Bioscience & Bioengineering 101: BB101

Lecture – 9

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BSBE, IIT Bombay

Development, Stem Cells & Cell Reprogramming

Outline

- **Development:** progressive changes in size, shape & function during life of an organism
- **Stem cells:** cells that can be made to form different types (have a property of being highly flexible)
- **Cell reprogramming and cloning:** two fascinating areas of biotechnology that have revolutionized our understanding of cellular behavior and potential applications in medicine and research.
- Different topics, but are connected…

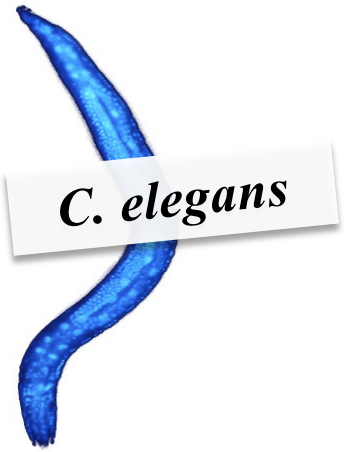
- Development ✓
- Stem Cells
- Cell Reprogramming

Development

How Did a Single Cell Develop Into Embryo?

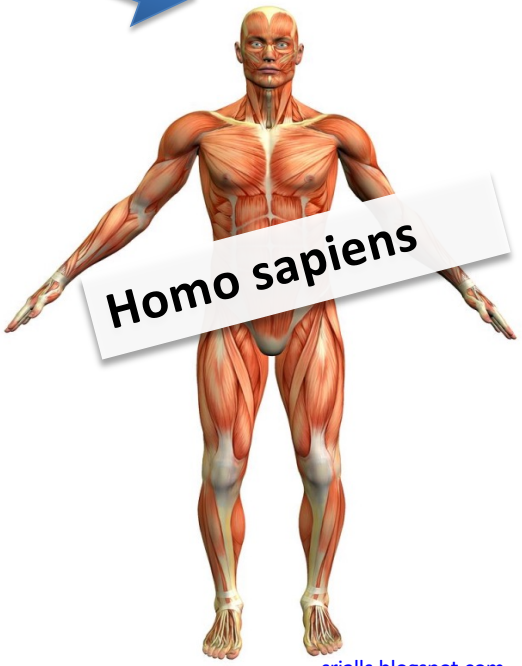
Development of Multi-cellular Organisms

www.stemcellsinc.com



www.nih.gov

www.tumblr.com



srialls.blogspot.com

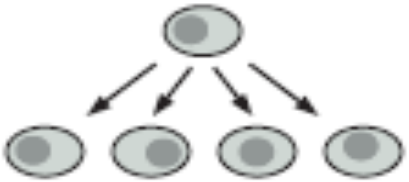
Development of Multi-cellular Organisms

- Four essential processes for multi-cellularity:

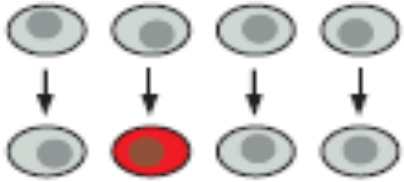
1) Proliferation (this lecture)
2) Specialization (regulation of gene expression lecture)
3) Interaction (communication lecture)
4) Movement (Ranjith's lecture)

- All processes are coordinated

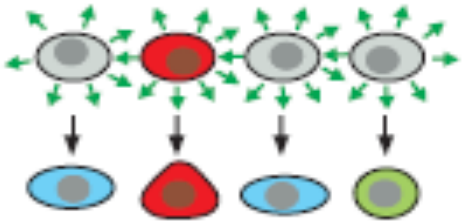
Proliferation



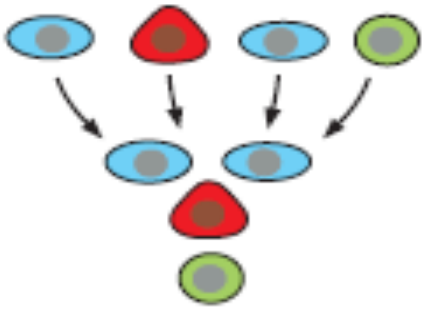
Specialization



Interaction

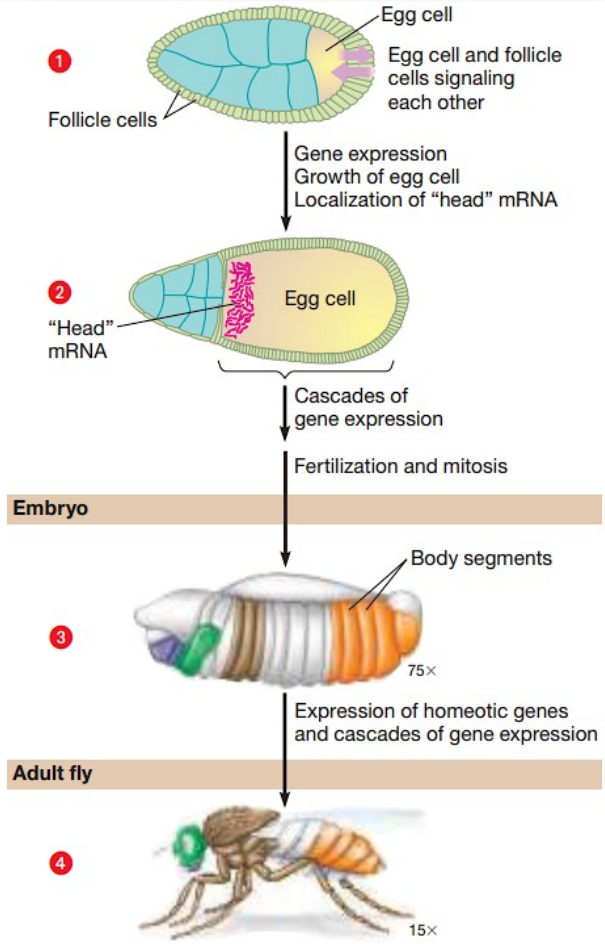


Movement



Understanding Development: Cell Division and Differentiation

- Development involves cells going from "undifferentiated" to" differentiated"
- Undifferentiated: no obvious phenotype, have the potential to become something else
- Differentiated: have a distinct form and function (hair, nails, liver, muscle, etc)
- During embryonic development, cells are given cues that depend on their position in the embryo
- These cues give rise to changes in gene expression that result in specific genes being expressed



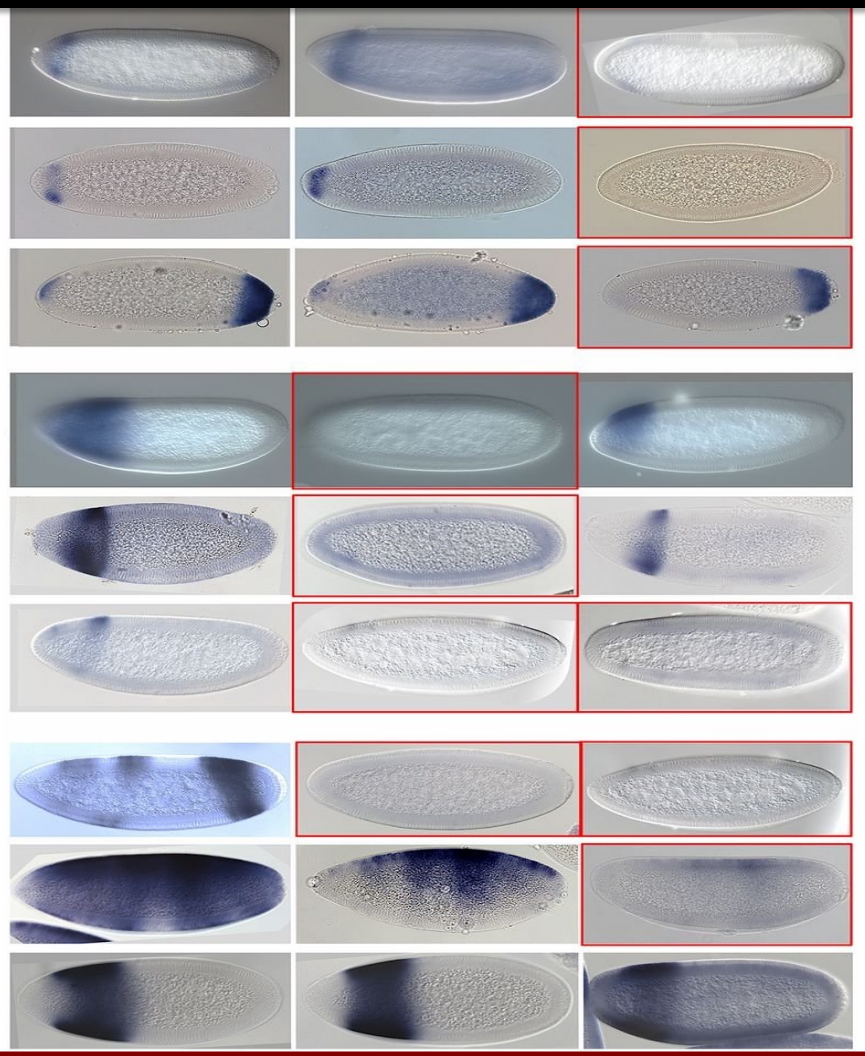
▲ Figure 11.8B Key steps in the early development of head-tail axis in a fruit fly

The early embryo looks like a blob, but it's not!

Expression of different genes at different positions
(there are different signaling molecules at different positions) …

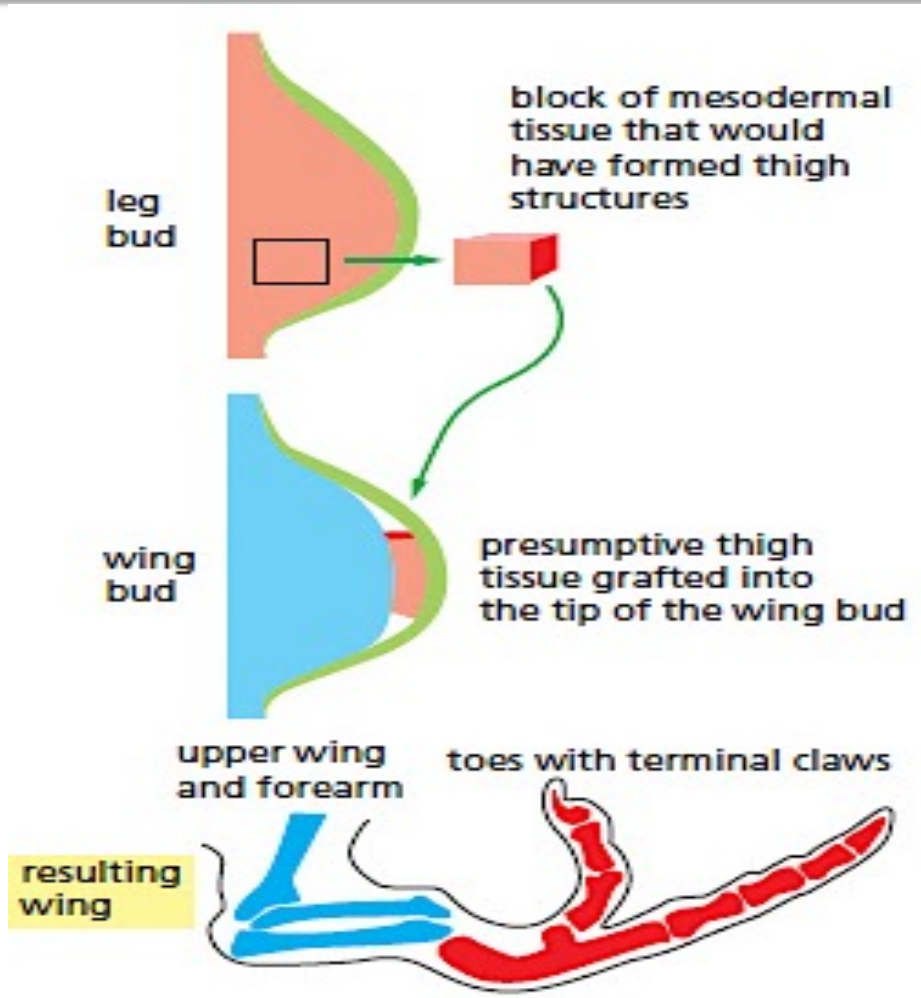
PNAS, 114 (31) 8295-8300

Cells move to reach their positions in the embryo (gastrulation)



