7.016 Recitation 22 – Fall 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

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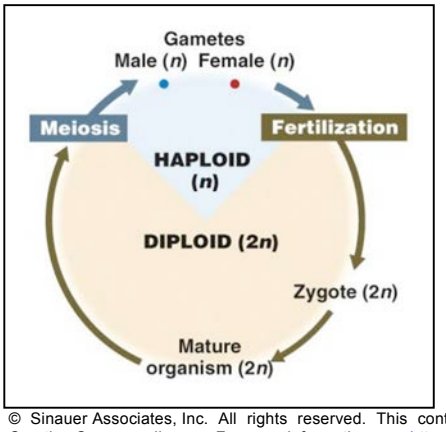
Summary of Lectures 34 (12/7) and 35 (12/10):

Human immunodeficiency virus (HIV): is a retrovirus that infects the T_H cells of the immune system. HIV gets into the T_H cells by docking onto the CD4 protein on the surface of T_H cells. CD4 protein normally helps T_H cells recognize the MHC class II molecules on the surface of antigen presenting cells (macrophages, dendritic cells and B cells). However, the HIV virus has evolved to have a glycoprotein (gp120) on its surface that binds to CD4, which targets HIV to T_H cells. This glycoprotein also has the ability to promote the fusion of the lipid bilayer of HIV with the cell membrane of T_H cells. This allows the virus to release its contents into the T_H cells. The HIV virus harms the T_H cells, thereby depleting the immune system and the ability to fight this virus or any other opportunistic infections.

HIV mutates rapidly due to its error prone reverse transcriptase that it uses to replicate its genome. This allows HIV to frequently change its amino acid makeup and subsequently the structure and shape of its viral proteins so that our immune system cannot gain immunity to the HIV.

Circle of life, fate and potency: Development is the process of forming a multicellular organism from a single fertilized egg (2n) through the process of Mitosis. As an adult, the organism produces gametes through meiosis, which fuse to form a zygote that develops into a newborn, which represents the next generation.



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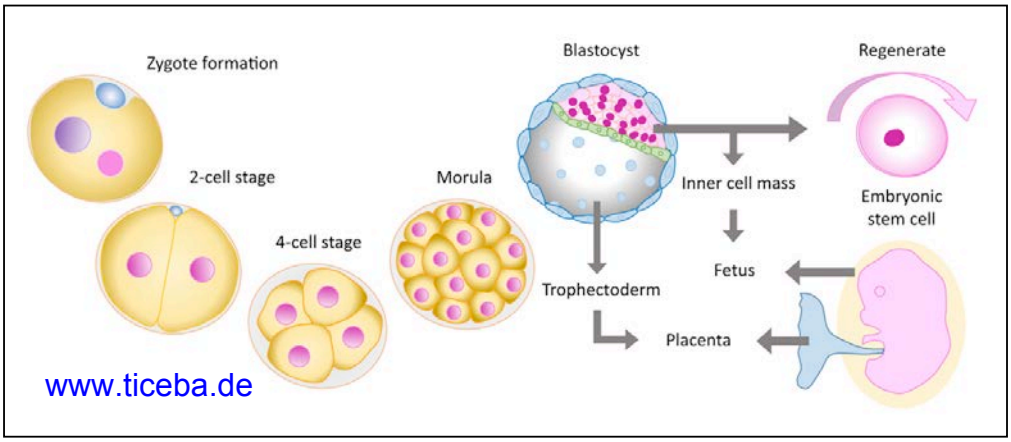
Development involves a balance between cell division and cell death, formation of cell types through cell differentiation, positioning different cell types at the correct locations, and organizing cells into the correct 3D- shapes to form tissues, organ, systems and hence the whole organism.

The functionally specialized or differentiated form of a cell is called its "fate". Cells make stepwise decisions to assume their fate. Undecided cells are termed "uncommitted" or "undetermined". These cells become determined or committed, once they have decided their fate, but have not yet assumed it. Subsequently, cells differentiate to assume their final fate and function in the body.

The number of differentiated cell types originating from a stem cell defines its potency. Potency decreases with developmental time.

Cell	Definition
Totipotent	Cell that can form ALL cell types including the placenta. The zygote (i.e. fertilized egg) is a totipotent cell
Pluripotent	A cell that can form all the cell types of the embryo proper but not all the cells of placenta. Embryonic stem cells, induced pluripotent stem cells
Multipotent	A cell that can form multiple cell types but NOT all cell types i.e. hematopoetic stem cells (HSC, which form all the blood cell types and cells of immune system, intestinal stem cells, which form all the cells of the intestine. Most adult stem cells are considered multipotent.
Unipotent	A cell that can form only one differentiated cell type
Differentiated	A cell that has attained its final fate i.e. neuron, muscle, B cells and many others

Embryonic Stem cells (ES) and induced pluripotent stem cells (iPS): ES cells are pluripotent stem cells that can be generated from the cells from the inner cell mass (ICM) of an early embryo. The ICM cells when cultured with appropriate nutrients can result in the formation of ES **cell lines,** which are pluripotent; they can be coaxed with proper signaling molecules to give rise to any cell type present in our body.



