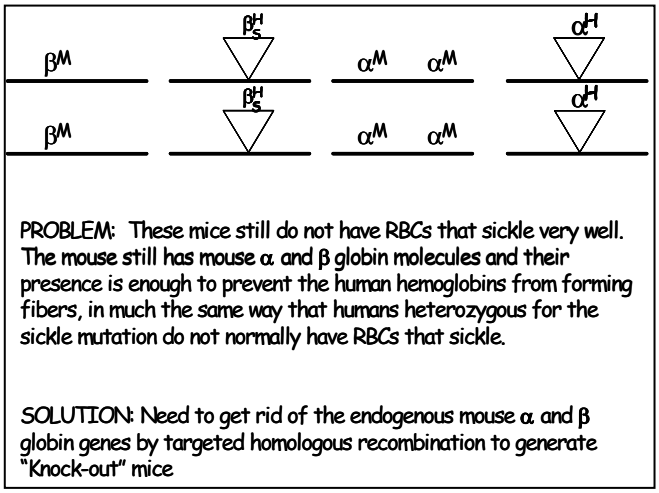
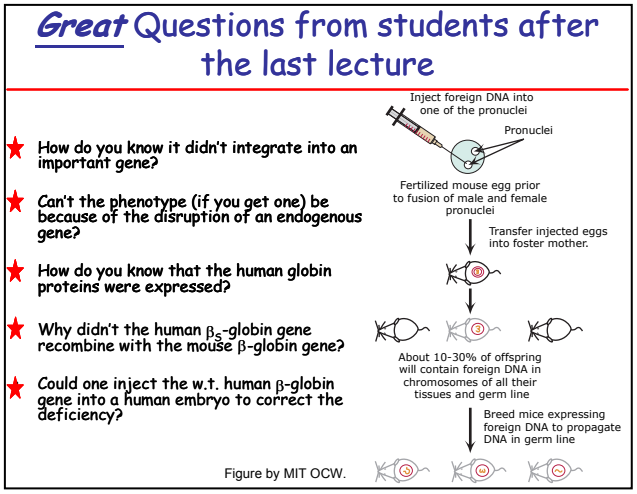
Lecture 24
Transgenes and Gene Targeting in Mice II

In the last lecture we discussed **sickle cell disease (SCD)** in humans, and I told you the first part of a rather long, but interesting, story describing how a mouse model for this human disease has been generated. I only got half way through the story…we will cover the rest today. In the last lecture we discussed how the **human β-globin** gene with the sickle mutation (β_S^H) was introduced as a **transgene** in mice, in the hope that it would cause the precipitation of hemoglobin and the sickling of mouse red blood cells (**RBCs**); had this happened this would have generated an animal model for **SCD.** If you recall, the transgenic mouse did not have sickling RBCs, and to try to fix this, the human **α-globin** gene was also introduced into the mouse genome…but still the doubly transgenic mouse did not have sickling RBCs. The solution to this was to inactivate the endogenous mouse **α-globin** and **β-globin** genes, and that's what we will cover today. BUT, before then, I want to share with you some great questions that I got after the last lecture, and some responses to those questions.





So…how do we "get rid of" the endogenous mouse **α-globin** and **β-globin** genes? Just like making **transgenic mice** this involves some manipulations of the mouse embryo…but this is a much more complex process, and some background about the preimplantation mouse embryo is needed. For about 4-5 days after fertilization, the mouse embryo is freefloating (and therefore accessible) and all of the cells that will eventually form the mouse remain **totipotent**, meaning that they have the potential to

Images removed due to copyright reasons.

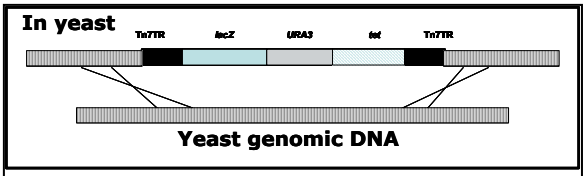
differentaite into any, and every, mouse cell type. This has been shown in various dramatic ways. For instance, if the four-cell embryo is dissected and each cell implanted into a different foster mother, four identical mice will be born. More interestingly, if cells from two genetically different pre-implantation embryos (e.g., embryos destined to produce mice with different fur colors) are simply mixed together (they are sticky) and implanted into a foster mother, a single **chimeric** mouse will be born. Essentially the two types of **totipotent** cells mix together and produce an animal that has a micture two types of cells in its body. This animal has four genetic parents!!

Images removed due to copyright reasons.