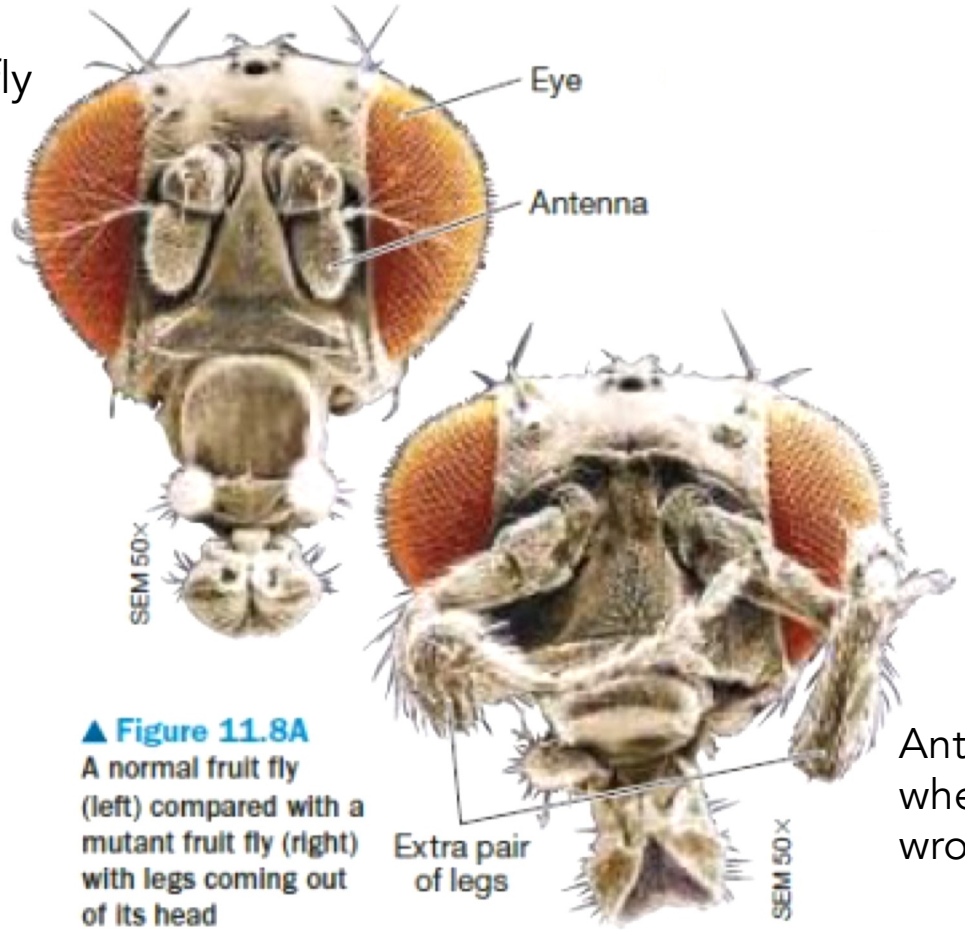
The position of a cell affects its differentiation

- Developmental decisions are made long before a visible change
- Cell's state of determination can be tested by transplanting it to altered environments
- Between extremes of the fully determined and the completely undetermined cell, there is a whole spectrum of possibilities
- POSITIONAL VALUES



Frankenstein flies: Proteins for "legs" expressed in the head

"Normal" fly



▲ **Figure 11.8A**
A normal fruit fly (left) compared with a mutant fruit fly (right) with legs coming out of its head

Antennapedia mutant (a monster), where proteins for legs are wrongly expressed in head

Common Stages of Embryonic Development

- **Fertilization** – fusion of sperm & egg
- **Cleavage** – series of cell divisions divide embryo into many cells & generate a hollow ball of cells "blastula"
- **Gastrulation** – Blastula rearranges into a multilayered embryo
- **Organogenesis** – last major stage of embryo development, which generates rudimentary organs

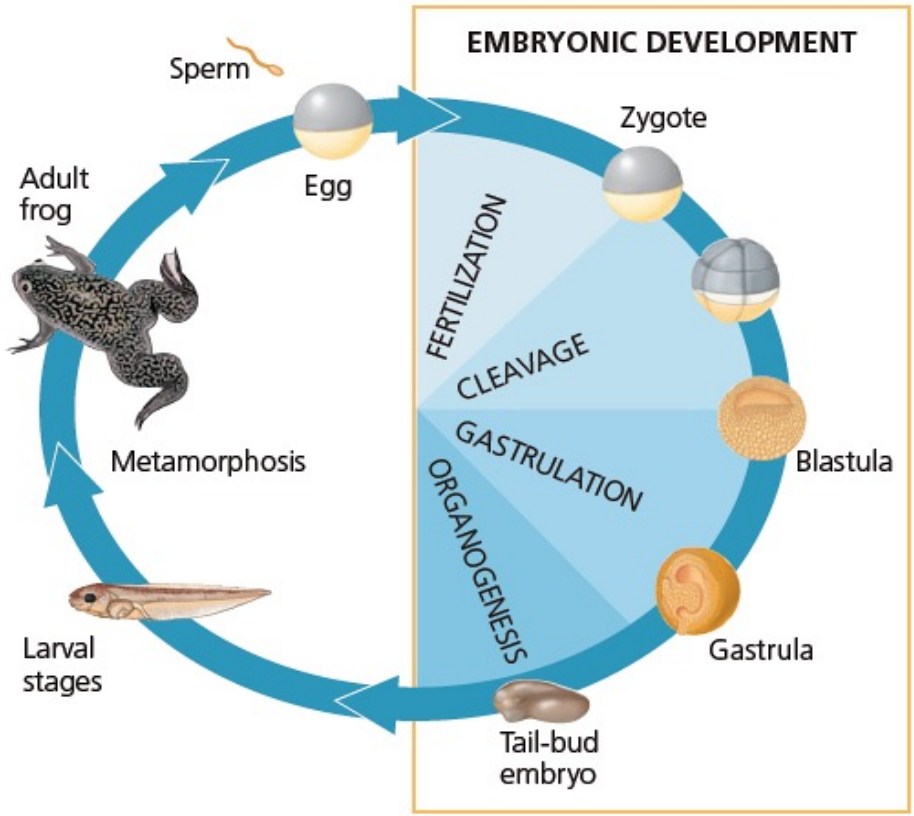


Figure 47.2

Metamorphosis - larva (tadpole) undergoes major changes in anatomy to become an adult

Fertilization

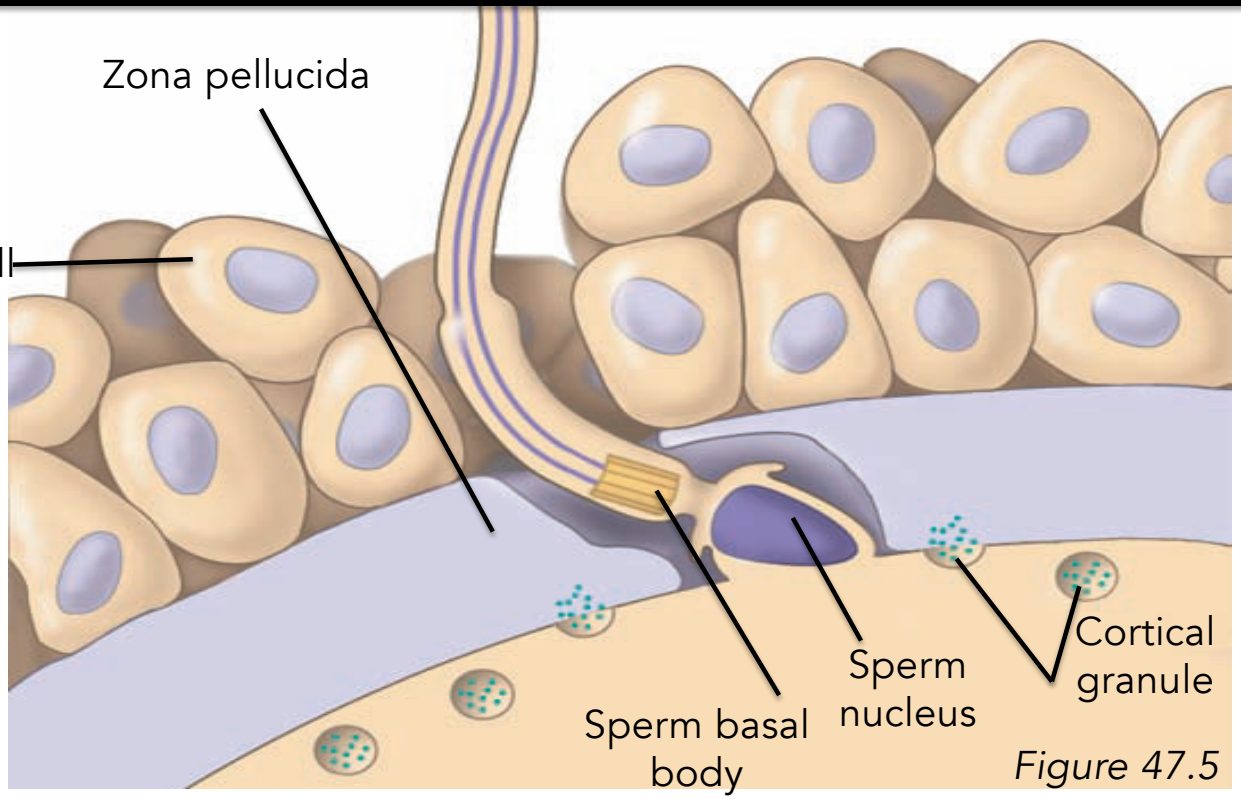


Figure 47.5

- Sperm travels through follicle cells & zona pellucida & fuse with egg
- Cortical reaction initiates events to ensure that only one sperm nucleus enters the egg

