Digestion of Oligonucleosomes with Micrococcal Nuclease

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Abstract

INI1 is an important protein in the Switch/Sucrose Non-Fermentable (SWI/SNF) complex. It is related to Malignant Rhabdoid tumors (MRT) that appears in infants. Its function is still a mystery, but in children that have MRTs, INI1 is missing or truncated. In this experiment, we digested chromatin from natural cells with Micrococcal nuclease (MNase) so that in future experiments we can run an EMSA to determine if INI1 binds to mono-nucleosomes. Two separate experiments were run with samples containing long oligonucleosome (LON) and different concentrations of MNase. After running titrations of differing concentrations of MNase, it is my conclusion that the proper concentration of MNase to digest chromatin to a single nucleosome is 1×10^{-5} units/microliter (units/µL). This concentration produced more mono-nucleosomes and less product of other species.

Keywords: Micrococcal nuclease, Oligonucleosome, Digestion, Titration

Introduction

Malignant rhabdoid tumor (MRT) is a very aggressive tumor that appears in infants, usually emerging in the child any time from birth to the age of two (Biegel *et al.* 1999). Typically, MRTs will appear in the infant's central nervous system or kidneys, but they can appear practically anywhere in the body (Jackson *et al.* 2009). Biegel *et al.* (2002) and Klochendler-Yeivin *et al.* (2000) both found that the INI1 protein (a tumor suppressor) within chromosome 22 is key for initiation of MRTs. INI1 is one of 9-12 proteins (Biegel *et al.* 1999) found in the switch/sucrose nonfermenting (SWI/SNF) ATP-dependent chromatin remodeling complex.

The SWI/SNF complex in particular makes it possible for the nucleosome DNA (chromatin) to be easily accessible (Becker and Horz 2002), and this makes it possible for transcription to happen (Biegel *et al.* 1999). Chromatin is a complex structure of DNA wrapped around histones (proteins), which connect together in fours and then connect to other structures, i.e. other histones, DNA, or nonhistone proteins. A nucleosome is the DNA wrapped around the histones; the individual nucleosomes are connected together with linker DNA. Chromatin is the collection of these nucleosomes and linker DNA (Tymoczko *et al.* 2010). The process of chromatin remodeling by the SWI/SNF complex is necessary for all cellular development (Becker and Horz 2002) and organogenesis (Klochendler et. al. 2000). Without the SWI/SNF complex, transcription activity would not happen properly, suggesting the function of SWI/SNF is more complex than a one single action during transcription (Sudarsanam and Winston 2000).

INI1 in particular is involved with early development of cells and organisms, as well as all other development in an organism (Klochendler *et al.* 2000). As well as being capable of nucleosome remodeling (Sudarsanam and Winston 2000). Children who are diagnosed with MRT will have SWI/SNF complex's that lack the protein INI1 or have mutations of the protein. The mutations of INI1 can either be heterozygous or homozygous, meaning the protein will either have the same mutations on both chromosomes, or the mutations will be different on each chromosome. Many doctors misdiagnose children that have MRT with medulloblastoma or primitive nueroectodermal tumor, because MRT's resemble primitive nueroectodermal tumors (Biegel *et al.* 1999). Since the function of INI1 is still unknown, further research on the protein will contribute greatly to the scientific world and help to decrease the misdiagnoses of MRTs, as well as increasing the effectiveness of treatment for MRTs. In the following research, the most effective concentration of MNase to use to digest chromatin to a single nucleosome is studied. The results from this experiment will lead to future studies on the ability of INI1 to bind to a mono-nucleosome.

Methods

LON were obtained from HeLa cells and 2 microliters (µL) were added to each sample. Each sample contained 22 µL of reaction buffer, 1 µL of MNase and 25 µL of water. The control sample with

no MNase contained 26 µl of water. The concentrations of MNase were from 5×10^{-2} to 5×10^{-6} units/µl. The stock reaction buffer contained 3.4 mM $CaCl_2$, 7.95mM $MgCl_2$, 0.22 mg/mL BSA, and 93.18 mM Tris. The MNase storage buffer contained 50 mM tris pH of 8, 50% glycerol, 0.05 mM $CaCl_2$, and 0.1 mg/mL BSA. The final concentrations of samples were 43.1 mM Tris, 1.3 mM $CaCl_2$, 0.099 mM BSA, 7.76% glycerol, and 3.02 mM $MgCl_2$. After adding all the reagents to the samples, the final concentrations of MNase were 5×10^{-3} to 5×10^{-7} units/µL. After all the reagents were added, the samples set in a water bath for 30 minutes at 30° C. The digestion was stopped with 1.6 µL of .5 M EDTA. After digestion was stopped, samples were made with 5 µL 50% glycerol and 13 µL of product. A sample of 5 µL of KB and 5 µL of loading dye was also made. Samples were made to run on a 2% argarose gel for 45 minutes.

I repeated digestion with different amounts of MNase with the concentrations of 5×10^{-4} and 5×10^{-5}. The resulting final concentrations were from 1×10^{-6} to 1×10^{-5} units/µL. Samples were made to run on the gel similar to samples made for the 2% agarose gel. The samples were run on a 6% native gel for 45 minutes. The native gel contained 3.53 mL of water, 0.8 mL of 50% glycerol, 2 mL 1x TBE, 1.59 mL 30%Acrylamide mix, 0.04 mL APS, and 0.016 mL TEMED.

