Lecture No.2: Chromatin structure and function

Hence this course is mainly focusing on gene regulation, on both levels (transcriptional and post transcriptional, we have first to be familiar with the structure and the packaging of the DNA. As structure of the DNA was previously taught early in your undergraduate stage, this lecture will focus on DNA packaging which is mainly achieved by what so called Chromatin.

Chromatin:

DNA is packaged into a small volume to fit in the cell and to strengthen DNA to protect it from damage. The main proteins which do this job are called histones. The combination between DNA and histones is known as **chromatin**. The primary functions of the chromatin is packaging of DNA to allow mitosis and meiosis and to control gene expression and DNA replication. Chromatin is only found in euokaryotic cells, while prokaryotic cells have a very different organization of their DNA which is referred to as a genophore (a chromosome without chromatin).

The structure of chromatin depends on several factors. The overall structure depends on the stage of the cell cycle: during interphase the chromatin is structurally loose to allow access to RNA and DNA polymerases that transcribe and replicate the DNA. The local structure of chromatin during interphase depends on the genes present on the DNA: DNA coding genes that are actively transcribed ("turned on") are more loosely packaged and are found associated with RNA polymerases (referred to as euchromatin) while DNA coding inactive genes ("turned off") are found associated with structural proteins and are more tightly packaged (heterochromatin). Epigenetic chemical modifications of the structural proteins in chromatin are responsible for the tightness of the DNA packaging and therefore they control gene expression. These modifications include primarily methylation and acetylation of histone proteins. As the cell prepares to divide, i.e. enters mitosis or meiosis, the epigenetic modifications of the chromatin make DNA packaging tighter to facilitate segregation of the chromosomes during anaphase. During this stage of the cell cycle this makes the individual chromosomes in many cells visible by optical microscope. [1]

There are three levels of chromatin organization; Euchromatin, Heterochromatin and higher level DNA packaging. When DNA wraps around histone proteins forming a structure known as nucleosome it is called euchromatin. However, when multiple histones wrap into a

fibre consisting of nucleosome arrays in their most compact form it is known as heterochromatin. The Higher-level DNA packaging represents the most compact form of DNA packaging and it occurs mainly during the metaphase of cell division to form chromosomes (metaphase chromosome).

Heterochromatin is highly condensed, gene-poor, and transcriptionally silent, whereas euchromatin is less condensed, gene-rich, and more easily transcribed. Nucleosome modifications distinguish heterochromatin from euchromatin. Euchromatin is typically enriched in acetylated histones H3 and H4 and H3K4 methylation (H3K4me) (which all are transcription activators), whereas heterochromatin is characterized by hypoacetylation of histones, H3K9me, association of heterochromatin protein-1 (HP1), and DNA cytosine methylation (5mC) (which are transcriptional repressors). It is usually stated that heterochromatin structure is more compact than euchromatin making the interaction of regulatory factors with their DNA targets difficult. However, very recently an interaction of general transcription factors with heterochromatin was shown (Morales, etal., 2001).

It appears that the three levels of chromatin organizations are based mainly on the formation of nucleosomes. Thus we will talk a bit more about the nucleosomes.

Nucleosomes

The nucleosome is the basic unit of an eukaryotic chromosome, consisting of 146 base pairs (bp) of DNA coiled around a core consisting of a histone octamer, H2A, H2B, H3, and H4. Histones H3 and H4 form a dimer, two H3-H4 dimers associate into a (H3-H4)2 tetramer. DNA wraps around this tetramer, forming a tetrameric particle. Histones H2A and H2B heterodimerize and heterodimers associate on each side of the tetrameric particle to form a nucleosome (Fig 3). In addition to the core histones, there is the linker histone, H1, which contacts the exit/entry of the DNA strand on the nucleosome. Nucleosomes are interconnected by sections of linker DNA, a far shorter arrangement than pure DNA in solution. Nucleosomes are organized forming a simple helix or solenoid with 6 nucleosomes per turn (Fig. 1), in which the linker DNA is folded between consecutive nucleosomes (Daban, etal., 2011). The nucleosomes bind DNA non-specifically, as required by their function in general DNA packaging. There are, however, large DNA sequence preferences that govern nucleosome positioning. Nucleosomes can be positioned on the DNA either precisely or randomly (*Fig 4*). Nucleosome positioning on the DNA has direct consequences

on the accessibility of DNA regulatory sequences to cognate regulators **(**Morales, *et al*., 2001; Daban, *et al*., 2011).

