Developmental Biology 3230 **6 Feb. 2004**

Midterm Exam 1

Answer 8 of the following 10 questions for full credit. You may choose which 8 to answer. Please put an X through the questions you do not want me to grade.

1. What are the major questions that developmental biologists want to answer about embryogenesis? What are the unique characteristics of animal development compared to say building a car? What do we mean when we say that development is epigenetic?

How does an embryo self regulate cell number? What are the molecular changes that drive cell determination and differentiation? How are the major AP and DV patterns established? How do the generated cells self organize into the correct tissues and organs (pattern formation or morphogenesis)? The embryo is an autonomous, self-organizing, self-constructing machine that functions from the moment of fertilization. The individual cellular components of embryos often have tremendous regulative abilities and can thus form many different "parts". A car is put together from specialized static parts that are unique and fixed. A car cannot put itself together. It needs an outside intelligence to assemble it. A car doesn't function until it is assembled. Even a perfectly functioning car cannot repair or replicate itself. Development is **epigenetic** because the linear strand a DNA in the zygote encodes proteins--it does not encode body parts. There is no place in the genome that encodes a hand. There is no point to point correspondence between the genome and the organism. The DNA exists in the complex environment of the embryo. The fertilized egg, even excluding the nucleus, is a very information rich environment, information that regulates the zygotic DNA

2. What is the theory of genome equivalence? Describe the techniques developed by John Gurdon to test the theory of genome equivalence. How did the results of his experiments either support or refute the theory? What modern day technique is a direct result of his pioneering work?

The theory of genome equivalence proposes that all cells of the body contain the same DNA (genetic material) and that cell differentiation occurs by reversible changes to DNA to repress the expression of some genes and activate others.

Amphibian Cloning (John Gurdon, 1975)

The techniques for nuclear transplantation were developed by John Gurdon. He chose an amphibian, the leopard frog ((Rana pipiens) as a model system because of the large size of the egg (the frog had long been a model system to study early development because of size, availability and ease of rearing animals in the lab). Using small glass micropipettes he developed techniques for enucleating eggs, isolation of donor nuclei, injection of donor nuclei into host enucleated eggs, and activation of eggs. He found that somatic cell nuclei could support normal development, but only rarely did he a normal adult (never fertile). This supported the theory of genome equivalence, but suggested that changes occur during differentiation that are difficult to reverse.

Modern techniques of reproductive and therapeutic cloning rely on the techniques of nuclear transplantation pioneered by Gurdon.

3. Why are the male and female pronuclei different (nonequivalent) in their ability to support development? Describe a specific example that illustrates this difference. Explain/describe the evolutionary argument that is given to account for this "irrational" design?

It turns out that during differentiation of the gametes the DNA is specifically altered in a sex (sperm and egg) specific way that leads to differential gene expression in the genes of the two gametes. The term for this is **imprinting** and is the result of differential methylation of the regulator regions of genes. Thus the patterns of methylation during gametogenesis are distinct in the in the sperm and egg nucleus.

One class of genes that are differentially regulated by imprinting are those involved in energy metabolism. On example that you should remember is **Insulin-like growth factor II (Igf2)** and **Insulin-like growth factor receptor (Igf2r)**. Expression of the Igf2 protein leads to an increase in glucose uptake by the embryo (from the mom). This gene is active in sperm derived genes and inactive in female derived genes (through methylation). The Igf2r protein binds to the Igf2 protein causing inactivation and premature degradation. Igf2r is active in female gamete DNA and repressed in the male gamete DNA. 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. Now the Igf2 produced by the male DNA causes the placenta to enlarge and glucose uptake from the Mom to increase. The pup dies late in embryogenesis in part because it is grossly enlarged (30%).

At least part of the explanation lies in the **genetic conflict between male and females**. 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, thus he contributes DNA that is imprinted in a way that will try to maximize the resources taken from the female for "his" embryo. 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. She can only have progeny with herself, while the male can have progeny with many females. Thus the female imprints the DNA of her gametes to counteract the "selfishness" of the male derived DNA. Remember, there can be a genetic conflict between the baby and the mom because the baby is only related to mom by 50%.

4. What are ES (embryonic stem) cells and where do they come from? Why is the scientific and medical community so interested in these cells? Describe the cloning technique made possible by the discovery of ES cells.

Embryonic Stem (ES) cells are totipotent cells derived from the inner cell mass of a blastula (blastocyst) stage mammalian embryo. ES cells can be grown to large numbers in culture and still maintain their totipotency. This was critical for developing the technique of gene targeting by homologous recombination, where the frequency of the targeting event is very low (1 out of 1 million). Now this rare event can be easily selected from targeting millions of cells. The targeted cell can be isolated and allowed to grow up in culture. Now the ES cell with a modified genome can be re-introduced into the ICM of a host blastocyst where it will contribute to normal development, and with luck, the germ line.

ES cells are also of interest because when exposed to the right culture conditions (growth factors) they can be coaxed into differentiating into many difference cell types that have potential therapeutic uses (Therapeutic cloning).

5. What is gene targeting by homologous recombination? Briefly describe why the technique is so important for the study of mammalian development and human disease.

