Biochemistry 201
Advanced Molecular Biology
Chromatin Remodeling
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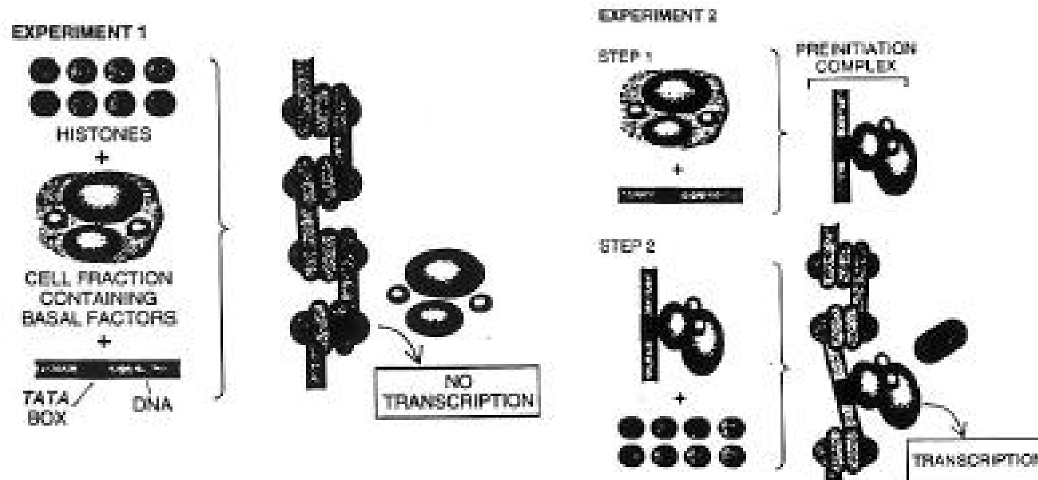
Introduction

The interactions between histones and DNA in chromatin must undergo tremendous changes in order to permit processes such as replication and transcription. Considerable evidence has been accumulated that histones are not passive structure determining elements, but rather, they interact with transcription factors, enhancer and silencer factors in the process of both regulating and permitting transcription. We will also review the changes in structure of nucleosomes that occur during transcription and the role of histones, regulatory elements and transcription factors in this process.

Histones as regulators or Genes

Experiments with in vitro transcription show that:



Histones block transcription in chromatin if they are assembled together with transcription factors or assembled on DNA prior to the presence of transcription factors.

If transcription factors are bound to DNA first, then histones associate with chromatin such that transcription can occur.

If upstream activator proteins are also present during a simultaneous assembly of DNA, histones, transcription factors, then again transcription can occur. Upstream activator

proteins appear to help assemble nucleosomes in a specific positions and also to recruit the basal transcription factors to the site of transcription initiation.

Using expression of the normally silent mating type loci as a test case, Grunstein's group examined mutations in histones and their effect on expression of the mating type genes at HML-alpha and HMRa sites which are normally strongly repressed. They use these two genes as models for bulk of chromatin in eukaryotic cells which is also silent. Deletion of amino acids 15 to 29 in the amino terminus of histone H4 caused these sites to become expressed. Single amino acid substitutions at positions 16 and 19 had a similar effect. A second site revertant of these histone mutants was observed in the sir3 locus, suggesting that H4 and sir3 product must interact during the transcription process.

The silent mating type loci reside near the telomeres of yeast chromosomes and many genes there can be affected by mutants in the sir loci. Even genes artificially inserted near the telomeres can be induced to be expressed by mutants in H4 and repression reestablished by SIR 3 mutants. This indicates that SIR3 and histone H4 help to determine proper chromatin structure and repression of proteins near telomeres.

