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Proteins Research Proposal
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Project Summary

Eukaryotes package DNA into nucleosomes, which further condense into chromatin fibers. Although packaging DNA in to chromatin allows it to fit in to the nucleus, the condensed chromatin structure also functions as a barrier to DNA transcription, replication and repair. In order for these functions to occur, the nucleosome must be altered [2]. Human SWI/SNF (hSWI/SNF), a nucleosome remodeling machine is required for transcription of several genes and influences tumor suppression [16]. Although many experiments have been conducted to clarify the mechanism for nucleosome remodeling by hSWI/SNF, the mechanism is still not fully understood [2]. I am interested in investigating the effects linker histones have on the stability of the hSWI/SNF remodeled state, and if modifications of linker histones affect this stability. We propose that linker histones will increase the stabilization of the remodeled state, and that modifications of the histones will negate this increase in stability. Learning more about the remodeled state will provide information about the mechanism by which hSWI/SNF functions, explaining the protein's role in transcription, and tumor suppression.

AIM 1: Investigate the effect linker histones H1 and H5 have on hSWI/SNF remodeled nucleosomes. Two studies, a supercoiling assay on a circular nucleosomal array and MNase digestion of a linear array, will be conducted to determine the effects linker histones have on the stability of the hSWI/SNF remodeled state.

AIM2: Characterize the effects that phosphorylated linker histones H1 and H5 have on hSWI/SNF remodeled nucleosomes. Similar studies to those proposed in specific aim 1 will be used to investigate the effects that phosphorylated linker histones H1 and H5 have on the stability of the hSWI/SNF remodeled nucleosomal array.

Background

DNA Packaging in Eukaryotes

Histones are some of the most conserved proteins across different species with histone H4 being almost 100% conserved in many species [9]. Eukaryotes have five histones: H1, H2A, H2B, H3 and H4, small proteins that contain a large percent of positively charged amino acids. Histones assemble with DNA to form nucleosomes, which further condense into chromatin fibers [19]. Although histones are small, they are abundant, and play an essential role in cell function [21].

In eukaryotes, DNA is compacted into chromatin, allowing DNA to fit into the nucleus. The basic structure of chromatin is the nucleosome, which consists of approximately 145 base pairs of DNA wrapped around a histone octamer and an additional 60 base pairs of linker DNA. The histone octamer or histone core consists of a $(H3)_2(H4)_2$ tetramer and two H2A-H2B dimmers [21]. The tetramer is at the center of the octomer with a dimmer at one face of the tetramers [9]. DNA wraps around the core histone about 1.75 times creating a 10nm nucleosome with about 14nm or 60 base pairs of linker DNA that tie the nucleosomes together [21]. However, the number of amino acid base pairs in the nucleosome varies depending on the species and tissue and can range from 150 to 250 base pairs [19].

Unlike the other histones, histone H1 has an elongated structure with both carbonyl and amino terminal arms extending form its otherwise globular core. H1 binds to DNA on the outside of the histone core and extends its arms along the linker DNA between the nucleosomes [21]. The structure that is formed when H1 binds is called a chromatosome and contains an additional 20 base pairs of DNA [4]. Although histone H1 is not needed in the nucleosome to condense DNA into the 30nm fibers that make up chromatin, H1 is required to achieve maximum compaction of DNA [3].

Nucleosome Mobility

Although the compaction of DNA into chromatin organizes DNA allowing it to fit into the nucleus, the chromatin structure also functions as a barrier to DNA replication, transcription and repair [2]. When DNA is condensed into chromatin, initiation factors cannot bind to packaged promoter sequences to start DNA replication, transcription or repair [9]. To allow for both adequate compaction of DNA into chromatin and access of DNA to transcription

machinery, the nucleosomes structure has to be disrupted in some way. Meersseman *et al* [10] demonstrated that nucleosomes themselves are mobile, sliding over short ranges of DNA in a temperature dependent manner with nucleosomes sliding further with higher temperatures. Because factors including linker histone, core histone modifications, and interactions with DNA binding proteins have been shown to affect transcription of DNA *in vitro*, Meersseman *et al* proposed that theses factors could affect the intrinsic mobility of the nucleosome by further freeing or fixing the nucleosomes [10].