Nucleosome modulation

The nucleosome structure can be modulated by the substitution of one of the histones for a variant counterpart. The most studied are the centromeric histone H3-variant CENP-A , histone H2A.Z found in the active chromatin and histone H2AX that is phosphorylated in response to DNA double strand breaks

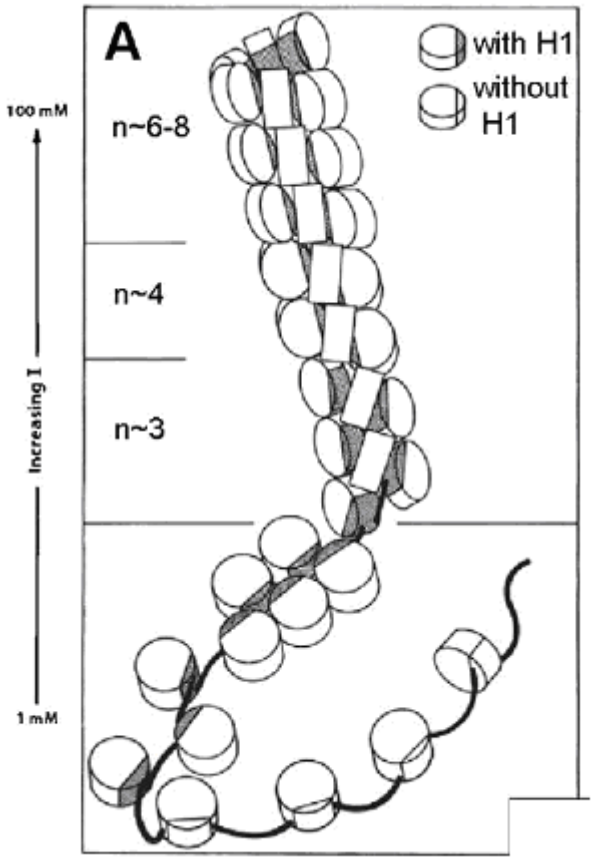

Fig1. (Daban, etal., 2011).

