

**BIO380HF—HUMAN DEVELOPMENT**  
**University of Toronto Mississauga**  
**First Midterm Test— September 21, 2011**  
Professor Danton H. O'Day

**Test Length: 50min.; 100 Marks Total;** No aids allowed.

Answer all questions as asked providing the best or most appropriate answer in each case. Point form and diagrams can be used where appropriate but they must be organized and clear. Marks are deducted or not given for misspelled key words or unclear and contradictory points and information.

**1. Define the Following Terms (4 marks each; 32 marks total)**

**a. Non-Disjunction:** Chromosomes don't pull apart so one cell gets both sister chromatids (that each become a chromosome) while the other cell doesn't get that chromosome. Non-disjunction can occur during either meiosis I or meiosis II as illustrated in the two following graphics.

**b. Apoptosis:** is the formal name for the controlled regulation of cell death. It involves the activation of specific genes and signal transduction pathways that underlie the cell death program. Unlike tissue necrosis caused by external damage, it is a controlled process in which cells show a precise breakdown in a series of well-defined steps that are under active study. Apoptosis allows the body to remove specific cells without damaging surrounding cells and tissues.

**c. Immunohistochemistry:**

-Embryos (normal, mutant, exposed to teratogens, etc.) are fixed, sectioned (if necessary) and probed with a primary antibody against a specific protein after which the bound antibody is visualized by binding to a secondary antibody. The secondary antibody is bound to a marker (e.g., horse radish peroxidase) that can be detected by specific methods (e.g., a colour reaction).

**d. In Situ Hybridization:**

-Messenger RNA in embryos can be detected by binding to complementary RNA molecules (also called riboprobes). The riboprobe has typically been labelled with a marker such as digoxigenin (DIG) that can be detected by anti-DIG using immunohistochemistry. In the past radioactive riboprobes were common but are used less frequently as safer, more sensitive probes are being developed.

**e. Knock-down experiments:**

-embryonic cells can be injected with morpholinos (stabilized mRNAs) or RNAi (interfering double-stranded RNA) which decrease gene expression rather than completely blocking it. The genetically manipulated embryos are then studied using the diversity of techniques open to cell and developmental biologists.

**f. Globospermia:**

Globozoospermia is a rare (incidence <0.1% in male infertile patients) form (teratozoospermia) resulting from abnormal spermiogenesis. It is mainly characterized by round-headed spermatozoa that lack an acrosome. Since the sperm lack acrosomal membranes and acrosin contents, they are unable either to penetrate the zona pellucida of an oocyte or to fuse with the egg cell membrane (oolemma).

**g. Ionophore oocyte activation:**

Calcium ionophore (e.g., A23187) treatment of oocytes helps in activating them during in vitro fertilization. Replaces a factor that normally regulates the flux of calcium ions allowing calcium ions to flow into the egg.

**h. Teratoma:**

Cancerous masses containing differentiated cells that are in a disorganized state

Teratomas look like tiny disorganized embryos

They arise due to PGCs getting "lost" in non-gonadal sites

Because of their "totipotent" nature, PGCs can differentiate into diverse cell/tissue types (hair, skin, cartilage, teeth, etc.)

**2. What is the difference between a descriptive and experimental embryology? (8 marks).**

**Descriptive embryology:**

- Understanding the normal and abnormal events that happen during embryological events through direct study of the embryos without experimental intervention
- Comes about through observation using confocal, light and phase contrast microscopy of living, fixed and stained tissues, electron microscopy for fine structural details.

**Experimental embryology:**

- Insight into normal and abnormal events that happen during embryological events through experimental intervention of embryos
- Use of removal and transplantation of tissues; also marking of embryos with dyes, genetic markers and seeing where they go after various experimental interventions.

**3. What is a cell marker? Describe how specific cell markers have been used to follow a cell type during embryogenesis. (8 marks)**