Pennings *et al* [12] later investigated Meersseman's proposal and showed that human linker histones H1 (from HeLa cells) and its avian subtype H5 suppress the short-range mobility of nucleosomes resulting in a more stable nucleosome structure. Histone H5 suppresses nucleosome mobility more effectively than H1 because it binds more tightly to chromatin. The immobilization of nucleosomes, observed by binding of H1, can be reversed by displacing the linker histone [12].

Histone Modifications

ATP independent post-translation modifications of the histones, mainly acetylation, methylation and phosphorylation can alter the stability of the histone-DNA interaction causing the chromatin structure to destabilize. Acetylation of histones by histone acetyltransferases (HATs) is associated with increased transcription. Acetylation occurs at specific lysines on the histone tails, which reduces the positive charge of the histone protein, weakening its bond to negatively charged DNA [17]. Although this proposal does not focus on nucleosome remodeling by HATs, interdependence between histone acetylation and ATP dependent nucleosome remodeling has been observed, suggesting that some of the same principals and mechanisms apply to both ATP independent and dependent nucleosome remodeling factors [2].

Nucleosome Remodeling

Nucleosome remodeling is defined as a process that alters the structure of the histone-DNA interaction of a nucleosome in an ATP dependent manner. Nucleosome remodeling proteins involve the use energy released from the hydrolysis of ATP do destabilize the DNA-histone interaction in the nucleosome making DNA more accessible. Many different nucleosome remodeling factors have been identified and shown to affect several types of processes

influenced by the structure of chromatin. These processes include transcription, core histone relocation and changes in DNA supercoiling [2]. These nucleosome remodeling factors have been divided into three major classes, the SWI/SNF (switch/sucrose nonfermenting) complexes, ISWI (imitation switch) complexes and complexes related to MI-2 [13]. This proposal will focus upon the SWI/SNF class, specifically human SWI/SNF.

The SWI/SNF family

The SWI/SNF nucleosome remodeling machine is a 2MDa complex, about half the size of a ribosome, and contains 11 subunits [17]. Originally, five subunits of the *Saccharomyces cervisiae* SWI/SNF (ySWI/SNF) complex encoded by the genes *SWI1, SWI2, SWI3, SNF5* and *SNF6* were identified to be initiators and positive regulators of the HO and SUC, two inducible genes. SWI/SNF has been found to regulate many other genes as well [14]. The SWI2/SNF2 subunit complex of the SWI/SNF protein is the motor of the complex possessing the ATPase activity. The SWI2/SNF2 subunit can hydrolyze up to 1000 ATPs per minute. The other subunits of the complex are less understood [2,13].

Because histones are conserved across species, it was speculated that SWI/SNF like proteins could also be found in higher eukaryotes. This is indeed the case. The protein brahma (brm) in *Drosophila* was found to be an activator of hoemotic genes is the closest relative to SWI2 of yeast [13]. Two distinct human SWI/SNF (hSWI/SNF) proteins are have been found to be related to yeast SWI2. One subunit is BRG1, also known as hSWI/SNFa or BAF and the other contains is hBRM (human brahma) [14].

Mechanisms of SWI/SNF

The mechanism for nucleosome remodeling by the SWI/SNF complex is only partially understood. Several mechanisms for SWI/SNF function have been proposed based on evidence found from DNAse I digestion assays, restriction enzyme accessibility assays, and topology alterations in nucleosome arrays. SWI/SNF can displace the histone octamer from DNA when the free DNA is in trans to the remodeling protein (figure 1d) [2,15]. Owen Hughes [11] supported this in an experiment that involved nucleosomes that contain the five binding sites of the yeast transcription factor GAL4 through mobility shift analysis, footprinting and a binding reaction. Naked DNA was observed in the binding reaction suggesting that in the presence of

SWI/SNF and ATP the histones are removed [11]. It is now thought that DNA binding transcription factors in addition to SWI/SNF enhances this displacement [15].

