

1. (10pts) What is a Fate Map? How would you experimentally acquire the data to draw a Fate Map? Explain what a Fate Map does and does not tell you about the mechanisms used to specify cell fate during development.

**-Fate map shows what every part of the embryo becomes at latter stages of dev, i.e., traces embryonic origins of specific cell lineages.**

**-Labeling and visually following cells.**

**-does: what cells end up as, where and what type,**

**- does not: how this occurs, ie what signals are utilized and how they are conveyed. Intrinsic vs. extrinsic cell determination.**

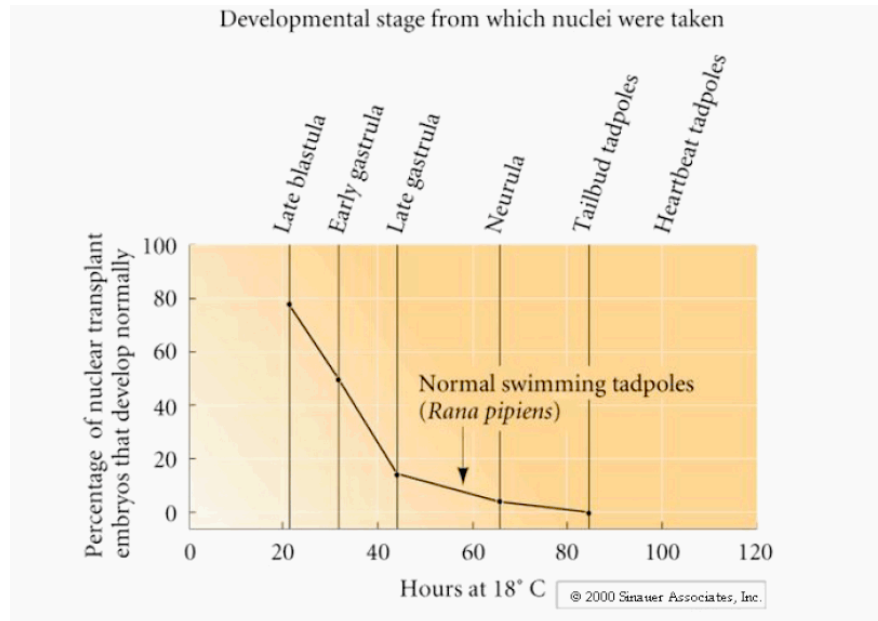
2. (15pts) What is SCIDs? Described the medical experiment that was used to attempt a cure. Describe what went right and what went wrong. How would you change the protocol to avoid the same problem?

**In the first trial they use “ex-vivo” infection of marrow cells from SCIDs children. The retroviral vector carrying the normal gamma C gene inserted “randomly” in the marrow cells. Unfortunately, because of the large number of cells infected (10 million or more), there was a high probability of the gene inserting near an oncogene. Because both the gamma C gene and the LMO-2 gave a selective growth advantage these few cells “took over” and gave rise the leukemia like disease.**

**There are many possible ways to avoid this problem. The simplest might be to use exactly the same protocol, but use a selectable marker to isolate and clone individual T and NK lymphocyte stem cells that receive the transgene. These cells could be tested for expression levels of the transgene AND FOR THE INSERTION SITE TO ENSURE IT LANDED IN A SAFE LOCATION WHERE IT WOULD NOT AFFECT A NEARBY ONCOGENE. Once the cell clone is deemed safe and effective it could be expanded in culture until the appropriate number of cells are available for reinjection into the patient.**

**Other possibilities include gene targeting by homologous recombination. This would require identification of the appropriate T and NK stem cell (or developing ES cells by therapeutic cloning and a protocol for inducing them to become T and NK lymphocyte stem cells), expansion of cell populations in culture, gene targeting, selection, and again characterization of the targeted gene and expansion of the correctly targeted cell line before finally injecting back into the patient.**

3. (10pts) Describe the experiment that was done to generate the data shown in the graph below. What were the implications of the results at the time the experiments were performed and what would be our current interpretation given more recent experiments. Briefly describe a way to increase the percentage of nuclear transplant embryos that develop normally from nuclei harvested from cells in later developmental stages cells.



**-Donor somatic nuclei at different stages of development, injected into an enucleated egg**

**-Tests genomic equiv.**

**-Showed that genomic equiv. is maintained in somatic cells of all stages examined**

**-Now understand that DNA becomes restricted and not all genes are “open”, methylation status etc.**

**Success can be increased by serially transplanting nuclei from somatic cells to eggs, allowing them to divide to the blastula stage, and then transplanting them back to an enucleated egg. This significantly improved the success rate and led to the development of adult frogs from transplanted intestinal nuclei.**

4. (10pts) The best strategy for reproductive success may be somewhat different for the male and female. The male wants to maximize the potential survival of embryos carrying his DNA. However, the best strategy for the female is to regulate the resources given to the embryo so that she can save enough resources for potential future offspring. How is this “genetic conflict” between male and female manifested on the DNA level? What is term for this DNA change? Where and when does it occur?

**-DNA based changes in gametes prior to fertilization (during gametogenesis) not altering sequence but changing methylation status to affect changes in gene usage (ie transcription) This is called “imprinting.**

5. Define the following 4 terms:

A. (5pts) Therapeutic cloning

**Using recombinant DNA and cloning techniques to generate genetically engineered or defined totipotent or pluripotent stems cells for treatment of diseases, or production of tissues for transplantation**

B. (5pts) Reproductive cloning

**Using nuclear transfer and cloning techniques to generate a genetically identical animals**

C. (5pts) Regulative development

**Regulative development suggests extrinsic determination and plasticity in determining cell fate, and predicts that removing a specific cell will be no different than removing any other cell and will produce little or no deficit in the resulting embryo.**

D. (5pts) Deterministic development

**Deterministic development is lineage based and predicts that removing a cell will result in a specific and predictable deficit.**

6. (10pts) How does Protein Kinase C affect intracellular pH shortly after fertilization? How is this effect on pH accomplished? Name three functional outcomes that this change in PH has on late events of egg activation.

**It phosphorylates specific serine and threonine residues on a Na/proton exchanger which causes the intracellular pH to increase from 6.7-7.2. Three functional outcomes of this event are stimulation of protein synthesis, stimulation of DNA replication, and cytoplasmic movements of morphogenic material.**

7. 10pts) Cleavage stage embryos of many species undergo very stereotyped patterns and rates of mitosis. Compare and contrast the human and fly (drosophila) cleavage stage of development. Suggest a theory for why they are so different.

**-Mammals: isolecithal, slow 1/day clev., rotational holoblastic clev., Asynchronous div., regulated by zygotic nucleus immediately from fert., no yolk.**

**-Dros: centrolecithal eggs, div. every 8 min., superficial clev., synchronous clev. Till 14th nuc. Div., maternal mRNA very important for dev., lots yolk.**

**-Life of fly is 21 days, humans say 70 years, flies employ a reproductive strategy to invest little energy in lots of quickly developing, relatively simple organisms. Mammals invest lots of energy in a few slowly developing complex offspring. Thus each employs a different route to evolutionary success.**

8. (15pts) Starting with a dish of isolated mouse embryonic stem cells, and the DNA for the BMP gene, outline how you could create a homozygous BMP knockout mouse using homologous recombination. (No need to draw figures, word description would be fine).

