Developmental Biology Exam 1 **KEY** Name
Feb. 4, 2011

1. (20 pts) Define "Reproductive" and "Therapeutic" cloning. Make sure your descriptions clearly distinguish the critical differences between them. Describe an example of each.

Reproductive cloning refers to the transfer of a somatic cell nucleus into an enucleated oocyte followed by implantation of the artificially fertilized oocyte into a host organism's uterus. Reproductive cloning results in the generation of a genetically identical animal to that of the nuclear donor. Probably the most famous example of a successful reproductive cloning attempt is Dolly the sheep. Dolly was the first mammal to be cloned from adult somatic cells. The process of nuclear transfer from somatic cells that had been arrested in G_0 stage of the cell cycle that Dolly's creators used was an improvement over the transplantation of actively cycling cell nuclei, and many other mammalian organisms have now been successfully cloned. Maternal twins arising from naturally occurring processes (Figure 1 A-C) can be considered reproductive clones as well.

Therapeutic cloning refers to the use of recombinant DNA to transform cultured embryonic stem cells in order to generate genetically engineered totipotent or pluripotent stem cells for treatment of diseases. Therapeutic cloning of stem cells can also be used for the production of isogenic tissues for transplantation. The use of recombinant DNA and the production of stem cells or tissues in addition to whole transgenic animals (Figure 2), distinguishes therapeutic cloning from reproductive cloning. Examples of therapeutic cloning include the treatment of SCID patients with therapeutically cloned transgenic lymphocytes, the woman whose trachea was grown ex-vivo and implanted, and many other stem cell-derived treatments discussed in class and in our text.

Figure 1

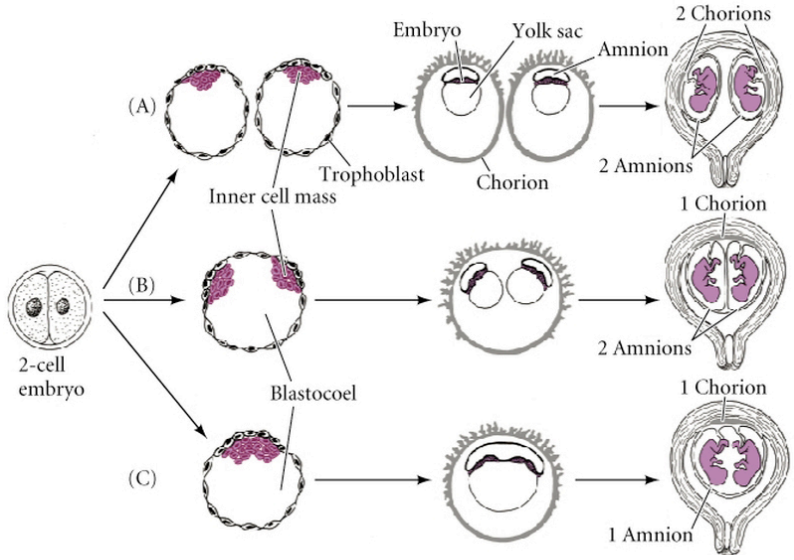
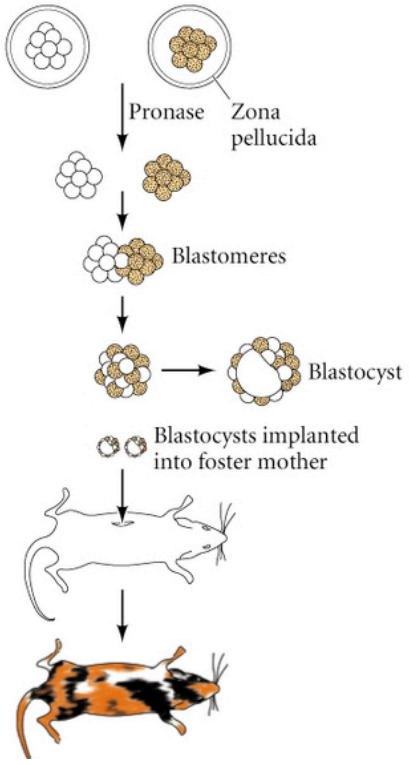


Figure 2



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2. (15 pts) What techniques did John Gurdon develop that were critical for testing the Theory of Genome Equivalence? Describe his early results and explain how they supported, but did not prove the theory. Explain how his techniques made possible the modern cloning technologies used today.