Early findings revealed that the preimplantation mouse embryo is remarkably malleable, and that cells in the the preimplantation embryo are TOTIPOTENT

The ability of these genetically different **totipotent** cells to mix together in the preimplantation embryo is crucial for the mouse gene **knock-out** technology.

In order to make a directed genetic change in a specific mouse gene we exploit **homologous recombination** just as we have discussed for *E. coli* and *S. cerevisiae*. However, this is much harder to do in mammalian cells than bacteria and yeast. In yeast, when a linear DNA duplex is introduced into the cell, about 90% of the time that that DNA is integrated into the yeast genome it is done by the homologous recombination machinery such that incoming DNA fragment is swapped for the endogenous gene. In mammalian cells the DNA that is integrated into the genome is almost always at a non-homologous site, and the frequency of homologous replacement of an endogenous sequence is about 10^{-3} to 10^{-5}. What this means is that we have to allow thousands of integration events to take place, and to be able to identify the integration event we want…namely an integration even that took place by **homologous recombination.**



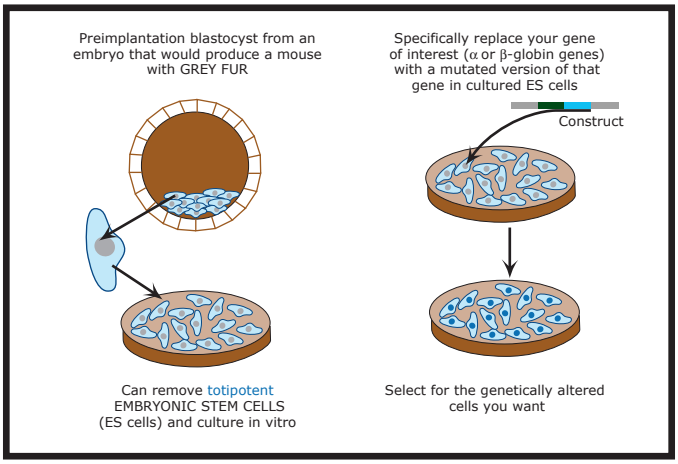
In yeast homologous recombination to replace an endogenous gene with the transfected DNA fragment occurs >90% of the time

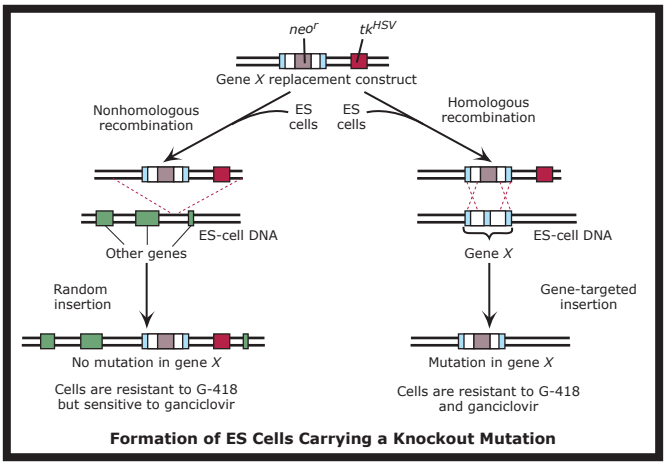
In mammalian cells such homologous recombination between genome and transfected DNA fragment is very rare (<0.01% of the time)

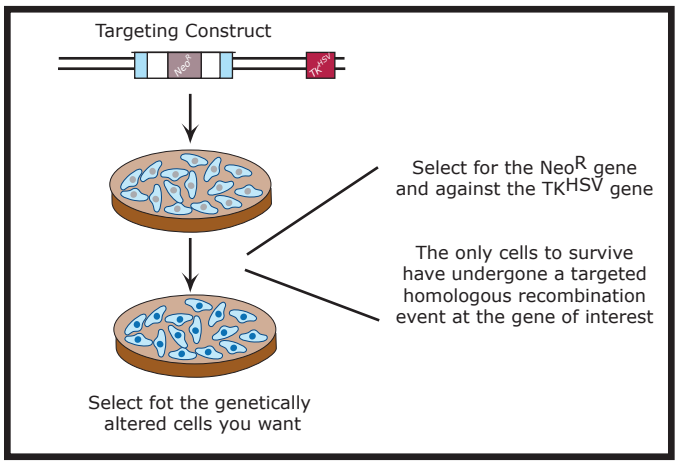
Have to have clever selection schemes to get the rare cells that integrated a transfected DNA fragment by **targeted homologous recombination**