Zygote formation & early Post-fertilization Events

From Ovulation to Implantation

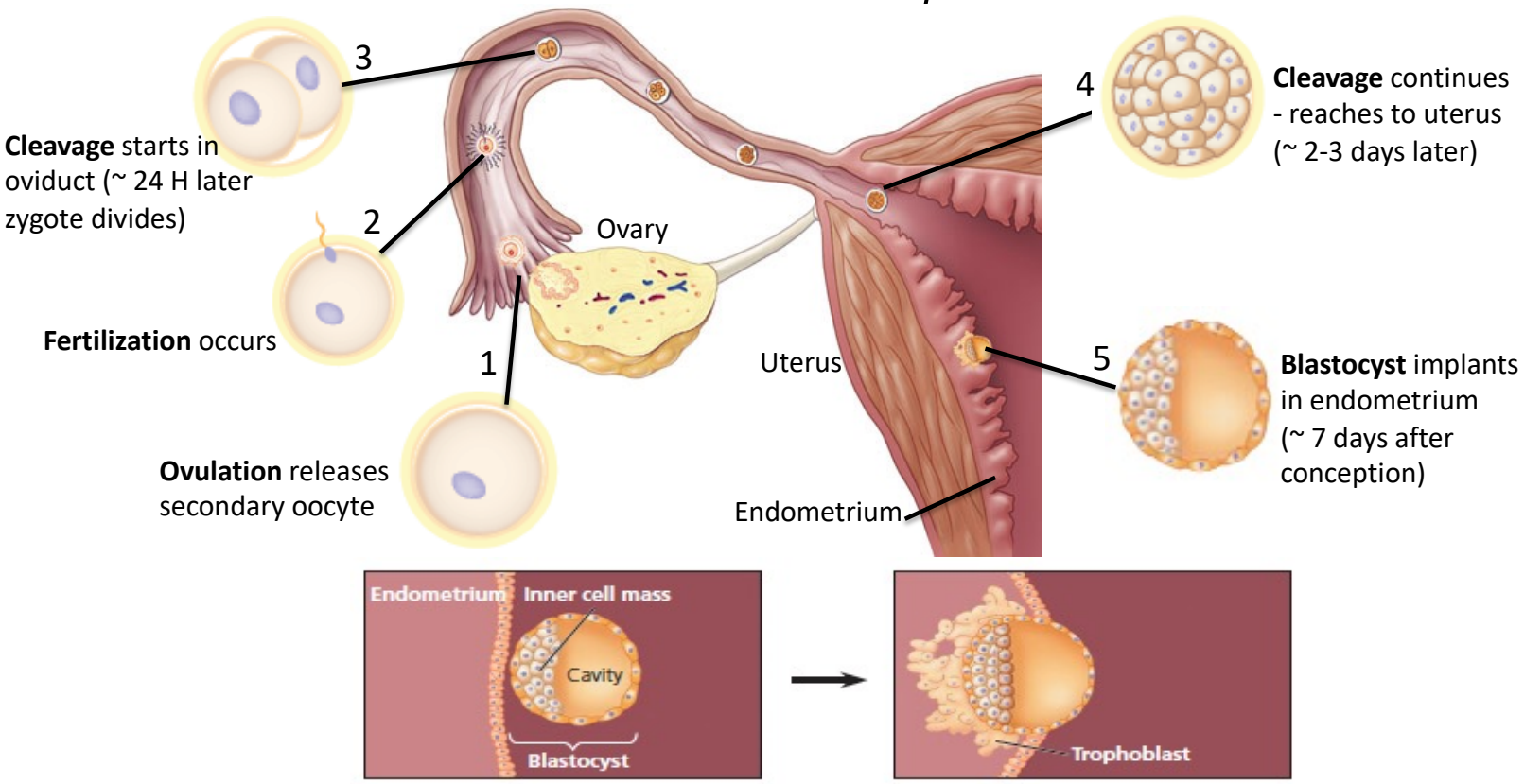


Figure 46.15

Implantation of Blastocyst

Several days after blastocyst formation, embryo implants into the endometrium; Human pregnancy averages 38 weeks

Stages of Human Embryo Development

- Placenta is combination of maternal & embryonic tissues
- It transports nutrients, respiratory gases & wastes between embryo or fetus & mother

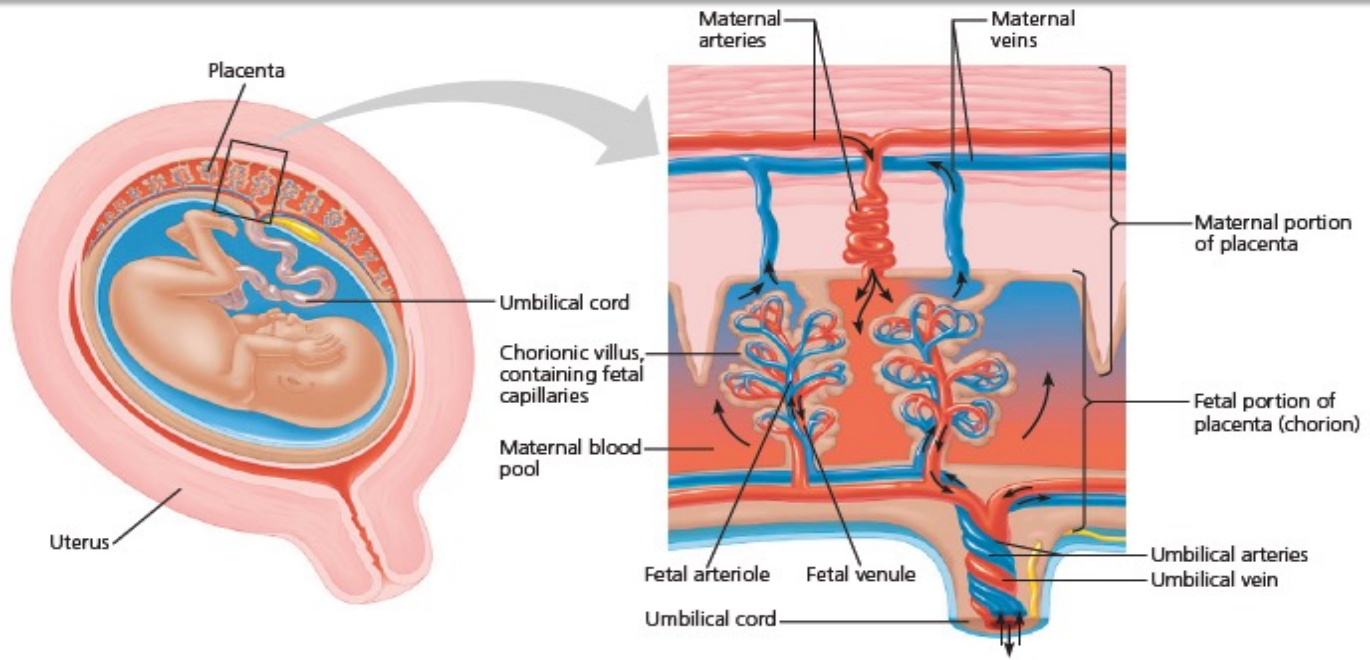


Figure 46.16

Morphogenesis

- Morphogenesis - cellular & tissue-based processes by which animal body takes shape
- Last two stages of embryonic development – gastrulation & organogenesis are responsible for morphogenesis
- ***Reorganization of cytoskeleton*** associated with morphogenetic changes in embryonic tissues
- ***Programmed cell death*** or apoptosis is common feature of animal development e.g. cells in tail of tadpole undergo apoptosis during frog metamorphosis

Extra-embryonic membranes

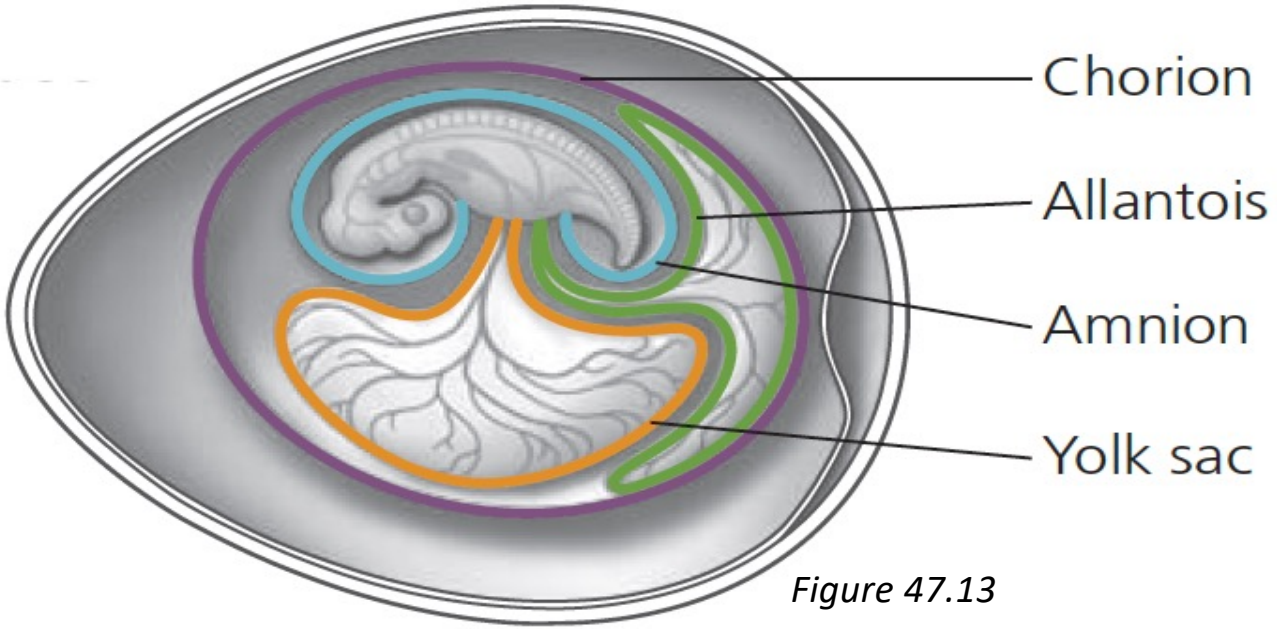


Figure 47.13

- Extra-embryonic membranes provides life support system for embryo development
- Mammals, reptiles and birds, are called **amniotes**
- Inside the shell or uterus, embryos of Reptiles and Mammals are surrounded by fluid within amnion layer.

Shaping the Vertebrate Body

Germ layers: Cell layers produced by gastrulation

Ectoderm (Outer layer of embryo)
Epidermis of skin
Nervous and sensory system
Jaws and teeth
Germ cells

Mesoderm (middle layer of embryo)
Skeletal and muscular system
Dermis of skin
Excretory and reproductive systems
Circulatory and lymphatic system

Endoderm (Inner layer of embryo)
Epithelial lining of digestive tract, respiratory tract and reproductive tract
Thymus, thyroid and parathyroid glands