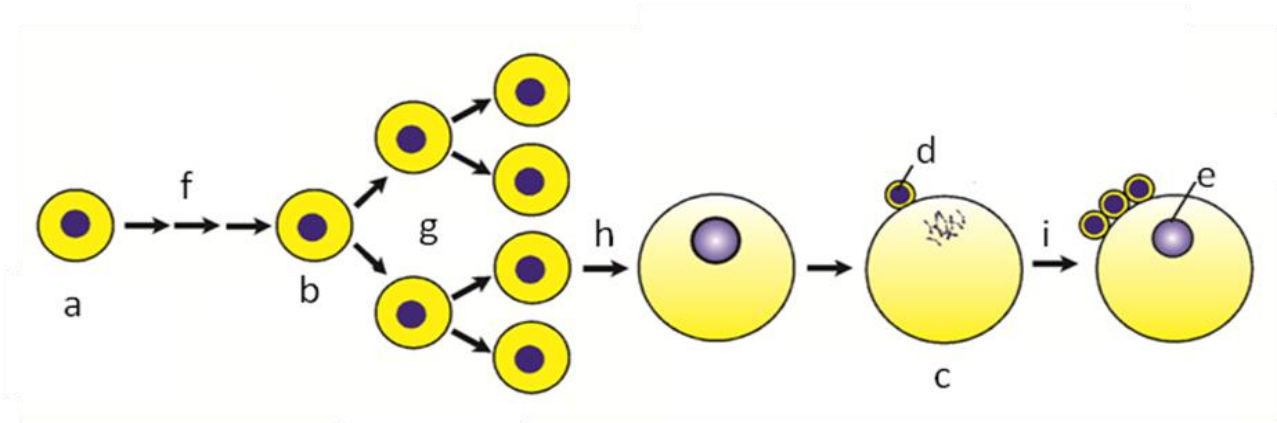
A cell marker is a specific attribute of a cell that lets you find it among a group of other cells. Alkaline phosphatase is a germ cell "marker enzyme" for PGCs

Early work using alkaline phosphatase staining was used to reveal the pattern of germ cell migration in various mammals including humans.

More recently cellular labeling (e.g., GFP; green fluorescent protein linked to a germ cell specific protein) Use of staining with monoclonal antibodies directed against germ cells has added further insight into this subject.

For example, Stromal cell derived factor-1 (SDF-1) and its chemokine receptor CXCR-4 are essential for germ cell migration.

**4. Provide the labels for the following figures and answer the questions about them. (1 mark each; 12 marks total)**



**Name the cells and structures indicated in the above picture:**

- a. \_\_\_\_\_ primordial germ cell \_\_\_\_\_
- b. \_\_\_\_\_ oogonium \_\_\_\_\_
- c. \_\_\_\_\_ secondary oocyte \_\_\_\_\_
- d. \_\_\_\_\_ polar body \_\_\_\_\_
- e. \_\_\_\_\_ germinal vesicle \_\_\_\_\_

**Name the events indicated in the above picture:**

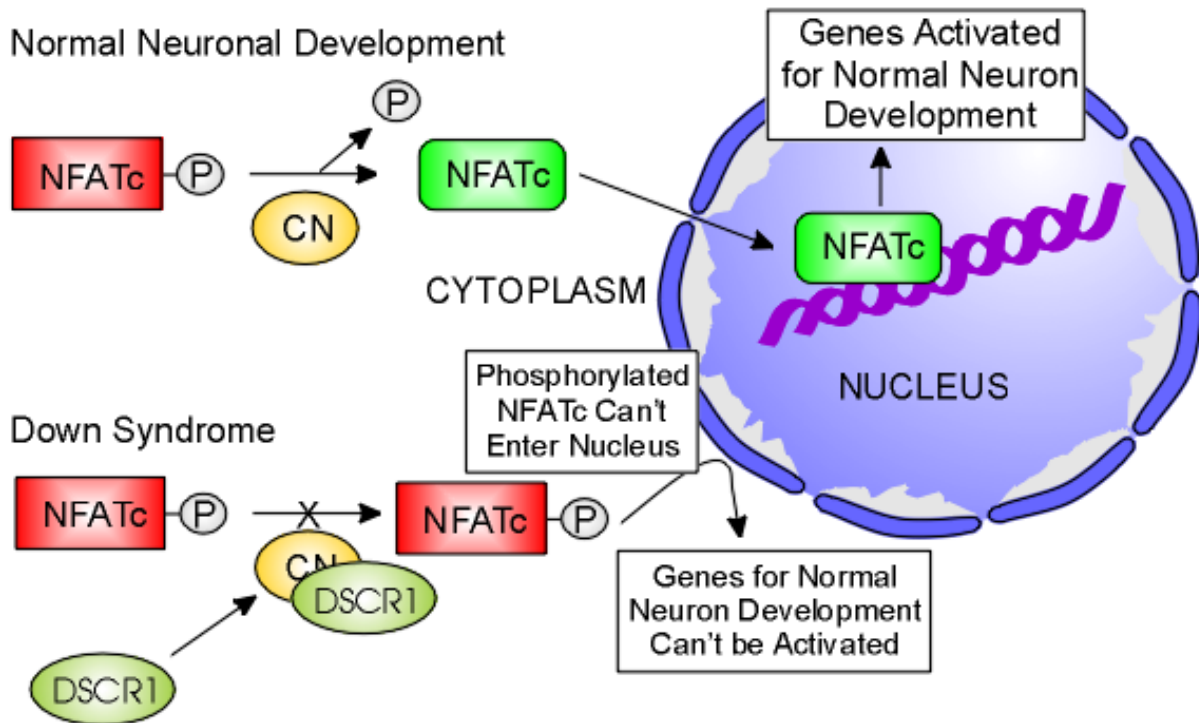
- f. \_\_\_\_\_ migration to gonadal ridges \_\_\_\_\_
- g. \_\_\_\_\_ mitosis \_\_\_\_\_
- h. \_\_\_\_\_ growth and differentiation \_\_\_\_\_
- i. \_\_\_\_\_ Meiosis I \_\_\_\_\_

