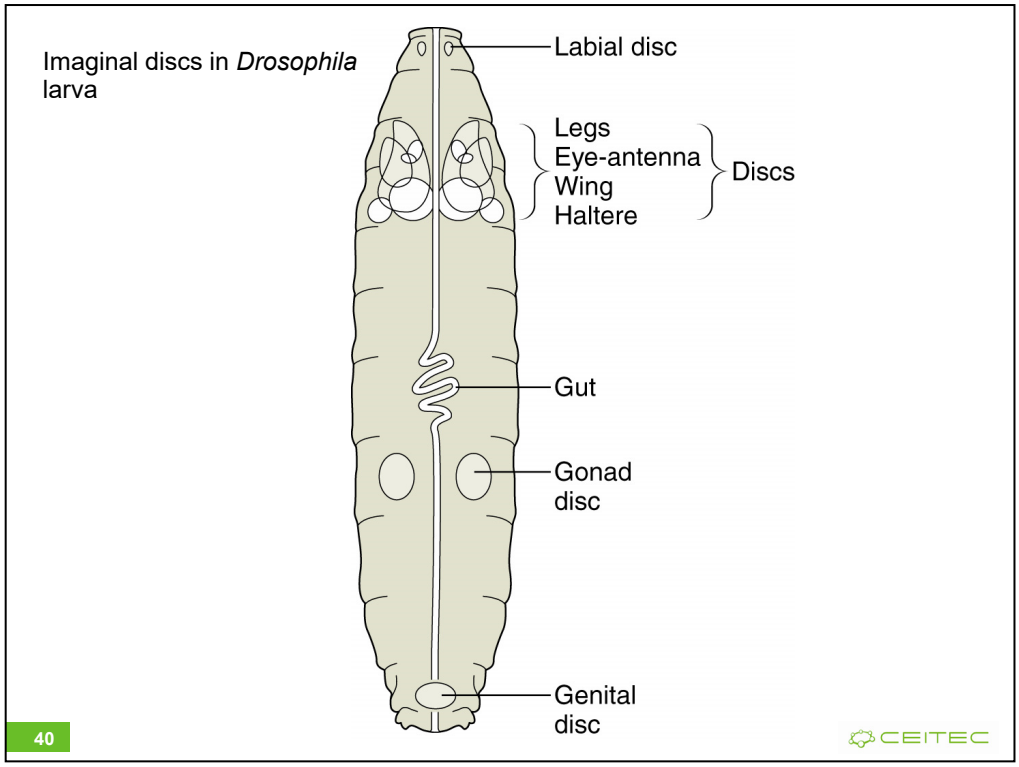
- Overview of metamorphosis



At the time when the germ band is fully extended, the so called *parasegments* appear at the surface of embryo. Three of them will develop into head and mouth, three into thorax and eight into abdomen.

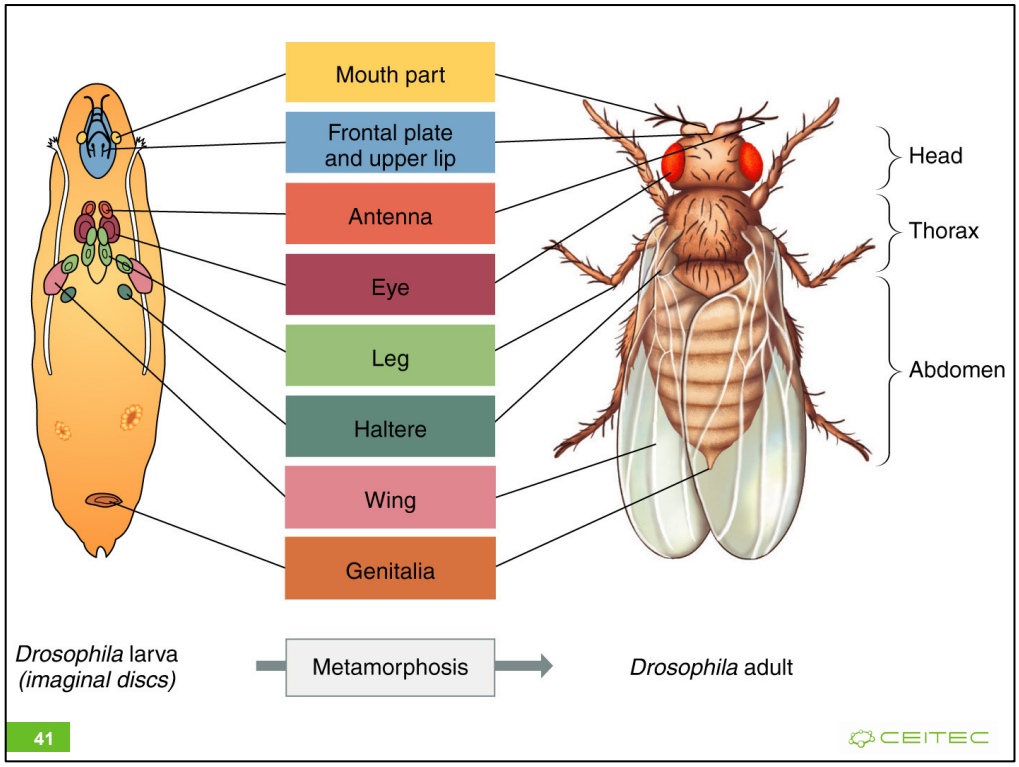
In comparison to parasegments, the segments of the adult are shifted slightly towards anterior end.

There are complex genetic interactions determning formation of individual parasegments in *Drosophila*. Their detailed description is a matter of the advanced lecture Bi0580 Developmental genetics by prof. Boris Vyskot.