SWI/SNF type nucleosome remodeling proteins also participate in sliding of the histone octamers on the DNA in the cis conformation (figure 1c). This occurs by a sliding mechanism rather that displacement because a 4-way junction in the DNA blocks movement of the histone core [15]. Sliding the histone octamer could occur by either a twisting or bulging model. Peterson and others favor a mechanism that uses the energy from ATP to twist the DNA helix relative to the histone octomer. The rotation of DNA would lead to disruption in the histone-DNA connection, which would allow transcription, replication and repair of the DNA [13]. Becker favors the bulging model where a small loop of DNA bulges limiting the rate of nuclear remodeling, because he found that nicking the linker DNA do not inhibit nucleosome sliding. In the bulging model, the remodeling protein needs to have two contacts, one to the nucleosome and one to the DNA [2]. However, not all cases of nucleosome remodeling can be explained by this model, including cases where the promoter is stretched across more than one nucleosome. This suggests that maybe SWI/SNF functions in a mechanism involving both displacement and sliding [2,8].

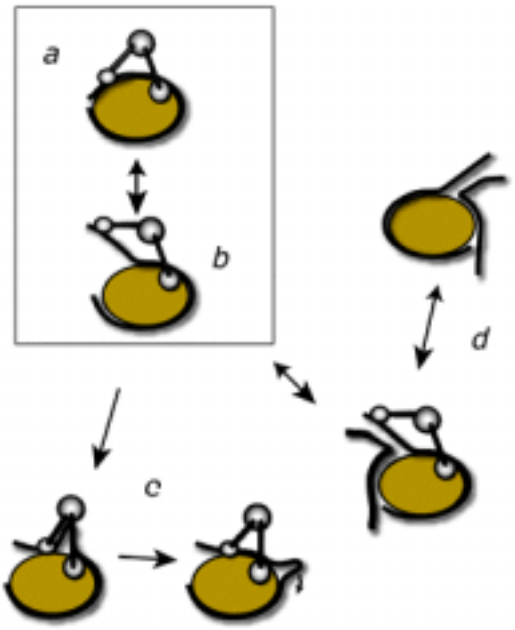

Figure 1. Hypothetical nucleosome remodeling pathways. (a) Interaction of nucleosome remodeling factor (gray spheres) with nucleosomes (shown as black DNA segment wound around brown spool of histone octamer). (b)Unpeeling of DNA segment from histone octamer surface. (c)Nucleosome sliding in cis. (d) Trans-displacement of histone octamer. (Figure modified from reference 2)

Human SWI/SNF

In mammals, several forms of SWI/SNF-like complexes including hSWI/SNF have been purified through a series of chromatographic purification steps. The mammalian complexes have between nine and twelve subunits depending on which cells they come from [16] and are able to remodel a nucleosome every 5.2 minutes [7]. Human SWI/SNF affects several functions including transcription, tumor suppression and HIV-1 nucleosome interactions [16]. Each

hSWI/SNF contains either the BRG1 or hBRM ATPase along with one of several BRG1-associated factors (BAFs). Even though BRG1 and hBRM have similar sequences as shown in figure 2, they remodel distinct nucleosomal arrays that regulate different cellular pathways. The BRG1 protein has a constant level of expression in most cells and its ATPase activity is required for embryonic development where as hBRM ATPase activity is not required for embryonic development and it increases in concentration during cell differentiation. It is still not clear why BRG1 and hBRM have such distinct activities.