- John Gurdon's team developed the techniques for generating enucleated frog eggs and transferring into these eggs the nucleus of another cell (Figure 1 A & B).
- Dr. Gurdon demonstrated that differentiated somatic cells from the early-late blastula stage were best at supporting normal development to the tadpole stage (Figure 2), but that normal tadpole development was rapidly and negatively affected as somatic cells differentiated.
- These nuclear transplantation experiments supported the Theory of Genome Equivalence (that all cells in an organism contain the same complement of DNA) by showing that differentiated somatic nuclei were capable of genetic reprogramming to a nearly totipotent state when transplanted into enucleated eggs.
    - These experiments also demonstrated that exposure to egg cytoplasm was sufficient to cause reprogramming of nuclei from differentiated somatic cells.
- Dr. Gurdon's data suggested that the changes occurring in the genomes of differentiating cells were cumulative and increasingly difficult to reset as development progresses.
- Dr. Gurdon's data could not be said to *definitively* demonstrate that differentiated somatic nuclei were identical at the level of DNA sequence, however, because none of the nuclear transplantation experiments yielded viable adults.
- Gurdon's techniques for nuclear manipulation paved the way for later groups' successes in both reproductive and therapeutic cloning.

Figure 1 A)

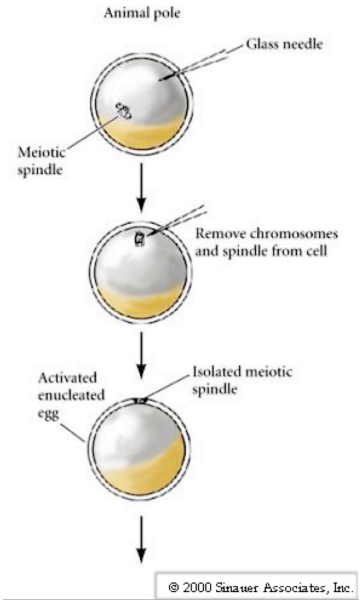


Figure 1 B)

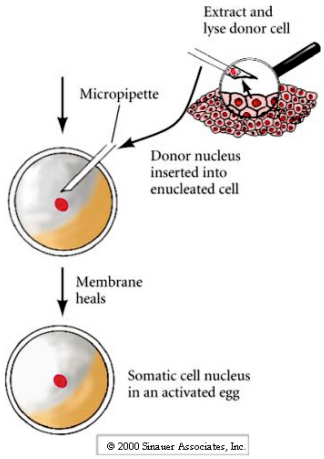
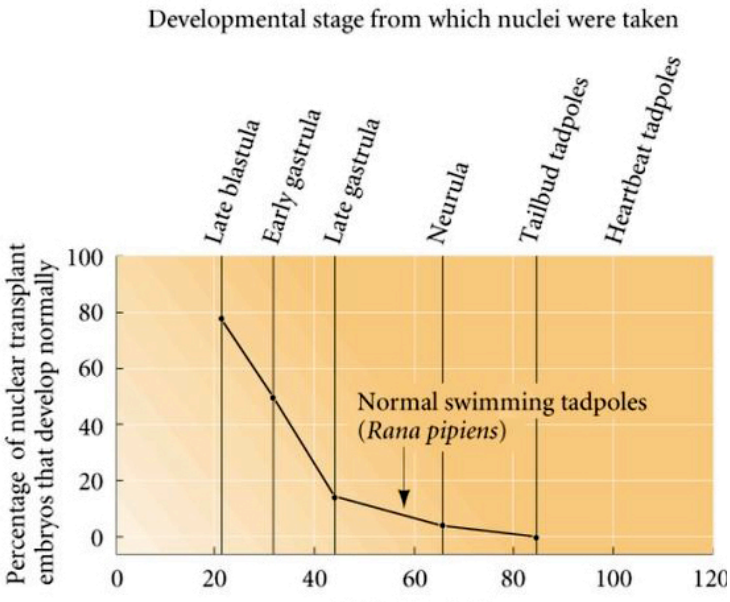


Figure 2



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3. (20 pts) When a mouse pup inherits a deletion of Igf2r from the father, the pup develops normally. However, when a pup receives the same mutation from the female the results are disastrous. Explain these odd results and describe the "Developmental Rationale".