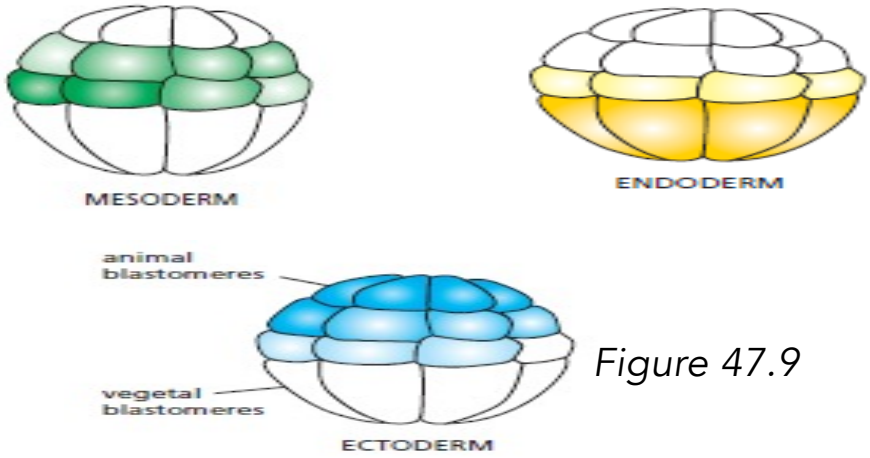
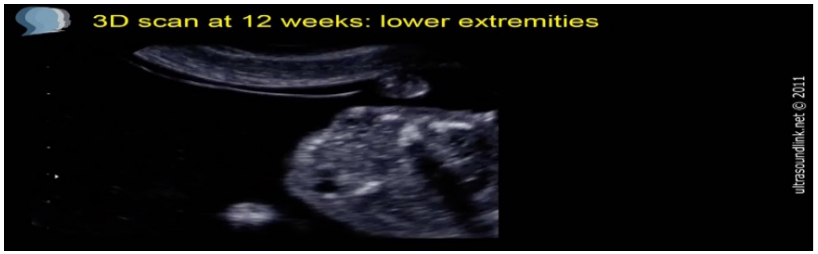
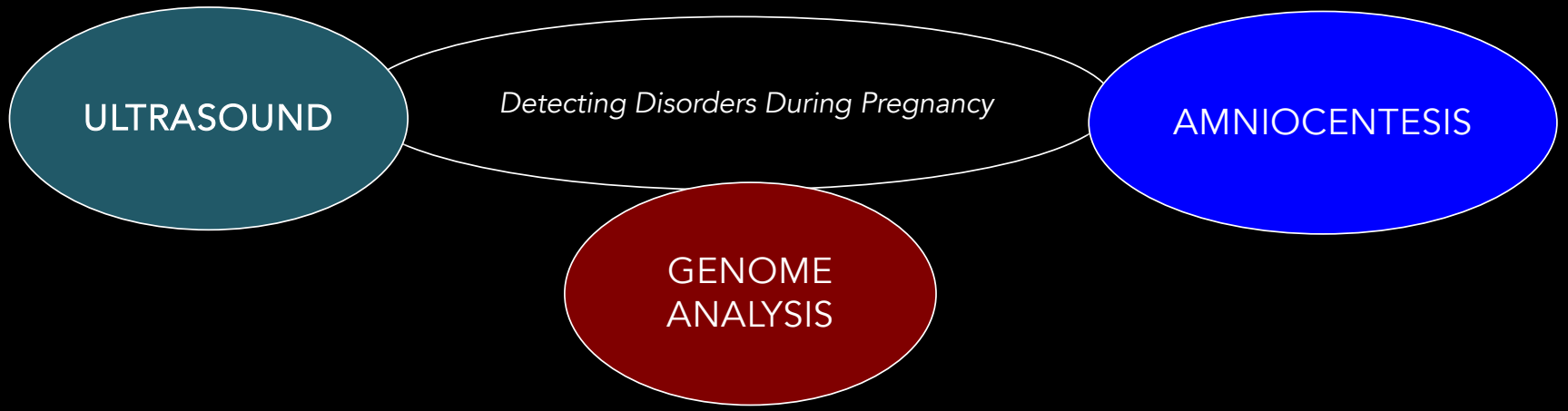


Figure 47.9



- Ultrasound imaging generates images which is used for analysis of fetus size & condition

Modern Reproductive Technologies



Chorionic Villus Sampling (CVS)

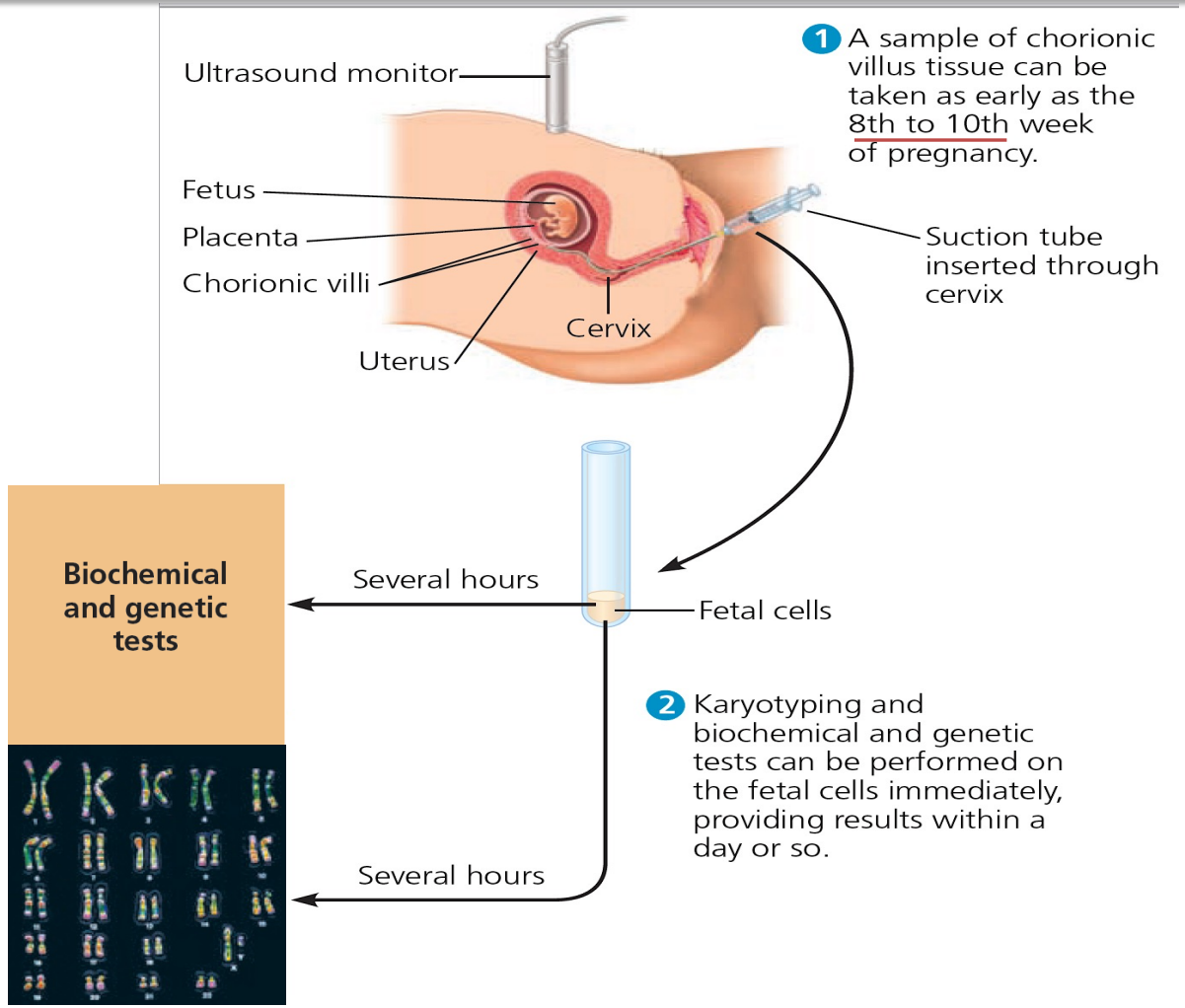


Figure 14.19

Testing a Fetus for Genetic Disorders: Amniocentesis

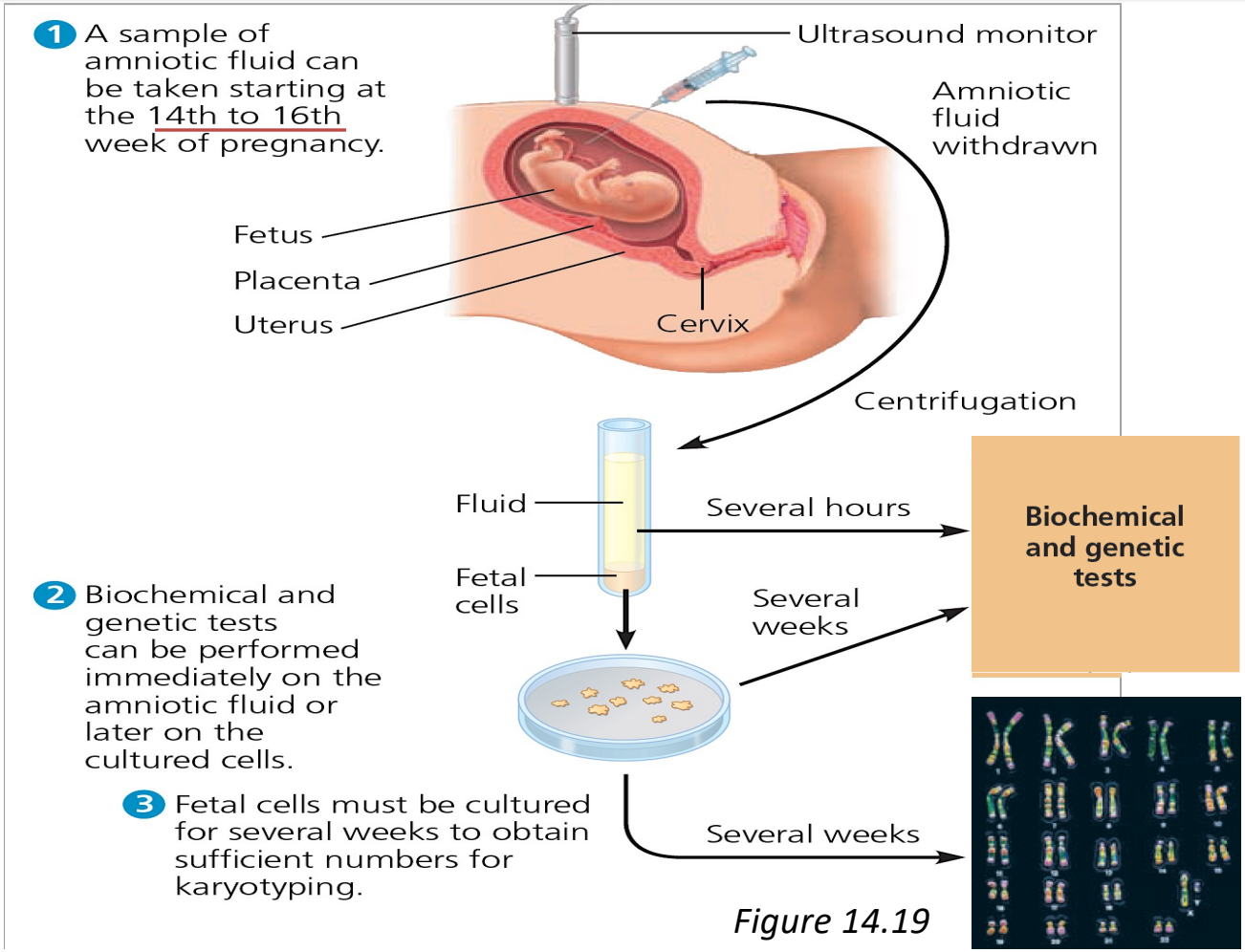


Figure 14.19

Analyzing fetus for genome analysis

- In the newest reproductive technology, a pregnant mother's blood is used to analyze the genome of her fetus
- Polymerase chain reaction (PCR) and high throughput sequencing can convert the bits of fetal DNA into useful information

Infertility and *In Vitro* Fertilization (IVF)

- IVF involves combining oocytes and sperm in laboratory
- Fertilized eggs are incubated until they have formed at least eight cells & are transferred to uterus for implantation

Figure 46.21

Hold the egg with a pipette and use a fine needle to inject one sperm into egg cytoplasm

- Development ✓
- Stem Cells
- Cell Reprogramming

Stem Cells

The major goal of cloning human embryos is not reproduction but to produce stem cells to treat human diseases