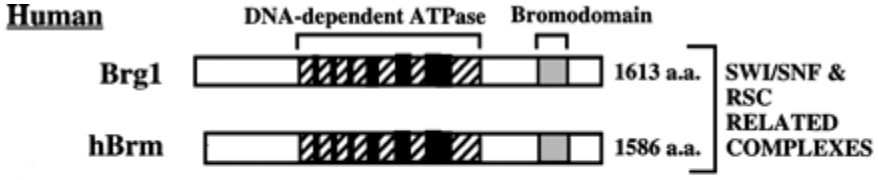

Figure 2. The ATPase subunits of human ATP-dependent chromatin remodeling complexes. SWI/SNF2 are homologous to BRG1 and hBRM in humans Complexes have been shown to alter some aspect of chromatin structure in an ATP-dependent manner. (Figure modified form reference 20)

hSWI/SNF Remodeled Nucleosome

The nature of a hSWI/SNF remodeled state is not fully known. The formation of accessible DNA through the disruption or sliding of histones by SWI/SNF nucleosome remodeling factors could occur with or without the presence of a remodeled state following ATP hydrolysis [2]. Recent experiments have provided evidence both supporting and discounting the existence of a stable hSWI/SNF remodeled state. In two studies, C.L Peterson and colleagues show that the accessibility to DNA by restriction enzymes is not detectable after remodeling. These results could have occurred because restriction enzyme assays primarily detect sliding of nucleosomes, which cannot return to a pre-remodeled state [2].

Several changes persist after remodeling has occurred and ATP has been removed. The characteristic mononucleosomal DNase 1 digestion pattern seen in a remodeled nucleosome, the MNase (micrococcal nuclease) digestion pattern, and topological changes in the nucleosomal array can be detected even after remodeling has occurred. This suggests that a stable remodeled state does exist [2,7]. Guyon *et al* [7] find that hSWI/SNF, in the presence and absence of topoisomerase I, is able to alter the topology of a closed nucleosomal plasmid. Toposiomerase I relaxes the twists that occur in the remodeled plasmid, increasing the stability the remodeled state. This remodeled state is stable, reverting back to its original state only hours after ATP

hydrolysis has stopped. They also find that histone tails are not required to maintain the remodeled state. Free plasmid DNA speeds up the reversion to the un-remodeled conformation suggesting that hSWI/SNF stabilizes the remodeled state and that the plasmid super-coiling is not due to the dissociation of the histone core. The stability of the remodeled state was also tested under MNase digestion. The remodeled state appeared to be more stable in this assay. This would be the case if hSWI/SNF functioned in both displacement and sliding mechanisms and MNase assays detected both of these remodeled states [7]

Influence of linker histones on hSWI/SNF nucleosome remodeling

In 1995 the influence of H1 linker histone on ATP dependent nucleosome mobility was tested, and it was concluded that the histone did not affect ISWI type nucleosome sliding [18]. However, because linker histone H1 suppresses nucleosome mobility as found by Pennings *et al* [12], and hSWI/SNF induces nucleosome mobility. Hill and Imbalzano [4] proposed that histone H1 would suppress the nucleosome remodeling ability of hSWI/SNF. Imbalzano and Hill demonstrated that the nucleosome remodeling activity by hSWI/SNF on mononucleosomes is inhibited in the presence of H1 by both DNase I degradation and restriction enzyme accessibility assays. H1 does not inhibit the hydrolysis of ATP, yet the remodeled state is formed slower in the presence of H1. This suggests that the presence of H1 is not coupled with the ATPase activity so H1 might bind to the nucleosome making it more stable in the un-remodeled state [4].

In a later study, Hill, Imbalzano and colleagues [5] concluded that linker histone has a global effect on all nucleosome remodeling classes including ISWI and MI-2. They also performed their experiment on ySWI/SNF nucleosomal arrays, which act similarly to hSWI/SNF. Their data shows that the inhibition of remodeling due to the linker histones is both a result of H5 stabilizing the chromatin array and stabilizing the individual chromatosomes, the latter being the major stabilizing effect. It was also demonstrated that modification of the linker histone, mainly phosphorylation by Cdc2/Cyclin B kinase reduces the linker histones affinity for the chromatin allowing the histone to exchange on and off the chromatosome [5]. Further study to investigate the relationship between linker histone and the hSWI/SNF remodeled state will clarify the role H1 has in ATP-dependent nucleosome remodeling and may offer insight to determine the mechanism of hSWI/SNF remodeling.

