Chapter 1

Review of Literature
Chapter 1

Introduction

Chromatin Structure

DNA forms the genetic material in all living organisms. Depending upon the complexity of the organism, the size of the genetic material varies. The eukaryotic genome size ranges from $12 \times 10^6$ base pairs in *S. cerevisiae* to $6 \times 10^9$ base pairs in humans (Rosa, A. et al. 2008). This huge length of DNA in higher animals needs to be packaged in such a way that it can be contained within the cell. Therefore, the eukaryotic DNA forms a complex structure along with various histones and non-histone proteins called chromatin. The organization of DNA into chromatin helps the genome not only to be stored in a highly compact form inside the cell but also keeps it protected from nuclease digestion and other physiological and environmental insults. The basic unit of eukaryotic chromatin is the nucleosome as shown in fig 1.1, which comprises of an octamer of core histone proteins, two each of H2A, H2B, H3 and H4 (Kornberg, R. D. 1974; Lorch, Y. et al. 1999). About 146 base pairs of DNA wraps around this histone octamer, forming 1.65 superhelical turns around the histone core (Kornberg, R. D. 1974). This results in a five- to ten-fold compaction of DNA. Each nucleosome is connected to its neighbor by ~10-80 bp of linker DNA forming a polynucleosome structure. The linker histone H1 associates with each nucleosome giving rise to a further 50-fold compaction. This compaction results in a 30 nm stabilized fiber (Wolffe, A. P. et al. 1997). The crystallographic structure of the histone octamer and the nucleosome has been deciphered (Arents, G. et al. 1991). Each histone molecule in the octamer is characterized by two domains: i) a structured domain known as histone fold; and ii) an unstructured NH$_2$ tail.
(Arents, G. et al. 1991; Luger, K. et al. 1997). The protruding N-terminal tail of the core histone contacts DNA, other histones, and non-histone proteins and thus, contributes to higher folding of the chromatin. Further level of compaction is contributed by the post-translational modifications of core histones and histone variants at their N- and C-terminal tails.

**Fig 1.1:** Schematic diagram of a nucleosome.

**Chromatin remodeling**

Compact DNA on one hand is economical to the cell in terms of space but at the same time it acts as a potential barrier for cellular machineries to find access to the target DNA sequences when needed. Processes like DNA replication, transcription, repair, recombination, and apoptosis are multistep events. They involve different sets of factors to initiate, maintain and accomplish these DNA metabolic processes by recruiting specific proteins on to the specific DNA sites. Therefore, the cell needs a mechanism that can modulate the chromatin environment such that the access of factors is facilitated or hindered as required. Such alteration in chromatin structure that regulates the condensation and relaxation of DNA is known as chromatin remodeling. Chromatin
remodeling is a dynamic process and there exist several mechanisms that regulate the chromatin structure within a cell. For example, in order to manipulate chromatin structure, histone proteins can undergo various modifications. Histone H3, for example, in response to Epidermal Growth Factor (EGF) has been shown to undergo phosphorylation and acetylation both of which are tightly coupled (Barratt, M. J. et al. 1994; Clayton, A. L. et al. 2000). Similarly, histone variants can be deployed in place of canonical histones as one of the major factors responsible for rearranging chromatin structure during specific cellular events (Sarma, K. et al. 2005). Histone variants differ from their canonical histones in their amino acid composition (Sarma, K. et al. 2005). Incorporation of such histone variants in the nucleosome results in the alteration in both the structure of individual nucleosomes and the ability of nucleosomes to form higher order chromatin structure, which in turn is responsible for bringing discernible changes in histone-DNA contacts (Sarma, K. et al. 2005).

Till date, two major classes of chromatin remodelers have been characterized. The first class consists of proteins that alter chromatin structure via covalent modification of histones in an energy-independent manner and hence constitute energy-independent remodelers (Kadonaga, J. T. 1998; Varga-Weisz, P. et al. 2001; Falsenfeld, G. 1996; Workman, J. L. et al. 1998). The N-terminal tails of histone proteins are subjected to various covalent post-translational modifications, including methylation by histone methyl transferases (HMTs), acetylation by histone acetyl transferases (HATs), deacetylation by histone deacetylase complexes (HDACs), and phosphorylation by kinases. Other modifications such as ADP-ribosylation, ubiquitination, and SUMOylation (Kouzarides, T. et al. 2007) by their respective effector proteins have also
been reported. Such modifications are reversible and act as switches to bring about a change in the interaction between histones and DNA to conduct specific downstream responses (Strahl, B. D. et al. 2000).

