Chromatin Remodeling Factors and DNA Replication

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Abstract Chromatin structures have to be precisely duplicated during DNA replication to maintain tissue-specific gene expression patterns and specialized domains, such as the centromeres. Chromatin remodeling factors are key components involved in this process and include histone chaperones, histone modifying enzymes and ATP-dependent chromatin remodeling complexes. Several of these factors interact directly with components of the replication machinery. Histone variants are also important to mark specific chromatin domains. Because chromatin remodeling factors render chromatin dynamic, they may also be involved in facilitating the DNA replication process through condensed chromatin domains.

1 Introduction

Inheritable traits are not only encoded in the sequence of DNA, but also determined by factors 'on top' of the DNA, the epigenetic information ('epi' is classical Greek for 'on top'). Epigenetic phenomena play an important role in the maintenance of gene expression patterns through cell generations, for example in tissue-specific gene expression. A striking example of epigenetic regulation is found in X-chromosome inactivation in mammalian cells, where one of the two X chromosomes is maintained in an inactive, highly condensed state throughout development. In many organisms epigenetic regulation is mediated by DNA methylation, but epigenetic phenomena are also found in organisms where DNA methylation does not take place. The eukaryotic genome is packaged and organized by a plethora of proteins forming the superstructure chromatin that is a major facet of epigenetics. It is very important, therefore, for chromatin structures to be faithfully duplicated during DNA replication to maintain epigenetic information. There is accumulating

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evidence that chromatin remodeling factors play a key role in facilitating and regulating DNA replication through chromatin and the propagation of epigenetic information during DNA replication. This chapter summarizes our current knowledge about chromatin remodeling factors that have been linked directly to the DNA and chromatin replication process.

2 Chromatin and Chromatin Remodeling Factors

2.1 Chromatin

The most abundant of chromatin proteins are the histones that together with DNA assemble the basic building block of chromatin, the nucleosome. The nucleosome is basically a spool, a histone octamer around which 147 base pairs of DNA are wrapped in almost two superhelical turns (reviewed in Luger 2003). The octamer is composed of two H2A-H2B dimers interacting with a core of an H3-H4 tetramer. In the human genome, nucleosomes occur on every 180 base pairs of DNA on average, forming periodic arrays ('beads-on-a-string' fiber). Histone H1 interacts with the linker DNA at the entry–exit point of the nucleosome and stabilizes higher levels of folding of nucleosome arrays. Little is known about the molecular mechanisms of further levels of compaction of the chromatin fiber, but proteins that regulate fiber–fiber interactions, such as heterochromatin protein 1 (HP1), cohesins, condensins and topoisomerases, play an important role in chromatin organization (reviewed in Gasser 1995).

The existence of different levels of chromatin folding or organization is evident at the microscope level in interphase nuclei, where one can differentiate between the highly condensed structures called heterochromatin and the more 'loose' euchromatin (Hennig 1999). Heterochromatin and related structures have been clearly linked to gene silencing (Wallrath 1998). One refers to a gene as silenced when it is shut-off through subsequent cell generations.

Nucleosomes occlude much of the surface of the DNA wrapped around them and limit the access of many factors. In this way, chromatin is involved in the regulation of many processes, including transcription activation. Our understanding of chromatin received a major boost with the discovery of enzymes that render chromatin highly dynamic and facilitate access of cellular factors to the DNA. These enzymes, chromatin remodeling factors, are involved in all processes of DNA metabolism and are integral parts of the transcription regulatory machinery (reviewed in Narlikar et al. 2002). Two major classes of chromatin remodeling factors can be distinguished: histone modification enzymes and ATP-dependent chromatin remodeling factors.

2.2
Histone Modification Enzymes

Histones are evolutionarily highly conserved proteins and yet there is clear evidence that nucleosomes act as mediators of epigenetic information (reviewed in Jenuwein and Allis 2001). Epigenetic information is stored via chemical modifications of the histones, especially in their N-terminal tails which span about 25 amino acids. The histone tails protrude from each histone out of the nucleosome core body and can be recipients of many alterations, including acetylation, phosphorylation, methylation, ADP-ribosylation and ubiquitination. These various modifications occur at multiple sites within the tails and result in a great nucleosome heterogeneity. Figure 1 illustrates some of these modifications in histones H3 and H4. An idea has been developed that histone modifications form a 'bar code' for each nucleosome, which defines and regulates its interactions with other chromatin components and carries information about the transcriptional status of the gene that it is part of (Strahl and Allis 2000).