Significance

The addition of linker histones in experiments aimed at clarifying the mechanism by which hSWI/SNF and other nucleosome remodeling factors has not readily been explored [3]. Because linker histones are present *in vivo*, it seems essential to include H1 in *in vitro* studies aimed at discovering the mechanism of SWI/SNF. To understand the mechanism of SWI/SNF protein, more experiments exploring the remodeled state are necessary. This proposal seeks to investigate the effects linker histones and modifications to the core histones have on the hSWI/SNF remodeled nucleosome. Investigation into whether the linker histone is able to bind and stabilize the remodeled state may provide information in determining the mechanism by which hSWI/SNF functions. Understanding the mechanism of hSWI/SNF will further explain the role it has in transcription and tumor suppression.

Research Design and Methods

Experiment 1A to address specific aim 1.

Rationale

Guyon *et al* [7] found hSWI/SNF remodeled nucleosome arrays to be stable as shown by a plasmid supercoilling assay and analysis of the DNA an agarose gel. I propose to use a similar technique to determine the affects linker histones H1 and H5 have on the stability of hSWI/SNF remodeled nucleosome arrays.

Hypothesis

I hypothesize that linker histones will increase the stability of hSWI/SNF remodeled nucleosome arrays. Because linker histone H5 binds more tightly to unremodeled chromatin, I also hypothesize that linker histone H5 will have a stronger stabilizing effect than H1.

Approach

To analyze the effects of linker histones on hSWI/SNF remodeled nucleosome arrays, hSWI/SNF, linker histones, tailless core histones, and tailless nucleosome arrays must be purified. Human SWI/SNF will be purified from HeLa cells by affinity chromatography and purified to at least 50% as judged by silver stain analysis [6]. Linker histones, H1 form HeLa cells and H5 from chicken erythrocyte nuclei, will be purified by first extracting chromatin from the cells and then depleting the chromatin of histones by sucrose gradient centrifugation. Fractions of the gradient containing the linker histone will be further purified by FPLC [12]. The

tails must be removed from the core histones because hSWI/SNF remodeling requires at least 2mM Mg^{2+}. However, concentrations of Mg^{2+} over 0.65 mM cause aggregation of the nucleosome arrays. [4]. Removal of the core histone tails still allows nucleosome remodeling by hSWI/SNF [7] and prevents aggregation of the nucleosome arrays in the presence of 2mM Mg^{2+}[5]. Core histones will be purified by hydroxyapatite chromatography [1]. Trypsinized nucleosomes will be made by digesting polynucleosomes with trypsin and purifying with hydorxyapatite chromatography [6].

Circular nucleosomal arrays will be made as described previously [1,7]. The plasmid pSAB8 will be linearized with *Eco*RI and separated from unincorporated nucleotides in a spin column. The plasmid will be constructed into nucleosomal array using *Xenopus* heat-treated assembly extract and trypinized histones followed by separation on a glycerol gradient. Circular nucleosome array + H1 and circular nucleosome array + H5 will also be made by adding either H1 of H5 during the addition of *Xenopus* heat-treated assembly extract and trypinized histones.

To determine if linker histones H1 and H5 are able to bind to the circular array, the circular array, circular array + H1, and circular array +H5 will be analyzed by SDS-Page [1]. If the linker histones are incorporated in to the array, I would expect these bands to travel a shorter distance on the gel than the arrays without linker histone. If linker histones are able to bind to the unremodeled nucleosome plasmid I would also expect it to bind to the remodeled state. If linker histones were unable to bind to the plasmid, a different assay involving a linear array (Experiment 1B) would have to be employed.

Topological changes to the circular remodeled plasmid can be measured by deprotenization of the DNA and analyzing it on agarose gel. To determine if linker histone affect the stability of the remodeled state, first hSWI/SNF, the array, topoisomerase I and ATP will be incubated for 30 to 45 minutes to allow remodeling to occur. The remodeling will be stopped with the addition of apyrase [7]. Histone H1, histone H5 or no linker histone will be added to the reaction mixture with the addition of apyrase or 30 minutes after the addition of apyrase. The mixtures will then be incubated for up to 16 to 20 hours depending on how long reversion to the pre-remodeled state takes. Additional topoisomerase I will be added as need, as it degrades over time [7]. Aliquots of the mixture will be taken every hour and run on a chloroquine gel to determine if reversion has occurred. Reversion rates will be measured by plotting the amount of remodeled array quantified with a PhosphorImager versus retention time.