It is a technique that allows us to modify the DNA of a chromosome in a precise way, by replacing an endogenous stretch of DNA with a piece that has been modified in the lab. The modified DNA can be placed (targeted) to a specific location in the genome to replace a defective gene with a functioning copy or replace a functional gene with a defective one. Because this technique can be performed on mammalian ES cells we can now generate germ line modifications that will be transmitted normally to progeny just like any other DNA.

It is the only way to study the effects of genetic mutations on mammalian development. This technique not only allow us to make "gene knockouts" of any mouse gene to see the effect on development and generate disease models, but it also makes possible the testing of genetic therapies by making "knockins". It is also the basis for any human engineering of the germ line or of stem cells.

6. Describe the technique used in the SCID gene therapy trial. What problem arose and how did the doctors try to explain it. Try to reconstruct the quantitative assumptions they must have made for their explanation to be reasonable.

Severe combined immunodeficiency–X1 (SCID-X1) is an X-linked inherited disorder characterized by an early block in T and natural killer (NK) lymphocyte differentiation. This block is caused by mutations of the gene encoding the a cytokine receptor subunit of interleukin-2, -4, -7, -9, and -15 receptors, which participates in the delivery of growth, survival, and differentiation signals to early lymphoid progenitors. A gene therapy trial for SCID-X1 was initiated, based on the use of complementary DNA containing a defective Moloney retrovirus–derived vector and ex vivo infection of CD34 cells from patient recovered bone marrow. Gene therapy was able to provide full correction of disease.

However, after 1-2 years 2 out of 10 children treated developed a leukemia-like disease. Both patient leukemias contained an IL2RG-containing retrovirus integrated in the proximity of LMO2,a known human T cell oncogene leading to aberrant transcription and expression of LMO2. It has been generally assumed that replication-defective virus-induced insertional mutagenesis would be extremely rare. However, if we assume that a frequency of 1 to 100,000 or even 1 million then the possibility of a few events is quite probable given the number of bone marrow stem cells transfected is easily estimated to be in that range. If we also assume that these rare events would be further selected by growth advantages of these cells we can see how the 2 instances of leukemias could be explained.

7. Describe the physical and molecular events in the egg that occurs during the normal egg activation that is triggered by sperm contact.

Increased Na current leading to depolarization within 1/10 of a second. The rapid depolarization of the egg plasma membrane prevents further sperm egg fusions----This is the fast block to polyspermy
Release of Ca from intracellular stores that raises Ca concentration within 10 secs. The increased Ca concentration causes the cortical granules (about 15000 at 1 um) to fuse with the egg plasma membrane and release their contents. The enzymes released by the cortical granules inactivates the BINDIN receptors on the vitelline layer and a peroxidase crosslinks tyrosine residues of adjacent proteins within the vitelline layer so that sperm can no longer attach to or penetrate to the egg. Additionally, attachments between the vitelline layer and the egg are digested releasing many osmotically active particles into the space between the vitelline and plasma membranes. Water rushes in and forces the vitelline layer away from the surface of the egg. It is now termed the FERTILIZATION ENVELOPE.

Egg activation is initiated by the rise in Ca concentration. Sperm cell ligands bind to egg membrane receptor proteins that either directly or indirectly activates a tyrosine kinase which in turn activates phosphoinositide specific phospholipase C and leads to hydrolysis of phosphatidylinositol bisphosphate (PIP2) in the plasma membrane to produce inositol trisphosphate (InsP3) and diacylglycerol. The InsP3 causes release of Ca from an internal store (specialized endoplasmic reticulum). Protein Kinase C is activated by diacylglycerol and Ca. It phosphorylates specific serine and threonine residues on target proteins. In this case a Na/proton exchanger which causes the intracellular pH to increase from 6.7-7.2. The low pH is thought to keep the egg metabolically inactive. Initial protein synthesis is due to transcription of maternal mRNAs and maternal rRNA. The increase in pH is thought to make the mRNAs available for translation and increase the efficiency with which rRNA move along the mRNA. DNA synthesis is also facilitated. Rise in intracellular pH in some organisms induces the late synthetic events of egg activation

8. What are the characteristic features of the cleavage stage of embryogenesis? Describe all the ways in which mammalian cleavage is atypical.

The cleavage stage of embryogenesis is generally characterized by very rapid and geometrically stereotyped mitotic cells divisions. There is often no cell growth between rounds of cell division because there is no G1 or G2. Cells go directly from S to M to S under control of maternally provided activated cyclins, Cleavage stage cells do not move relative to one another. They often form a blastocoel. The pattern and rate of cleavage is often controlled by the maternal genome and independent of the zygotic genome.

Mammalian cleavage is unusual because cleavage in mammals can be very slow---1/day. Additionally, the cleavage planes are somewhat different from other animals. First cleavage is meridional just like sea urchin and frog. However, the second cleavage division sees one of the blastomeres dividing meridionally and the other equatorially! This type of cleavage is called **ROTATIONAL HOLOBLASTIC CLEAVAGE**. Another unique feature of mammalian cleavage is that the blastomere cleavages are asynchronous. (compared with the synchrony of sea urchin and frog up till the midblastula transition). Cleavage of the mammalian embryo is regulated by the zygotic nucleus from the very start.

