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Proteins Research Proposal
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Background

DNA Packaging in Eukaryotes

Eukaryotes have five histones: H1, H2A, H2B, H3 and H4 which form repeating units that compact DNA. Histones are small proteins that contain a large percentage of positively charged amino acids, usually lysine and arginine [16]. Although these proteins are fairly small, they are abundant with a weight approximately equaling the weight of DNA in the nucleus [18]. Histones are some of the most conserved proteins across different species with histone H4 being almost the same in many species [7].

In eukaryotes, DNA is compacted into chromatin to allow it to fit into the nucleus. The basic structure of chromatin is the nucleosome, which consists of about 145 base pairs of DNA wrapped around a histone octamer [18]. However, the number of amino acid base pairs in the nucleosome varies depending on species and tissue and cam be anywhere from 150 to 250 base pairs [16]. The histone octamer or histone core consists of a $(H3)_2(H4)_2$ tetramer and two H2A-H2B dimmers. The tetramer is at the center of the octomer with a dimmer at each of the ends of where the DNA wraps [7]. 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. Histone H1 or the linker histone has an elongated structure, unlike the other histones, with both carbonyl and amino terminal arms extending form its otherwise globular core. H1 binds to DNA in the histone core and extends its arms along the linker DNA between the nucleosomes [18]. The structure that is formed when H1 binds is called a chromatosome and contains an additional 20 base pairs of DNA [3]. 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 the maximum compaction of DNA [2].

Nucleosome Mobility

Although the compaction of DNA into chromatin organizes DNA allowing it to fit into the nucleus, the chromatin structure also acts as a barrier to DNA replication, transcription and repair [1]. 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 demonstrated that nucleosomes themselves are mobile, sliding 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 virto, Meersseman et al proposed that theses factors could affect the intrinsic mobility of the nucleosome by further freeing or fixing the nucleosomes [8].

Pennings et al later showed that linker histones H1 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 [9]. Histone modifications and DNA binding proteins have also been shown to affect the intrinsic mobility of chromatin.

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 unfold. 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, weakening its bond to negatively charged DNA [14]. 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 [1].

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. These factors 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 tested and shown to affect several types of processes associated with the structure of chromatin including transcription, core histone relocation and changes in DNA supercoiling [1].

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 [10].

The SWI/SNF family

The SWI/SNF nucleosome remodeling protein is a 2MDa protein, about half the size of a ribosome, and contains 11 subunits [14]. 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 regulators of the HO and SUC genes [11]. The SWI2/SNF2 subunit complex of the SWI/SNF protein, which contains a bromodomain, is the motor of the complex, having the ATPase activity. The SWI2/SNF2 subunit can hydrolyze up to 1000 ATPs per minute. The other subunits of the complex are less understood [1,10].

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 activation of hoemotic genes is the closest relative to SWI2 found in yeast [10]. Two distinct human SWI/SNF (hSWI/SNF) are related to yeast SWI2. One contains the subunits BRG1, also known as hSWI/SNFa or BAF) and the other contains hBRM (human brahma) [11].

Mechanisms of SWI/SNF

The mechanism for nucleosome remodeling by the SWI/SNF complex is only partially understood, so several mechanisms for which SWI/SNF works 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 in trans. Owen Hughes showed this in an experiment that involved nucleosomes that contain the five binding sites of the yeast transcription factor GAL4. It is now thought that DNA binding transcription factors in addition to SWI/SNF enhances this displacement. SWI/SNF type nucleosome remodeling proteins also participate in sliding of the histone octamers on the DNA in the cis conformation. This occurs by a sliding mechanism rather that displacement because a 4-way junction in the DNA blocks movement of the histone core [12]. 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 [10]. 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 done 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 [1]. 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, suggesting that maybe SWI/SNF functions in a mechanism involving both displacement and sliding [1,6].

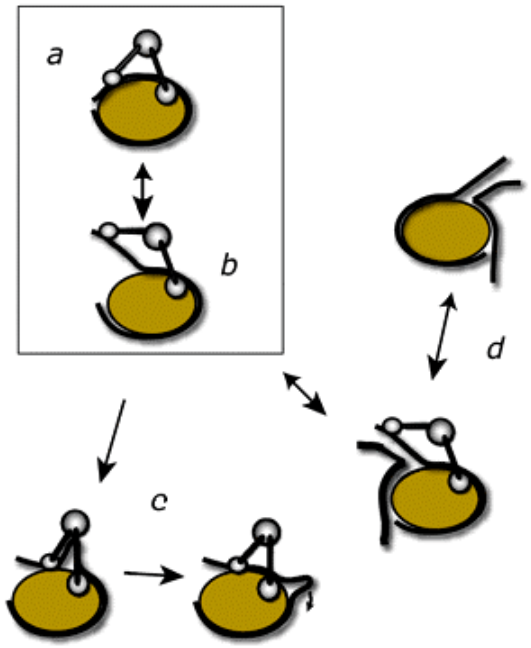

Figure 1. Hypothetical nucleosome remodeling pathways. (a) Interaction of nucleosome remodeling factor (gray spheres) with nucleosomes (shown as black DNA segment wound around brown spool of histone octamer). (b)Unpeeling of DNA segment from histone octamer surface. (c)Nucleosome sliding in cis. (d)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

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 form. Human SWI/SNF have been found to be involved in transcription, tumor suppression and interaction with HIV-1 nucleosome [13] and are able to remodel a nucleosome every 5.2 minutes [5]. 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 APTase 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.

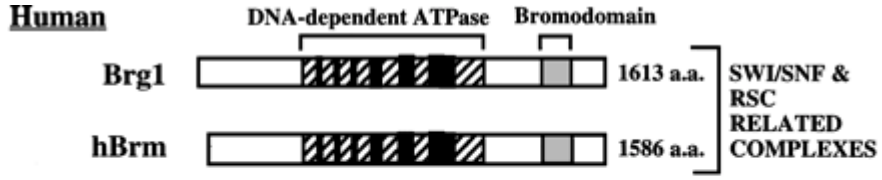

Figure 2. The ATPase subunits of human ATP-dependent chromatin remodeling complexes. SWI/SNF2 are homologous to BRG1 and hBRM in humans Complexes have been shown to alter some aspect of chromatin structure in an ATP-dependent manner. (Figure modified form reference 17)

hSWI/SNF Remodeled Nucleosome

Although the formation of accessible DNA through the disruption or sliding of histones by SWI/SNF nucleosome remodeling factors could occur without the presence of a remodeled state following ATP hydrolysis [1], evidence supporting and discounting the existence of a stable hSWI/SNF remodeled state has been found. 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. Several changes including the characteristic mononucleosomal DNase 1 digestion pattern seen in a remodeled nucleosome, Mnase (micrococcal nuclease) digestion pattern, and topological changes in the nucleosomal plasmid can be detected even after remodeling has occurred suggesting that a stable remodeled state does exist. Guyon et al find that hSWI/SNF, in the presence of topoisomerase I, is able to alter the topology of a closed nucleosomal plasmid and that the altered state is stable, reverting back to its original state 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 [5]

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 [15]. However, because linker histone H1 suppresses nucleosome mobility as found by Pennings et al, and hSWI/SNF induces nucleosome mobility, Hill and Imbalzano 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 [3]. In a later study, Hill, Imbalzano and colleagues concluded that linker histone has a global effect on all nucleosome remodeling classes including ISWI and MI-2. The were also able to perform 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 [4].

The addition of linker histones in experiments aimed at clarifying the mechanism by which hSWI/SNF and other nucleosome remodeling factors has not been readily explored [3]. It would be interesting to see if H1 also stabilized 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.

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.

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