**Indicate the ploidy of the following cells or structures**

- j. a: \_\_\_\_\_ diploid \_\_\_\_\_
- k. b: \_\_\_\_\_ diploid \_\_\_\_\_
- l. e: \_\_\_\_\_ haploid \_\_\_\_\_

**5. Explain why trisomy 21 affects neuron development and its significance to human development. (20 marks).**

The role of DSCR1 in neuronal development is just one aspect of Down Syndrome. The genes for DSCR1 on chromosome 21 regulate the phosphorylation of transcription factors that regulate brain nerve cell development. By inhibiting normal brain cell development, Down Syndrome individuals have impaired intellectual functioning.



- Gene: *DSCR1* (Down Syndrome Critical Region 1) is found on chromosome 21
- The extra copy of chromosome 21 leads to over-expression of DSCR1 in developing brain cells (neurons)
- DSCR1 protein is an inhibitor of calcineurin (CN), a calcium and calmodulin-dependent protein phosphatase
- In normal neurons, CN removes phosphate groups (i.e., dephosphorylates) NFATc (Nuclear Factor of Activated T cells) allowing it to enter the nucleus to regulate genes required for normal development
- Increased levels of DSCR1 lead to the inhibition of CN which prevents it from dephosphorylating the critical transcription factor NFATc.
- Phosphorylated NFATc can't enter the nucleus to regulate specific genes required for normal brain development

**6. Using point form and diagrams, describe the origin of primordial germ cells in humans with particular emphasis on the genes involved. (20 marks).**

**Germ Cell Formation in Mammals**

In humans the PGCs, the germ cell lineage is not established in the same way as in many lower animals. For one thing, "germ plasm" does not appear to exist in mammals and the germ line is not predetermined. In the rat and mouse a similar material called "nuage" appears in germ cells but could not be detected earlier in the embryo. PGCs are derived from the posterior epiblast but transplantation experiments have shown that this material is not determined early. For example, in the mouse the germ cell lineage only becomes defined around the time of gastrulation not during oogenesis or early cleavage as it does in lower animals. Grafting experiments have shown that many regions of the mouse embryo are capable of forming germ cells when they are transplanted to the extraembryonic mesoderm region of the epiblast prior to gastrulation. Until more is known, it is assumed the human germ cell population arises in a similar way. The topics of the important topic of determination of cells and tissues and cellular interactions (e.g., induction) that mediate the process will be covered at many times throughout this course.

**BMP & DAZ Genes & Human Germ Cell Formation**

Various factors seem to be important such as bone morphogenetic protein (BMP; originally revealed as a factor involved in bone morphogenesis) since mice with null mutations for *Bmp4* lack primordial germ cells. While germ-line determination is likely to differ from other animals in many specific ways, work on lower forms has guided the direction of human studies. For example, over the last few years, another gene first identified in *Drosophila* as being important in germ cell development has also been shown to function in germ cell formation in humans. Mutations in the human DAZ gene (Deleted in Azoospermia) and/or its homologs can result in the absence of either eggs or sperm cells. The exact role of DAZ in human spermatogenesis is under analysis. Oct4, a nuclear transcription factor, also appears to be critical for the origin of PGCs since it is expressed in cell lineages that give rise to PGCs as well as in PGCs and oocytes but not in sperm once they are in the testes. How these different proteins interact still remains to be revealed.

The following figure summarizes what we know about the origin and formation of the human sex cells.