Results

In Figure 1, lanes three and four display banding at the bottom of the lanes, indicating that a species has run through the gel very quickly and is collecting at the bottom. Digestion has occurred in these two lanes and mono-nucleosomes are left at the bottom of the lane. In Figure 2, there are bands at the bottom of nearly all the lanes. Mono-nucleosomes are collecting at the bottom of these lanes. However, lane four exhibits less streaking and does not have a band at the top of the lane. The majority of the species in the lane has collected at the bottom.

Discussion

In Figure 1, lanes one and two show that there are little to no nucleosomes. This was expected because MNase was added in such a high concentration. Therefore, the MNase digested all of the chromatin including the nucleosomes. However, it was unexpected that lane six did not show up. This is a result of an error made in the experimental process.

In Figure 2, it was expected to see bands on the bottom of nearly all the lanes. because the concentrations used were between two concentrations

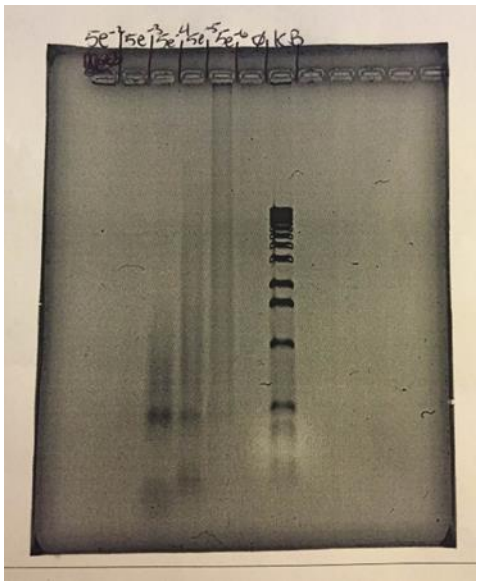

Figure 1 *- Ten-fold titration*
Left to right: 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, $5x10^{-6}$ units/uL of MNase, no MNase, KB ladder

that were known to produce mono-nucleosomes. The bands that are at the top of the lanes were expected as well because native gels are more selective about what can go through them. Smaller species are able to go through better than larger items. Therefore, the bands at the top are more likely larger fragments of nucleosomes. In lanes two and five, there appears to have been another problem with either loading of the gel or the experimental process.

The bands in the bottom of lane three and four on Figure 1 indicates that the oligonucleosomes were digested to mono-nucleosomes. In lane three and four, the concentration of MNase used was 1×10^{-4} and 1×10^{-5} units/µL. Therefore, the best MNase

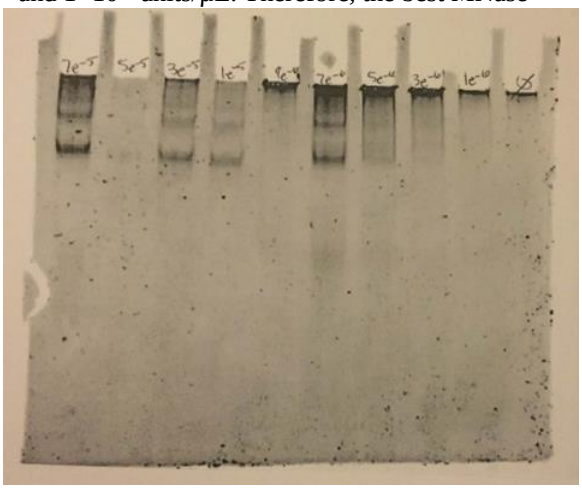

Figure 2 *- Narrow MNase concentration for digestion*
Left to Right: 7×10^{-5}, 5×10^{-5}, 3×10^{-5}, 1×10^{-5}, 9×10^{-6}, 7×10^{-6}, 5×10^{-6}, 3×10^{-6}, 1×10^{-6} units/uL of MNase, no MNase.

concentration to use to digest oligonucleosomes to mono-nucleosomes is somewhere between these two concentrations. Therefore, I ran another titration between these two concentrations. The bands on the bottom of the lanes in figure two indicated the oligonucleosomes were digested to mono-nucleosomes. However, the best concentration of MNase to use is 1×10^{-5} units/µL, because the lane shows less streaking and does not have a band at the top the lane. This indicates that the MNase digested the oligonucleosomes to completion. It did not leave many species that were not mono-nucleosomes. The majority of the species at the bottom of the lane are mono-nucleosomes.

The goal for this project is to determine if INI1 will bind to a mono-nucleosome. This is important for understanding the function of INI1. Currently, it is known that INI1 binds to naked DNA and artificial mono-nucleosomes (Steele and Ruhl 2016). However, if it can be proven that INI1 binds to true nucleosomes, then this will support the theory that INI1 is involved with the remodeling of DNA. The next step in this project to run an electrophoretic mobility shift assay (EMSA) to determine if INI1 will bind to natural mono-nucleosomes.

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