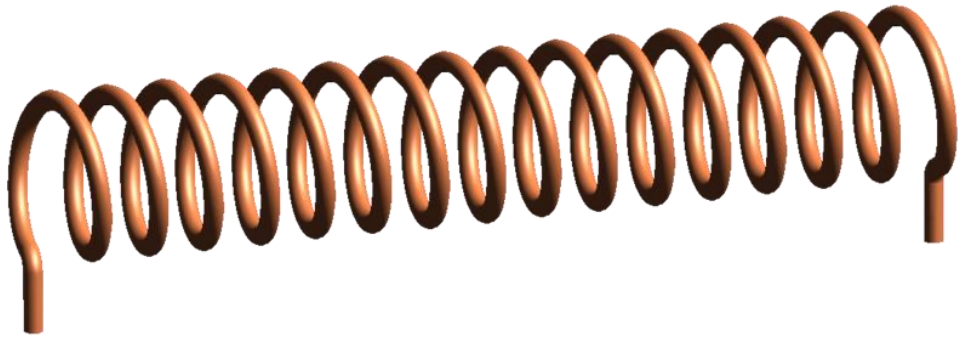

Fig2 : Selenoid shape

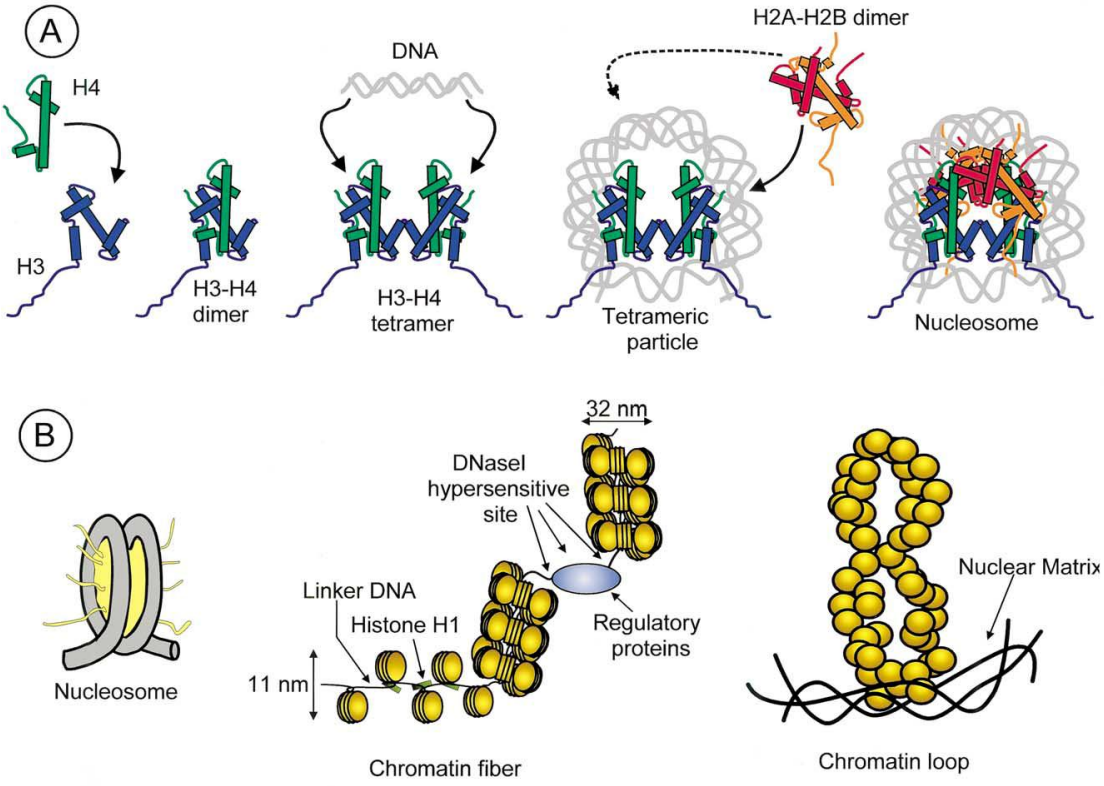

Fig3 (Morales, etal., 2001)

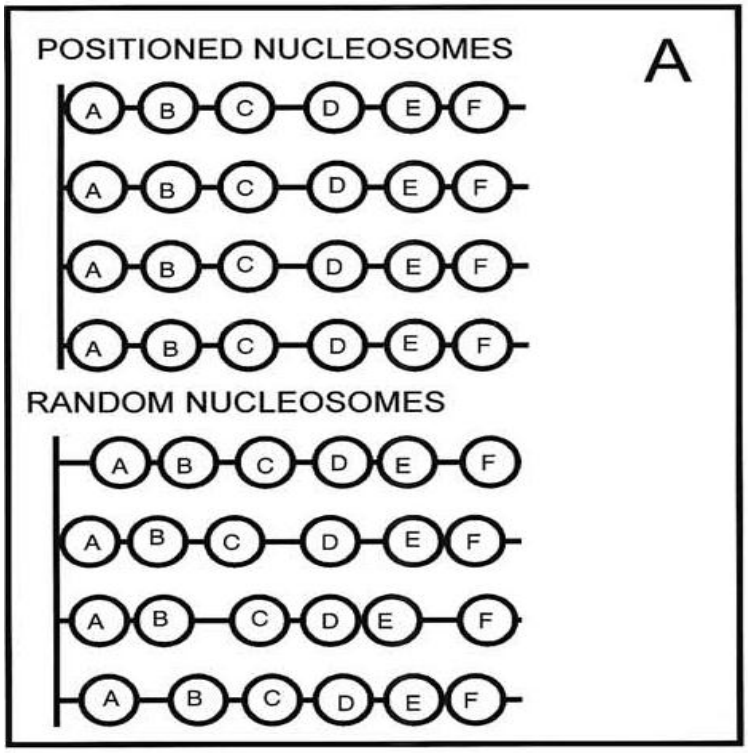

Fig 4: nucleosome positioning (((Morales, etal., 2001)**.**

Chromatin Remodelling

DNA transcription, replication, repair and/or recombination require DNA accessibility to factors involved in the initiation of such processes. This requires sequential changes into chromatin structure.

To achieve such chromatin structural changes, two major mechanisms have been proposed: 1) the posttranslational modification of histones; and 2) the action of ATP-dependent chromatin remodeling complexes (Morale, etal., 2001).

1. Post-translational modifications of histones

Histones can undergo post-translational modifications such as acetylation, methylation, phosphorylation, poly-ADP ribosylation, and ubiquitination of histone amino termini (*fig 5*). The affinity of histones for DNA and chromatin-associated proteins is controlled by these modifications, which along with the positioning of histones organize the genome into either open or condensed chromatin and thus regulate the accessibility of DNA for transcription, recombination, replication, and repair. The positioning and modification of the histones form a histone code or epigenetic ‘memory’ that is passed from mother cell to daughter cells. It is

proposed that the combinatorial use of histone modifications may work as a marking system that is recognized/read by regulatory proteins. On the other hand, these marks may directly affect chromatin structure.