Eukaryotic cells, in addition, also deploy a second class of chromatin remodelers to actually alter histone-DNA contacts to mobilize the nucleosomes in the context of chromatin. These remodelers are ATP-dependent factors that utilize energy derived from ATP hydrolysis to alter the structure of the chromatin making it accessible to various proteins (Kingston, R. E. et al. 1999). SNF2, RSC, ISWI, NURD, and INO80 complexes are some of the well-characterized ATP-dependent chromatin remodelers. In addition to these two classes of chromatin remodelers, non-histone proteins like Heterochromatin Protein 1 (HP1), High Mobility Group Nucleosomal (HMGN) protein family and scaffold proteins also play a critical role in altering chromatin structure. These non-histone proteins form important components of the chromatin and can interact with other proteins to regulate the chromatin organization. HP1 proteins are highly conserved in almost all eukaryotes from yeast to humans. Originally they were thought to be a constitutive component of heterochromatin (Eissenberg, J. C. et al. 1990) and were supposed to be involved in gene repression by forming heterochromatin. Later reports strongly suggested that HP1 proteins do also have euchromatic distribution (Horsley, D. et al. 1996; Minc, E. et al. 2000). In addition, HP1 proteins interact with many other proteins such as histones H1, H3, and H4 to mediate transcriptional activation or repression. They have also been shown to sequester genes to the nuclear periphery during heterochromatization leading to the inactivation of the genes (Polioudaki, H. et al. 2001; Bannister, A. J. et al. 2001). HMGN proteins are present in the nuclei of all
mammalian cells. They are the only group of nuclear proteins that bind to core nucleosomal particle without any sequence specificity (Shirakawa, H. et al. 2000). This protein family is composed of 3 members: HMGN1, HMGN2 and HMGN3. They have been reported to bind to the nucleosomes as homodimers and alter the chromatin structure to facilitate several DNA-dependent activities (West, K. L. et al. 2001).

In this chapter, I will be focusing on the ATP-dependent chromatin remodelers.

ATP-dependent chromatin remodelers

The ATP-dependent chromatin remodeling proteins play diverse role in cells (Narlikar, G. J. et al. 2002). Most of these factors exist as large multi-subunit complexes. The characteristic feature of all these complexes is the presence of a highly conserved ATPase subunit required for the binding and hydrolysis of ATP, which belongs to the SNF2 family of proteins (Eisen, J. A. et al. 1995).

The yeast SWI/SNF complex containing the Snf2 protein was the first remodeling complex to be described. It was first isolated from yeast as a ~2 MDa complex and was found to be conserved from yeast to humans (Cairns, B. R. et al. 1994). The name SWI/SNF was derived from the discovery of the yeast mutants through genetic screens for expression of the HO gene, involved in regulation of mating type switching (the name Swi is derived from SWitching defective) and expression of the SUC2 gene, required for sucrose fermentation (the name Snf is derived from Sucrose Non-Fermenters) in yeast. The SNF2 protein family belongs to Superfamily2 (SF2) of helicases (Gorbalenya, A. E. et al. 1993). These proteins are known to possess helicase-like motifs, which are small stretches of amino acid sequences found to be conserved in helicases (Eisen, J. A. et al. 1995). These motifs are shared by a group of enzymes that hydrolyze ATP in the
presence of DNA including the SNF2 family proteins (Pazin, M. J. et al. 1997). The helicase motifs are required for DNA binding, ATP binding, as well as for ATP hydrolysis (Koonin, E. V. et al. 1993; Korolev, S. et al. 1998). The variation within the helicase motifs as well as its organization among this family of proteins confers phylogenetic diversity to this group of proteins and forms the basis of classification of helicase family into four superfamilies (Hall, M. C. et al. 1999), as shown in fig 1.2.

Superfamilies 1 and 2 (SF1 and SF2), both contain seven conserved helicase motifs and form the largest and closely related groups. The superfamilies 3 and 4 are much smaller, with the SF3 proteins containing only three conserved motifs and SF4 proteins containing five conserved motifs (Hall, M. C. et al. 1999). Though none of the family members of SNF2 family possess DNA unwinding helicase activity yet these proteins have been placed in the helicase-like superfamily 2 (SF2) (Pazin, M. J. et al. 1997).

![Fig 1.2: Classification of ATPases into three different superfamilies of helicases. The SNF2-like family of ATPases belonging to SF2 is further classified into distinct subfamilies. (Adapted from Gorbelnya and Koonin. 1993)](image)

**SNF2 family of proteins**

As stated earlier, the proteins of the SNF2 family possess seven helicase-related motifs. These are motifs I, la, II, III, IV, V and VI. Structural and functional studies of different motifs of prokaryotic as well as eukaryotic helicases have highlighted upon the biochemical functions of individual ATPase motifs. Motif I and II are believed to form
the nucleotide binding region (Koonin, E. V. et al. 1993). Motifs IV, V and VI are involved in DNA and ATP binding (Korolev, S. et al. 1998). Though SNF2 family proteins do not have helicase activity, their function is defined by their ability to utilize the energy released by ATP hydrolysis to alter histone-DNA interactions and thus facilitate chromatin remodeling. In this context, the role of individual motifs has not been completely elucidated. The conserved ATPase motif is often flanked by relatively non-conserved sequences suggesting the potential involvement of SNF2 proteins in diverse cellular functions (Flaus, A. et al. 2006). The SNF2 family is divided into several subfamilies (Eisen, J. A. et al. 1995; Flaus, A. et al. 2006). Based on the presence of additional motifs in the ATPase subunit, SNF2 related proteins have been broadly classified into 24 subfamilies. This classification also defines the distinct functions of these subfamilies. Many of these subfamilies have been assigned with some biological role while the physiological role of some still remains to be explored. SNF2 family includes subfamilies such as SNF2, ISWI, Mi-2, CHD1, SWR1, INO80, RAD54, ATRX, Mot1, ERCC6, and SMARCAL1 (Flaus, A. et al. 2006). SNF2 (SWI2/SNF2), ISWI, CHD, and INO80 constitute the four best-studied subfamilies of ATP-dependent chromatin remodeling complexes and will be discussed in brief in the following sections.

