

Epigenetic inheritance, imprinting, and chromatin remodeling

Reading: Chp 5: 117-118, sidelights and speculations: p. 207

The remaining classes in the course will deal with some related, medically relevant and important aspects of mammalian development: imprinting, stem cells, and cloning. Along the way, we will review some concepts from earlier classes.

Learning Goals

Explain the process of imprinting, comparing it to other kinds of local or chromosomal inactivation that we have studied.

Predict whether a gene is imprinted in the male or female germline dependent on the outcome of a cross.

Explain reasons why imprinting may have been selected for in mammals.

Epigenetic inheritance

Epigenetic inheritance (EI) refers to information that is not encoded in the DNA sequence but can be passed through mitosis from one cell generation to another, or meiosis, from one individual to another. A simple example of mitotic EI is maintenance of the gene expression pattern characteristic of a differentiated state in a tissue for many cell generations, after the transcription factors originally involved in specifying its fate are no longer synthesized (another example of this is how anterior boundaries of Hox gene expression are maintained in the *Drosophila* embryo after gap and pair-rule transcription factors are gone). Dosage compensation, discussed in the previous class, is a more extreme example of EI, involving most of the entire X chromosome in females. In imprinting, an epigenetic change introduced during gametogenesis affects gene expression in the next generation.

Until recently, EI was a mysterious phenomenon, hard to explain with the classical molecular biology of DNA replication and gene expression. It is still incompletely understood and is an area of intensive current research, but we now know that it involves chromatin remodeling, which is an important general control mechanism for gene expression.

Imprinting

Imprinting was revealed by the unusual behavior of alleles at a few unusual genetic loci in mice and humans. Alleles at these loci violate the general rule that the phenotypic consequences of an allele should be the same regardless of which parent it is inherited from. About 75 mammalian imprinted loci are now known (see list at <http://www.mgu.har.mrc.ac.uk/research/imprinting/>).

For example, in mice the *Igf2* gene encodes a growth factor, IGF-2, which acts to stimulate growth of the placenta, causing the fetus to be larger. If an embryo receives a deletion allele of *Igf2* (*Igf2* Δ) from its father, through the sperm, and a wild-type allele from its mother, through the egg, the placenta will be underdeveloped and the embryo will not survive. However, if the *Igf2* Δ allele comes from the mother and the wild-type allele from the father, the embryo will develop normally. In interpreting the results of crosses like this that involve imprinting, it is helpful to focus on which parent the wild-type allele is coming from, since the deletion allele is already non-functional. In this case, the wild-type allele must be somehow substantially or completely inactivated (imprinted) during oogenesis, but not during spermatogenesis. Consequently, in the first embryo above, there is essentially no *Igf2* function, while in the second, there is one active copy of the gene. As is true for most X chromosome genes because of X-inactivation, individuals only have one active copy of imprinted genes, since the other copy has been inactivated in either the sperm or the egg that gave rise to the embryo.

A more complex example from humans is a pair of diseases found in individuals who are heterozygous for deletion of a locus on one end of human chromosome 15. This loss can cause two quite different sets of symptoms, depending on whether the wild-type chromosome came from the father or the mother. If it came from the mother, offspring are obese and mildly mentally retarded with short stature (Prader-Willi syndrome). If it came from the father, offspring are severely mentally retarded and suffer from seizures and lack of speech (Angelman syndrome). This pattern suggests that there are at least two developmentally important genes at

this locus, one imprinted during spermatogenesis and the other during oogenesis. Loss of the gene imprinted in sperm causes the more severe phenotype.

Evidence for maternal and paternal epigenetic differences from pronuclear transplantation experiments.

Independent early evidence for imprinting came from early nuclear transplantation experiments on wild-type mouse embryos, when scientists developed procedures for removing one of the two pronuclei from a newly fertilized embryo, replacing it with a pronucleus from another embryo, and then reimplanting the embryo into the uterus for development. So long as the manipulated embryo contained a male and a female pronucleus, it developed normally. But surprisingly (at that time) embryos containing two male pronuclei (called androgenetic) had unusually large placentas and arrested embryonic development at the somitogenesis stage. Conversely, embryos with two female pronuclei (gynogenetic) had very small placentas and arrested embryonic development at a later stage. This result indicated that even though the genomes in the two pronuclei were wild-type and identical, they must have *epigenetic* differences in their ability to express some genes important for growth and development of the embryo.

In a final example: a mouse gene called H19, which is linked to Igf2, is imprinted oppositely to Igf2, i.e., it is imprinted during spermatogenesis. Embryos from an Igf2 Δ sperm and an Igf2⁺ egg resemble gynogenetic embryos (small placenta) in their developmental defects, while embryos from an H19⁺ sperm and an H19 Δ egg are more similar to androgenetic embryos (enlarged placenta). From this description, you should be able to infer the normal general function of the H19 gene. Interestingly, the H19 gene does not encode a protein, exerting its effects on development as an RNA.