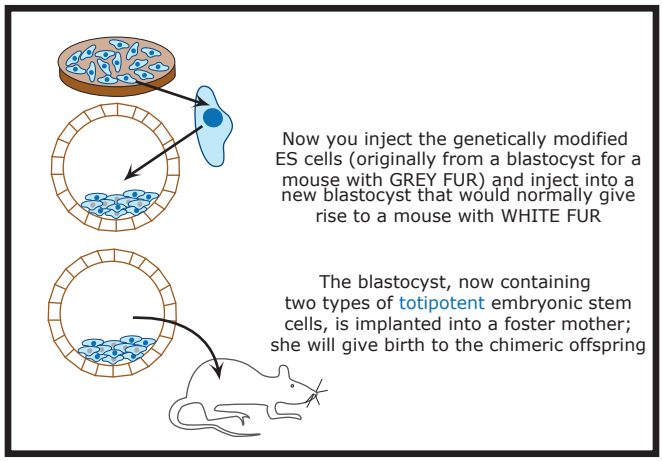
The first crucial development for this technology was being able to grow the **totipotent** cells from **preimplantation embryos** in culture in the lab; these are called **mouse embryonic stem cells (ES cells);** the crucial development was to devise a clever way to select integrated a DNA construct by **homologous recombination**.

Cells from the inner cells mass of a **preimplantation embryo** at the **blastocyst** stage could be removed and cultured in the lab without the cells losing their **totipotency**; i.e., even after being cultured in the lab for many years these cells can still be introduced back into a preimplantation embryo and go on to make all the tissues of a mouse. What this means, is that the cells can be genetically manipulated whilst in culture…and then put back into a mouse preimplantation embryo!!



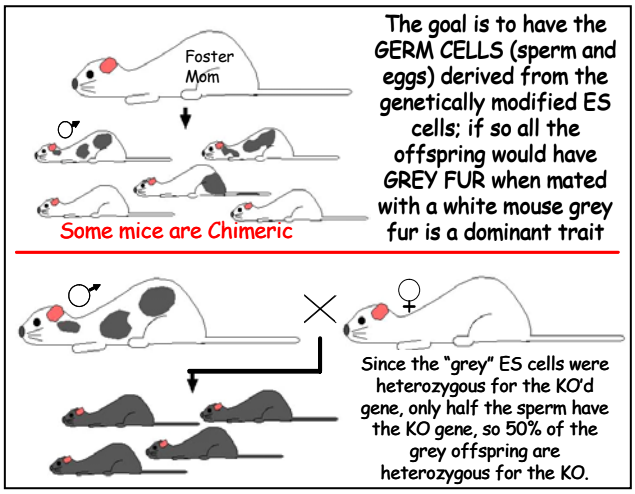


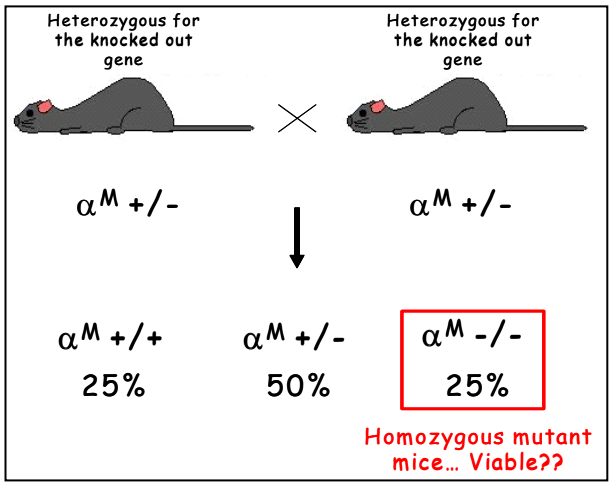




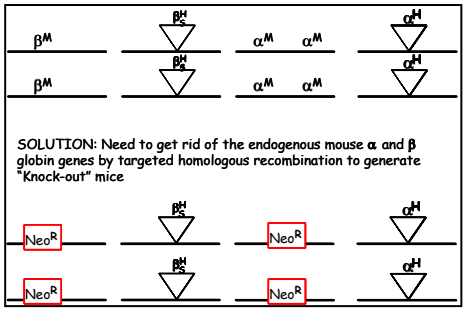
Figures by MIT OCW.