Through the third cleavage the blastomeres form a ball of loosely associated cells just like the other animals we've studied. Before the fourth cleavage the cells of the blastula dramatically change their behavior towards one another. They rapidly try to maximize their contacts with the other blastomeres and in doing cause the blastula to compact. This **COMPACTION** results in part from the production of an novel adhesion molecule UVOMORULIN (E-Cadherin) and is stabilized by the formation of tight junctions between the outer cells which like in the sea urchin seals off the interior of the blastula from the exterior. The cells also form gap junctions among themselves that allows the passage of small molecules, such as ions and some second messenger molecules such as Ca++ and C-AMP. The compacted 16 cell morula consists of an outer rind of cells and a few cells (1-2) completely internal. Most of the external cells give rise to the **TROBLASTIC OR TROPHECTODERMAL CELLS**. These cells do not contribute to the embryo proper, but instead are necessary for implantation of the embryo in the uterine wall and form the tissues of the CHORIAN, an essential component of the placenta.

9. What were the experiments that won Spemann the Nobel Prize in Medicine in 1935? Describe the interpretation and significance of the experiments. (Mangold died in a tragic accident before receiving recognition for her work).

The dorsal lip region of the late blastula/early gastrula has unique properties. The transplantion of this tissue by Spemann and Mangold let to a dramatic result. The transplanted dorsal lip tissue induced the surrounding host tissue to form a new embryonic axis.

The dorsal lip of the blastopore is the only self-differentiating region in the early gastrula because when transplanted it can initiate gastrulation and affect the surrounding host tissue. Heteroplastic (interspecies) transplantation with two species of newt, one darkly pigmented and the other non pigmented allowed Spemann and Mangold to separately follow host and donor cells derived cells during development. Transplantation of the dorsal lip of the blastopore at EARLY gastrula stages into novel locations in another early gastrula resulted in the formation of new blastopore, nearby host tissues were induced to gastrulate, and form a new body axis. Thus, the normal fate of cells could be completely changed by association with cells of the dorsal lip of the blastopore. The result was often a double embryo connected at the belly. This is very different from the other transplants that showed cells were not determined until the late gastrula. This is a dramatic example of **embryonic induction**. The dorsal lip of the blastopore is called the **ORGANIZER**.

10. Describe mammalian gastrulation. Draw a "top" view and a cross sectional view of a mammalian embryo half way through gastrulation. Be sure to label all the major cell types generated during gastrulation.

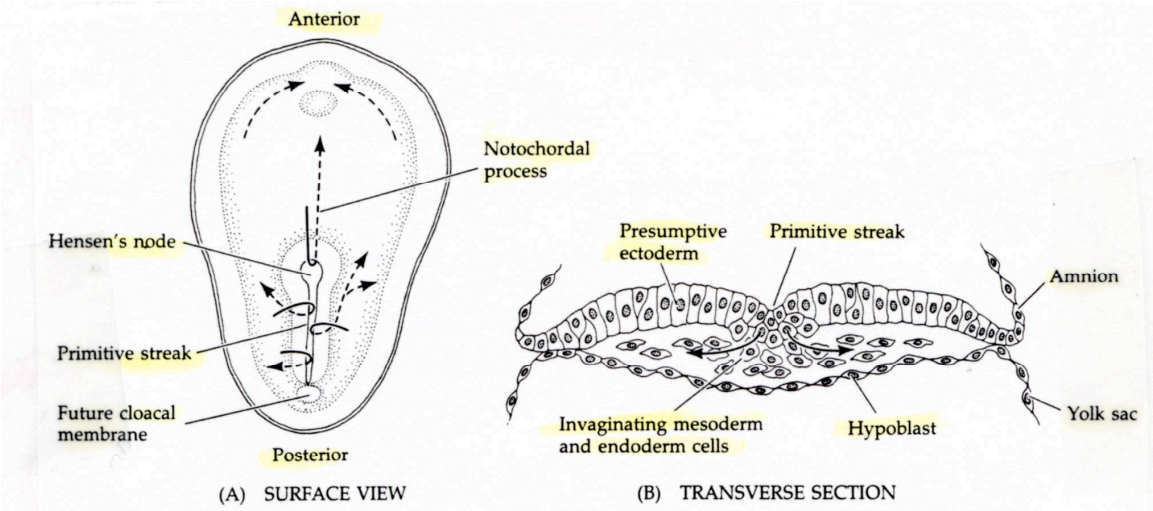

FIGURE 42
Cell movements during mammalian gastrulation. (A) Schematic diagram showing the dorsal surface of the embryonic epiblast (amniotic ectoderm removed). As in chick embryos, cells migrating through Hensen's node travel anteriorly (cephalad) to form the notochord, while the remaining cells traveling through the streak migrate laterally to become the mesoderm and endoderm precursors. Dotted lines indicate internal migrations. (B) Transverse section of the embryo. (After Langman, 1981.)