**SNF2 remodeling complexes**

![Fig 1.3: A Representative diagram of SNF2 family remodeling complex.](image-url)

The SNF2 class remodelers possess a central ATPase domain and a bromodomain at the C-terminus to mediate protein-protein interaction (Horn, P. J. et al. 2001) as represented
by fig 1.3. This class of chromatin remodelers are highly conserved and are present in the ySWI/SNF and Sth1 based RSC complexes in yeast, BRM based dSWI/SNF complexes, BAP (Brahma associated proteins) and PBAP (Polybromo associated BAP) in Drosophila. Similar examples of human SNF2 complexes are BAF (BRG1 or BRM-associated factors), and PBAF. The ySWI/SNF complex containing Snf2 ATPase subunit is the most thoroughly studied chromatin-remodeling complex. The complex, initially isolated from yeast, contains at least 11 subunits including the ATPase subunit (Imbalzano, A. N. et al. 1998). The role of ySWI/SNF complex in chromatin remodeling was confirmed by showing the ability of the biochemically-purified complex to alter the structure of the chromatin in an \textit{in vitro} reaction (Peterson, C. L. 1992). Recent evidences have shown that Snf2 subfamily members also interact with additional proteins such as glucocorticoid and retinoic acid receptors to modulate transcription of these genes (Yoshinaga, S. K. et al. 1992). The ySWI/SNF and RSC complexes have been reported to regulate transcriptional activation by mediating nucleosome sliding such that the histone-DNA contacts are altered. Despite of the structural similarity between ySWI/SNF and RSC complexes, they drastically differ in their functions. Yeast SWI/SNF is found to be less abundant in a cell and not essential for viability whereas RSC is more abundant and essential for cell viability (Cairns, B. R. et al. 1996). Drosophila BAP plays an important role in cell cycle regulation by directly regulating CDC25 expression, which is required for a cell to enter into the mitotic phase (Moshkin, Y. M. et al. 2007). Human SNF2 complex such as BAF and PBAF contain BRM and BRG1 as the catalytic subunit respectively (Becker, P. B. et al. 2002). BRM and BRG1 play distinct roles in the cell. BRM is expressed more in non-proliferating cells whereas
BRG1 is highly expressed in cells undergoing constant proliferation (Reisman, D. N. et al. 2005). hSNF2 has been found to interact with histone acetyl transferases (Poch, O. et al. 1997) and histone methyl transferases to facilitate diverse cellular functions, suggesting that nucleosomal remodeling and histone modifications are interdependent phenomena. The histone methyl transferase associated with BRG1-containing SNF2 complexes is reported to methylate and downregulate the tumor suppressor gene ST7 (Suppressor of Tumorigenicity). Similarly BRG1 and the HATs CBP/p300 are also found to associate with active Pol II suggesting a role in transcriptional elongation (Kouskouti, A. et al. 2005).

**ISWI remodeling complexes (Imitation Switch)**

Fig 1.4: A Representative diagram of ISWI family remodeling complex.

The ISWI remodeling complexes were first isolated from drosophila embryo extract and include ACF (ATP-utilizing chromatin assembly and remodeling factor), NURF (Nucleosome remodeling factor), and CHRAC (chromatin remodeling and assembly complex) in Drosophila (Tsukiyama, T. et al. 2002; Varga-Weisz, P. D. et al. 1997). The ISWI complexes contain the highly conserved ISWI ATPase and are characterized by the presence of C-terminal SANT-like domains (Grune, T. et al. 2003) as shown in fig 1.4. The ISWI-based complexes contain fewer subunits (2 to 4) as compared to the SNF2 containing complexes (Martinez-Balbas, M. A. et al. 1998). In mammals, two ISWI homologs have been identified and are known as SNF2H and SNF2L. Chromatin remodeling activity of ISWI subfamily proteins is based on their ability to loosen
histone-DNA contacts causing the nucleosomes to slide along the DNA in an ATP-dependent manner (Varga-Weisz, P. D. et al. 1997). ISWI containing complexes are reported to have diverse functions including transcriptional regulation of RNA polymerase II (Becker, P. B. et al. 2002) replication, chromatin assembly and maintenance of chromatin structure. These complexes have been found to enhance the stability of chromatin structure (Kadonaga, J. T. et al. 2002). In humans, the ISWI containing hACF complex is required for DNA replication (Collin. et al. 2002) whereas in mouse, ISWI containing NoRC (Nucleolar remodeling complex) complex represses transcription of rRNA (Strohner, R. et al. 2001).