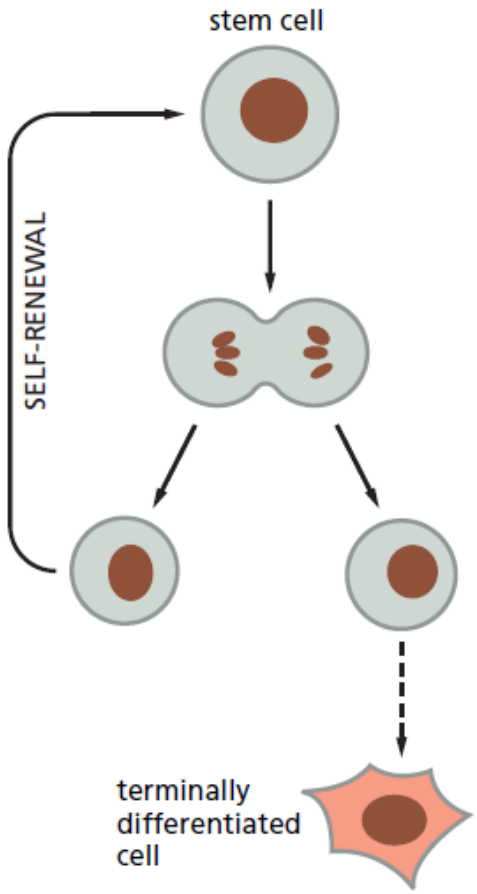
Stem Cells: cells that have not yet achieved their final fate/identity

Properties:

- Able to divide
- Capable of differentiation*
- Not terminally differentiated
- Daughter cells can remain in undifferentiated state or differentiate

Examples: Epidermal stem cells, hematopoietic stem cells (found in adults) and many embryonic cells (found in the developing embryo)

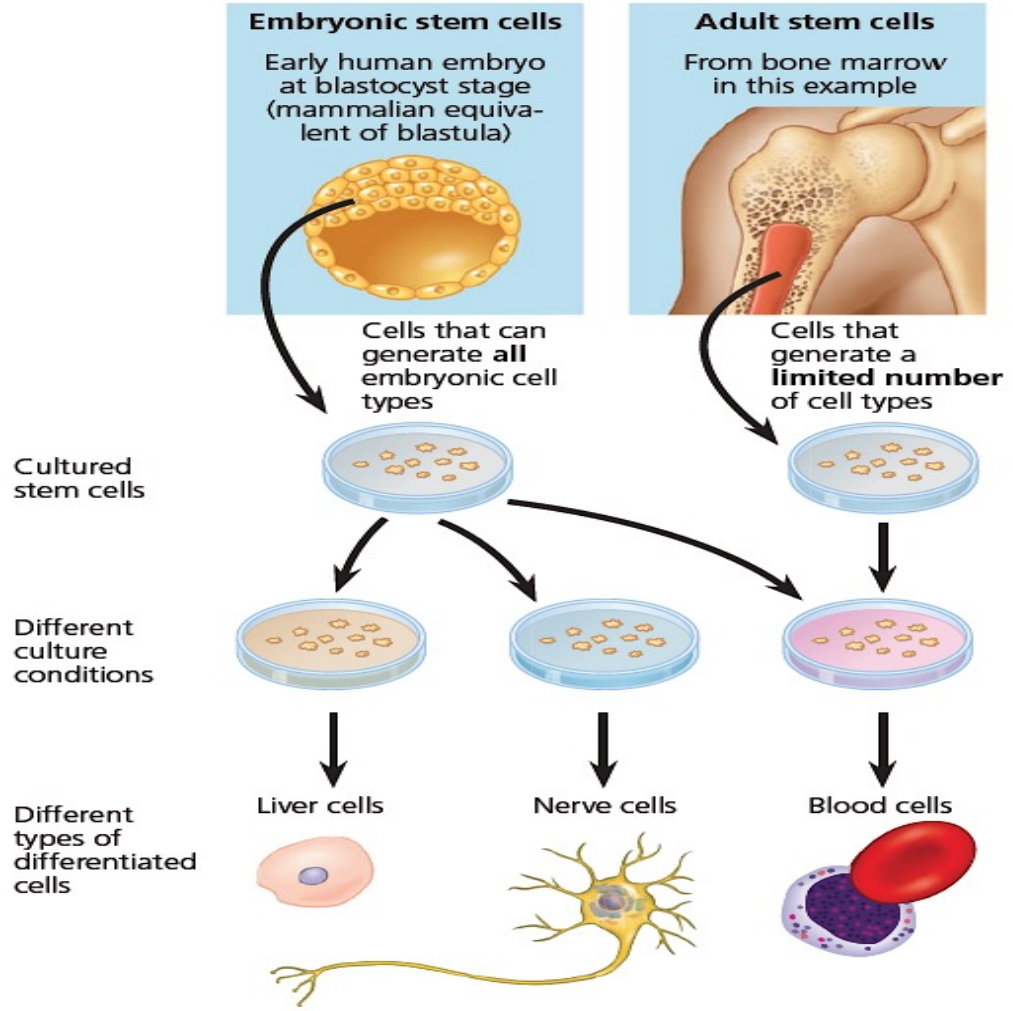
*Differentiation: expressing genes that give form and function



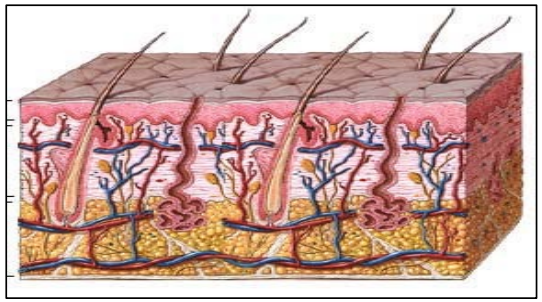
Embryonic Stem (ES) Cells

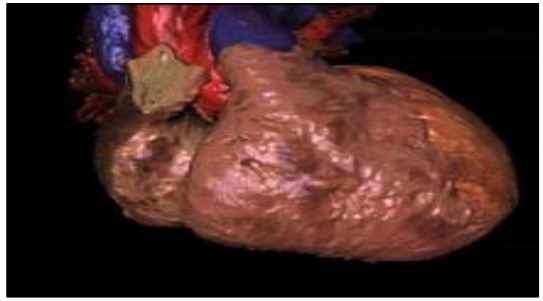
- ES cells can be derived from early human embryos and from fetal germ cells
- ES cells can be induced to differentiate into a wide variety of cell types in culture, by treatment with combinations of signal proteins and growth factors
- Conversion of somatic cell to ES cell by manipulating gene expression

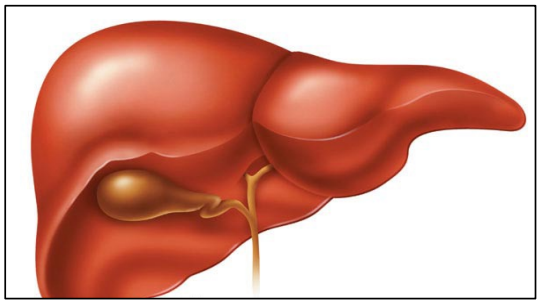
Each type of specialized cell has a memory of its developmental history and seems fixed in its specialized fate.

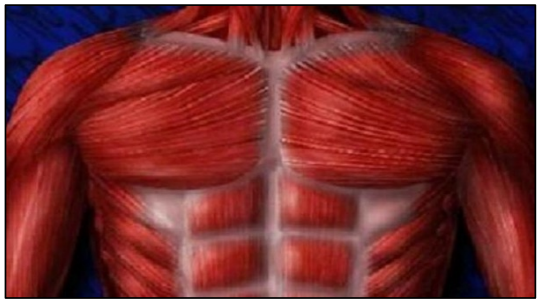


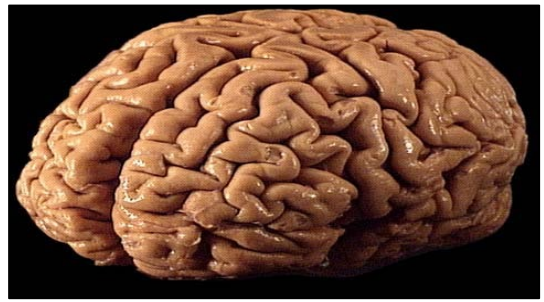
Stem Cell Engineering











Maximum regeneration

Moderate regeneration

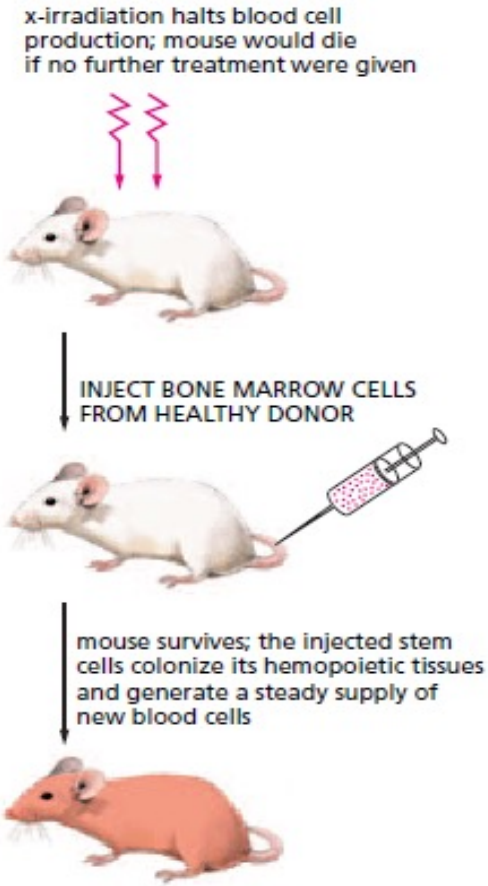
Minimal regeneration

Experiments showed that stem cells can be transplanted

- X-ray irradiated mouse can be saved by transfusion of cells taken from the bone marrow of a healthy, immunologically compatible donor

- Stem cell population in bone marrow is low (~ 1 in 10000)

- Hematopoietic stem cells can be isolated from bone marrow using Fluorescence Activated Cell Sorter (FACS)



How can Nathaniel's umbilical cord cells be used to cure his brother Nicolas?

What information is relevant?

- Umbilical cord cells have sub-populations of ES cells
- They can be enriched and differentiated into many different cell types
- T-cell lymphoma is a cancer of immune cells (T-cells)

- Strategy: remove all cancerous T-cells (chemotherapy and radiation) followed by repopulating the body non-cancerous T-cells derived from ES cells

How are Nicolas and Nathaniel "blood brothers"?

