Final Project

BIOL 463 – Gene Regulation in Development

Is DNA Polymerase α, a component of the eukaryotic replisome, necessary for the deposition of H2A and H2B onto nascent DNA?

Background Information:

Replisome Proteins

The replisome is a complex of proteins in eukaryotic cells that carries out the replication of genetic material in eukaryotic cells. It consists of many different proteins, only a few of which are of interest to this paper and will thus be discussed in this section. DNA polymerase α (Pol α) is a component of the replisome in eukaryotic cells that initiates the formation of Okazaki fragments during lagging strand synthesis. The FACT complex (FAcilitates Chromatin Transcription) allows transcription of the DNA, and is believed to help stabilize the nucleosomal structure throughout the process of replication. CMG Helicase assists in the unwinding of the DNA to allow replication proteins to continue through the replication fork. The amino terminus of Pol α (Pol1) binds to the mcm2 subunit of CMG Helicase and FACT, connecting them in the replisome. FACT and the mcm2 subunit have both been shown to bind Histone proteins H3 and H4.

Introduction

A recent study has shown that Pol1 also contains a highly-conserved binding motif that is specific for Histone proteins H2A and H2B, and is also necessary for maintaining gene-silencing in telomeric and mating-type loci following replication (Evrin et al. 2018.) This was shown by conducting microarrays of different gene expression in *Saccharomyces cerevisiae* cells that were WT for the histone-binding ability of Pol1, and in cells that were mutants for the histone-binding ability. It was further shown that disrupting the histone-binding motif is not necessary for propagating DNA synthesis. One inference that can be made from this is that the histone-binding abilities of Pol1 are used at some point after the replication of DNA.

It is still unclear how this process works to maintain gene-silencing following replication. As Pol α is bound to the mcm2 subunit and FACT, and one possible inference is that Pol1 binds histones after replication, it is possible that these three proteins assist in the formation of

nucleosomes following transcription by assisting in the deposition of histone complexes. The purpose of this experiment is to explore whether Pol α is necessary for the proper formation of nucleosomes following transcription.

Relevance and Impact:

Although some histone chaperones have been shown to deposit histones onto newly-replicated DNA (Park et al. 2008), there is still much that is unknown about this complicated process. It is possible that the DNA replication machinery (e.g. Pol α) plays a dual role in stripping histones and proteins off the DNA during replication, and replacing them immediately after. There is little research done in this field, although it is an important process to understand.

Understanding of the functions of the replisome machinery will help pave the way for future studies that investigate the role of chromatin in mediating epigenetic inheritance. Characterizing the proteins responsible for the reassembly of nucleosomes after replication will allow future researchers to study modes of inheritance, and investigate how epigenetic markers are conserved across generations of cells. This experiment should provide a deeper understanding of the functions of the replisome components in *S. cerevisiae*, which will provide a useful foundation for exploring other eukaryotic systems with more complex regulatory systems, such as plants and eventually humans.

In turn, understanding the mechanisms behind epigenetic inheritance and the formation of chromatin architecture may allow future researchers to develop a wide variety of techniques and concrete applications. For example, future studies may investigate how to modify epigenetically silenced genes or prevent these genes from being silenced by modifying the proteins responsible for their silencing.

Hypothesis and Predictions:

It has been established that proper binding of the Pol1 subunit of Pol α to histones is not necessary for the proper replication of DNA strands, but it is instead necessary for the proper silencing of genes at telomeric and mating-type loci (Evrin et al. 2018). As such, one logical inference would be that the histone-binding ability of the Pol1 subunit is necessary for a step in the replication process that occurs after the strands have been replicated.

It is also known that the Pol1 subunit of Pol α contains a conserved binding site for histone proteins H2A and H2B, and also binds to FACT and the mcm subunit of CMG helicase (both of which have been shown to bind H3 and H4) (Evrin et al. 2018). Histone complexes were also found to be bound *in vivo* to Pol α, FACT, and CMG helicase (Martin et al. 2018). As these three replisomes proteins are linked together in the replisome and have been found to simultaneously bind histone complexes, it is possible that the three of them work in concert to deposit histones

onto nascent DNA following replication. The proper deposition of histones is required for the formation of nucleosomes, and is thus the foundation of chromatin architecture.