- The "Developmental Rationale" for these results becomes apparent in the context of differential reproductive strategies male and female mice. From the male perspective, the goal is to maximize the potential survival of embryos carrying his DNA. Males can fertilize multiple females, and do not share the same metabolic burdens associated with bringing offspring to term that females do. From the perspective of the female, then, it is better to limit the resources available to the embryo in order to balance the energy needs between herself and her current and future offspring.
- Differential expression of the Igf2 protein leads to an increase in maternal glucose uptake by the mouse embryo. Expression of the Igf2r protein causes the inactivation and premature degradation of Igf2, which reduces embryonic glucose uptake (Figure 1). Igf2r is unmethylated and active in female oocytes, but methylated and inactive in male sperm (Example of methylation at a different locus is shown in Figure 2).
- In light of the scenario outlined above, it becomes apparent that male sperm would decrease the expression of the Igf2-inhibiting Igf2r protein in an attempt to maximize embryonic glucose uptake. Similarly, female oocytes express Igf2r in order to limit glucose uptake by the embryo and thus conserve energy.
- If a mouse pup inherits a paternal Igf2r deletion (which has been "turned off" to begin with) it has no effect on new Igf2r production, since the intact female Igf2r gene is properly expressed. However, if the mouse pup inherits only the paternal version, which is methylated and inactive, then Igf2 (and thus embryonic glucose uptake) is uncontrolled. The resulting increase in glucose uptake causes embryos that carry only the methylated paternal Igf2r gene to die in late embryogenesis because they become grossly enlarged. This is one of many examples of genetic imprinting (Figure 2).

Figure 1

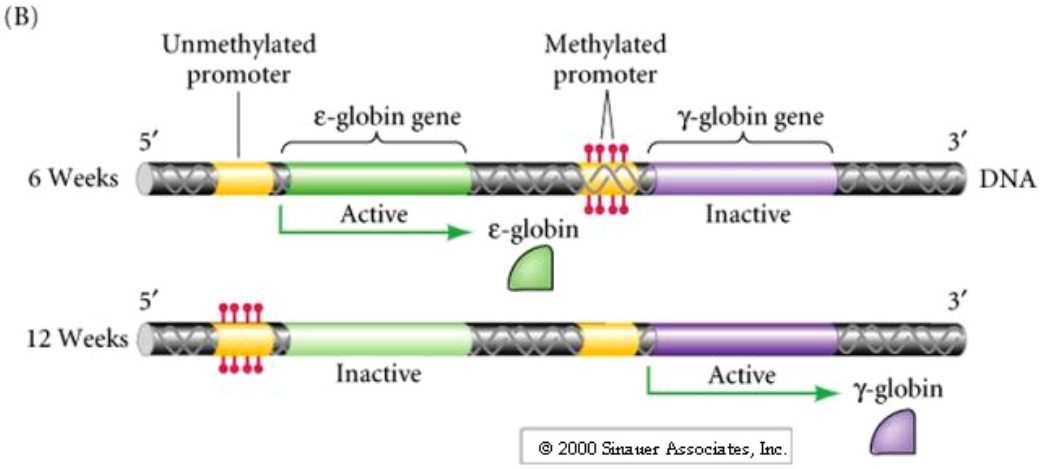

Figure 2. Effects of methylation and genetic imprinting at the globin gene locus

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4. (10 pts) Who was Martin Evans and what was his contribution to our understanding of early development? How did his observations and techniques enable another powerful molecular-genetic technique developed here at the University of Utah?

- Martin Evans's specific contributions were the development of the techniques for culturing mouse embryonic stem cells.
    - Importantly, Evans contributed to our understanding of early development by demonstrating that his cultured embryonic stem cells were indeed totipotent.
- The availability of these cultured embryonic stem cells made possible the introduction of specific gene alterations into the germ line of mice and the creation of transgenic mice to use as experimental models for human illnesses (Figure 1).
- Martin Evans was awarded the Nobel prize in Physiology or Medicine along with Mario Capecchi and Oliver Smithies for their work in discovering a method for introducing homologous recombination in mice employing embryonic stem cells.