(1) Treat Nicolas to destroy his bone marrow cells (chemotherapy and radiation therapy)

Nicolas

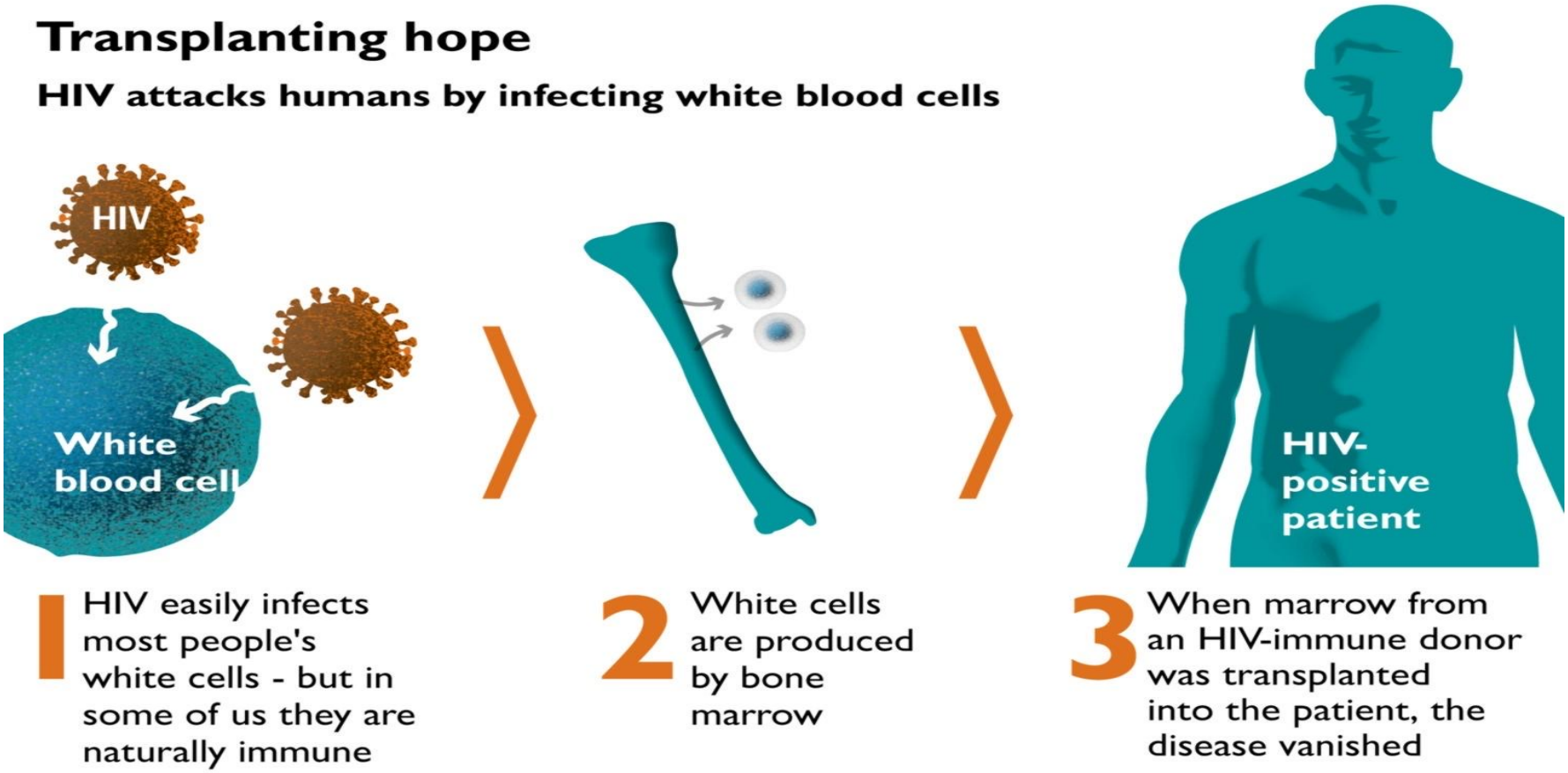
(3) Transfer Nathaniel's ES cells into Nicolas and they will repopulate the bone marrow

(2) Collect embryonic stem cells (hematopoietic cells) from Nathaniel's umbilical cord blood

Nathaniel

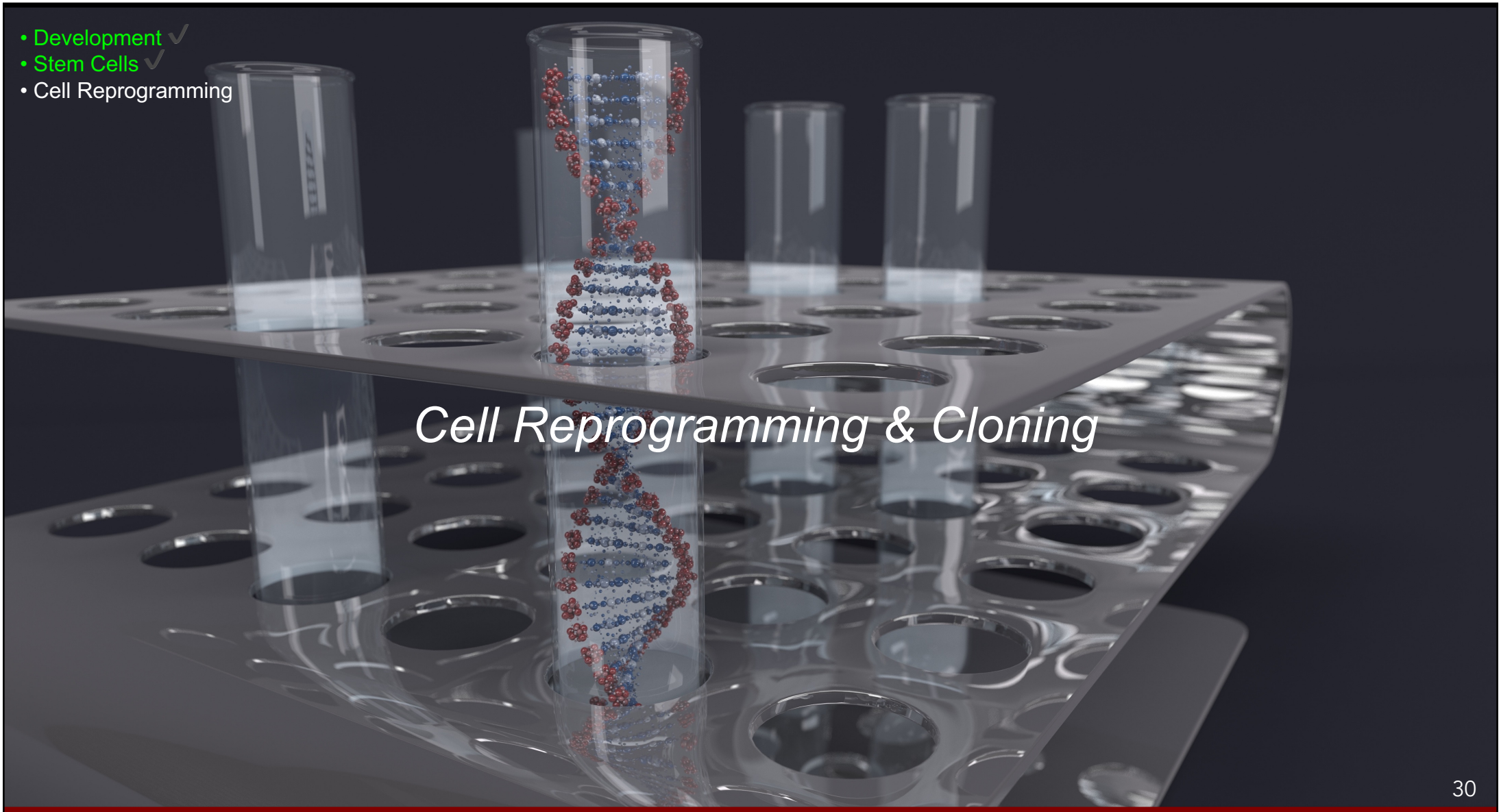
https://www.lls.org/treatment/types-of-treatment/stem-cell transplantation/allogeneic-stem-cell-transplantation

Cure of an HIV patient using this technology



https://www.thetimes.co.uk/article/yes-i-cured-a-man-of-hiv-but-now-i-need-to-pick-up-my-daughter-from-school-qlmxh5r70

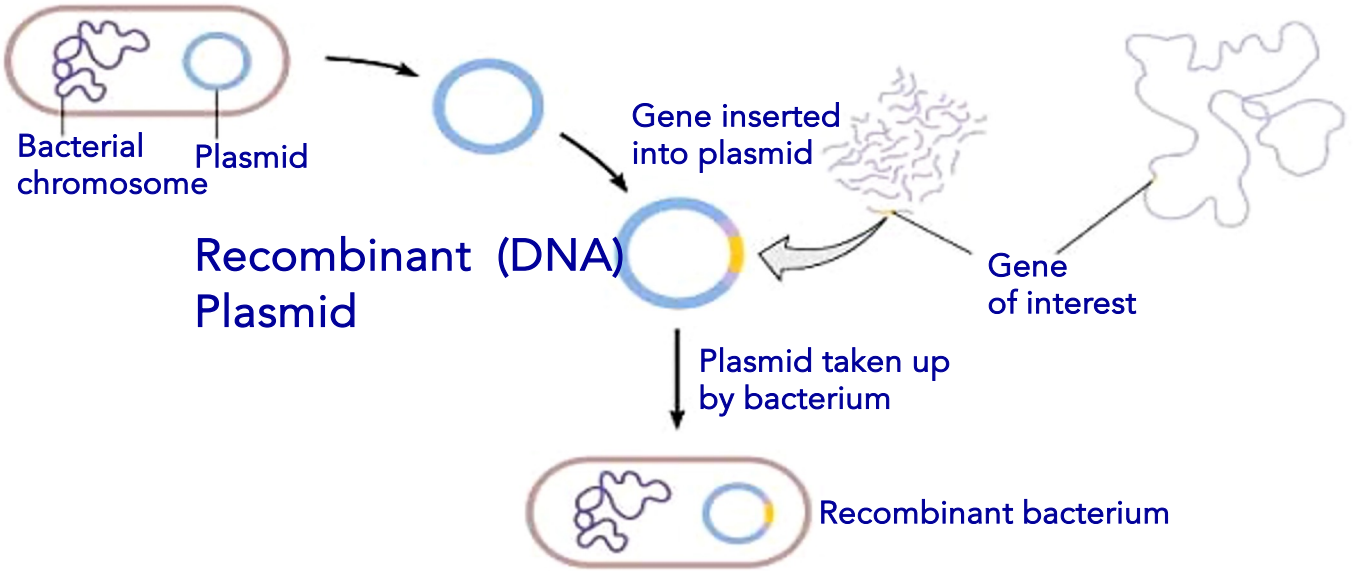
- Development ✓
- Stem Cells ✓
- Cell Reprogramming