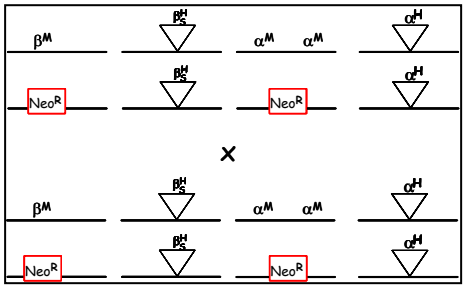
Essentially, once you have identified mouse ES cells (originally from a grey furred mouse) that have been genetically altered the way you wish…these cells can be used to generate a living animal that contains descendents from these **totipotent ES cells**. Lets see how you get from there to a mouse in which every cell contains that genetic alteration.

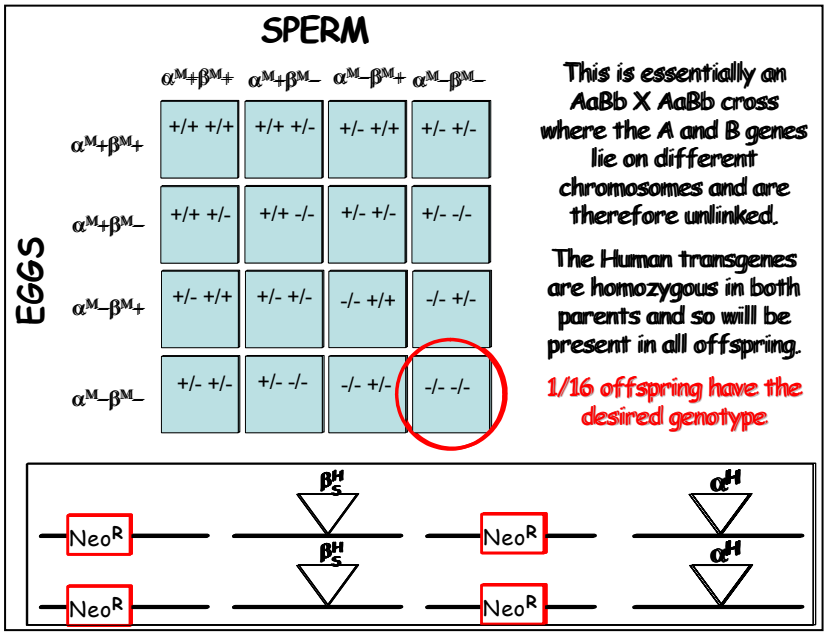




The blastocyts implanted into the foster mother will produce animals with varying contributions from the "white fur ES cells" and the "grey fur ES cells", the latter having been genetically manipulated to have an altered gene, e.g., a mutated **α-globin** gene. The crucial step is that the gonads be derived from the genetically altered "grey fur ES cells", because then the genetic alteration can be passed on to an offspring (which will have grey fur) in which every cell carries the genetic alteration. These offspring can then be crossed to generate a mouse that is homozygous for the altered gene. This can be done for generating mice with deletion mutations in the **α-globin** gene and then again for deletion mutations in the **β-globin** gene.

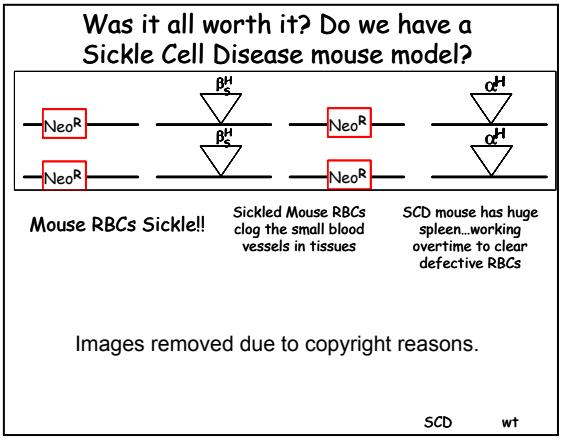






There are many different mating schemes that one could use to generate mice that are homozygous for deletions in both the **mouse α-globin** gene and the

mouse β-globin gene, and that also carry the **trangenes** encoding the **human α-globin** gene and the **human β-globin** gene with the sickle cell mutation. What I have shown you is just one way to obtain this mouse. It should be noted that after birth, this mouse ONLY expressed **human hemoglobin**, and the mouse is therefore said to be **humanized.**



The outstanding news is that this mouse does indeed represent an excellent model of **Sickle Cell Disease** which is now being used to explore therapies for SCD that are very difficult to carry out on human populations. So far, these mice have been used to explore the effectiveness of new drugs in ameliorating the tendency of RBCs to sickle. Moreover, the mouse has been used to test out **Gene Therapy** approaches to treating the disease. Both of these approaches have been successful in the mouse, paving the way for trying out these treatments in people.