**CHD remodeling complex (Chromodomain Helicase DNA-binding)**

![CHD (Mi-2) class](image)

**Fig 1.5**: A Representative diagram of CHD family remodeling complex.

Members of the CHD remodeling complexes are characterized by the presence of two N-terminal chromodomains, a central DNA-dependent ATPase domain and a C-terminal DNA-binding motif (Delmas, et al. 1993) as shown in fig 1.5. CHD proteins are evolutionarily conserved and have been characterized in various organisms from yeast to mammals. The chromodomain is thought to play a role in chromatin compaction (Paro, R. et al. 1991). The most prominent member of this subfamily is the ATPase Mi-2. (Wang, H. B. et al. 2001). In Drosophila, mutations in CHD are found to cause arrest in second stage of larval development, suggesting an involvement in the regulation of normal development (Zhang, Y. et al. 1999). In humans, CHD4 has been shown to be important for normal T-cell development (von Zelewsky, T. et al. 2000). This complex
possesses histone deacetylase activity and mediates transcriptional repression (Becker, P. B. et al. 2002). Though the Mi-2 complex exists as a multisubunit complex in *D. melanogaster*, in *S. cerevisiae*, CHD1 has been shown to exist as a monomer. Mi-2-containing complexes are known to catalyse nucleosome sliding *in vitro* (Brehm, A. et al. 2000).

**INO80 and SWR1 remodeling complexes**

![INO80, SWR1 class](image)

**Fig 1.6:** A Representative diagram of INO80 and SWR1 family remodeling complex.

Ino80 protein constitutes the ATPase subunit of a large INO80.com complex. The conserved ATPase domains of Ino80 and Swr1 are split into two segments by a spacer sequence and thus, are known as split ATPases (Shen, X. et al. 2000) as represented by fig 1.6. hINO80 complex possesses ATP-dependent nucleosome remodeling activity similar to the yeast homolog. The INO80.complex contains 15 subunits including Ino80, Rvb1, Rvb2, Arp4, and actin (Shen, X. et al. 2000). Rvb1 and Rvb2 proteins are highly conserved from yeast to human. Rvb protein shows homology to bacterial RuvB which is known to be a Holliday Junction DNA Helicase (Jonsson, Z. O. et al. 2001). Yeast cells lacking Ino80 or any of the core subunits (Arp5 and Arp8) are hypersensitive to DNA damaging agents suggesting its involvement in DNA repair (Shen, X. et al. 2000).

In addition, it has been shown that the phosphorylated H2AX recruits INO80 complex to double-strand DNA breaks (DSB) at the HO locus and its chromatin remodeling activity is required for the processing and repair of DSB (Shen, X. et al. 2000). The novelty of SWR complex (Krogan, N. J. et al, 2003, Mizuguchi et al, 2003) lies in its role as a histone exchanger that exchanges canonical H2A with H2A.Z variants. SWR1 complex
contains 14 polypeptides and shares some of the subunits like Arps, actin, Rvb1 and Rvb2 etc with the INO80 complex (Krogan, N. J. et al. 2003). Both yINO80 and hINO80 complexes have DNA-dependent ATPase activities and ATP-dependent nucleosome sliding activity *in vitro* (Shen, X. et al. 2000).

**Other subfamilies**

Besides the above mentioned subfamily proteins, there are other groups like SSO163-like subfamily, Rad 54-like subfamily, Rad5/16-like subfamily and SMARCAL1, which is a distant member of the family. Though most SNF2 family members exist as multi-protein complexes, these proteins do not exist as complexes. Rad54 is a 900 amino acid long protein that belongs to *RAD52* group of SNF2 family proteins. The N-terminal region of Rad54 is required for interaction with Rad51, while the C-terminal region contains the helicase motifs required for ATP hydrolysis. Rad54 in cooperation with Rad51 (related to bacterial RecA protein) functions to remodel the chromatin during DNA repair process (Sugawara, N. et al. 1995). Genetic experiments have shown that human RAD54 is a functional homolog of *S. cerevisiae* RAD54. Disruption of RAD54 in mouse embryonic stem cells has been shown to impair homologous recombination resulting in increased sensitivity to ionizing radiation (Essers, J. et al. 1997).

**Mechanism of Chromatin remodeling**

Despite the fact that all remodeling factors possess related ATPase subunits to derive energy from hydrolyzing ATP to alter nucleosome conformations, yet it is not necessary that these machines act by a single common mechanism. As the nucleosomal organization may range from tightly packed nucleosomes to regions having regularly
spaced nucleosomes, the cell has evolved different classes of ATP-dependent remodelers that can use different mechanisms to remodel the chromatin (Becker, P. B. et al. 2002; Lusser, A. et al. 2003). For example, SNF2 complexes cause nucleosome disruption, whereas the ISWI complex catalyses nucleosome assembly or enhance the stability of chromatin structure (Lusser, A. et al., et al. 2003). Similarly, ATPase activity of SNF2 complexes is stimulated equally by nucleosomal DNA and naked DNA whereas ATPase activity of several ISWI complexes is stimulated only by nucleosomal DNA (Laurent, B. C. et al. 1993; Tsukiyama, T. et al. 1995). Further, the process may involve repositioning of nucleosomes by sliding histone octamers to other sites on the same DNA molecule (cis-displacement) or may involve transfer of histone octamers to other DNA molecules (trans-displacement) (Whitehouse, I. et al. 1999, Lorch, Y. et al. 1999). Under in vitro conditions the cis or trans relocation is determined by the ratio of Swi/Snf to the octamer.

