ChIP Analysis of Hepatic Gene Silencing

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ABSTRACT

A better understanding of the complex mechanisms responsible for activating and silencing cellular genes is required to develop strategies to prevent and treat disease. We have been developing the chromatin immunoprecipitation (ChIP) assay to examine the binding of liver-enriched transcription factors to liver-specific gene promoters in cell culture model systems. To test the applicability of this assay, we have used formaldehyde to crosslink the DNA to the bound protein in the hepatoma cell cultures followed by isolation and sonication of the DNA-protein complexes. The complexes are then immunoprecipitated using antibody to the transcription factor HNF1α and the attached DNA amplified using PCR. Using this technique we show optimization of key steps in the procedure and verify HNF1α binding to the human alpha-1 antitrypsin promoter in hepatoma cells. We will extend our studies identify which transcription factors are binding to liver gene promoters in cells which fail to express liver function despite forced expression of liver-enriched transcription factors. Thus, the ChIP assay can be utilized to study protein-DNA interactions on the regulatory regions in our cell culture system to ask questions concerning the mechanisms of gene silencing and activation.

INTRODUCTION

HNF1α is a liver-enriched transcription factor that activates a large number of liver-specific and b-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 are required for 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 are required for silencing that was not previously available (5-6, and see fig. 2).

RESULTS

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.

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. DNA was deproteinized 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.

CONCLUSIONS

REFERENCES