Cell Reprogramming & Cloning

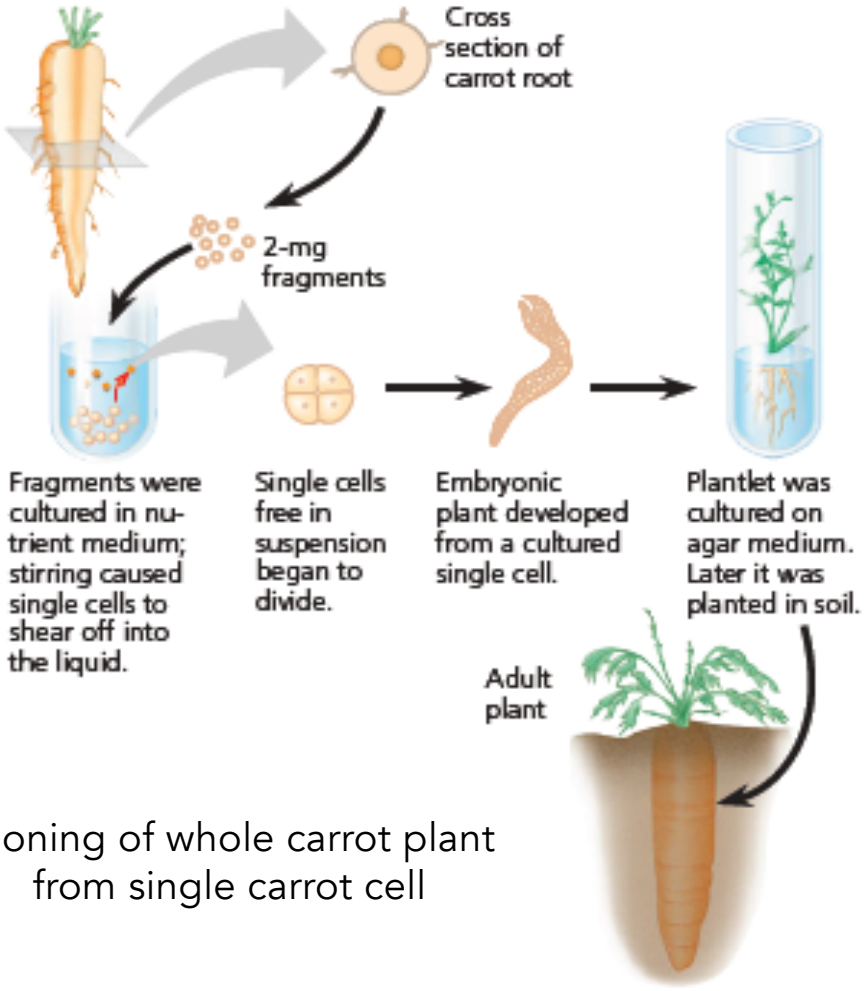
Gene Cloning

- Construction of recombinant DNA molecule
- Introduction into a host cell
- Selection using antibiotics



Cloning of Plants

- Cloning plants using root cells
- Grow root cells in culture
- Cells dedifferentiate and form callus
- Stimulation of callus with plant hormones



Cloning of whole carrot plant from single carrot cell

Cloning

- Cloning: "producing a cell or organism with the same nuclear genome as another cell or organism" (Human Genetics Advisory Commission)
- Clone: a genetic duplicate of a person or creature

Sir John B. Gurdon

- *Hypothesis:*

  Specialized cell genome might contain information needed for differential development of organism
- *Experiment:*
  - Replaced cell nucleus of frog egg with that of mature, specialized cell
  - Subsequently, egg developed into fully functional tadpole

Gurdon, J.B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. Journal of Embryology and Experimental Morphology 10:622-640.

Can Nucleus from a Differentiated Animal Cell direct Development of an Organism?

- Gurdon destroyed nuclei of frog (*Xenopus laevis*) eggs by exposing eggs to UV light. Transplanted nuclei from cells of frog embryos & tadpoles into enucleated eggs

- Results:

(a) when transplanted nuclei came from an early embryo, most recipient eggs developed into tadpoles

(b) when nuclei came from fully differentiated intestinal cells of a tadpole < 2% eggs developed into normal tadpoles

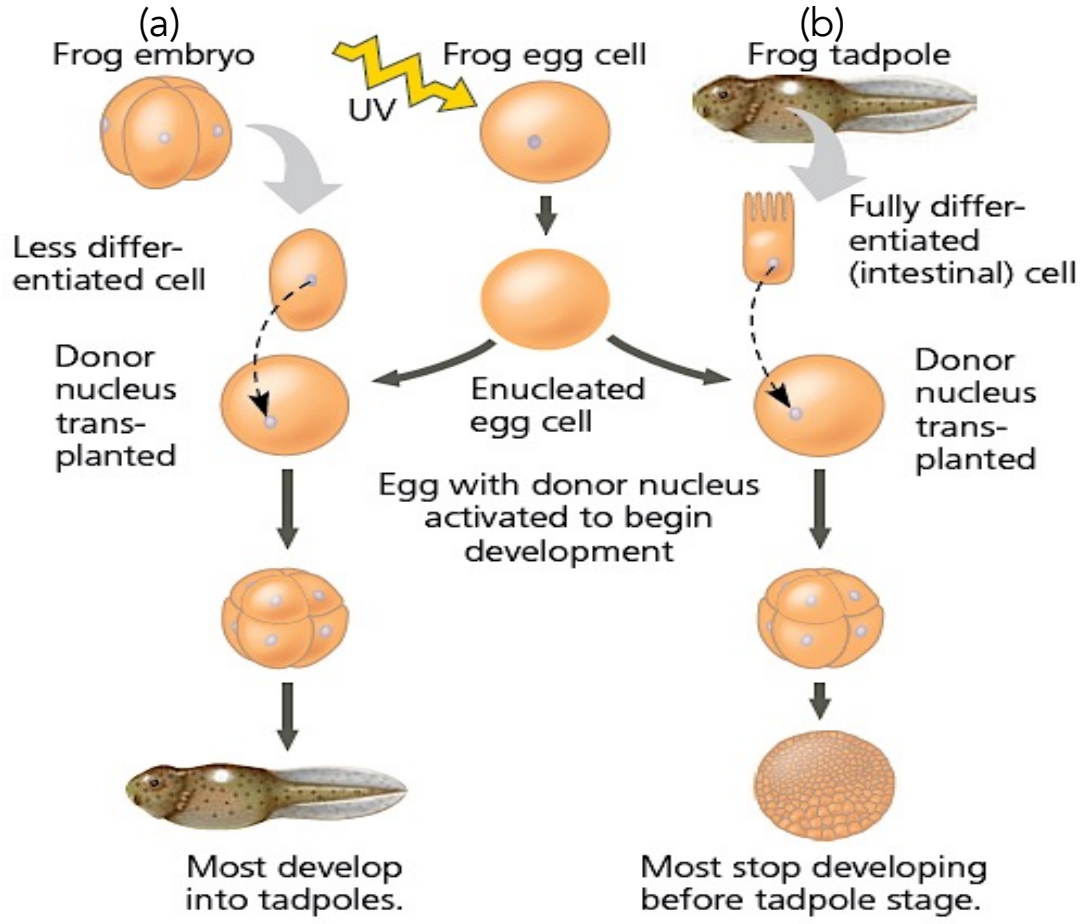
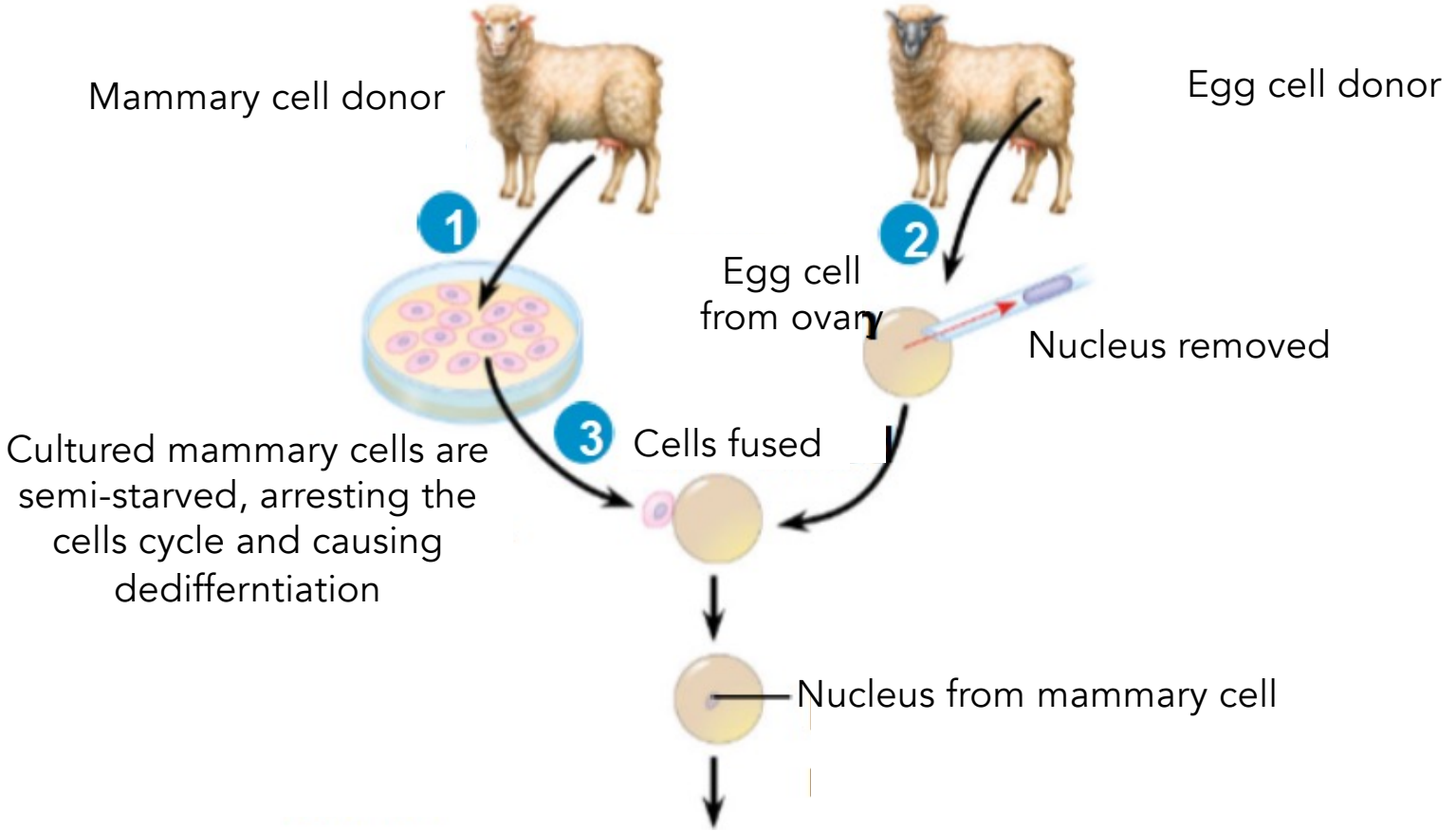