Enzymes that mediate histone modifications have been identified only relatively recently, and many of them are important transcriptional regulators. Histone acetyltransferases (HATs) have usually been linked to transcriptional

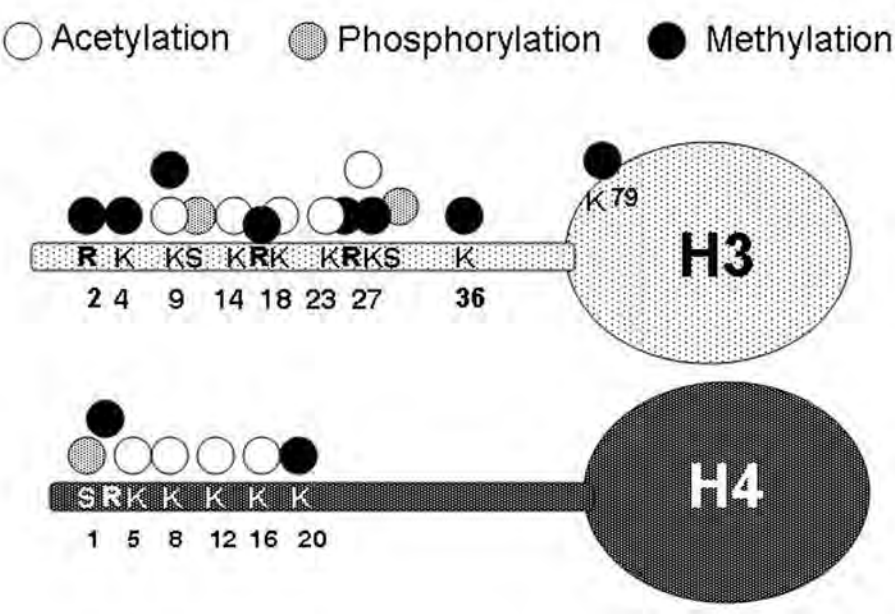

Fig. 1. A summary of characterized modifications of residues on mammalian histones *H3* and *H4*. The extended rod symbolizes the N-terminal tail-domain. Lysine residues can be mono-, di- and tri-methylated. Lysine 9 methylation of histone H3 is associated with transcriptional repression and is not compatible with acetylation of the same residue (which is linked to transcriptional activation)

activation, whereas histone deacetylases (HDAcs) have a major role in transcriptional repression. However, this is an oversimplification, and there are examples where a HAT is involved in gene silencing (see below) or an HDAc in activation (de Rubertis et al. 1996). Often, these enzymes are found in complexes with other proteins that facilitate histone acetylation or deacetylation within the nucleosome (Grant et al. 1997; Tong et al. 1998).

The complexity of the histone code is illustrated by the different functional associations of histone methylation. Methylation of lysine 9 (K9) of histone H3 by the histone methyltransferase (HMT) SU(VAR) 3–9 and its homologues is linked to heterochromatin formation and gene silencing, whereas methylation of lysine 4 (K4) is linked to transcriptional activity (see, for example, Noma et al. 2001, reviewed in Grewal and Elgin 2002). In addition, in budding yeast, dimethylation of K4 is linked to potential transcriptional activity, whereas trimethylation of the same residue occurs when the gene is actually actively transcribed (Santos-Rosa et al. 2002).