Given these, the predicted outcome of this experiment is that Pol α is necessary for the proper formation of nucleosomes. Mutations of Pol α will result in a lack of deposition of histones onto nascent DNA, and thus prevent the proper condensation of chromatin. It is also known that linker DNA (DNA not bound by histones) is much more accessible to these enzymatic proteins, and is thus much more susceptible to digestion (Heins et al. 1967). As such, if Pol α is necessary for the proper formation of nucleosomes by assisting in histone deposition, genetic material extracted from cells with a mutation in the histone-binding ability of Pol α will be more susceptible to digestion by nuclease proteins.

Experimental Procedures:

I will use a line of *S. cerevisiae* cells as the system to investigate whether Pol α is necessary for the formation of nucleosomes their replisome has been well-characterized, and they have been the system of choice for the previous studies that this experiment is founded on. They are a practical choice as they are eukaryotic cells with a short turnover time, and provide a suitable model for more complex eukaryotes that can be studied further down the line.

To study the necessity of Pol α, I will develop two lines of cells: one cell line that is WT for Pol α as the control, and one cell line with a mutation in Pol α as the variant. The WT cell line is an important control as it will set the base level of nucleosomes present in *S. cerevisiae*. A decrease in the amount of nucleosomes present in the variant line can thus be attributed to the mutation of Pol α.

All cells will be arrested in the G1 phase through treatment with alpha factor, and be allowed to proceed through S phase synchronously. After the cells have had sufficient time to replicate their DNA, they will be arrested in the G2 phase. Allowing cells to proceed through S phase and arresting them at the same point in time will minimize error caused by cells having different amounts of DNA because of asynchronous DNA replication. After the cells are arrested in G2 phase, cells will be cross-linked, lysed, and their genetic material extracted. Cross-linking will ensure that the DNA is bound to histones and remains in the same form that it would have been *in vivo*. Lysing the cells is necessary for extracting their genetic material, which will then be further analyzed.

MNase (Micrococcal nuclease) is a nuclease commonly used to map nucleosome position on *S. cerevisiae* by digesting DNA that is not bound by histones. DNA that is bound by histones or other regulatory proteins are protected from MNase digestion (Herrmann et al. 2017). Incomplete MNase digestion results in a DNA ladder on an agarose gel where the bands indicate the amount of mononucleosome, dinucleosome, trinucleosome, etc. If the reaction is carried to completion, only the band indicating the mononucleosome would be present on the agarose gel

analysis. A typical MNase digestion is pictured below. If the genetic material is more accessible to the MNase (e.g. if there is a higher concentration of MNase, if the reaction is allowed to proceed longer, if there are less histones etc.), then you would expect less of the higher number of nucleosome fragments, and more mononucleosomes (as the DNA would have been much further digested.)