In each of the parasegment there are small number of specific cells. These are:

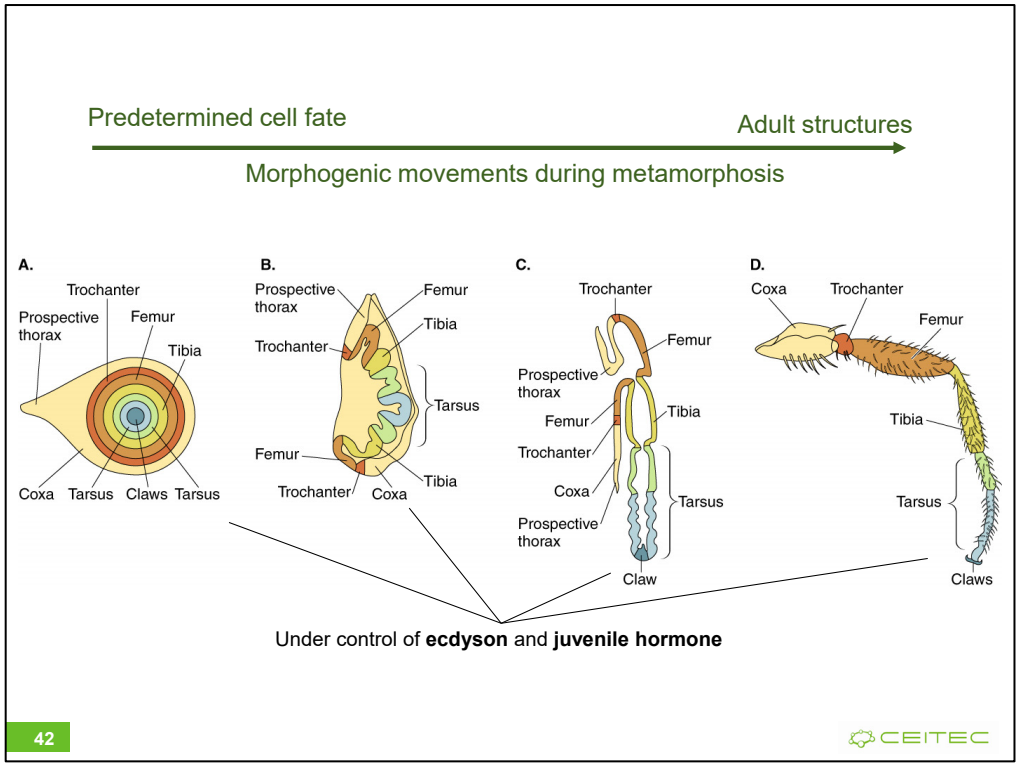
1. *Histoblasts* that retain the capacity of cell division and replace dying larval epithelium in the pupa
2. ***Imaginal discs*** that will evert and form many different organs during *metamorphosis*



At the and of larval stage of development, *Drosophila* undergoes postembryonic developmental stage, called **metamorphosis**.

During metamorphosis, most of the larval structures are destroyed and some of them, e.g. neural system or gut are substantially reorganized. New organs of adults develop mostly from the imaginal discs.

Imaginal discs contain special cells that proliferate and differentiate during larval development.



During metamorphisis, the developmental fate of cells in imaginal discs is predetrermined and during metamorphosis, the cells undergo series of intensive **morphogenetic movements**, leading to **formation of adult structures** (see above).

The proces of metamorphosis is under tight hormonal control, mostly of *ecdyson* and its derivatives and *juvenile hormone*.

The genetic regulation and more detailed description of metamorphosis in *Drosophila* is a matter of the advanced lecture Bi0580 Developmental genetics by prof. Boris Vyskot.

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- Overview of metamorphosis
- Introduction into genetic and molecular mechanisms of the early embryogenesis in *Drosophila*