Expected Results, Interpretations, Limitations

I **expect** the reversion rates of hSWI/SNF remodeled nucleosome arrays + linker histones to be slower than the reversion rates without linker histones. If this occurs, I will **interpret** our results to be consistent with our hypothesis. If this does not occur, our results may be interpreted in several ways. If reversion rates with linker histones are similar to those with out I would interpret the result to mean that linker histones do not slower down the rate of reversion. I could also conclude that the linker histones were unable to bind to the remodeled array. This could be due to alterations in the nucleosome-linker histone binding site caused by remodeling. If reversion rates are faster, I would interpret this to mean that linker histones do not stabilize the remodeled state. Linker histones may favor stabilization of the unremodeled state speeding up reversion. I also expect nucleosome arrays + H5 to have a lower rate of reversion that nucleosome arrays + H1. If this does not occur I would propose that the binding sites of H1 and H5 are not the sole factor contributing to in the stabilization of the nucleosome-remodeled array.

One **limitation** of this experiment includes the possibility that the array will not revert back to the unremodeled state in a timely fashion. If this happens, topoisomerase can be excluded from the reaction. This results in a less stable remodeled state as shown by Guyon *et al* [7]. Another limitation is the possibility that the histones are unable to bind to circular remodeled nucleosomes. If this is the case, using a linear array, as proposed in experiment 1B, may be more effective.

Experiment 1B to address specific aim 1

Rationale

Continue to determine the effect linker histone have on the stability of the hSWI/SNF remodeled nucleosome array through MNase digestion analysis of a linear nucleosomal array.

Hypothesis

I hypothesize that linker histone H1 and H5 will bind to the hSWI/SNF remodeled nucleosome, stabilizing the structure.

Approach

The p2085S-G5E4 plasmid linear array containing 5S nucleosomes will be made or obtained. The 5S-G5E4 sequence will be cut from the plasmid with restriction enzymes and the linear DNA will be assembled into a polynucleosome array by gradient dialysis [1, 7].

Chromatin arrays containing H1 and H5 will also be made by adding the linker histones during polynucleosome assembly. To determine if linker histones H1 and H5 are able to bind to this linear array, the nucleosomal array and two chromatin arrays will be analyzed by SDS-Page as described in experiment 1A. Since linker histones have successfully been incorporated into a 5S nucleosome linear array [5], we can be fairly certain that they will also be incorporated into our array. A mixture of the array, hSWI/SNF and ATP will be incubated for 30 to 45 minutes to allow for remodeling to take place. Remodeling will be stopped with the addition of ADP. At this time no linker histone, H1 or H5 will be added to the reaction mixtures. Incubation of the samples will continue with aliquots taken at 0, 24, 48 and 72 hours. The aliquots will be digested with MNase and analyzed on an agarose gel. The unremodeled state will show a pattern of digestion and the remodeled state will appear as a smear. A control containing no hSWI/SNF will also be analyzed at these times for comparison.

Expected results, Interpretations, Limitations

I **expect** the array + linker histones to stay remodeled longer that the array lacking histones. If this occurs, I will **interpret** our results to be consistent with our hypothesis. If results show that array + linker histones are equally stable or less stable than the array lacking linker histone, our results can be interpreted similarly to the interpretations stated in experiment 1A.

A major **limitation** of this experiment is the possibility that the reversion of the arrays cannot be measured using the MNase assay. The MNase assay measures twisting of the DNA and sliding the nucleosomes in cis [2]. The sliding in cis may take longer to revert or may not revert at all. If sliding of the nucleosomes does not revert, but the twisting does, the MNase assay will show results that would be interpreted to mean that reversion had not occurred.