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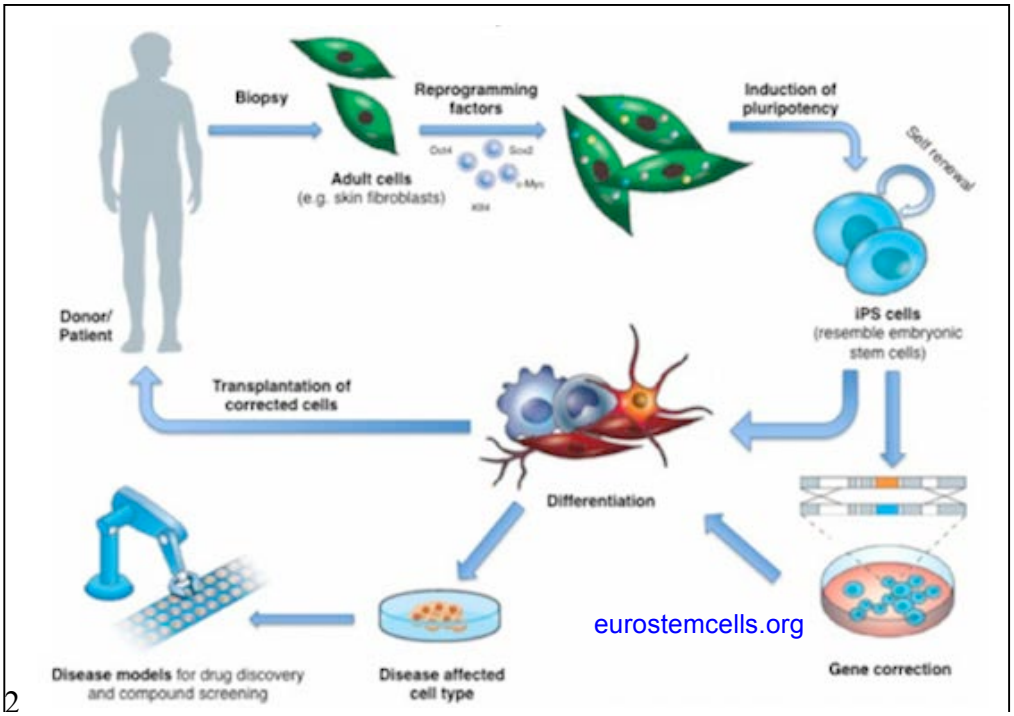
To establish the ES cell line you start with an early embryo. Because these cell lines are not autologous (from self) there is a chance that they can be rejected, if transplanted into an individual. In order to avoid rejection one could make a clonal embryo from the nucleus of a patient's somatic cells (.somatic cell nuclear transfer). ES cells can be obtained from the resulting embryo and reprogrammed for use in the patient. For human embryos, this raises ethical concerns, because it involved making as embryo that in theory, is capable of generating a human, if it was implanted into a mother.

Another possible way of creating compatible tissue is by using the **iPS cells**. Here you start with an adult differentiated cell (such as cell from the patient's body having the same MHC-I to prevent the chance of immunological rejection) and introduce 3-4 genes that encode specific transcription factors (*sox2, klf4, myc, oct4*) and then culture them in the presence of nutrients to generate pluripotent autologous cell lines. These could then be differentiated (*in vitro*) into cell types that would be used for organ repair or study different diseases.



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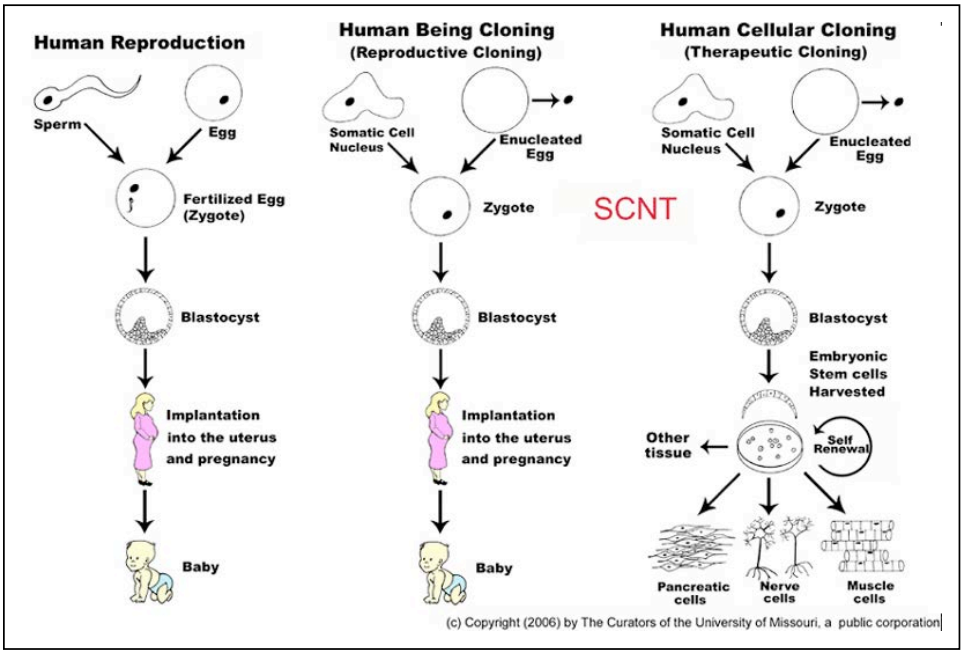
Somatic cell nuclear transfer (SCNT): Here the idea is similar to "cloning of a gene into a plasmid" as you observed in your recombinant DNA lectures. However, here it applies to the whole organism i.e. you try to make a replica of yourself with the same genetic makeup as yours. This may be of significance in agriculture and may have therapeutic implications. Note that this is not a way to replace a lost relative or a pet, because nothing can reproduce the experiences and memories we have growing up, which influence our behavior (i.e. nature versus nurture).