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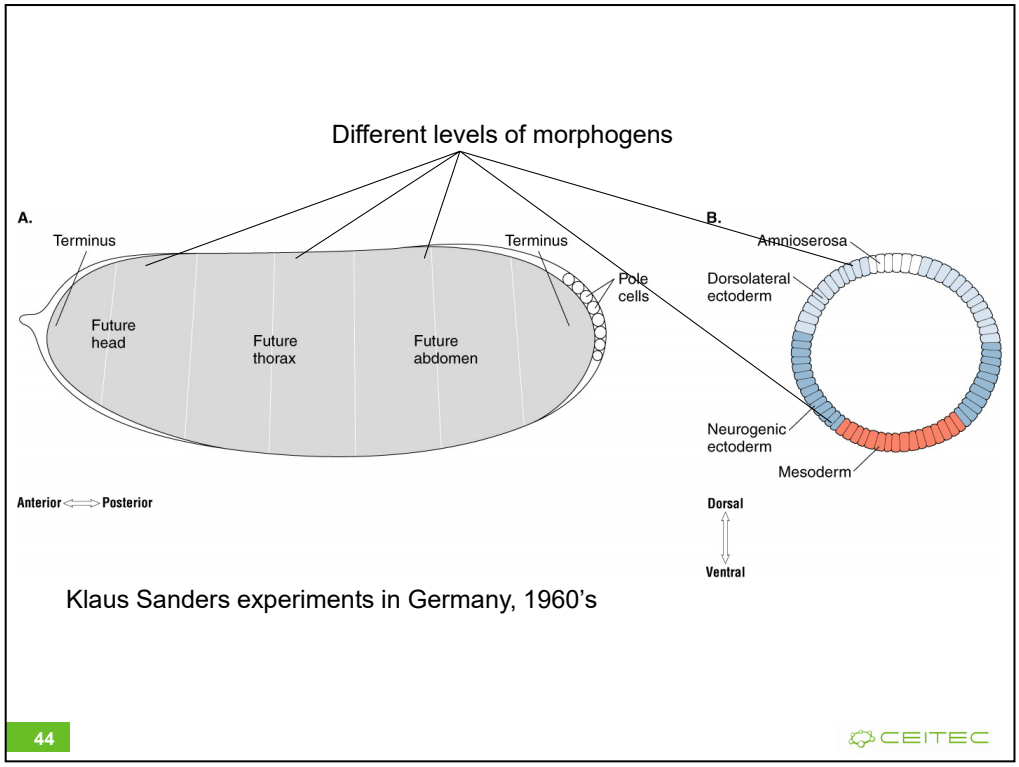
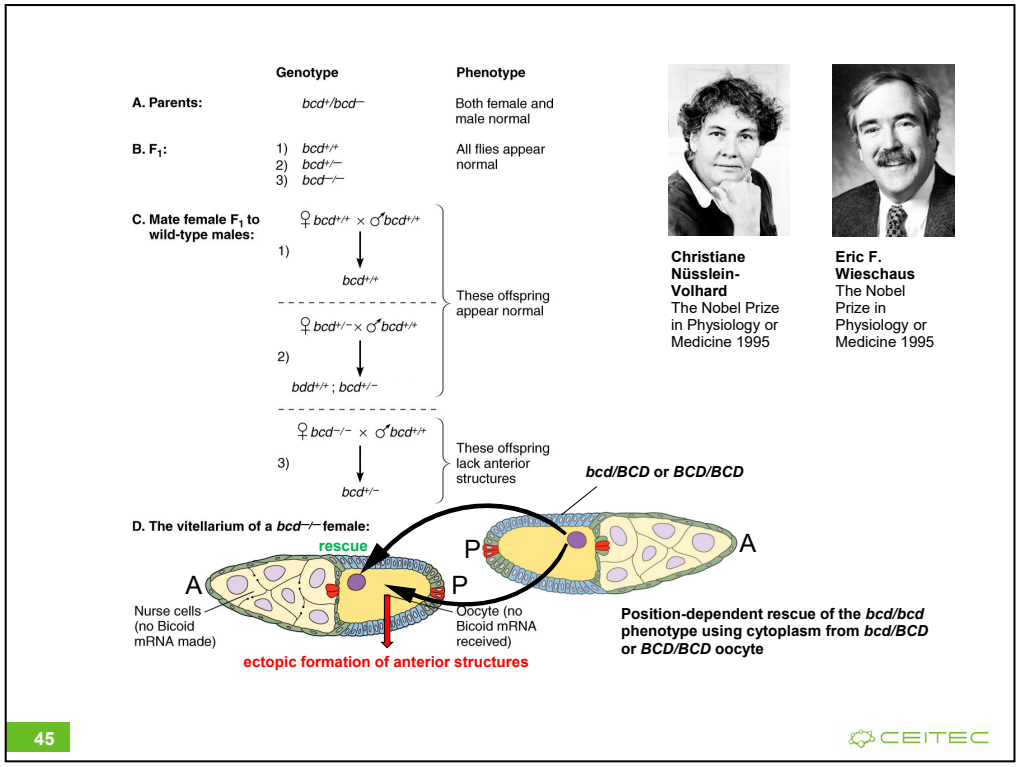


The regions in embryo of *Drosophila* are predetmined by their **position** in both anterio-posterior and dorso-ventral axes.

By the classical embryonic experiments done by Klaus Sanders in Germany in 1960's with leaf hoppers, he shown that the different regions contain different amounts of substances that favor either anterior, posterior, dorsal or ventral development.

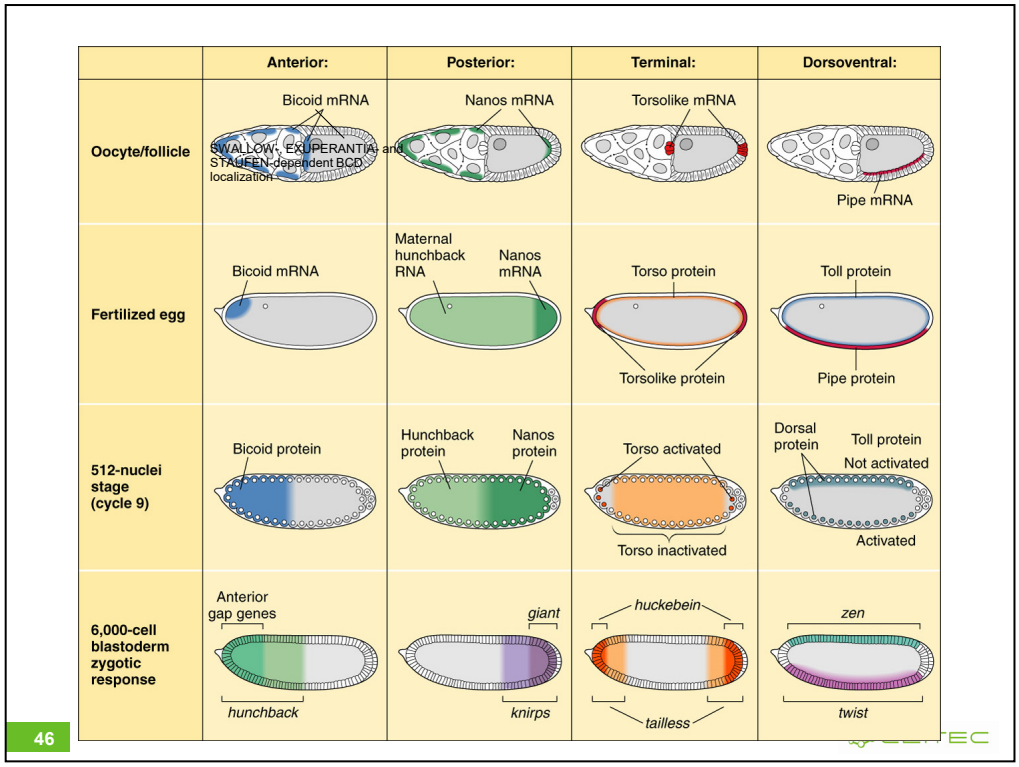
Now we know that these substances are so called **morphogens**.



The molecular nature of morphogens was discovered by genetic experiments of Eric Wieschaus and Christiane Nusslein-Vollhardt, the German Nobel prize winners.