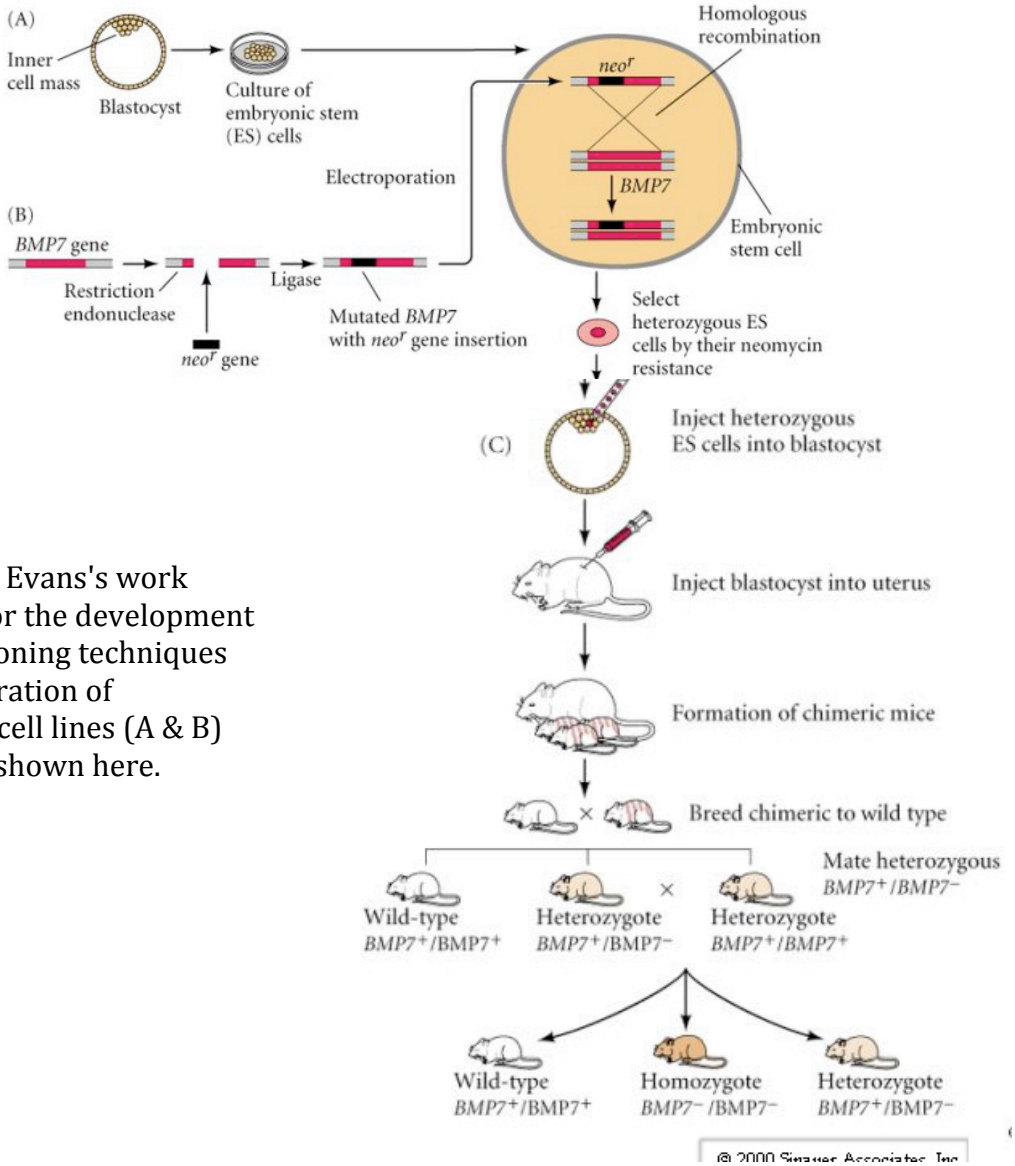

Figure 1. Martin Evans's work paved the way for the development of therapeutic cloning techniques such as the generation of transgenic stem cell lines (A & B) and animals (C) shown here.

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5. (15 pts) Describe the signaling events mediated by sperm contact with a mature egg. A diagram is fine, but label it so that you describe the important events occurring in the fertilized egg.

- Figure 1 shows the initial events stimulated by sperm binding to the egg include the fast block to polyspermy.
- Figure 2 shows the downstream signaling events that occur in response to increased Ca concentration and initial sperm-mediated kinase stimulation.