Experiment 2A to address specific aim 2

Rationale

Phosphorylation of linker histones reduces the histone's affinity for the nucleosome [5]. I propose to characterize the effects that phosphorylated linker histones H1 and H5 have on the stability of hSWI/SNF remodeled nucleosome arrays.

Hypothesis

I hypothesize that phosphorylation of linker histones H1 and H5 will negate the stabilization effects that linker histones alone have on remodeled nucleosome arrays.

Approach

hSWI/SNF, linker histones, tailless core histones, tailless arrays and the circular nucleosomal array will be made as described in experiment 1A. Linker histones H1 and H5 will be treated with Xenopus Cdc2/Cyclin B kinase, a kinase known to phosphorylate linker histone [5]. To determine if the phosphorylated linker histones are able to bind to the array, the array, array + H1 and array + H5 will be analyzed by SDS-Page [1]. If binding occurs, the array + linker bands will travel a shorter distance on the gel.

Experiments to determine if the phosphorylated linker histones affect the stability of the remodeled state will be preformed as described in experiment 1A. hSWI/SNF, the array, topoisomerase ATP and I will be incubated for 30 to 45 minutes to allow remodeling to occur. Remodeling will be stopped with apyrase. No linker histone, phosphorylated H1, or phosphorylated H5 will be added to the reaction mixture when the apyrase is added or 30 minutes later. The mixtures will be incubated for up to 10 hours to allow the remodeled nucleosome array to revert. Aliquots of the mixture will be taken every hour and run on a chloroquine gel to determine if reversion has occurred. Reversion rates will be measure by quantifying the amount of remodeled array using a PhosphorImager and plotting the amount of remodeled array versus retention time [7].

Expected Results, Interpretations, Limitations

I **expect** to find that the reversion rates of hSWI/SNF remodeled nucleosome arrays + phosphorylated linker histones to be less stable that nucleosome arrays + linker histones. In fact, I would expect reversion rates to be similar to reversion rates in which no linker histones were used. If these results occur, we will **interpret** them to be consistent with our hypothesis. If the reversion rates are slower, I will interpret this to mean that the binding of the phosphorylated linker histone itself has a stabilizing effect.

Limitations of this experiment include the possibility that phosphorylated linker histones will not bind to the circular plasmid using the technique suggested. A different technique would have to be developed if this was the case.

Experiment 2B to address specific aim 2

Rationale

Continue to determine the effect of phosphorylated linker histones have on the stability of the hSWI/SNF remodeled nucleosome array by using MNase digestion analysis of a nucleosome linear array.

Hypothesis

I hypothesize that phosphorylated linker histones will not cause further stabilization the remodeled state.

Approach

The linear array used in experiment 1B and phosphorylated linker histones used in experiment 2A will also be used in this experiment. Mixtures of the linear array, hSWI/SNF and ATP will be remodeled for 30 to 45 minutes. Remodeling will be halted with the addition of ADP. Phosphorylated linker histones H1 or H5 or no linker histone will be added to the reaction mixtures at this time and the mixture will be incubated. Aliquots will be taken every 24 hours for three days, digested with MNase and analyzed on an agarose gel as was done in experiment 1B. A control lacking hSWI/SNF will also be run and used for comparison of reversion rates.

Expected Results, Interpretations, Limitations

I **expect** to find the remodeled array + phosphorylated linker histones to revert at the same time that the array + no linker histone reverts. I will **interpret** this result to be consistant with my hypothesis. Otherwise, I will interpret the results to mean that phosphorylation of linker histones does not affect the stability of a hSWI/SNF remodeled array

A **limitation** of this experiment could be that the remodeled state reversion cannot be measure by MNase digestion as discussed in experiment 1B.

Overall Summary of Experiments

I have proposed two experiments, a supercoilling assay and MNase digestion, to study the effects that linker histone H1 and H5 and phosphorylation of these histones may have on the hSWI/SNF remodeled state. I have considered how the results of these experiments could be interpreted, and also took into consideration the limitations that each experiment may have.

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