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Here you take the nucleus from a somatic cell and implant it into an enucleated egg so that now the ploidy of the egg is 2n. You let it develop into an embryo, you implant the embryo into a surrogate mother so that it develops into a newborn (**reproductive cloning**) or you grow the embryonic cells in cell culture plates under appropriate conditions to establish ES cell lines (**therapeutic cloning**).

The cloning efficiency of SCNT is very low i.e. 0.001% with adult somatic cell nucleus and 10% with ESC or iPS. Furthermore the newborn can have many physiological problems since it is very difficult to completely reprogram the genetic and epigenetic information.

Interesting links:

- Dolly the sheep: http://novaonline.nvcc.edu/eli/evans/HIS135/events/dolly96/Dolly_Module.html
- First gene-edited baby: https://www.theatlantic.com/science/archive/2018/11/first-gene-edited-babies-have-allegedly-been-born-in-china/576661/
- Ethical and policy issues of human cloning: http://science.sciencemag.org/content/277/5323/195.full
- Three parents baby explained: https://www.sciencenews.org/article/three-parent-babies-explained

Questions

1. Combination therapy is the new treatment strategy for treating HIV patients.

a) Why combination therapy is more successful in preventing the emergence of the disease resistant clones?

b) If the combination therapy involves a drug that prevents…

- HIV binding to the target cell, what protein does this target? _____
- Integration of HIV genome into host cell genome, what protein does this target?

  _____
- Prevents the cleaving of viral polypeptide into individual proteins, what protein does this target?

  _____

2. Draw a graph that shows the correlation between cell potency and time during the development of an organism starting from the zygote or 1-cell stage.

3. Any somatic cell can be used for SCNT. What does this tell you about the genome of somatic cells??

4. You decide to clone animals by somatic cell nuclear transfer (SCNT) by transferring the nucleus from a mature B cell. Would this change the B cell receptor diversity and the functioning of the immune system?

Solution key

1. Combination therapy is the new treatment strategy for treating HIV patients.

a) Why combination therapy is more successful in preventing the emergence of the disease resistant clones?

Multiple separate mutations in different viral components are far less likely to occur than the single mutations thus reducing the chances of generation of drug resistant strains of HIV.

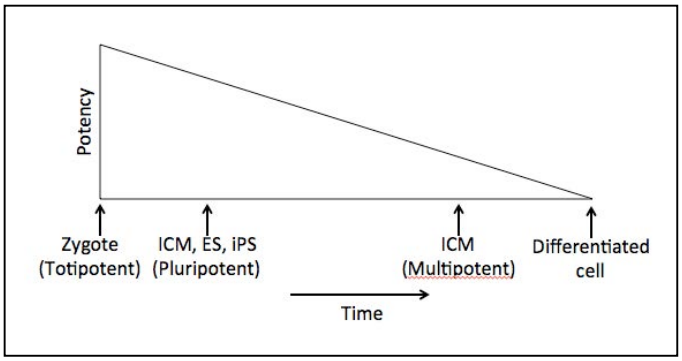
b) If the combination therapy involves a drug that prevents…

- HIV binding to the target cell, what protein does this target? *GP120 and / or GP41 and CCR5 co-receptor on target cell*
- Integration of HIV genome into host cell genome, what protein does this target?

  Reverse transcriptase or integrase
- Prevents the cleaving of viral polypeptide into individual proteins, what protein does this target?

  Viral protease

2. Draw a graph that shows the correlation between cell potency and time during the development of an organism starting from the zygote or 1-cell stage.



3. Any somatic cell can be used for SCNT. What does this tell you about the genome of somatic cells?

It shows that all the cells have the genomic information that is necessary to form an entire organism.

4. You decide to clone animals by somatic cell nuclear transfer (SCNT) by transferring the nucleus from a mature B cell. Would this change the B cell receptor diversity and the functioning of the immune system?

Yes, since the heavy (H) and the light (L) polypeptide chain genes of the antibody are already rearranged in the mature B cells due to VDJ gene segment recombination. So this clone can produce only one type of antibody receptor or B cell receptor specific to one antigen. This clone will be immuno-compromised.

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