MOLECULAR ANALYSIS OF HEPATIC GENE SILENCING

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ABSTRACT

It is well known that genes are turned on (activated) by specific biochemical processes. Recent results suggest that, for some genes, mechanisms exist to turn off (silence) gene expression. Our hypothesis is that these silenced genes are unable to interact with essential proteins. We wish to test this hypothesis using a powerful newly developed technique called the Chromatin Immunoprecipitation (or "ChIP") assay. The ChIP assay provides information that allows one to construct testable models to explain gene silencing.

We are developing the ChIP assay in order to determine whether a liver protein termed Hepatocyte Nuclear Factor 1 (HNF1) is allowed to bind to the silenced liver specific alpha 1 antitrypsin (a1AT) gene. HNF1 is known to be required to express the a1AT gene in the liver, but HNF1 is incapable of activating expression of the a1AT gene in certain cultured rat cells that our laboratory has developed. A critical step in the ChIP assay requires that the DNA extracted from our desired cell line be sonicated into fragments that between 200 and 1000 base pairs in length. In order to optimize DNA sonication for the ChIP assay, samples were sonicated, deproteinated and then analyzed by agarose gel electrophoresis to determine their size. The most optimal sonication treatment found was eight rounds of ten second pulses. This optimized procedure is now being used to optimize additional steps in the ChIP assay. These results will lead an understanding of mechanisms of gene silencing in mammals.

INTRODUCTION

HNF1α is a liver-enriched transcription factor that activates a large number of liver-specific and β-cell specific genes (1, see Fig. 1). Control of gene expression is the foundation of life as organisms develop and respond to their environment (2). The generally accepted reason for the silencing of gene expression is simply that they have never been activated by the body. However newer evidence points out that this is far too simplistic to explain gene control in many situations. The most likely explanation for gene silencing is that missing factors that are required for activation are not present (1). Previous introduction of these factors has not resulted in gene activation (3-4). However, the ChIP assay allows an opportunity to study to mechanism of gene silencing that was not previously available (5-6, and see fig. 2). It allows one to determine what proteins are interacting with a gene at any given point in time. It will allow us to determine which specific factors able to bind to a silenced gene. In order to analyze the a specific gene, genomic DNA has to be sheered to 200-1000 base pair sizes.

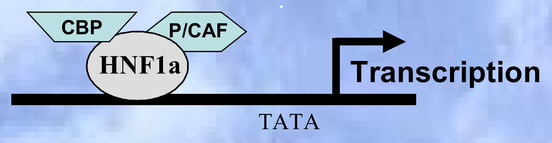
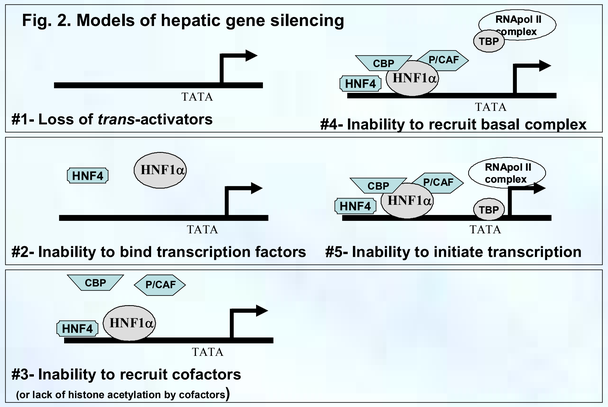


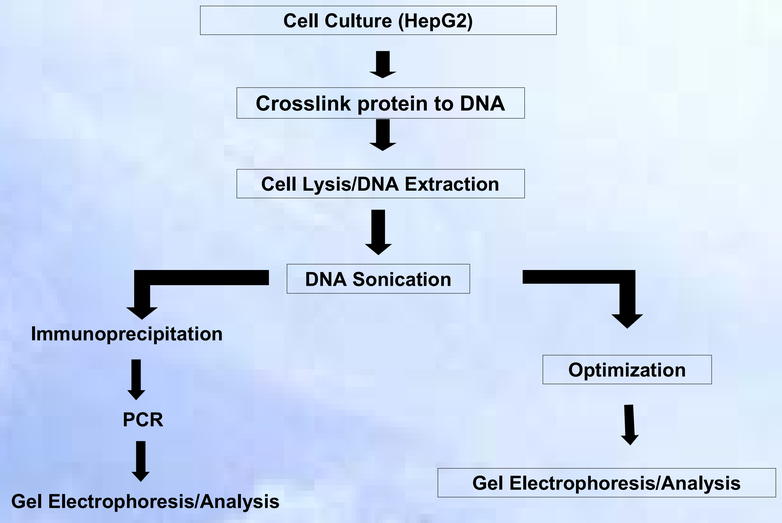
Fig. 1. Multiple coactivators interact with HNF1α to activate transcription of the a1-antitrpsin gene



METHODS

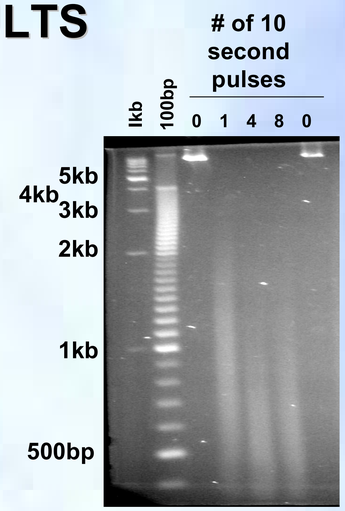
To carry out the ChIP assay, cultured cells were grown to 80-90% confluency in 100cm culture dishes. DNA was covalently crosslinked to proteins using formaldehyde. The cells were then lysed using SDS lysis buffer and scraped into a microfuge tube. Cell lysates (500ul) were then sonicated using a variety of conditions. Aliquots were taken after each sonication protocol. In order determine the correct number of sonication pulses, aliquots were taken after zero, one, two, four and eight rounds of 10-second sonications. Samples were deproteinated with protease, RNA removed with RNase A, then the aliquots were run out on an agarose gel with 1000bp and 100bp ladders. The gel was then analyzed to determine optimal sonication parameters.

Fig. 3. CHIP ASSAY PROGRESSION



RESULTS

Fig. 4. DNA Size Fragments After Variable Rounds of Sonication. Crosslinked DNA was either untreated or sonicated for 1, 4 or 8 rounds of 10 second pulses. DNA was deproteinated, treated with RNase A then analyzed by agarose gel electrophoresis



CONCLUSIONS

DNA sonication is an reliable method for sheering DNA to the size desired range for use in the ChIP assay. Results suggest that the optimum DNA length was produced by sonicating the DNA for eight ten second pulses at one third the maximum power level. Further experimentation is being conducted to ensure that the sonication procedure is adequate for immunoprecipitation and final steps of the ChIP assay.

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