Evolutionary implications of imprinting

Imprinting appears to be restricted to vertebrates, though invertebrates have related epigenetic mechanisms. Why should it have evolved? Evolutionary biologists have proposed a plausible model for evolution of imprinted genes in placental mammals, in which many (but not all) imprinted genes encode factors that affect growth of the embryo in the uterus. The argument is that males benefit from larger offspring, which will be more likely to thrive, reproduce, and spread their genes in the population. Since there are no demands on the males to carry the embryo, the larger the embryo, the better. Since females carry the offspring, smaller offspring lessen the physical and physiological demands of pregnancy and birth, therefore allowing them to produce more progeny to spread their genes. Therefore, males have evolved mechanisms for imprinting genes that limit fetal growth, while females have evolved mechanisms for imprinting genes that promote fetal growth. Under normal circumstances, the effects of these imprints balance out to produce normal sized embryos.

Molecular mechanisms of imprinting and chromatin remodeling in general

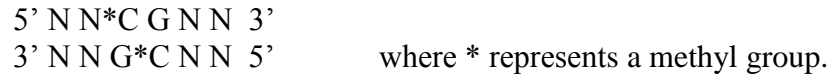
Imprinted genes remain inactivated throughout the lifetime of an animal in somatic tissues. The imprinting is erased only in the germ line, prior to gametogenesis in both sexes, so that during gametogenesis, the appropriate sex-specific imprints can be newly applied. For example, in a male mammal, all of its maternally derived chromosomes will carry maternal imprints until early in germ line development. At that point, all imprints are erased, and during spermatogenesis, paternal imprints are applied to all chromosomes.

At the molecular level, imprinting is simply a special case of the chromatin remodeling that occurs during gene silencing in development. We discussed these mechanisms briefly near the beginning of the course, and this is a good chance to review them.

The molecular basis of gene silencing in vertebrates is methylation of DNA and accompanying histone modifications that increase the local packing density and stability of nucleosomes, which in turn prevents transcription initiation of (i.e. silences) nearby genes. When developmentally regulated genes are silenced, their promoter regions are highly methylated and the local chromatin is condensed. Because DNA methylation and

histone modifications at a silenced locus can be propagated from one cell generation to the next, they represent epigenetic changes in the DNA.

How are these changes maintained? As you probably remember from previous courses, the cytosine CG dinucleotide sequences in double-stranded DNA can be methylated on opposite strands by DNA methylases, to give dimethylated sequences:



Methylation of a previously unmethylated sequence will occur only if a methylase is specifically recruited to this sequence. How recruitment works is not known, but we can guess that it occurs when inhibitory transcription factors bind to a silencer sequence (negative response element). Such a sequence will maintain its methylated state when the DNA is replicated, because although the two new DNA strands will initially have unmethylated Cs at these positions, there are additional ubiquitous methylases that recognize hemi-methylated GC sequences and add a methyl group to the unmethylated strand.

These methylases are thought to recruit other enzymes that modify histones so as to stabilize nucleosomes. These enzymes include histone methyl transferases (HMTs), which modify chromatin conformation by adding methyl groups to specific histone residues, and histone deacetylase (HDAC), which removes acetyl groups from histone lysine and arginine side chains, increasing the binding affinity for DNA. DNA methylase, histone methyl transferases, and HDAC may act as a complex to assure that the condensed state of a silenced region is re-established immediately after DNA replication. Finally, these complexes recruit Polycomb-like proteins (related to the Polycomb class of proteins active in *Drosophila* development) that somehow help to maintain the condensed state.

Many genes for specialty proteins are silent throughout development except during the differentiation process in a specific tissue, e.g. the β -globin gene in reticulocytes of the blood-forming system. The “un-silencing” of such genes generally occurs one cell generation before they are expressed, and is mediated by a demethylase that removes methyl groups from the DNA of the promoter region and histone acetyl transferase (HAT), which acetylates the charged side chains in histones to decrease their affinity for DNA.

In much of this course, we have emphasized short-term regulation of gene expression by combinatorial control involving activating and inhibitory transcription factors. However, keep in mind that this control operates primarily while cells are becoming determined or differentiated during early embryogenesis. Longer-term controls, such as maintenance of a determined or differentiated state in a particular tissue, are locked in more permanently by the epigenetic changes of chromatin remodeling.

X-inactivation in mammalian dosage compensation is an extreme case of remodeling, in which almost an entire chromosome is converted in the early embryo to heterochromatin, which is inactive in somatic tissues for the lifetime of the animal and is reactivated only in the germline during oogenesis.

Imprinting is a special case of remodeling which involves silencing of a few genes during gametogenesis in both sexes, in such a way that the silencing persists throughout the lifetime of somatic tissues in the next generation, and is reversed (erased) only in the germline prior to gametogenesis.

Imprinting and animal cloning

Although the cloning of a variety of different mammals by insertion of somatic nuclei into enucleated eggs represents a spectacular accomplishment of applied biology, the success rate of such experiments is generally only a few percent. One reason for this may be that the chromosomes in the inserted somatic chromosomes will carry parental imprints from the previous generation, which have not gone through the normal process of erasure and re-imprinting during gametogenesis. Better understanding of the imprinting process may reveal ways to increase these success rates.