Two potential mechanisms have been proposed to explain the chromatin remodeling driven by ATP-dependent molecular machines (Lusser, A. et al. 2003).

**Lateral cross-transfer mechanism**

For the SNF2 subfamily complexes, a lateral cross-transfer mechanism has been suggested as shown in fig 1.7. In this model, the small segment of DNA undergoes twisting or untwisting in an energy-dependent manner to disrupt histone-DNA interactions at the nucleosomal entry sites. This results into a cross-transfer and a net DNA translocation of about 80 bp relative to the octamer (Kassabov, S. R. et al. 2003). This model explains how SWI/SNF related complexes remodel mouse mammary tumor promoter that has closely spaced nucleosomes (Deroo and Archer, 2001) without requiring sliding of the histone octamers. This model is also found to be consistent with
the results obtained in the photocrosslinking analysis of the yeast SWI/SNF complex (Kassabov, S. R. et al. 2003), where they have demonstrated that the yeast SWI/SNF complex unwraps the nucleosome core, causing it to slide and then rewraps the nucleosome back into its original state resulting in net displacement of the nucleosome. Further, this model also explains the property of SNF2 subfamily factors to disrupt nucleosomes instead of assembling the nucleosomes (Fan, H. Y. et al. 2003). In this mechanism, a dinucleosome structure is formed as a reaction intermediate. The formation of a dinucleosome intermediate is characteristic feature of the SNF2 subfamily complexes and is not mediated by ISWI complexes (Langst, G. et al. 2001). The dissociation of a significant length of the nucleosomal DNA has also been shown to promote transfer of the histones from one DNA fragment to a separate DNA template _in trans_. This is again a characteristic feature of SNF2 subfamily complexes but not of ISWI subfamily complexes (Langst, G. et al. 2001).


Fig 1.7: A lateral cross-transfer model for nucleosome remodeling by SNF2 remodeling complexes.
ATP-driven DNA translocation model

ISWI subfamily complexes have been proposed to remodel nucleosomes in an ATP-driven DNA translocation manner (Whitehouse, I. et al. 2003). According to this model, ACF and ISWI complexes have the ability to translationally reposition nucleosomes along the DNA (Whitehouse, I. et al. 2003; Fyodorov, D. V. et al. 2002). This model is in accord with the general property of ISWI subfamily complexes to assemble nucleosomes rather than to disassemble nucleosomes (Ito, T. et al. 1997). In this model a small loop is formed to translocate or slide the DNA along the chromosome. The formation of the loop explains the inability of ISWI subfamily complexes to catalyze the transfer of histones from one template to another template or to form dinucleosome like structures from mononucleosomes, commonly observed with the SNF2 subfamily complexes (Whitehouse, I. et al. 2003; Fyodorov, D. V. et al. 2002). The ISWI subfamily can efficiently remodel nucleosomes containing nicked DNA (Langst, G. et al. 2001). In fact, nucleosomes containing nicked DNA where the nicks are present at the edge of the nucleosome are supposed to be a better substrate than intact DNA (Langst, G. et al. 2001). The model is represented by fig 1.8.

![DNA translocation model](Ref: Lusser and Kadonaga, 2003.)

**Fig 1.8:** A DNA translocation model for nucleosome remodeling by ISWI remodeling complexes.
Various roles of SWI2/SNF2 complex

SWI/SNF complexes are known to mobilize and slide nucleosomes along the DNA template to increase or decrease the accessibility of the DNA to nuclear proteins (Xiao, H. et al. 2001; Whitehouse, I. et al. 2003). Such alteration in accessibility leads to facilitation or repression of various chromatin-dependent processes such as transcription, replication, repair, recombination and other nuclear processes, some of which are discussed in brief in the following section.

SWI/SNF complexes and transcription

The process of transcription requires DNA to become accessible to specific transcription factors and RNA polymerases. The ATP-dependent chromatin remodelers regulate transcription by regulating accessibility of DNA to transcription factors (Cote, J. et al 1994). Remodeling factors themselves do not have sequence-specific DNA binding sites; rather they are directed to the specific site by transcription factors (Dimova, D. et al 1999). Such remodeling factors are found to disrupt nucleosome structure at promoters and thus facilitate the binding of transcription factors to it (Kingston, R. E. et al. 1996; Narlikar, G. J. et al. 2002). Recent studies suggest that SWI/SNF complexes may alter transcription cascade by working with the gene specific activators or repressors resulting in appropriate remodeling of chromatin that would later be bound by transcriptional factors thus, suggesting that SWI/SNF is required for transcriptional activation as well as repression of some genes. For example, the role of SWI/SNF in transcription activation in vivo has been demonstrated by the increased nuclease sensitivity of the SUC2 promoter (Wu, L. et al. 1997). The mammalian SNF2 complex containing BRG1 has been found to interact with transcriptional activators such as glucocorticoid receptor to
activate transcription (Fryer, C. J. et al. 1998). Similarly, Swi5 acts as a recruiter of Swi/Snf to the HO promoter (Yudkovsky, N. et al. 1999) to promote its function. Microarray experiments have shown that INO80 complex regulates transcription of at least 20% of the yeast genes (Jonsson, Z. O. et al. 2001). Thus, Swi/Snf recruitment by transcriptional activators is probably conserved throughout eukaryotes. The SNF2 protein are also involved in transcription repression. For example, RSC is essential for repression of the CHA1 gene (Moreira, J. M. et al. 1999). NuRD is another complex that mediate gene repression. In this complex the Mi-2 ATPase subunit interacts with sequence-specific transcriptional repressors (Kehle, J. et al. 1998; Kim, J. J. et al. 1999) to perform its repressive role. Also, NuRD has been found to interact with CpG-methylated DNA and may thus participate in gene inactivation (Wolffe, A. P. et al. 1999). Furthermore, SNF2 is shown to have role in tumor suppression by interacting with retinoblastoma protein (Dunaif, J. L. et al. 1994) to regulate cellular growth. Human BRG1 and hBRM bind to retinoblastoma (Rb) gene products to inhibit the activity of transcriptional activator E2F (Muchardt, C. et al. 1999). Mot1 also belongs to SNF2 family proteins and is known to dissociate TBP bound to TATA boxes from a TBP-DNA complex in an ATP-dependent manner and repress transcription (Auble, D. T. et al. 1994).

