

Epigenetics, What Is Wrong With Cloned Embryos and Levels Of Gene Regulation

(supplement to 7.013 lecture 26, April 13, 2007)
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A. Epigenetics.

In lecture, we developed the following ideas.

- Young embryonic nuclei are totipotent, while older nuclei lose potency, including somatic cell nuclei used for SCNT.
- Potency loss is not because the DNA sequence has changed, since even adult nuclei can be totipotent when put into the egg cytoplasm, and can make a normal embryo, albeit at low frequency.
- Nuclear potency is under EPIGENETIC control. “Epigenetics” means control of gene expression (specifically transcription), that it is not dependent on DNA base sequence.
- Epigenetic gene regulation is a very big deal in biology, and the subject of much current research, as it means that two organisms can have the identical DNA base sequence, and not express the same genes!!
- Epigenetics explains why “identical twins” are not entirely identical, and why cloned animals are not identical and are abnormal.
- Epigenetic control is due to modulation of chromatin structure, and whether genes are accessible for transcription (see B. below).
- In vertebrate animals (fish, frogs, chickens, mice, humans), DNA methylation is crucial to altering chromatin structure, and whether a gene is transcribed.
- Which genes are methylated and on which cytosines, changes during development. Thus the DNA of an embryo has a different methylation pattern than that present in each adult cell type.
- DNA methylation is also different in male and female germ cells, and in genes derived from the paternal or maternal genome later in development. This is referred to as “IMPRINTING”.
- (If you have heard of X chromosome inactivation, that is under epigenetic control also.)

So.....

- A normal embryonic DNA methylation pattern is required for normal development. This is because it allows transcription to occur at the appropriate time and in the appropriate cells, and thereby regulates correct embryonic gene expression.

B. Why animals derived by SCNT are abnormal.

Our discussion concerns vertebrate animals, on which SCNT and cloning has focused.

- Adult methylation patterns do not normally revert to embryonic patterns.
- SCNT requires that the adult nucleus acts as an embryonic nucleus, and therefore the adult nucleus needs to be reprogrammed.
- When the older nucleus is put into the egg cytoplasm the cytoplasm partially modifies (“reprograms”) the methylation pattern, but usually cannot “reset” it completely to the normal embryonic pattern.
- Thus, the chromatin structure of the donor nucleus is abnormal, and transcription is also abnormal.
- The resulting inappropriate gene expression in the cloned embryo cannot direct normal development, and cloned embryos are therefore abnormal.
- The goal of current cloning research is to figure out how to reprogram an adult nucleus to an earlier, embryonic state, and so to increase potency of that nucleus.

C. Levels of Gene Regulation.

How can we put together the idea of regulation of gene expression that is dependent on the DNA sequence, with the idea of epigenetic regulation and other levels of gene control? Strategies of gene control form a hierarchy, summarized below.

Level 1: Most important: DNA base sequence!!

- Mutations in genes can render them non-functional or incorrectly functional.
- These mutations can be in the promoter, coding sequence, or can affect splicing or translation of any gene.

Level 2: Second in importance: EPIGENETIC CONTROL.

- Chromatin (DNA packaged with histone proteins) must be “opened” in order to allow transcription of the DNA.
- This “opening” includes whether transcription factors can bind their target sequence, whether they can direct transcription initiation and elongation, and/or whether the DNA double helix can be unwound.
- In vertebrates, chromatin structure is largely dependent on methylation of cytosine in nuclear DNA.
- DNA methylation inhibits transcription, because it prevents modification of histones that would allow opening of the chromatin and transcription.
- (Don't need to know this for exam.) DNA methylation patterns can be created de novo, or maintained through multiple cell cycles by DNA methylases. How genes are chosen to be methylated de novo is not clear.

Level 3: Third in importance: all the other mechanisms we have discussed.

- These include transcriptional controls that regulate whether a specific transcription factor is present in a specific cell type.
- Post-transcriptional controls regulate whether the final gene product (usually a protein) is made from a transcript.
- These can include choice of splice sites, RNA stability, transport to cytoplasm, translation, protein stability, protein modification and export to the correct place.