2.3 ATP-Dependent Chromatin Remodeling Factors

The nucleosome is a relatively stable entity. A class of enzymes use the energy gained by ATP-hydrolysis to move or disrupt nucleosomes efficiently. These enzymes are usually complexes of diverse proteins, but they have in common ATPases that resemble a specific class of DNA helicases. Helicase activity has not been demonstrated for any of these ATPases, but there is evidence that they function by distorting DNA structure to some degree (Havas et al. 2000). Figure 2 illustrates the major classes of well-characterized nucleosome remodeling ATPases and some of their complexes. SWI2/SNF2-type ATPases are highly conserved and are involved in transcriptional regulation, the *Drosophila* homologue is called Brahma, and mammalian cells contain two closely related homologues, Brg1 and Brm. The nucleosome remodeling ATPase Mi2 and its related proteins have been linked to transcriptional repression; they are found in complexes containing histone deacetylases. ISWI (Imitation Switch) was originally identified in *Drosophila* where it is the core of the NURF, CHRAC and ACF chromatin remodeling complexes. In mammalian cells there are two isoforms of ISWI called SNF2H and SNF2L. Several recent reviews cover the biology and biochemistry of ATP-dependent nucleosome remodeling factors (Becker and Hörz 2002; Narlikar et al. 2002).

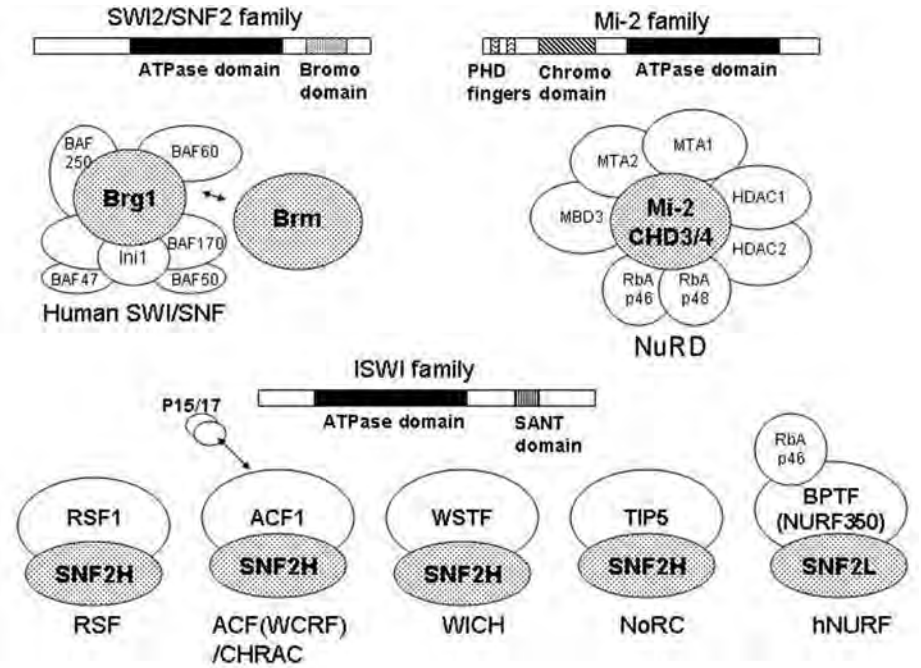

Fig. 2. Summary cartoon of the best characterized human nucleosome remodeling ATPases with their specific domain architecture and their respective complexes. *Brg1* and *Brm* are highly related to the yeast SWI2/SNF2 and STH1 ATPases. The complexes formed by Brg1 and Brm are very similar. However, Brg1 is also found in a complex containing a protein called Polybromo (BAF180) instead of BAF250 and this complex may be the mammalian counterpart of the yeast RSC complex (reviewed in Muchardt and Yaniv 2001). ISWI interacts with a number of different large proteins, forming distinct complexes with diverse biological functions. This scheme does not include recently identified ISWI complexes with cohesin subunits (Hakimi et al. 2002) and with TBP-related factor (Hochheimer et al. 2002). The CHRAC complex differs from ACF by the presence of a pair of histone-fold proteins that enhance the nucleosome sliding and chromatin assembly activity of the ACF1/ISWI core complex. (Kukimoto et al. 2004)

3
Chromatin Structure and DNA Replication

Chromatin limits the accessibility to DNA, and this raises the questions: How does the replication machinery interact with chromatin? Does replication occur through nucleosomes? Does it disrupt chromatin structure? These questions have been studied extensively in the SV40 system. SV40 is a double-stranded DNA animal virus whose DNA is packaged by nucleosomes. Firing of DNA replication in this system is dependent on the binding of T-antigen to the origin of replication from where replication initiates. If the origin is occupied by a nucleosome, DNA replication firing is prohibited and necessitates