Together with their students and colleagues, they have identified 34 genes that are critical during early embryo development-the mutants are embryo lethal or show maternal effects (e.g. *BICOID (BCD)*, see the scheme).

bcd/bcd embryos are lethal, they are missing anterior structures. When the anterior cytoplasm is replaced from the anterior region of the b*cd/BCD* embryo, it can rescue the phenotype. The effect is even stronger in case of *BCD/BCD*. However, when the anterior cytoplasm is placed to the middle portion of *bcd/bcd* embryo, that leads to formation of anterior structures there.



BCD was identified to encode transcription factor (TF) containing a domain that is called *homeobox*.

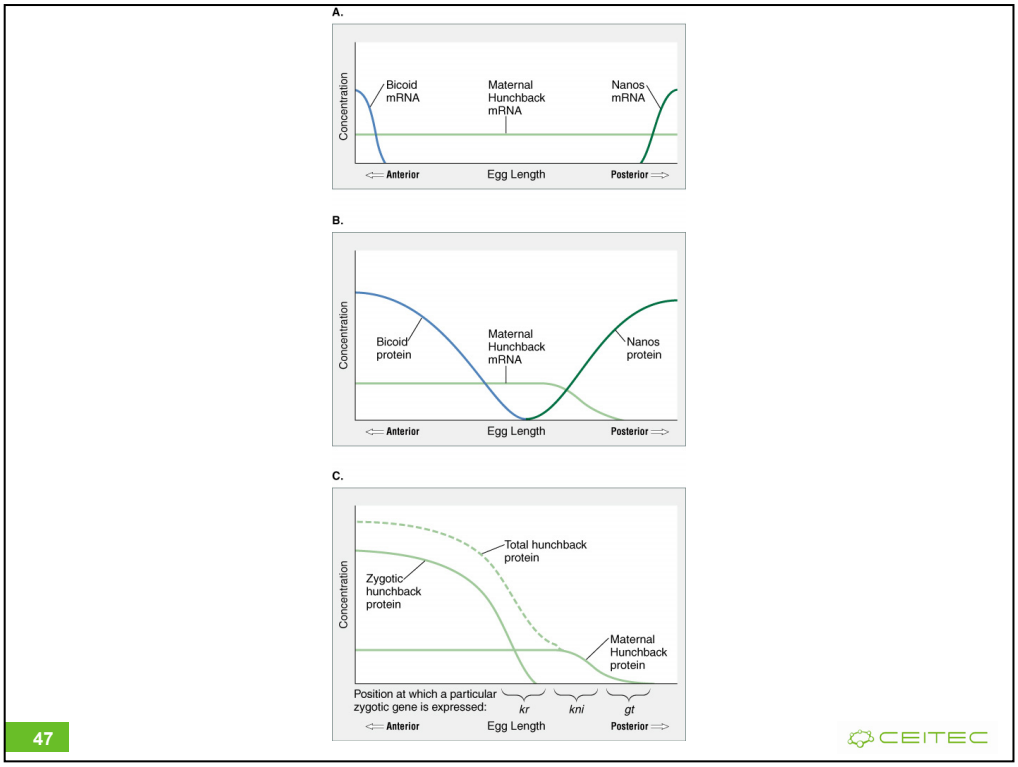
The more detailed description of development regulations via homeobox genes is a matter of an advanced lecture “Bi0580 Developmental genetics” by prof. Boris Vyskot.

BCD is the **major anterior determinant**. *BCD* mRNA is produced by the nurse cells and transported into the egg via ring canals. *BCD* mRNA is tethered to the cytoskeleton in the anteriror portion of the egg. The localization of *BCD* is affected by other gene products, e.g. *SWALLOW, EXUPERANTIA* and *STAUFEN*.

The **major determinant of the posterior embryo parts is NANOS**. NANOS shares some similarities with BCD in terms of origin of expression (nurse cells) and the regulation of action (localization, in this case posterior). Similarly to BCD, there are other genes necessary for the proper NANOS localization. Interestingly, 3’ UTR is necessary for the proper localization of NANOS.

However, in contrast to BCD, the molecular mechanism of NANOS action is more complicated. NANOS acts as a negative regulator of translation of few particular

mRNAs, particularly *HUNCHBACK*. mRNA. HUNCHBACK is a TF that activates some genes that are needed in the anterior portion of the embryo for the proper head development. However, it inhibits the expression of other genes necessary for the development in the posterior portion, e.g. *GIANT* (see further slides).

