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Detection of Acetylated and Non-Acetylated Histones in Chromatin

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The control of gene expression is fundamental for the differentiation of cell lineages and for these lineages to maintain their identity throughout the lifespan of an individual. Maintaining cellular identity is of primary importance in humans if continued functions are to be carried out correctly. The transcriptional state of a gene in a particular cell is usually determined by the presence or absence of appropriate activator and repressor proteins, termed transcriptional factors, in the promoter region of that gene. Two complementary mechanisms control whether a gene is receptive or not to these factors. These mechanisms involve methylation and acetylation. Methylation of DNA occurs at CpG islands where it allows epigenetic (something that influences the behavior of a cell without directly affecting its DNA sequence) control of gene expression. Methylation occurs on cytosine residues of DNA; the product 5-methylcytosine results in gene silencing. The methylated regions attract proteins, which catalyze the removal of acetyl groups from histone tails resulting in the condensation of DNA around the nucleosome. This puts the gene in a state that cannot be accessed by RNA polymerase and other proteins required for transcription, (the first step in gene expression) [1,2]. Thus, for many differentiation-specific genes, transcriptionally inactive chromatin can be chemically distinguished from active chromatin on the basis of acetylation on lysine residues of core histones and by the presence of methylated CpG islands in the gene itself. In-activation of an entire chromosome is linked to substantial decreases in the acetylation state of core histones. This can be demonstrated by the inability of acetylated histone antibodies to bind to an inactive X chromosome versus the avid binding to the active X chromosome [3].

In contrast to differentiation-specific genes where DNA methylation status is stable and determined early in embryogenesis, many other genes, whose expression levels need to be constantly adjusted in response to environmental conditions, histone acetylation alone appears to be a major mechanism for transcriptional activation. Here transcriptional factors activated by, for example hormones, attract histone acetyltransferase which adds an acetyl group to the amino group of lysine residues in core histones, blocking the positive charge and relieving DNA condensation.

It is now well established that cancer is due to the activation of genes known as oncogenes, and the inactivation of genes known as tumor suppressor genes. Inactivation of tumor suppressor genes frequently involves classical mutations leading to loss of function but in an increasing number of cases, has been shown to involve epigenetic mechanisms of gene silencing via methylation of CpG islands [4]. The ability to identify regions of chromatin at high resolution which are transcriptional active or silent on the basis of these chemical modifications could have a major impact on cell biology and may have clinical use.

Analysis of pure samples of both acetylated and non-acetylated histones with a Thermo Nicolet dispersive Raman spectrometer using wavelengths of 532 nm and 785 nm reveals spectral differences exist. Figure 1 shows the acetylated histone has a left shifted peak centered at 2940 cm^{-1} compared to the non-acetylated peak occurring at 2932 cm^{-1}, under 532 nm excitation.

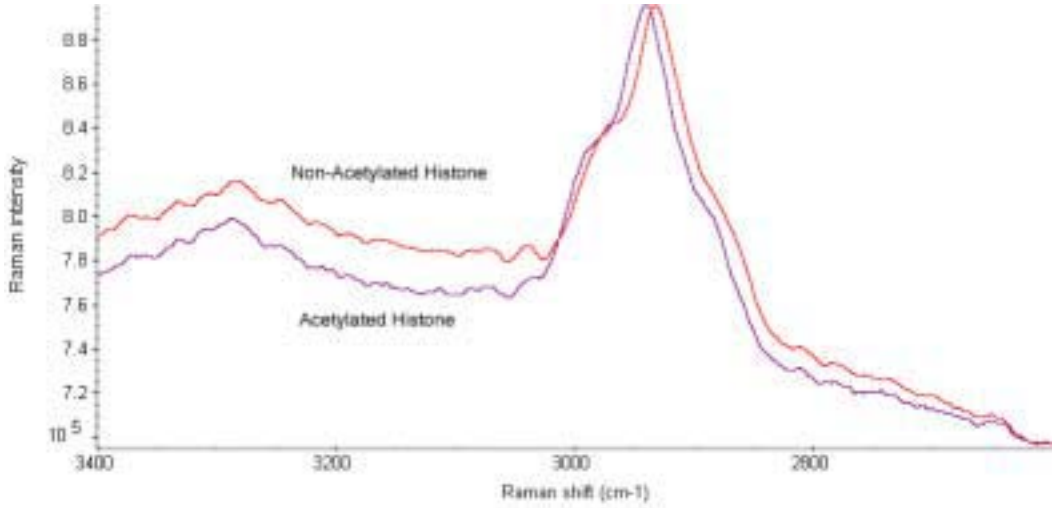

Fig 1. Raman spectra of acetylated and non acetylated histones under 532 nm laser excitation.

Figure 2 shows the same two samples but exposed to the 785 nm laser wavelength. Numerous Raman peaks, centered around 2932 cm^{-1}, 1666 cm^{-1}, 1434 cm^{-1}, and 1236 cm^{-1}, were detected indicating reduced Raman cross-section due to acetylation. Using the Thermo Nicolet confocal Raman spectrometer, we will show this spectral differentiation can be applied to detecting active from inactive genes.

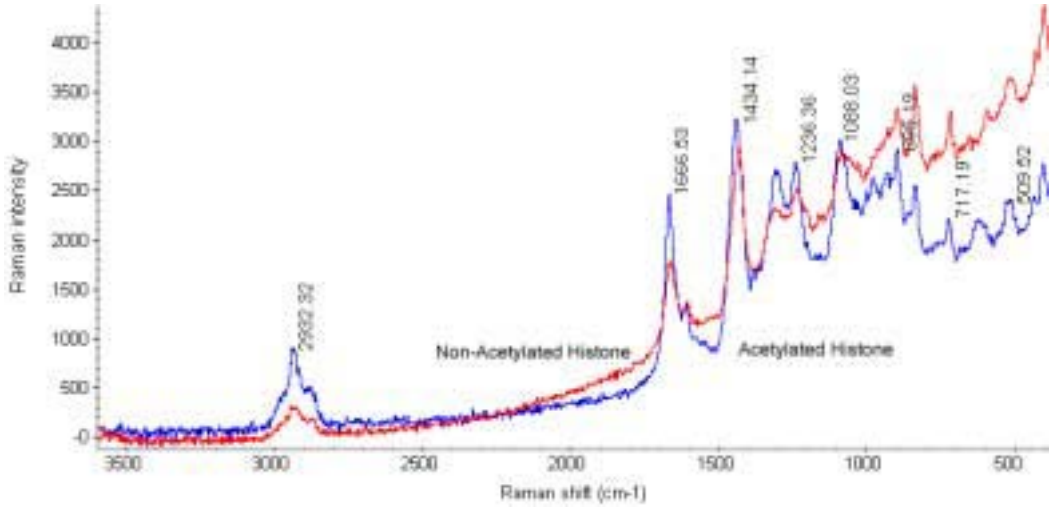

Fig 2. Raman spectra of acetylated and non acetylated histones under 785 nm laser excitation.

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