**Chromatin Immunoprecipitation**

|  |  |
| --- | --- |
| Chapter title and time code | Transcript |
| 0:00: Overview | Chromatin immunoprecipitation, or “ChIP,” is a technique used by researchers to assess protein-DNA interactions. Protein factors play important roles in the gene regulation; not only do they organize DNA in chromosomes, but they also bind to specific DNA sequences—called regulatory sites—to activate or repress expression. During ChIP, chromatin—or the “packaging” of DNA by its associated proteins—is “immunoprecipitated,” meaning that it is isolated through the use of antibodies. With this method, researchers can assess which proteins associate with which DNA sequences.  In this video, we will review chromatin modifications and their roles in epigenetic regulation, and how ChIP can assay these modifications. We will then describe a generalized procedure for this technique, and finally discuss how scientists are using ChIP in research today. |
| 1:08: Chromatin Modifications and ChIP | Let’s begin by reviewing what chromatin modifications are, and how to study them with ChIP.  In eukaryotes, DNA is stored in nuclei by being “wrapped” around protein complexes. These proteins are histones, and each DNA-wrapped histone complex is referred to as a “nucleosome.” Gene expression is regulated by “nucleosome occupancy,” or whether a stretch of DNA is packed into nucleosomes. Transcribed DNA tends to be located in “nucleosome-free” regions, which allow proteins to associate with a gene’s regulatory sites, and enable RNA polymerase to carry out transcription.  Current evidence suggests that changes in chromatin structure that regulate gene expression are mediated by chemical modifications made to histones, usually in their freely moving “tails.” The most common of these are acetyl, methyl, and phosphate groups that are added to, or removed from, specific amino acids, and these different histone modifications are observed to be associated with different levels or modes of gene expression.  For example, the addition of three methyl groups to the 27th lysine residue in histone subunit H3—a modification termed H3K27me3—has been linked to gene silencing. Alternatively, the H3K9ac modification, where an acetyl group is added to the 9th lysine residue on histone H3, has been associated with gene activation.  Histone modifications are hypothesized to play a role in the epigenetic regulation of gene expression by marking regions of chromatin as “active” or “silent.” One mechanism by which histone modifications are believed to exert their effects is to recruit transcription factors or chromatin “remodeling” enzymes, the latter of which physically move the positions of nucleosomes.  With ChIP, specific histone modifications can be targeted by antibodies, which can be “pulled down” along with the surrounding DNA. Researchers can then employ PCR, microarrays, or sequencing to identify DNA regions associate with histone modifications of interest. By varying the antibodies used during ChIP, this technique can also help pinpoint DNA regions bound by transcription factors and other regulatory proteins. |
| 3:47: Generalized ChIP Procedure | Now that you know the principles behind ChIP, let’s go through a generalized procedure for this technique.  To begin, cells of interest are collected and treated with chemicals like formaldehyde, which act as “cross-linking” reagents and help affix proteins to the DNA sequences they associate with by facilitating the formation of covalent bonds between them. Care must be taken to not “over treat” cells with formaldehyde, as this can impact the ability of antibodies to recognize their target histone modifications at later ChIP stages. To stop the cross-linking process, glycine is added to the formaldehyde solution with which cells are being treated. The cells are then collected and lysed to release the chromatin.  To solubilize chromatin and precisely define the DNA regions that associate with modified histones, chromatin is mechanically “sheared” into smaller pieces using sound waves—a process called sonication. Typically, scientists aim to create chromatin fragments 200 to 1000 base pairs in length. Once chromatin fragments of the desired size are generated, an antibody is added to the solution, and the mixture is incubated to give the antibody time to recognize its target histone modification.  Magnetic beads to which the antibodies can bind are then introduced into the mixture, immobilizing the antibody-associated chromatin complexes. The beads are collected through the use of magnetic racks, and washed several times to rinse off any unbound chromatin or antibodies.  To release the chromatin from them, beads are placed in a solution containing the detergent SDS, and after collecting the beads with a magnet, the supernatant is kept. The enzyme proteinase K is then added to this solution to degrade all proteins, including histones, so that the DNA component of the chromatin can be isolated. The resulting DNA is then purified and analyzed. |
| 5:52: Applications | Let’s now take a look at how scientists are currently using ChIP in their labs.  Many researchers use ChIP to evaluate changes in histone modifications brought about by extracellular signals. Here, cultured human cells were treated with a specific cytokine, or signaling molecule, and changes in histone methylation were assessed. By assaying ChIP DNA with real-time PCR, researchers determined that—in response to treatment—the transcription factor-encoding gene *IRF1* gained an activating histone mark, H3K36me3. This modification enabled both a regulatory protein and RNA polymerase II to associate with *IRF1*, resulting in its transcription.  ChIP can also be used to gain insight into changes in gene expression that occur during tissue injury and regeneration. In this experiment, researchers damaged a component of the peripheral nervous system in mice—the sciatic nerve—and then used ChIP to look for novel DNA-protein interactions in other peripheral nervous system structures, like the ganglia that flank the spinal cord. Scientists concluded that following nerve injury, the p53 protein, which is a regulator of DNA repair, became associated with a gene implicated in tissue regeneration, *GAP43*.  Finally, some scientists are working towards streamlining ChIP procedures to increase the throughput and efficiency of experiments. Here, researchers were able to “automate” ChIP, having a machine perform many of the steps in the protocol. This allowed researchers to simultaneously assess DNA associated with several different histone modifications, using a relatively small number of cells—10,000—yielding results similar to those seen with larger cell numbers, or with standard, “manual” ChIP techniques. |
| 7:59: Summary | You’ve just watched JoVE’s video on chromatin immunoprecipitation. In this video, we’ve discussed how DNA and proteins together form chromatin, and the steps of a protocol, called ChIP, that can be used to identify DNA sequences associated with specific chromatin states or proteins. We also explored how researchers are using and modifying ChIP to better understand the role of DNA-protein interactions during gene regulation. As always, thanks for watching! |