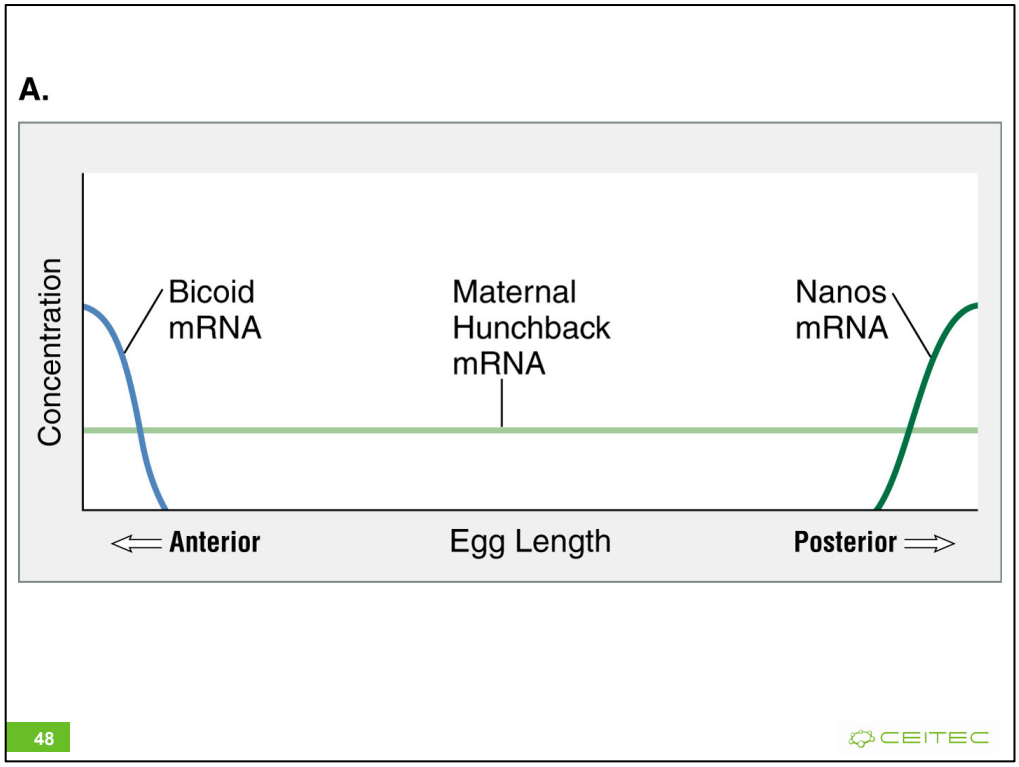


There is feed back regulation between the genes driving the anterior and posterior development of the *Drosophila* embryo.

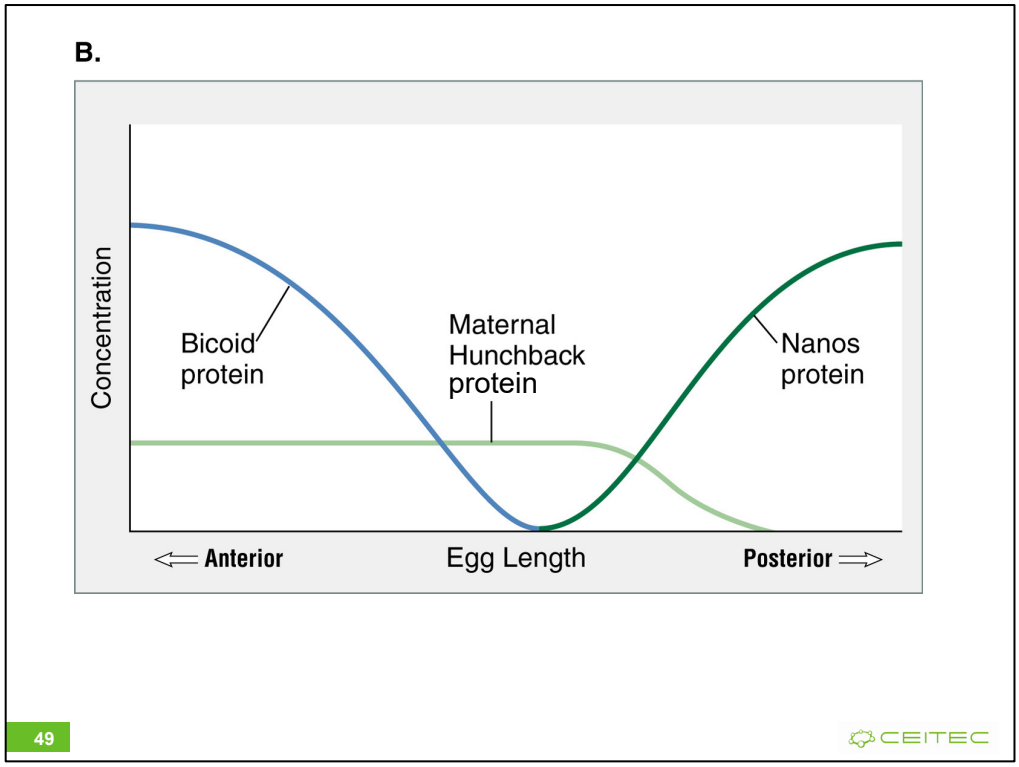
While *BCD* and *NANOS* are the major determinants of the anteior and posterior portions of the embryo, respectively, they also regulate at the transcriptional or translational level the expression of other target genes.

The situation is more complicated particularly in the case of *NANOS*, that regulates translation of dual (positive and negative|) regulator of the gene expression (TF) *HUNCHBACK*.

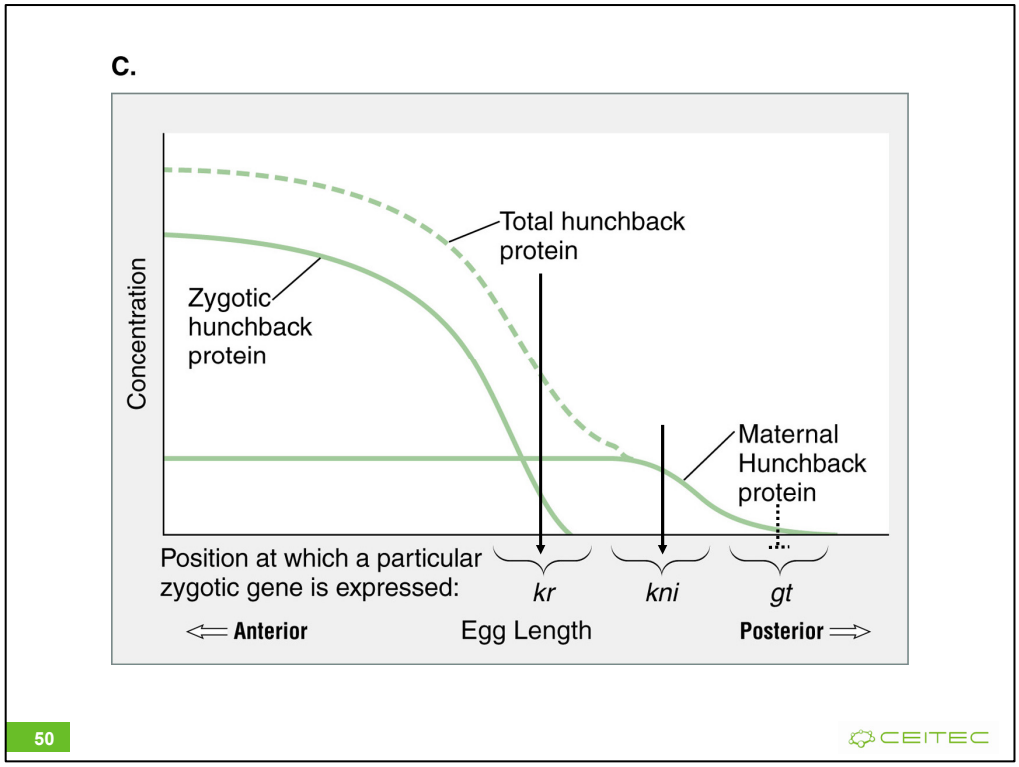
There is some level of maternal *HUNCHBACK* that is however not allowed to localize at the posterior end. Further, the level of maternal HUNHBACK is not sufficient for the proper regulation of target genes, especially in the anterior portion of the embryo (e.g. KRUPPEL (KR)).