**SWI/SNF complexes and Replication**

Besides transcription, chromatin remodelers also play a role in DNA replication. However, their involvement other than in transcription might be indirect in a way that chromatin modification influences the transcription of genes that are required for DNA replication (Karina, B. et al. 2006) thus affecting the process. In yeast, the SWI/SNF
remodeling complex has been shown to be required for initiation of replication (Falbo, K. B. et al. 2006) providing the evidence for the need of chromatin remodeling to move nucleosomes around the replication origin so as to allow the binding of replication factors. In addition, the dyad symmetry (DS) region of the origin of replication was found to be flanked by nucleosomes that undergo remodeling at the G1/S boundary. This event correlates with MCM binding at the G1/S border, suggesting changes in the chromatin structure in relation to replication (Karina, B. et al. 2006). Further, SNF2h was found to be enriched at the DS region in G1/S arrested cells, and mutation of SNF2h inhibited binding of MCM and hence inhibition of replication (Karina, B. et al. 2006).

These observations emphasize the role of chromatin remodelers in replication. Another important chromatin remodeler INO80 is well known in yeast for its role in replication. Recently, it has been reported that INO80 plays role in DNA replication in higher eukaryotes and humans as well, where it has been shown that hINO80 binds to chromatin at the replication forks. Further they have shown that INO80 is required for efficient DNA synthesis during the S-phase and is important for normal cell cycle progression (Hur, S. K. et al. 2010).

**SWI/SNF complexes in DNA Repair**

Damaged DNA sites need to be exposed to the repair factors and therefore, alteration in chromatin structure is essential. Chromatin remodelers that are known to alter the chromatin structure have also been reported to be playing an important role in DNA repair besides having a role in replication and transcription. In fact the same remodeling factor can also be involved in regulating all of the above three processes (Eberharter, A. et al. 2005; Hassan, A. H. et al. 2001), as loosening of the chromatin structure is required
for all such DNA metabolic processes. Smerdon et al (Smerdon, M. J. et al. 1999 and Green, C. M. et al. 2002) have proposed a useful model for the DNA damage repair. The "access-repair-restore" suggests that the chromatin structure is altered to expose damaged DNA to repair factors and the original structure is restored once the repair is complete.

ATP-dependent chromatin remodelers have been found to be involved in both double-stranded breaks repair (DSB) and nucleotide excision repair (NER) (Jagannathan, I. et al. 2006). NER takes place more rapidly in linker DNA between the nucleosomes than in core particles, suggesting the repair factors have less access to chromatin than to a naked DNA (Evans, D.H. et al. 1984). A number of in vitro studies have shown that excision steps in NER are enhanced by the SWI/SNF ATPase (Hara, R. et al. 2002). Further, it was reported by Hara et al. that there exist a cooperative relation between repair and remodeling activities, suggesting that SWI/SNF proteins increase the accessibility of repair factors to the DNA and in turn NER repair factors increase the remodeling activity of SWI/SNF (Hara, R. et al. 2002). Rad54, INO80, SNF2, SWR1, RSC, dTIP60 and BAF are some of the ATP-dependent remodelers that are involved in DSB repair as well, in addition to their respective roles in chromatin remodeling (Downs, J. A. et al. 2004).