More than 30 residues within each of the four octameric histone partners comprising a nucleosome are described as sites that can be modified in the context of chromatin. Individual histones may acquire a series of modification marks in close proximity to each other. The way these modifications interact with each other and the way they correlate with the transcriptional states are currently object of significant research efforts. The emerging view is that enzymes that catalyze histone modifications and proteins that are able to read the ‘‘code’’ act in a concerted and highly interdependent fashion. These ‘‘translators’’ or effector proteins bind to specific modifications and recruit other regulatory or remodelling factors which, in turn, will help to nucleate or maintain a particular chromatin structure, thus dictating transcriptional activity. The dynamic modifications of the chromatin structure are mostly observed during alterations in the transcriptional activity. But the implications of these modifications may extend to nuclear processes as diverse as cell cycle progression, and DNA replication and repair. In this series of lectures we will discuss histone post-translational modifications (Quina, etal.,2006).

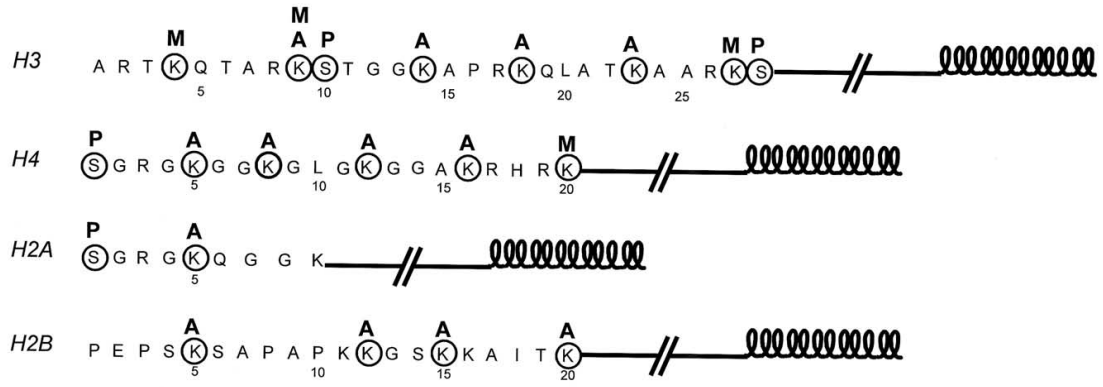

Fig 5 (Morales, etal., 2001)

1.1. Histone acetylation

Histone acetylation is the most frequent post-translational histone modification, consisting of the addition of acetyl groups to lysines mostly from the amino-terminal tails of core histones. Histone hyperacetylation is usually correlated with transcriptional activation. Histone N-terminal tails protrude outside the nucleosome. Their positive charge due to high lysine content may favor histone-DNA contacts, either within a nucleosome or between

nucleosomes. It is usually proposed that histone acetylation results in an opening of the chromatin fiber, although this has not been demonstrated. Acetylation could neutralize the positive charges of the histone tails and release the putative histone-tails DNA interactions. The influence of histone tails acetylation on the structure of a single nucleosome has been investigated in vitro. Histone tail removal or acetylation does not change the structure of the nucleosome. However, it has a dramatic effect on the structure of the tetrameric particles (nucleosomes without H2A-H2B), that undergo a structural transition. It has been demonstrated that H2A-H2B are mobile, because they are easily exchanged in vivo. In such a situation, dissociation of H2A-H2B dimmers from an acetylated nucleosome within a constrained chromatin fiber may cause a transition to a more locally 'open' conformation to facilitate processes such as transcription. In their natural chromatin context, histone tails engage interactions with numerous chromatin components. They mediate inter-nucleosomal interactions and play a role in maintaining a chromatin condensed state in vivo. However, the data on the effect of histone tail acetylation on chromatin fiber condensation/decondensation remain contradictory. Histone tail modifications can also play an indirect role on chromatin structure, allowing the recruitment of specific proteins to the modified histones. It has been proposed that the repertoire of histone-tail modifications constitutes a code (as discussed above). A role for histone tails modification in the recognition of specific protein is supported by the observation that proteins containing a bromodomain recognize specifically acetyl lysines. Altogether, data from the literature suggest that histone tail modifications may play a direct role on chromatin fiber compaction and/or an indirect role through the recruitment of chromatin remodeling complexes.

A number of enzymes harboring a histone acetyltransferase activity (HATs) has been characterized (for a brief summary see *table I*). Most of the HATs are part of large multiprotein complexes. These complexes are necessary to achieve correct histone acetylation within a nucleosome in vitro. Histone deacetylation is catalyzed by histone deacetylases (HDAC) and is a rapid phenomenon.