The levels of BCD and NANOS at the time of fertilization.



After fertlization, the proteins are translated. NANOS inhibits *HUNCHBACK* translation posteriorly.

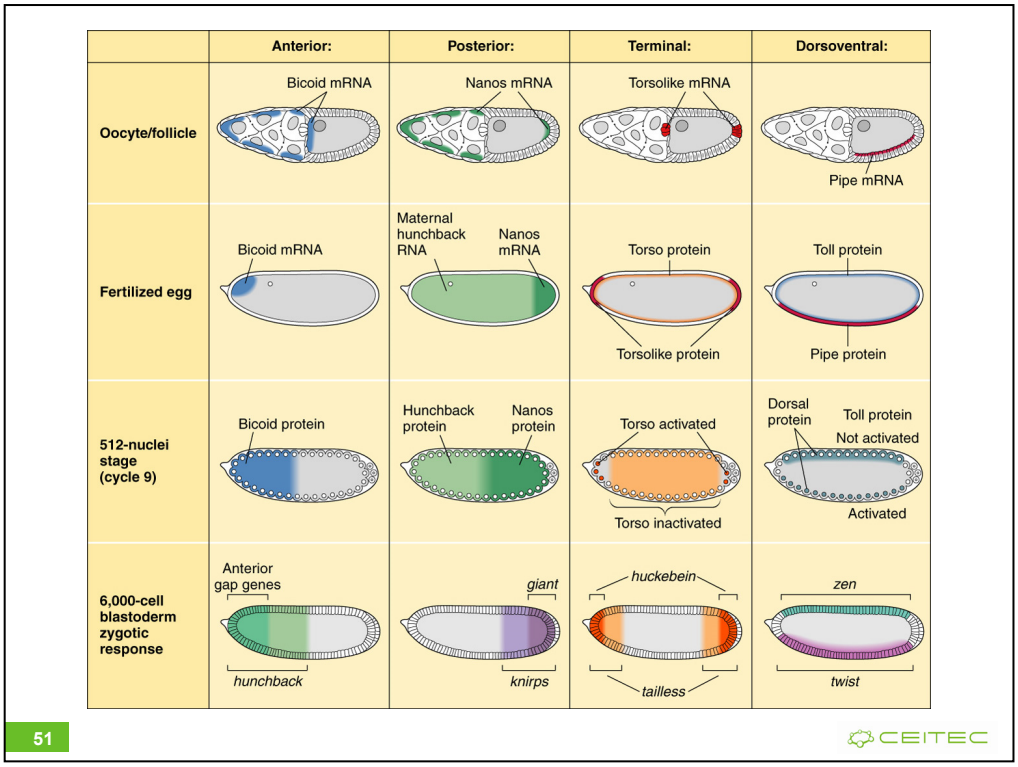


Later in development, *HUNCHBACK* is induced anteriorly by *BCD*.

The gradient of HUNCHBACK regulates the position of *KRUPPEL (KR), KNIRPS (KNI)* and *GIANT (GT).*

HUNCHBACK upregulates *KR* and *KNI,* where *KR* requires higher than maternal level of *HUNCHBACK* .

GT is repressed by *HUNCHBACK*.



There are 6 genes in the *Drosophila*, involved in the development of terminal structures, i.e. acron (anetrior) and telson (posterior). One of them is *TORSO*.

Similarly to *BCD* and *NANOS*, *TORSO* mRNA is produced by nurse cells, but TORSO protein is localized throughout the fertilized egg. The position-specific activation of TORSO is mediated via terminally located TORSOLIKE protein that activates the Ser/Thr kinase activity of TORSO. That allows expression of downstream genes, e.g. *TAILESS*, acting in the specification of acron and telson.

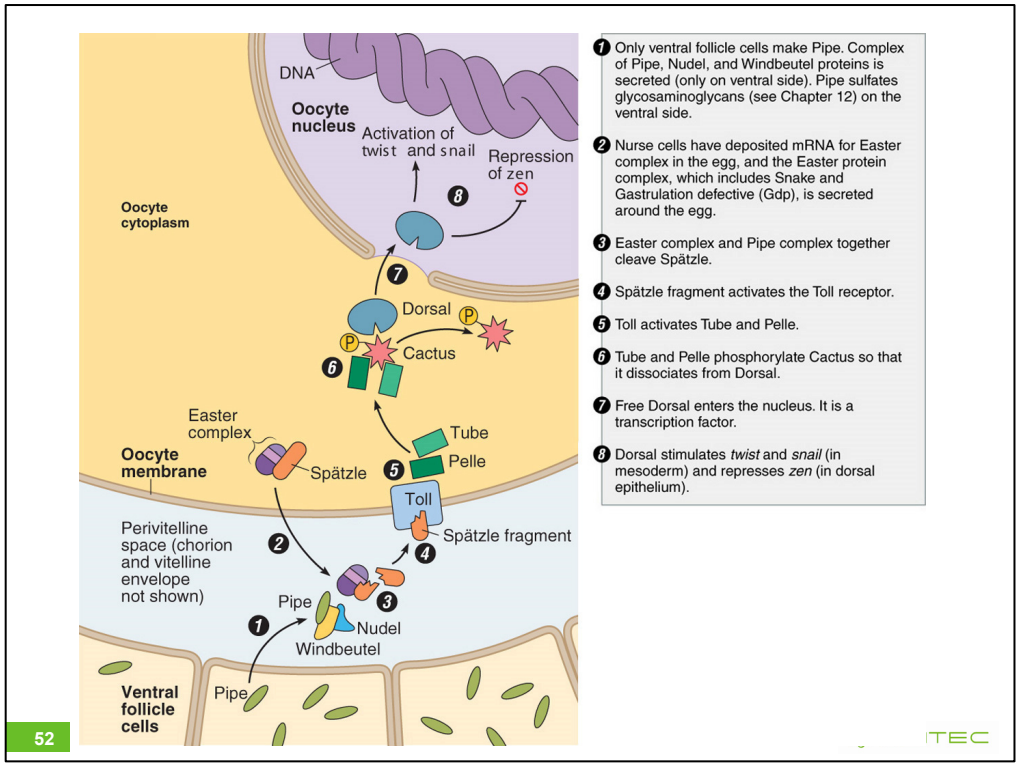
The dorsoventral patterning of the embryo is ensured via action of 16 genes, mutations in 13 of these genes are strictly maternal, 2 of them are expressed in the zygote after fertilization.