Figure 1

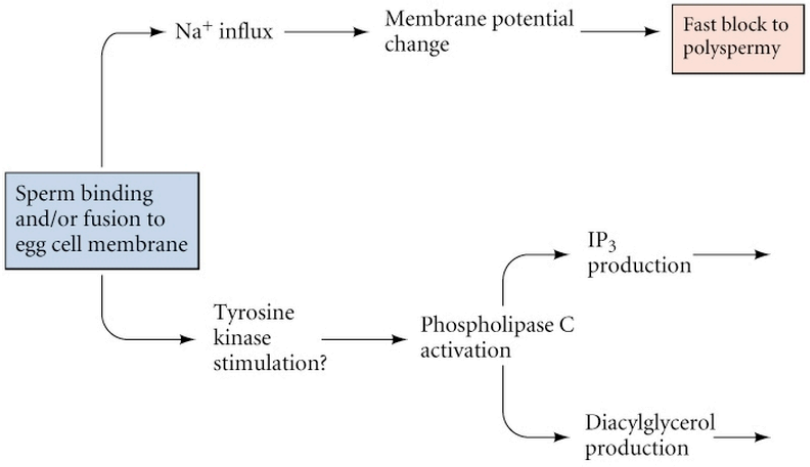
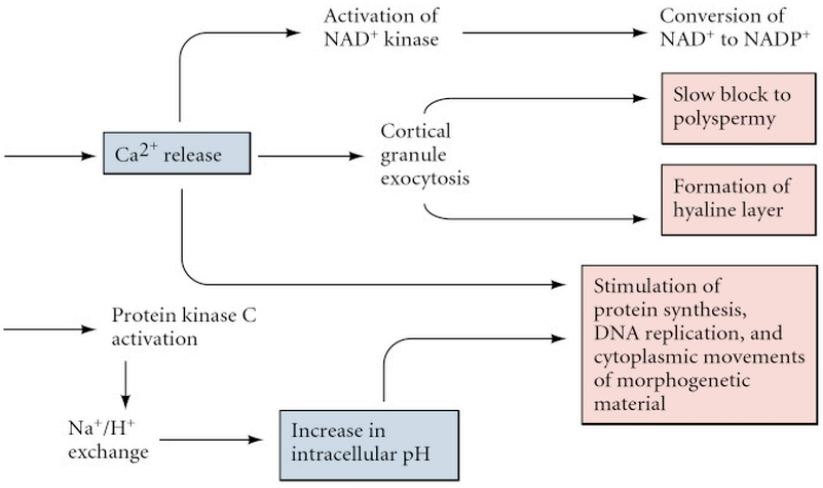


Figure 2



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6. (20 pts) Describe the experiments Horstadius performed on the sea urchin embryo to support **both** regulative and mosaic development. Briefly describe the results and the developmental model that best explains the results.

- Horstadius showed that isolated cells from the 4 cell stage could all give rise to fully formed larvae, in agreement with a regulative theory of development where cells maintain developmental plasticity as they develop.
- However, when Horstadius separated 16 cell stage blastomeres into animal and vegetal halves, he saw that only the vegetal half would give rise to micromeres, gastrulate, and form skeletons (Figure 1 A & B).
    - *These* observations agree with the mosaic theory of development, where cytoplasmic determinants from earlier embryonic stages specify later cells' differentiation and limit their regulative potential.
- He also showed experimentally that in a 16 cell stage embryo all tiers of blastomeres except the micromeres will take on different fates when transplanted into different positions in chimeric embryos.
    - When micromeres were isolated at this stage they were shown to always give rise to spicules and thus are definitively specified by cytoplasmic determinants from earlier embryonic stages, also supporting a mosaic developmental model.
- Finally, when Horstadius transplanted micromeres to animal half blastulae they regained the ability to differentiate into recognizable larvae (Figure 1 C), while micromeres transplanted into the animal hemisphere of intact 16 cell embryos could induce ectopic endodermal differentiation (Figure 2).
    - The ability of the micromeres to inductively determine the fates of neighboring blastomeres, which in turn interact with their neighbors, supports a regulative model of development, where cells are again shown to display a degree of plasticity in their developmental fates.

Figure 1

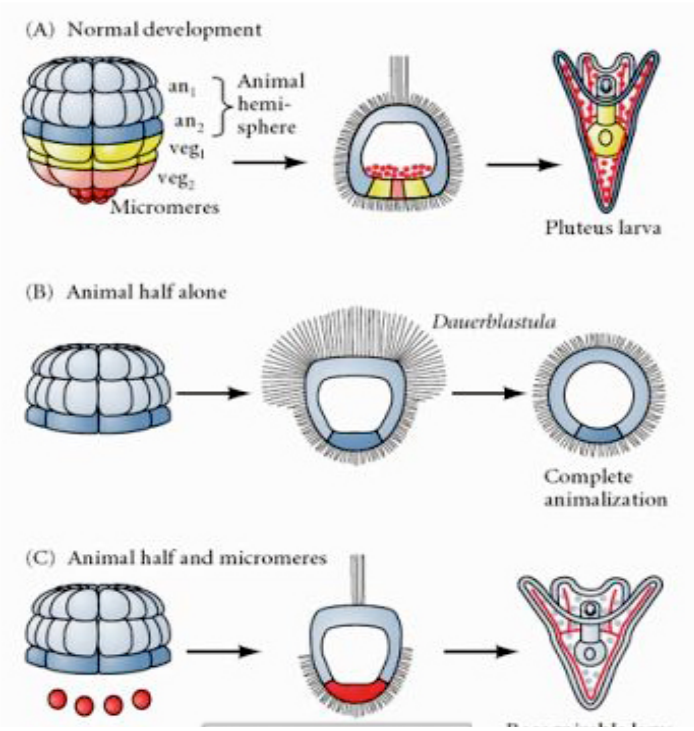

Figure 2