Histone acetyltransferases

Three main groups of HATs have been characterized based on sequence similarity, namely: GNAT, MYST and p300/CBP (Table 1). The GNAT (Gcn5-related N-acetyltransferase) group members function as co-activators for a subset of transcriptional activators. This group includes GCN5 and p300/CREB-binding protein-associated factor (PCAF). GCN5 acts as a

transcriptional adaptor and preferentially acetylates histone H3 and, to a lesser extent, histone H4. The MYST (monocytic leukemia zinc finger protein) family contains several members, namely, MOZ, Ybf2-Sas3, Sas2, Tip60 (homologue of yeast Esa1), HBO1 and MORF (MOZ related factor). All of them have a particular highly conserved 370 residues MYST domain, which uses an acetyl-cysteine intermediate in the acetylation reaction. They are involved in a wide range of regulatory functions including transcriptional activation. Finally, p300 and CBP acetylate the amino-terminal tails of all four histones.

Histone deacetylases

At present, 18 mammalian HDACs have been described. They are classified into classes depending on their structural homologies to yeast HDACs, enzymatic activities and sub-cellular localization (Table 1). Class I HDACs (HDAC1, -2, -3 and -8) are generally nuclear proteins that are widely expressed in different tissues, deacetylating both histones as well as other nuclear proteins . The members
of this class are crucial in controlling the proliferation state of mammalian cells. HDAC1 and HDAC2 often heterodimerize and have been found in the same co-repressors complexes. Whereas HDAC1 is involved in cell cycle regulation, influencing the ability of tumour cells to undergo mitosis, HDAC2 seems preferentially to prevent apoptosis and does not take part in cell cycle control. HDAC3 is necessary for normal mitotic progression.

On the other hand, class II HDACs are larger than class I HDACs, have tissue specific expression and can be located either in the nucleus or in the cytoplasm, depending on their phosphorylation state and subsequent binding with chaperone proteins. According to sequence homology and domain organizations, this class has been further divided into two classes. Class IIa is integrated by HDAC4, -5, -7 and -9, and class IIb includes HDAC6 and -10. (Lafon-Hughes, etal., 2008; Morale, etal., 2001; and Daban, etal., 2011).

Table I. Summary of the major histone acetyltransferases (HATs).

Table 1
Human enzymes involved in DNA methylation, histone acetylation and poly-ADP-ribosylation of histone and non-histone proteins

DNA methylation		Histone acetylation		Poly-ADP-ribosylation	
DNMTs [17,237]	DNA demethylases [17]	HATs families [3]	HDACs [39,84,129]	PARPs [174]	PARG [174]
DNMT1	MBD2	GNAT	Class I: HDAC1, HDAC2, HDAC3, HDAC8	PARP-1	
				PARP-2	
				PARP-3	
DNMT2	5-MCDG				
DNMT3A		P300/CBP	Class II (*IIa*): HDAC4, HDAC5, HDAC7, HDAC9	vPARP	PARG
DNMT3B	G/T MMR enzyme			Tankyrase-1	
DNMT3L	MBD4	MYST	Class II (*IIb*): HDAC6, HDAC10	Tankyrase-2	
			Class III: SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7	TiPARP	
			Class IV: HDAC11		

DNMT: DNA methyltransferases; MMR: mismatch repair; MBD: methyl CpG-binding domain; meCs: methyl citosines; HAT: histone acetyltransferases; GNAT: GCN5-related *N*-acetyltransferases; CBP: CREB-binding protein; MYST: named for the founding members of this HAT family MOZ, Ybf2/Sas3, Sas2, and Tip60; HDACs: histone deacetylases; SIRT: sirtuins; PARP: poly-ADP-ribose polymerases; PARG: poly-ADP-ribose-ribose glycohydrolase.

2. ATP-dependent chromatin remodeling complexes

ATP-dependent chromatin remodeling Complexes bind DNA and use the energy from ATP hydrolysis to move the histone octamers among and along DNA molecules. ATP-dependent chromatin remodeling complexes specifically recognize histones tail modifications (which have been discussed above), and through ATP hydrolysis unwrap, mobilize, exchange or eject the nucleosome, subsequently recruiting the transcriptional apparatus to nucleosomal DNA (Fig 6). The first step consists in the binding between the remodeler and the nucleosome. The translocase domain of the remodeler, which has been proposed to be composed of a torsion sub-domain and a tracking sub-domain, binds a specific location of the nucleosomal DNA. Upon ATP hydrolysis, the torsion subdomain carries out a directional DNA translocation. This event destroys histone-DNA contacts and creates a transient DNA loop that propagates around the nucleosome and resolves when it reaches the exit site on the other side of the nucleosome resulting in nucleosome repositioning. The tracking domain ensures that the waves of DNA loops can move only in one direction blocking any backward

movement. The remodeler then resets its original position ready for a new remodeling cycle (Fig 8). During the remodeling process the contacts between histones and DNA need to be broken and reformed along the length of the nucleosome. In this manner, chromatin structure simultaneously provides a packaging solution and a sophisticated apparatus for regulating gene expression.