Mutants in those 13 maternal genes are missing ventral structures (e.g. mesoderm and ventral nerve cord).

Mutation in remaining genes, e.g. *CACTUS* lead to partially or completely “ventralized” (i.e. missing dorsal structures) embryos.

The entire set of genes necessary for the dorso-ventral patterning are member of

specific signaling pathway, where the intracellular localization of DORSAL protein is critical.



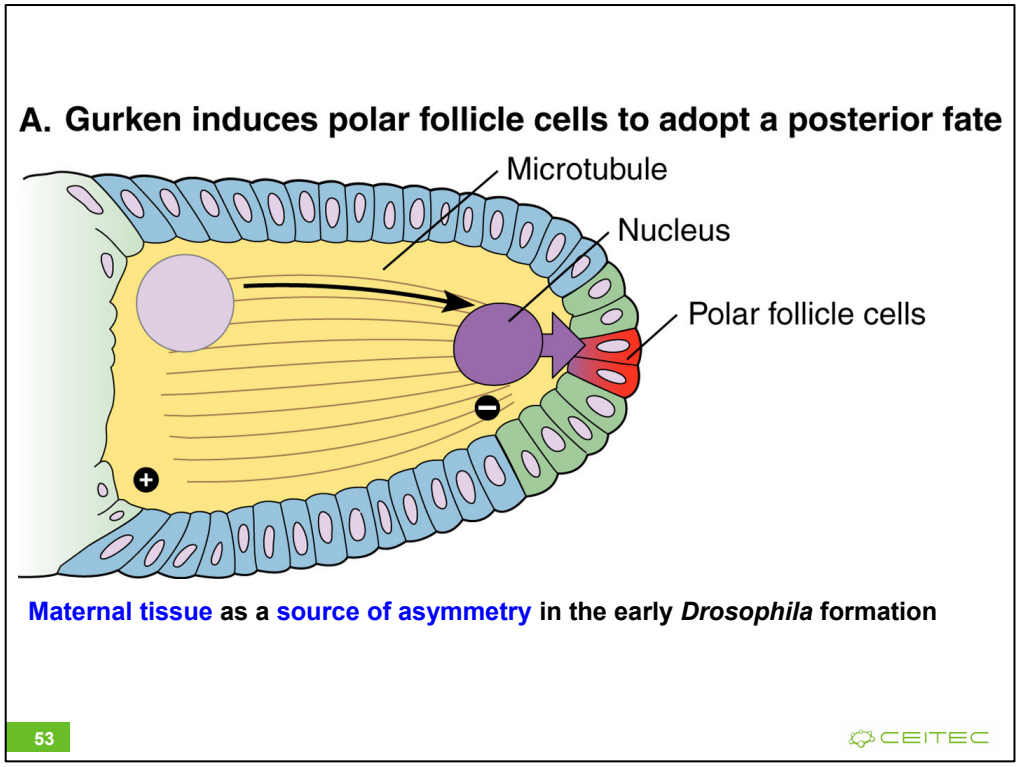
After cellularization, DORSAL becomes nuclear localized in the ventral portion of the blastoderm. This is achieved via complicated interplay of members of the DORSAL signaling pathway.

Ventral follicle cells express proteins that form a protein complex “X” (PIPE, NUDEL, WINDBEUTEL) in the perivitelline space.

PIPE modifies (sulfonates) protein from the EASTER complex (protein complex consisting of EASTER, SNAKE, GDP and SPATZLE).

That leads to the release of SPATZLE fragment that is a ligand of TOLL.