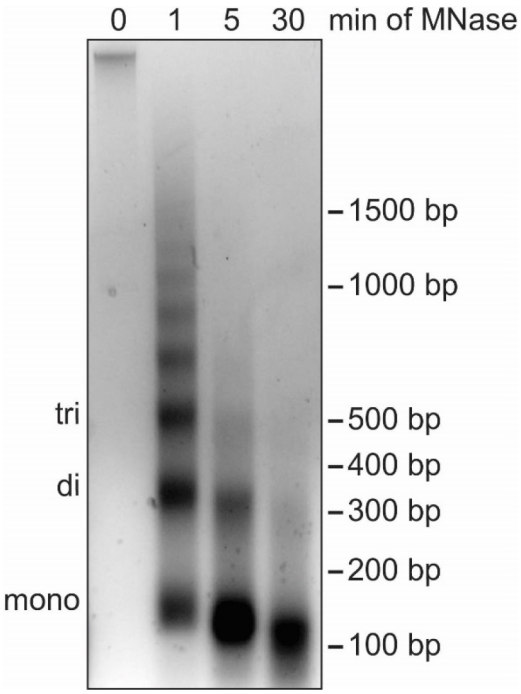

Figure 4 from Herrmann et al. showing an agarose gel analysis of MNase-digested DNA. Undigested DNA (0min MNase digestion, lane 1) shows that the DNA is not degraded by any endogenous enzymes. The position of the bands on the gel in the succeeding lanes corresponds to the mono-, di-, tri-, and polynucleosomes. Bands are located within intervals of approximately 150bp (the length of one nucleosome.) With increased time of MNase digestion (increased exposure to MNase,) less of the higher-order polynucleosomes are present, and more of the lower-order or mononucleosomes are present.

The genetic material extracted from the *S. cerevisiae* cells will then be subject to MNase digestion. Genetic material from each sample will be subject to MNase digestion for different time points (0, 1, 2.5, 5, 10, 20 minutes), and one sample will also be left without any MNase. The 0-minute sample is an important control to show that the starting genetic material is present and undigested. The sample without any MNase is a control to show that the genetic material is being digested only by the MNase added as part of the experiment, and not by any endogenous nucleases present in the cell. DNA from both lines will be digested for varying lengths of time to show how quickly the DNA is being digested, giving us an idea of how accessible the DNA is to the MNase (and therefore whether or not the nucleosomes were properly formed).

Results and Interpretations:

Result #1: There is an increase in DNA digestion in the Pol α mutant when compared to the WT cell line. This would be indicated by a faster disappearance of the heavier bands on the gel, and a higher abundance of the mononucleosome bands at the bottom of the gel.

This would indicate that the DNA in the Pol α mutant is more susceptible to MNase digestion. As such, we can infer that the DNA in the Pol α mutant is less densely packed than in the WT cell line. Pol α therefore is necessary for the proper formation of nucleosomes following replication.

The histone-binding motif of Pol1 would likely be responsible for depositing H2A and H2B onto the nascent DNA. Since Pol α was also shown to be bound to FACT and the mcm2 subunit of CMG helicase, it is likely that these three proteins would work in concert to deposit the histone octamer onto nascent DNA. This result would agree with previous studies that found that Pol α, FACT, and the mcm2 subunit are necessary for maintaining gene-silencing following replication, as the deposition of histone proteins and formation of nucleosomes would be vital to chromatin architecture, and thus for maintaining epigenetic modifications required for gene-silencing.

Result #2: There is no difference between the Pol α mutant line and the WT cell line.
This would indicate that there is no change in the susceptibility of the two cell lines to MNase digestion. As such, we can infer that there is no change to nucleosome formation nor chromosome architecture. Pol α therefore is not necessary for the proper deposition of histones onto nascent DNA. It is possible that there is a redundant protein that would deposit these histones onto the nascent DNA, and are adequate in the absence of Pol α. Another possibility is that Pol α plays no role in the deposition of histones at all. The histone-binding motif on Pol1 may function later on in the DNA maturation process. Since Pol α is necessary for maintaining gene-silencing following replication, it is also possible that Pol α recognizes histones after replication, and recruits methylases or other histone writers that would induce epigenetic silencing after replication.

Result #3: There is a decrease in DNA digestion in the Pol α mutant when compared to the WT cell line. This would be indicated by a greater amount of the heavier bands on the gel, and a lower abundance of the mononucleosome bands at the bottom of the gel.
This would indicate that the DNA in the Pol α mutant is less susceptible to MNase digestion. As such, we can infer that the DNA in the Pol α mutant is more densely packed than in the WT cell line. This would be an unexpected result as Pol α has been shown to be necessary for maintaining gene-silencing following replication, and a result that suggests that the Pol α mutant results in a more densely packed chromatin would be contradictory to this. One possibility is that WT Pol α recruits methylases to the chromatin after replication, and these methylases were cross-linked to the DNA during the experiment. If a significant enough amount of methylase were cross-linked to the chromatin, it could make block the DNA from the MNase, thus making it less susceptible to digestion.

References:

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