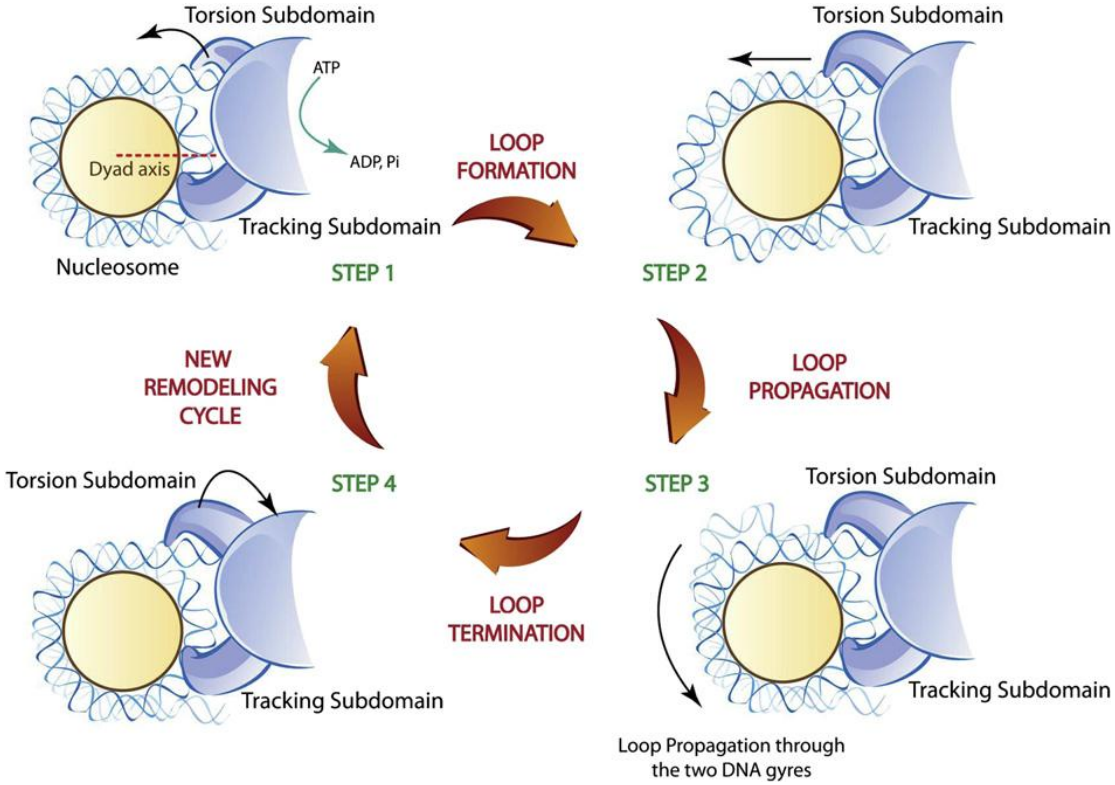

Fig. 6. Schematic representation of the SWI/SNF dependent nucleosome remodeling process. The nucleosome is illustrated as spherical disc surrounded by the two DNA gyres (indicated with two different brightness based on the relative distance to the reader). Steps 1e4 indicate the different stages proposed to occur during the remodeling process. During Step1, the translocase domain binds the nucleosome two. Upon ATP-dependent hydrolysis, the torsion sub-domain generates a DNA loop that translocates through the tracking sub-domain, continuing in the second gyre (Step 2e3). The loop resolves when it reaches the exit site on the other side of the nucleosome (Step 4). The combination of these steps results in nucleosome repositioning. The complex is then ready for a new remodeling cycle (Step 1) **(Tang, etal., 2010).**