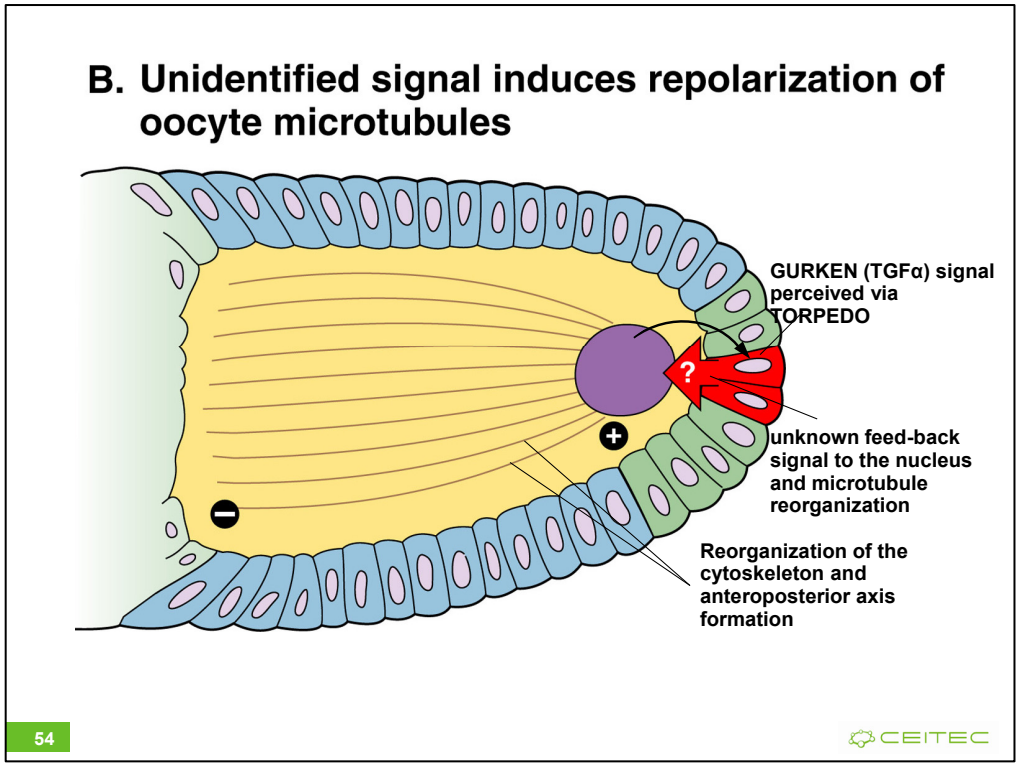
The activation of TOLL triggers the downstream cascade that allows nuclear localization of DORSAL via removal of the CACTUS from DORSAL.



What is the mechanism that allows predetermination of folicle cells and allows later spatial RNA/protein distribution and/or localized activation of pathways?

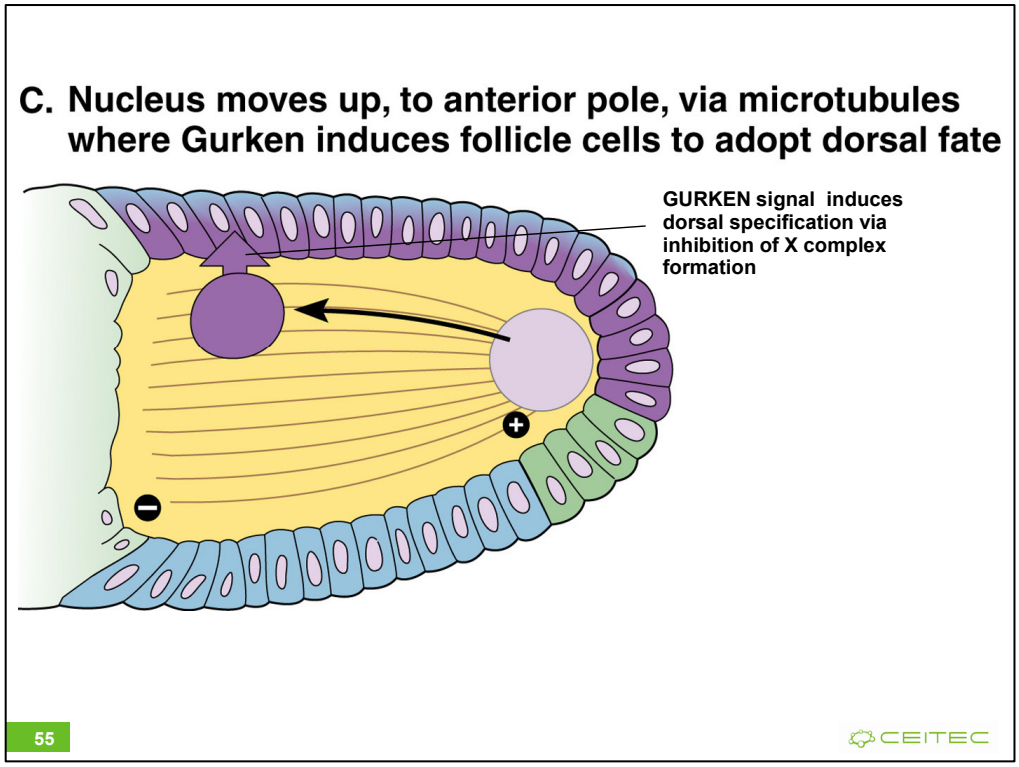
There is reciprocal interaction between developing oocyte and the folicle cells. Crucial is the microtubule-driven position of the oocyte nucleus.

Remember-follicle cells are a part of the maternal organism (it is diploid, *sporophytic* tissue).



When nucleus gets into contact with posterior portion of the folicle, GURKEN, a protein from the TGF-α (transforming growth factor-alpha) family is released from the oocyte nucleus. This signal is perceived by the folicle cells via TORPEDO receptor, which causes the folicle cells to signal back to the oocyte.

That leads to the reorganization of the oocyte cytoskeleton and the anteroposterior axis is laid down.



That allows nucleus to move to the opposite (anterior) pole and similar event via GURKEN signalling allows specification of anterior pole.

This second type of signaling induce nearby folicle cells to adopt a dorsal specification. This inhibits formation of X complex (which induces ventral identity) and induces complicated circuit that drives formation of bilaterally suited dorsal respiratory organs of the oocyte.

Key Concepts

- **Axis formation** is based on the **asymmetrical distribution of molecular determinants**, mostly proteins and RNAs. Processes ensuring the asymmetrical distribution of those process occur **very early in the oogenesis**.
- The **cytoplasm may contain factors** (TFs) that regulate the expression of **target genes in the nucleus** as shown in pole plasm transplatation experiments.
- During early development, the **developmental potential of cells** or group of cells **changes**.
- **Gastrulation** comprises a suite of **coordinated cell divisons and movements** that results into movement of specific cells from the surface to the interior.
- The **cytoplasm of embryo** in *Drosophila* and almost all other animal embryos **is heterogenic** in terms of the distribution of factors that regulate developmental fate of adjacent cells. The **origin of this heterogeneity is in the maternal tissue** and its **mutual interaction with the oocyte**.

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Discussion