**Introduction to Epigenetics**

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| Chapter title and time code | Transcript |
| 0:00: Overview | The field of epigenetics, whose definition is highly contested, broadly refers to the study of heritable differences in gene function that cannot be explained by DNA sequence changes. The term “epigenetics” was first introduced by Conrad Waddington in the 1950s, to explain how diverse cell types in the body could arise from one set of genetic material. Researchers have identified many processes thought to have an epigenetic basis, but there is still significant debate about many fundamental principles of the field.  In this video, we will highlight important discoveries in epigenetics, key questions being debated by epigeneticists, common tools used to answer these questions, and finally, some current research in the field. |
| 0:56: Historical Highlights | First, let’s review several key moments in the history of epigenetics.  In the 1930s, Hermann J. Muller observed a phenomenon known as position-effect variegation in *Drosophila*. He found mutant flies with mottled eyes, and linked this phenotype to the variable spread of condensed “heterochromatin” that silenced the gene responsible for eye color. This would be the first identified “epigenetic” phenomenon where a phenotypic change was observed without a corresponding change to the genetic sequence.  In 1959, Susumu Ohno observed in female rat liver cells that one of the two X-chromosomes was condensed. Two years later, Mary Lyon hypothesized that this condensed X-chromosome is genetically inactivated, that the choice of which X chromosome to be inactivated is random, and that this inactivation is stably inherited by the cell’s offspring. This process, now called X-chromosome inactivation or XCI, causes females to be biological mosaics.  In 1964, Alfred Mirsky published the earliest work on the role of histone modifications in gene regulation. Histones form the core of nucleosomes, which are the basic repeating unit of chromatin in eukaryotic cells. Mirsky studied how methylation and acetylation of histones affected RNA synthesis, and it is now known that numerous modifications alter the “activity state” of nearby chromosomal regions.  In 1975, Robin Holliday and his student John Pugh, and independently Arthur Riggs, proposed that methylation of CpG dinucleotides in DNA might be involved in stable epigenetic silencing, for example during XCI. Adrian Bird and colleagues lent further credence to this idea in 1985 by identifying clusters of unmethylated CpG sites throughout the genome that were later associated with transcriptionally active promoters. He would later also discover regulatory proteins that bind methylated DNA, eventually repressing transcription.  In 1984, Davor Solter, Azim Surani, and others observed that mouse embryos containing only maternal or paternal genetic material—created via nuclear transplantation experiments—did not develop normally. This marked the discovery of genomic imprinting, or parent-of-origin specific gene expression.  The first imprinted genes were discovered in 1991, where only the copy inherited from either the father or mother is ever expressed. One of these genes, H19, turns out to be rather unusual—its final product is a 2.3 kilobase RNA that does not get translated into proteins.  More of these “long noncoding RNAs” or lncRNAs were soon discovered, including Xist, which is required for shutting down the X-chromosome during XCI. Current evidence suggests that these RNAs may function as scaffolds to recruit regulatory factors. Today, researchers continue to work out how interactions among lncRNAs, DNA methylation, and histone modifications regulate epigenetic processes. |
| 4:34: Key Questions | Now, let’s turn to some questions being asked by epigeneticists.  At the most basic level, scientists are still actively studying the mechanisms by which epigenetic marks, such as histone modifications and DNA methylation, are created, removed, and interpreted. Researchers continue to characterize the enzymes that carry out these functions, as well as how the marks interact with the transcription machinery to activate or repress gene expression.  A deeper question that arises is whether there exists an “epigenetic code,” analogous to the well-defined “genetic code,” that dictates how information in DNA is translated into protein sequence. Researchers are trying to determine if the combination of epigenetic marks form a similarly predictive code that will one day make it possible to deduce the expression pattern of every gene.  Recently, scientists have been interested in the biological roles of lncRNAs. While a prevailing model is that lncRNAs help recruit epigenetic factors to specific genomic locations, their exact mechanisms, and whether all lncRNAs function similarly, are still being studied.  Finally, because epigenetic marks are chemical “add-ons” that are not simply replicated along with DNA, scientists are still trying to learn how the marks continue through cellular generations. Even more controversial is the potential trans-generational inheritance of certain epigenetic processes. Because it is observed that epigenetic marks are dramatically erased or “reprogrammed” early in embryogenesis, and again during gamete formation, how and whether these trans-generational phenomena actually occur remains hotly debated. |
| 6:30: Prominent Methods | Let’s now look at some tools being used in the study of epigenetics.  DNA methylation is most commonly detected by bisulfite analysis, a process to change unmethylated cytosine residues to uracil, which are then detected as thymine in sequencing reactions. Comparing sequences before and after bisulfite treatment allows researchers to identify the locations of methylated DNA. Another method to assay DNA methylation status is to digest DNA with methylation-sensitive restriction enzymes, which can only cut unmethylated DNA.  Immunoprecipitation, or pull-down techniques, are used to identify DNA or RNA sequences associated with specific features. Chromatin immunoprecipitation, or ChIP, isolates DNA bound by particular protein factors or histone modifications, whose sequence information can then be analyzed by PCR or sequencing.  On the other hand, methylated DNA immunoprecipitation, or MeDIP, is used for isolating and enriching methylated DNA. RNA immunoprecipitation, or RIP, and Chromatin Isolation by RNA Purification, or ChIRP, can respectively determine the protein partners of a non-coding RNA or its genomic binding locations. |
| 8:00: Applications | After reviewing the tools of epigenetics research, let’s look at some of their applications.  5-hydroxymethylcytosine, or 5hmC, a recently discovered, alternate form of cytosine methylation, has garnered much scientific interest. In this experiment, researchers performed a chemical reaction that specifically adds a glucose molecule to hydroxymethylated DNA, and then treated the DNA with restriction enzymes that either only cut unmethylated DNA, or cut all methylated DNA except when modified to be glucosylated-5hmC. This analysis, followed by PCR, allowed discrimination between types of methylation at specific DNA bases.  To understand the role of lncRNAs in epigenetics, researchers can perform ChIRP to identify their genomic binding sites. Here, DNA-RNA-protein complexes in cells were first tightly crosslinked with chemicals. Chemically tagged antisense oligonucleotides were then allowed to bind to the lncRNA, and antibodies against the tag were used to immunoprecipitate the complexes. DNA sequences that interact with the lncRNA were then identified by sequencing.  Finally, epigenetic processes such as X-chromosome inactivation can be studied at the single-cell level using microscopy-based techniques. In this experiment, fluorescence *in situ* hybridization for Xist RNA was combined with immunofluorescence against known histone modifications. The lncRNA and histone marks could then be “co-localized” to reveal possible functional relationships. |
| 9:50: Summary | You’ve just watched JoVE’s overview of epigenetics. In this video, we looked at the history of the field of epigenetics, some of the prominent questions and tools of the field, and specific examples of epigenetic research. As always, thanks for watching! |