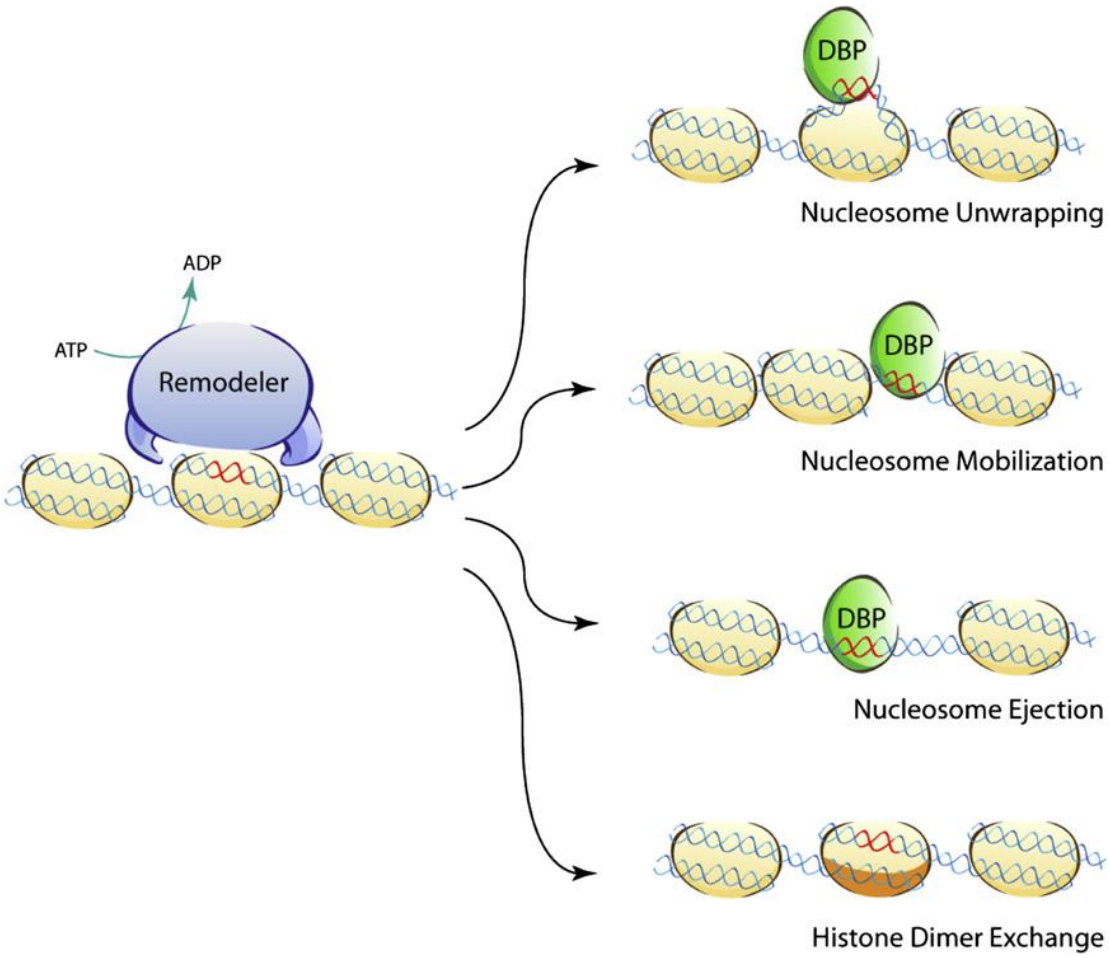

Fig. 7. Different effects of the ATP-dependent chromatin remodeling activity of remodelers on nucleosomal DNA: upon hydrolysis of ATP, a protected region of chromatin can become available to DNA binding protein complexes, such as transcription factors (in green). Nucleosomes can be unwrapped, mobilized or ejected to allow these processes. In some cases ATP-dependent remodeling complexes can use ATP to introduce histone variants within the nucleosome by a process called dimer exchange. (DBP stands for DNA-binding protein). **(Tang, etal., 2010)**

ATP-dependent chromatin remodeling complexes are large (>1 MDa) multi-component complexes (consisting of between 4 and 17 subunits) that are highly conserved within eukaryotes. They are characterized by the presence of an ATPase subunit belonging to the superfamily II helicase-related proteins. Proteins belonging to this class contain an ATPase domain that is itself comprised of two parts, the DExx and HELICc regions, which are separated by a linker. This class can be further classified into at least 4 different families (SWI/SNF, ISWI, NURD/Mi-2/CHD and INO80) based on the additional presence of unique domains within or adjacent to the ATPase domain (Fig. 7).