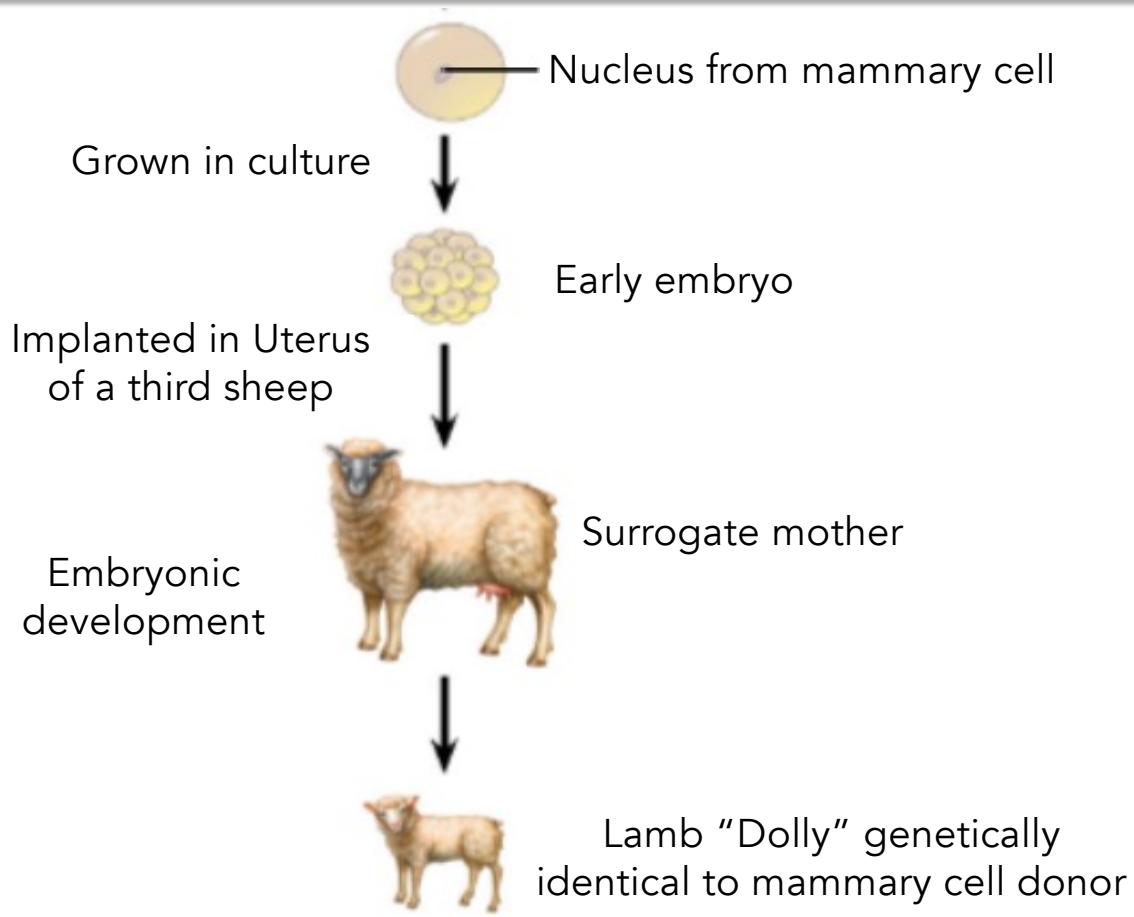
Figure 20.16

Cloning Animals



- 1997 Dr. Ian Wilmut cloned a sheep "Dolly"

Cloning Animals (2)



- First large cloned animal from somatic cells

Dr. Shinya Yamanaka

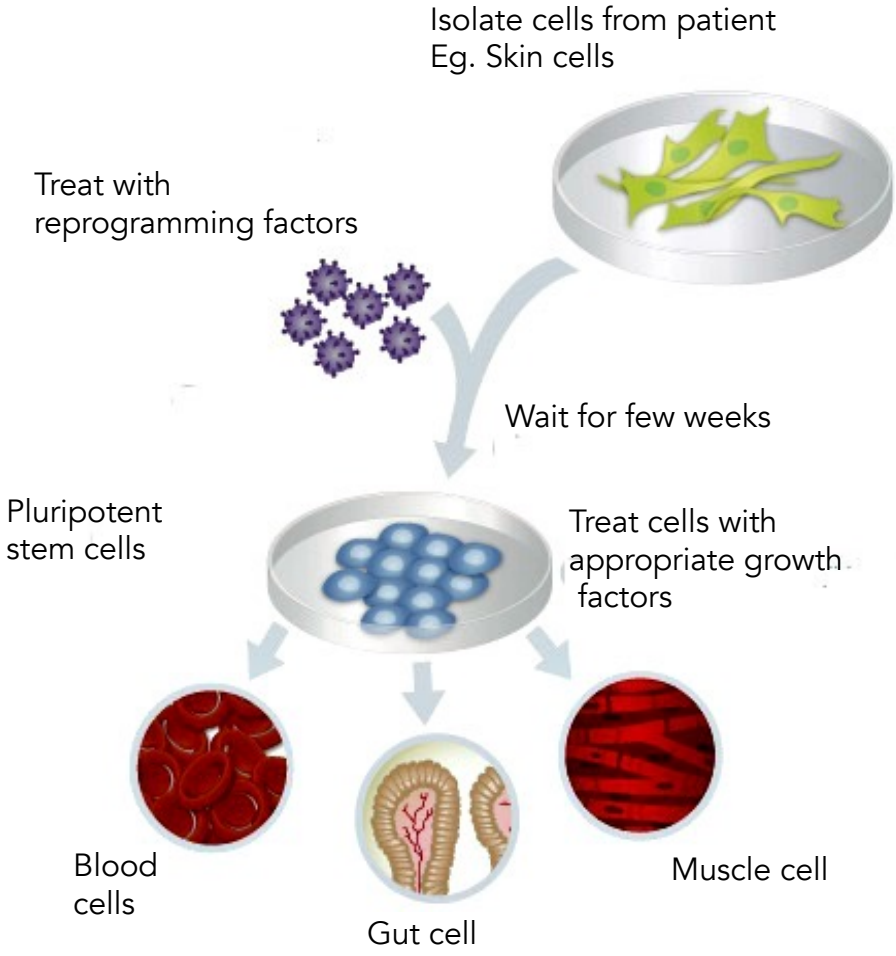
- *Hypothesis:*

  Inducing large number of transcription factors involved in pluripotency can revert a mature differentiated cell
- *Experiment:*
  - Demonstrated induction of pluripotent stem cells from mouse embryonic or adult fibroblasts

Takahashi, K., Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663-676.

Induced Pluripotent Stem Cells (iPS)

- These are generated from adult cells
- Shinya Yamanaka: Introduction of 4 specific genes converted adult cells to stem cells
- Patient match as they are derived from autologous sources



Cloning Star "Pride of Korea"

Dr. Hwang Woo-Suk

South Korean researcher
Professor at Seoul National University

Evidence of a Pluripotent Human Embryonic Stem Cell Line Derived from a Cloned Blastocyst

Woo Suk Hwang,[1,2*] Young June Ryu,[1] Jong Hyuk Park,[3] Eul Soon Park,[1] Eu Gene Lee,[1] Ja Min Koo,[4] Hyun Yong Jeon,[1] Byeong Chun Lee,[1] Sung Keun Kang,[1] Sun Jong Kim,[3] Curie Ahn,[5] Jung Hye Hwang,[6] Ky Young Park,[7] Jose B. Cibelli,[8] Shin Yong Moon[5*]

www.sciencemag.org SCIENCE VOL 303 12 MARCH 2004

- This study showed the feasibility of generating human ES cells from a somatic cell isolated from a living person

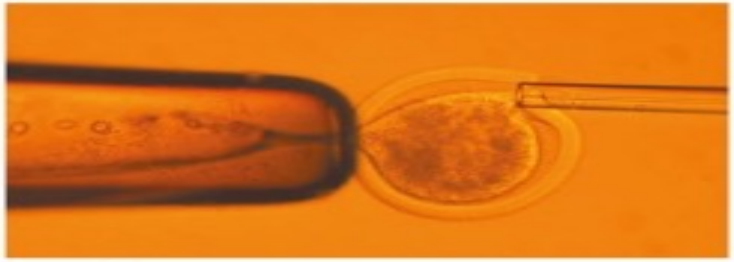
Patient-Specific Embryonic Stem Cells Derived from Human SCNT Blastocysts

Woo Suk Hwang,[1,2*] Sung Il Roh,[3] Byeong Chun Lee,[1] Sung Keun Kang,[1] Dae Kee Kwon,[1] Sue Kim,[1] Sun Jong Kim,[3] Sun Woo Park,[1] Hee Sun Kwon,[1] Chang Kyu Lee,[2] Jung Bok Lee,[3] Jin Mee Kim,[3] Curie Ahn,[4] Sun Ha Paek,[4] Sang Sik Chang,[5] Jung Jin Koo,[5] Hyun Soo Yoon,[6] Jung Hye Hwang,[6] Youn Young Hwang,[6] Ye Soo Park,[6] Sun Kyung Oh,[4] Hee Sun Kim,[4] Jong Hyuk Park,[7] Shin Yong Moon,[4] Gerald Schatten[7*]

www.sciencemag.org SCIENCE VOL 308 17 JUNE 2005

- Patient-specific, immune-matched human embryonic stem cells (hESCs) established by somatic cell nuclear transfer

The Rise and Fall of Hwang Woo-Suk



FEBRUARY 2004
Woo Suk Hwang describes the first stem-cell line, NT-1, derived from a cloned human embryo.

MAY 2005
Hwang's group publishes a second paper reporting 11 further human embryonic cell lines.

AUGUST 2005
Hwang's group is the first to clone a dog.

NOVEMBER 2005
US collaborator Gerald Schatten splits with Hwang, citing ethical problems in getting human eggs.

DECEMBER 2005
Pushed by increasing evidence, Seoul National University (SNU) launches an investigation.

JANUARY 2006
Hwang's human-cloning research is deemed fraudulent by SNU. His dog-cloning claims are upheld.

JULY 2006
Sooam Foundation starts up, with US$3.5 million from Hwang's supporters.

2007
The Korean health ministry grants Sooam the right to do human-embryo and cloning research.

OCTOBER 2009
Hwang is found guilty of embezzlement and bioethics violations. Appeal continues.

2011
Canada grants Hwang a patent for the NT-1 cell line.

2012
Sooam scientists clone a coyote using a dog egg-cell donor and surrogate mother.

2013
Court tells the Korean Centers for Disease Control and Prevention to register the NT-1 cell line.

Dr. Haruko Obokata

ARTICLE

doi:10.1038/nature12968

Stimulus-triggered fate conversion of somatic cells into pluripotency

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LETTER

doi:10.1038/nature12969

Bidirectional developmental potential in reprogrammed cells with acquired pluripotency

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Cell Reprogramming: Rise & Fall of Stars

Sir John B. Gurdon

Dr. Shinya Yamanaka

Nobel laureates

Dr. Haruko Obokata

Dr. Hwang Woo-Suk

Fraudulent research

Summary

- Fertilization and cleavage initiate embryonic development
- Modern reproductive technologies help in detecting disorders during pregnancy
- Stem cell engineering: promising as it is capable of giving rise to differentiated embryonic cells of any type
- Common theme for all cloning: product is identical to parent
- The biggest landmark studies in stem cell research resulted into biggest prize as well as biggest scientific scandals (discussion for "*ethics in research and publications*")

References

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- Wilmut, I., et al., Nature, 385: 264-267 (1997)
- *Acknowledgment*
  - Cover images – getty images


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Animal Development

KEY CONCEPTS

47.1 Fertilization and cleavage initiate embryonic development

47.2 Morphogenesis in animals involves specific changes in cell shape, position, and survival

47.3 Cytoplasmic determinants and inductive signals contribute to cell fate specification

▲ Figure 47.1 How did a single cell develop into this intricately detailed embryo?

A Body-Building Plan

The 7-week-old human embryo in Figure 47.1 has already achieved a remarkable number of milestones in its development. Its heart—the red spot in the center—is beating, and a digestive tract traverses the length of its body. Its brain is forming (at the upper left in the photo), while the blocks of tissue that will give rise to the vertebrae are lined up along its back.

By combining molecular genetics with classical embryology, developmental biologists have learned a great deal about the transformation of a fertilized egg into an adult. Examining embryos from a range of species, such as the chick embryo shown at the left, biologists have long noted common features of early stages, as evident by comparing the human and chick embryos shown on this page. More recently, researchers have demonstrated that specific patterns of gene expression in a developing embryo direct cells to adopt distinct fates. Furthermore, even animals that display widely differing body plans share many basic mechanisms of development and often use a common set of regulatory genes. For example, the gene that specifies heart location in a human embryo (such as the one in Figure 47.1) has a close counterpart with a nearly identical function in the fruit fly *Drosophila melanogaster*. Researchers dubbed the fly gene *tinman*. Why? Embryos in which this gene is defective lack a heart, much like the Tin Man in *The Wizard of Oz*.

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Next Lecture…

Proteins