Rad54 is an evolutionarily conserved chromatin remodeler with double-stranded DNA-stimulated ATPase activity and has an interesting role in homologous recombinational repair (HR) depending upon the stage of repair. At an early stage of HR, Rad54 helps recruiting Rad51 to the damaged site for which the need of ATP is found to be dispensable whereas at the late stages of HR, it promotes branch migration of Holliday junctions to stabilize DNA joints for DNA polymerase to bind which is found to be an ATP-dependent process (Wolner, B. et al. 2005). INO80 complex is another ATP-
dependent remodeler that is well known for its activity in DSBs. It is recruited to the DSB site created by the HO endonuclease at the yeast MAT locus (van Attikum, H. et al. 2004) where INO80 based remodeling is required to disrupt the nucleosomes at the yeast MAT locus. In support of this it has been observed that the deletion of catalytic ATPase subunit from INO80 complex leads to the increased sensitivity to agents causing DSBs (Tsukuda, T. et al. 2005). The ySWI/SNF complex too plays a role in DSB repair. Like INO80 complex, ySWI/SNF is recruited to the HO-induced DSB at the yeast MAT locus to repair DSB by HR pathway (Chai, B. et al. 2005). The Snf2 protein associates with both the recipient locus (MATα) and the donor locus (HMLα) to cause synapses that is blocked in the absence of the chromatin remodeling. Though the remodeling mechanism of ySWI/SNF complex in vivo is not well characterized but nucleosome reconstitution experiment suggests that the remodeling activity of ySWI/SNF complex alters histone-DNA contacts to remodel nucleosomes; transfer histone dimers; and slide nucleosomes in cis (Mohrmann, L. et al. 2005). hSNF2 has a direct role in V(D)J recombination where it stimulates V(D)J cleavage by RAG proteins and the catalytic subunit of BRG1 of hSNF2 is seen associated with immunoglobulin loci for the rearrangement (Patenge, N. et al. 2004). SWR1 and TIP60 are two ATP-dependent chromatin remodelers that are known to incorporate histone variants into chromatin (Narlikar, G. J. et al. 2002; Becker P. B. et al. 2002). The ATPase activity of SWR1 is used to exchange H2A-H2B dimers with H2AZ-H2B dimers in the nucleosomes (Mizuguchi, G. et al. 2004). SWR1 is known for its potential role in transcription but it also appears to have role in DSB repair, where it is found to be recruited to HO-induced DSB at the MAT locus by γH2AX and a mutant swr1 makes yeast cells sensitive to DSB causing agents (Papamichos-Chronakes, M. et
A recent finding suggests that INO80 complex acts antagonistic to SWR1 at HO-induced DSB at the MAT locus. It has been observed that in the absence of INO80, level of γH2AX goes down and signals the incorporation of H2AZ near the breaks (Papamichos-Chronakes, M. et al. 2006). Similarly, TIP60 is also known for its multiple activities such as DNA-binding, ATPase, helicase and histone acetyltransferase activity (Ikura, T. et al. 2000). TIP60 appears to have at least two functions in DSB repair. The HAT activity of hTIP60 acetylates H4 at DSBs, which promotes the recruitment of repair factors to damage site. In Drosophila, TIP60 is involved in exchanging the pH2Av variant for unmodified H2Av (Kruhlak, M. J. et al. 2006). The RSC chromatin remodeling complex too has been reported to play a role in DSB repair (Chai, B. et al. 2005). RSC plays important role in HR repair of a MAT DSB and like the γSWI/SNF, it associates with both recipient and donor chromatin to recruit factors for strand invasion (Chai, B. et al. 2005).

In addition to alterations in chromatin structure, post-translational modifications of histones also occur during repair of DSBs. DSB repair is characterized by the phosphorylation of C-terminus of histone H2AX, a H2A variant (Rogakou, E. P. et al. 1998). Formation of phosphorylated H2A also known as γH2AX occurs within minutes after a DSB is formed and spreads over a large chromatin domain (Rogakou, E. P. et al. 1998). γH2AX is required for the accumulation and retention of repair factors and check-point proteins at the site of damage. These evidences suggest that the two mechanisms of chromatin remodeling: histone modification and nucleosome disruption are linked to the DSB repair pathways.
SWI/SNF complexes and cancer

Mutations in ATP-dependent chromatin remodeling factors are linked to various cancers, BRG1 has been found to be mutated in multiple human cancer cell lines (Wong, A. K. et al. 2000). Studies with mammalian cell lines suggest that the ATPase subunit of hSNF2 complexes BRG1 and hBRM physically interacts with retinoblastoma (Rb). Rb plays important role in regulating cell proliferation and differentiation therefore, hBRG1 and hBRM function as tumor suppressor gene in an Rb-dependent manner (Dunaief, J. L. et al. 1994). In addition, hBRG1 ATPase also interacts with cyclin E that in turn associates with Cdk2 to control G1/S checkpoint of the cell cycle. Cdk2-cyclin E complex phosphorylates both hBRG1 and BAF155, which might regulate the activity of hSNF2 (Shanahan, F. et al. 1999). The SNF2 complex associates with BRCA1 to cause cell-cycle arrest, either by downregulating E2F target genes like cyclin E, or by up-regulating cyclin-dependent kinase inhibitors (Bochar, D. A. et al. 2000). Besides this, yeast RSC is found to be involved in centromere remodeling during G2/M arrest and mutations in RSC cause arrest in G2/M leading to cell death (Angus-Hill, M. L. et al. 2001).