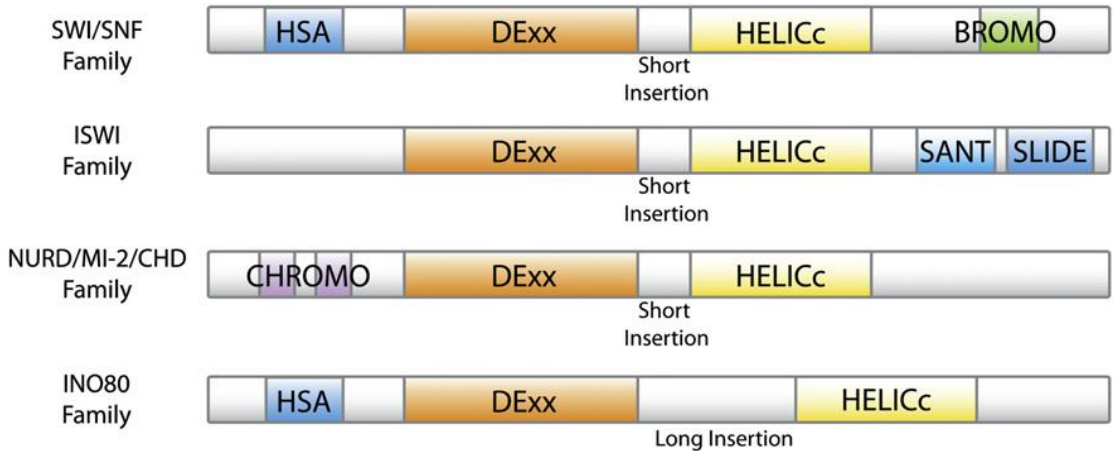

Fig. 8. Classification of ATP-dependent chromatin remodeling complexes: The ATPase subunit of all the remodeling complexes belongs to the superfamily II helicase group. The ATPase always contains a DExx and a HELICc domain, spaced by a linker. The remodelers are classified into different families based on the presence of additional domains on their ATPase subunits. The SWI/SNF family contains an HSA domain, involved in actin binding, and a bromodomain important for the binding of acetylated lysines. The ISWI family contains the SANT and SLIDE domains, important for histone binding. The CHD/NURD/Mi-2 family contains a tandem Chromo domain, also used for histone binding. The INO80 family, like the SWI/SNF family, comprises an HSA domain but it is also characterized by the presence of a longer insertion between the DExx and the HELICc domains. **(Tang, etal., 2010)**

Chromatin remodeling and diseases

Mutations affecting the function and targeting of chromatin-remodeling complexes generally cause cancers or multi-system developmental disorders. The multisystem nature of these single-gene disorders can be explained by deregulation of chromatin structure at many loci. The disruption of the acetylation/deacetylation balance by alteration of HATs and HDACs activities because of mutation, amplification, over-expression or translocation, have been found in various cancers, especially those of haematological and epithelial origin, contributing to tumour initiation and progression (Table 2).

Table 2:

Epigenetic alteration	Cancers
Histone acetylation	
Histone hypoacetylation	Prostate
HATs over-expression	Breast, ovarian
PCAF	
Mutation	Ovarian, colorectal
P300	
Missense or truncating mutation	Colorectal, gastric
Translocation	Leukemia
CBP	
Mutation or deletion	Lung
Translocation	AML
MOZ/MYST3	
Translocation	AML
HDAC1 over-expression	Gastric Prostate Colon Breast
HDAC2 over-expression	Gastric
HDAC6 over-expression	Breast (ERα-positive)
HDAC10 reduced expression	Lung
SIRT7 over-expression	Thyroid

Three possible mechanisms of acetylation deregulation have been postulated. On one hand, histone hypoacetylation at promoter regions induced by both, lack of HAT function or increased HDAC activity, results in silencing tumour suppressor genes, such as p21WAF/Cip, which expression was abolished by the hypoacetylation of histones at its promoter in some tumours. Inversely, abnormal histone hyperacetylation at certain promoters through increased HAT activity or reduced HDAC function could activate genes normally repressed, leading to the inadequate expression of proteins and cancer development. Lastly, the aberrant recruitment of HATs or HDACs activities could act as a carcinogenesis trigger.

The reversibility of epigenetic alterations opens new targets for therapeutic intervention in carcinogenesis [3,94,144]. As several types of cancers present mutations or chromosomal translocations that result in repression of transcription by overexpression or abnormal recruitment of HDACs, these enzymes are regarded as one of the most promising targets for epigenetic treatment. Thus, the use of HDAC inhibitors (HDACis) is considered a potent strategy in cancer therapy as they can induce growth arrest, differentiation and/or apoptosis in transformed cells in culture as well as in tumours. The increase in acetylated proteins after the treatment with HDACis, particularly histones, results in transcription induction of some genes and the up-regulation of others that have become epigenetically

repressed. For this reason, inhibitors of histone deacetylases, such as butyrate, Trichostatin A (TSA) or trapoxin are currently used to increase the level of histone acetylation in the cell (Laphon-Hughes, etal., 2008; Morales, etal., 2001).

References

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