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1. (15 pts) Nuclear cloning by John Gurdon was rarely successful in producing fertile adults. Why not? Explain why serial transplantation improves the success rate. What else could you do to improve the success rate, short of in vitro molecule genetic manipulations?

   Was not successful because he took nuclei from rapidly dividing cells, transplanted them into enucleated eggs and immediately activated egg development. Lead to rapid mitotic divisions that caused chromosomal abnormalities because of missing DNA damage checkpoint controls. Maternal genome controls mitotic cycle because it supplies active cycllins to drive M to S to M. Serial transplantation selects for the nuclei that are most successful at supporting cleavages without abnormalities. You could improve success rate by starving cells to push them into Go before transplanting into egg. Wait as long as possible to activate egg to prolong the time the DNA is exposed to the egg cytoplasmic reprogramming conditions, e.g., imprinting.

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2. (20 pts) Yamanaka identified the minimal set of transcription factors needed to reprogram differentiated cells to pluripotency. How was the Fbx15 promoter driving β-geo expression used in his experiment? How did he verify the pluripotency of the reprogrammed iPS cells?

Fbx expression is strongly correlated with stem cell fate. Using the Fbx promotor to drive β-geo expression he could use drug selection to identify cells that were induced by his transcription factor cocktail (delivered by viral vector) to produce Fbx and thus exhibit one hall mark of ES cell identity.

He checked for pluripotency by 1. injecting candidate iPS cells into mice and seeing if they formed teratomas, 2. Inject candidate iPS cells into inner cell mass of host embryo and determined if iPS cell lineage contributed to all cell types in resulting chimera, 3. Examined methylation state of ES cell specific gene promoters in candidate iPS cells.

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3. (20 pts) What is Cas9 and how is it used in a new gene knockout technique? How was it discovered and what is its normal cellular function? Why is there so much excitement about this new technique?

Cas9 is the nuclease used in the CRISPR technique of making gene knockouts. Cas9 paired with a guide RNA targets a double stranded DNA cut to a specific DNA sequence specified by the guide RNA. It was discovered in bacteria (mainly Archea) as a adaptive immune defense against DNA viruses. Scientists are excited because compared to other techniques (TALENS or gene targeting by homologous recombination) it is much easier and much more efficient, 80% or more. Other techniques efficiencies 0.001%?

4. (15 pts) Assuming the techniques that we have discussed become safe and routinely applicable to humans, describe an experimental protocol that would lead to the safe and permanent cure of SCID--X1 without the use of bone marrow extraction or transplantation. (Not a germ cell cure, just curing kids already born.)

Using Yamanaka's findings one could use a skin fibroblast from a SCID-X1 patient and induce pluripotent stem cells (iPS cell). Then using CRISPER (Capecchi and Smithies technique of homologous recombination and selection could be used, but would require larger expansion of iPS cells because of low targeting efficiencty) we could replace the defective *interluken-2-receptor* (il2r) gene with a functional copy. The cells harboring a functional copy of *il2r* could now be enriched for (could propose selection system, but efficiency of CRISPER is so great that PCR of 10 or 20 clones would likely work), and differentiated into hematopoietic stem cells (HSC) for injection back into the patient. Sequence DNA of clone to be injected to ensure proper targeting and no off target changes. This technique would lead to a repopulation of their bone marrow with HSC's harboring a functional copy of *il2r* and cure the individual. This method would also avoid the dangers associated with bone marrow transplantation, the tragedy associated with random integration of the transgene, and the possibility of rejection routinely seen with imperfectly matched bone marrow transplantation.

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5. (10 pts) A women comes to an IVF clinic because she can not get pregnant. The IVF doctors harvest several of her eggs and try IVF. They suspect she has a defect in activation of Phospholipase C. What did they observe during IVF that lead them to suggest a defect in Phospholipase C activation? What modification of the IVF technique could be used to generate a blastocyst that could successfully be implanted?

Polyspermy and an absence of egg activation. Likely a defect in TK leads to no activation of PLC. Thus no Ca increase and no cortical granule exocytosis or egg activation. Could directly inject single sperm and Ca (or activated PLC) through a glass micropipette to achieve fertilization and egg activation.

6. (15pt) Time lapse recordings were made of early cleavage stage human embryos to improve the success of IVF in producing healthy babies. Explain why they did these experiments, what they found, and how it might improve the IVF technique.

Extra Credit (up to 10 pts) What would you expect their results to be if they had done time lapse of sea urchin cleavage stage embryos? Why?

Time lapse recordings were made of early human embryo cleavages to determine if there was a correlation between cleavage characteristics and chromosomal abnormalities. They found that abnormal dynamic cleavage behavior was somewhat predictive of chromosomal abnormalities. Chromosomal abnormalities are thought to be the leading cause of IVF failure (failure to implant or spontaneous abortion). Thus “non-invasive” time lapse recording could be used to screen for the “best” embryos for implantation.

Extra Credit:

The results of time lapse recording of sea urchin cleavage stage embryos would likely be uninformative because, unlike human embryos, cleavage stage mitotic divisions are controlled by maternal factors that might not respond to chromosomal abnormalities at the 4 cell stage.

7. (5 pts) Why are there 128 cells in a zebrafish, sea urchin, and tunicate blastula?

Cleavage stage mitotic divisions are synchronous in these animals up till the midblastula transition. If they all divide synchronously then 2x2x2x2x2x2x2= 128 blastomeres.