SWI/SNF complexes in cell viability and development

Not all SWI/SNF proteins are essential for viability. For example, gene knockout experiments show that BRG1 cannot be deleted suggesting that the BRG1 complex is essential for viability (Bultman, S. et al. 2000; Sumi-Ichinose, et al.1997). However, BRM on the other hand is dispensable for cell growth since deletion of both alleles does not affect viability (Reyes, J. C. et al. 1998). Besides this, many of the SWI/SNF complexes have been shown to be involved in developmental pathways by directly interacting with promoter or enhancer binding proteins to mediate glucocorticoid and
stress induced apoptosis (Han, S. et al. 2001). These complexes have also been shown to contribute towards genomic recombination during T-cell differentiation by homologous recombination (Patenge, N. et al. 2004).

**SMARCAL1:**

Fig 1.9: Schematic diagram of SMARCAL1 showing seven helicase domains.

hSMARCAL1 (human SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a-like1) is one of the members of the SNF2 family of proteins. The protein was first isolated as DNA-dependent ATPase A, a 105-kDa protein from calf thymus tissues (Mesner, L. D. et al. 1993). The amino acid sequence analysis of this protein showed that it belonged to the SNF2 family of proteins due to the presence of the seven-helicase motifs in the C-terminus. The human homolog of this protein is known as hSMARCAL1 and was isolated from cDNA clones (Coleman, M. A. et al. 2000). The hSMARCAL1 transcripts were shown to ubiquitously express in different human and mouse tissues, suggesting its role as a housekeeping gene (Coleman, M. A. et al. 2000).

To understand the mechanism of ATP hydrolysis by SMARCAL1, the 82-kDa fragment named Active DNA-dependent ATPase A Domain (ADAAD) was overexpressed in E.coli. ADAAD was found to hydrolyze ATP only in the presence of DNA molecules possessing double-stranded to single-stranded transition regions (Muthuswami, R. et al. 2000). The stem-loop DNA molecules with 12 base pair long stem and 13 base loop were found to induce maximal ATP hydrolysis (Nongkhlaw, M. et al. 2009). In order to understand the underlying mechanism of how different DNA structures are recognized by
these proteins to bring about ATP hydrolysis, extensive biophysical studies have been done. Fluorescence spectroscopy studies have now shown that ADAAD has an affinity towards both DNA and ATP individually in the absence of the other ligand. The resulting [protein.ATP] or [protein.DNA] complex then interacts with the second ligand to form a ternary complex. Binding of both ATP and DNA brings about a conformational change resulting in ATP hydrolysis. This conformational change was found to be effected only by DNA molecules with a double-stranded to single-stranded transition region (Nongkhlaw, M. et al. 2009).

Mutations in hSMARCAL1 have been reported to cause a rare autosomal recessive disorder known as Schimke Immunoosseous Dysplasia (SIOD) (Boerkoel, C. F. et al. 2002). SIOD results from loss of hSMARCAL1 function that can happen by multiple mechanisms such as frameshift, deletion and nonsense mutations. Missense mutations too have been reported and these mutations result in altered subcellular localization, protein level, chromatin binding capacity and enzymatic activity of hSMARCAL1 (Elizondo, L. I. et al. 2009). The syndrome is characterized by chronic renal dysfunction, T-cell immunodeficiency, hypothyroidism and spondyloepiphyseal dysplasia suggesting that mutations in hSMARCAL1 affect multiple cellular functions (Boerkoel, C. F. et al. 2000). Microarray analysis has suggested that the protein interacts with nucleosomes and not with isolated histone proteins (Coleman, M. A. et al. 2000). Recently Elizondo et al have demonstrated the interaction of hSMARCAL1 with chromatin using polytene chromosomes. Further, it has been shown that the severity of the disease was inversely proportional to the overall hSMARCAL1 activity in Drosophila (Elizondo, L. I. et al. 2009). Interestingly, hSMARCAL1 has no yeast homolog.
suggesting, that the protein evolved only in higher eukaryotes to play a role in a process that occurs only in these organisms. Recently, Yusufzai and Kadonaga have shown that the protein might be an ATP-dependent reverse helicase, which catalyzes the annealing of single-stranded DNA bubbles stably bound by RPA (Replication protein A) to form dsDNA (Yusufzai, T. 2009). Further, it was shown that the conserved N-terminal motif is sufficient for binding to RPA and is required for the recruitment of hSMARCAL1 to the sites of DNA damage to induce DNA repair (Yusufzai, T. 2009). Recently, knockdown of hSMARCAL1 in zebrafish has been shown to cause cell cycle arrest at the G0/G1 stage, induce apoptosis, and developmental abnormalities (Huang, C. et al. 2009). This goes in accord with the earlier observation that SIOD is accompanied by defects in a large number of tissues and organs, suggesting its role in various developmental pathways.

HYPOTHESIS

Mutation in hSMARCAL1 leads to multiple system defects, suggesting hSMARCAL1 might play a multi-faceted role in a cell. We hypothesize that the unique N-terminal region which is present only in higher eukaryotes, could be involved in protein-protein interactions enabling it to link multiple cellular processes, and thus might have evolved to provide a unique role to such higher eukaryotic organisms. Therefore, we hypothesized that deciphering the protein partners of hSMARCAL1 might help in unravelling the biological role of hSMARCAL1.
Approach of my work:

- Biochemical methods were used to identify the interacting partners of hSMARCAL1.
- Physiological role of hSMARCAL1 in cell cycle and apoptosis was explored using cell biological techniques in HeLa cells.