Nucleosomes and repression of cis-acting transcription sequences

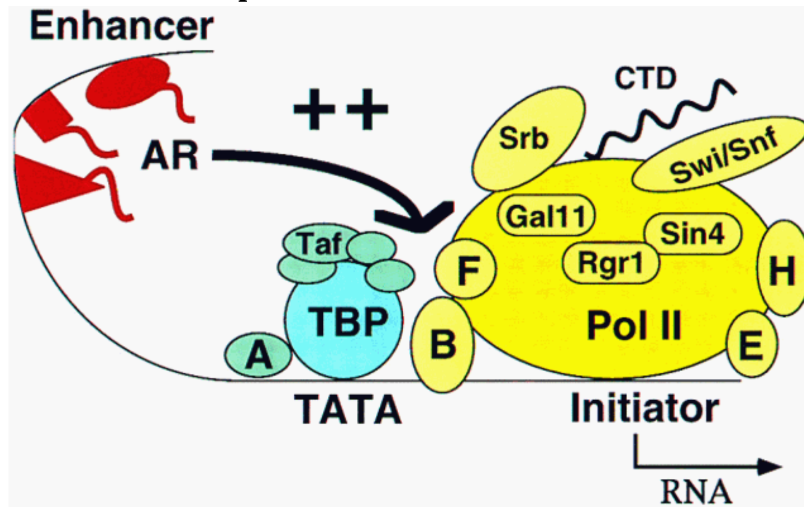
Promoter and enhancer sequences near and in eukaryotic genes can be blocked from functioning by the presence of histones. Proper orientation of histones near these regulatory sites can only be obtained if histones are deposited in a physiological manner on these sites.

Histones are normally deposited on newly replicated DNA in a very specific order. Tetramers of highly acetylated H3/H4 bind first. All newly synthesized histones have acetyl groups on several of the basic residues in the amino terminus of the histones. H3/H4 tetramers do not block transcription factor binding or transcription, either in the acetylated or non-acetylated form. The next histones to arrive, are two pair of histone H2A and H2B. When these histones join to form an octamer, then transcription of the promoters is abolished, unless, the enhancer proteins bind to the upstream activator sequences prior to the deposition of H2A and H2B.

The deposition of histone octamers and the formation of nucleosomes can be controlled by two processes simultaneously. First, certain DNA sequences naturally bind to sequences that show specific flexibility and bending due to their DNA sequence. This has been demonstrated most clearly on the 5S ribosomal gene from Xenopus laevis. Nucleosomes deposited on or near important cis acting sites can still permit access to transcription factors based on the translational positioning of the nucleosome along the DNA as well as the rotational exposure of the site on the surface of the nucleosome.

Transcription of Xenopus 5S Ribosomal chromatin is inhibited by the presence of histone octamers covering the gene (promoter), but not by tetramers of histones H3-H4. Both tetramers and octamers of histones bind to the Xenopus 5S chromatin at specific sites. The H2A and H2B proteins block the binding site for TFIIIA but the H3H4 tetramer do not.

Activation of Repressed Chromatin



Replication is one process that can reposition nucleosomes allowing genes to become expressed. This is certainly due to the helicase activity of replication which separates the strands of DNA. Histones fail to bind to single strands and must reform nucleosomes after the replication fork passes. Also transcription through an enhancer and promoter region may reestablish proper expression of a gene region. Interestingly, one transcription factor, TFIIH is a helicase itself.

Other factors that can stimulate proper transcription are general transcription factors, the SWI/SNF proteins discussed below.

Nucleosomes can also stimulate transcription from promoters that depend on up stream activating sequence by folding the DNA and bringing the activator proteins in close apposition to the promoter region itself. Examples include the Drosophila bicoid gene and yeast Gal 4 gene.

Activation of chromatin is also associated with acetylation of the N-terminal ends of the core histones. Nucleosomes containing acetylated histone core octamers do not block transcription of the Xenopus 5S genes and TFIIIA can function.

There is both structural and sequence similarity between histones and transcription factors. Two of the subunits of TFIID have dramatic sequence similarity to histone H3 and H4. Interactions between TFIID and DNA are as stable as that of those histones. The central core of histone H5 from chickens has a structure similar to the eukaryotic transcription factor family known as the forkhead family.

SIR3 and SIR4 are proteins that bind the telomeric DNA of Yeast to the nuclear membrane. SIR3 has a strong structural similarity to Histone H1.

Role of Specific Activator Proteins in Remodeling Chromatin Structure

Experiments with the MMTV (mouse mammary tumor virus) promoter, which depends on glucocorticoid receptors (GR) show that a nucleosome forms in a very specific position on the GR target site on the DNA. There are five finding sites for the GR and

two are accessible and two hidden by the nucleosome. When GR binds to the two accessible sites, it then recruits a large complex of proteins referred to as general transcription activators (see below).

Similarly in the Drosophila hsp26 promoter, a single well positioned nucleosome brings heat shock transcription factor binding sites together with TFIID and GAGA factor binding sites near the promoter of the gene.

Replication may be required to establish repressed state.
The proper deposition of core histones on transfected DNA apparently requires at least one round of replication. Transiently transformed cells do not show efficient assembly of chromatin and poor expression. DNA transfected on episomes do replicate and normal nucleosome arrangements occur.

General Activator Complexes

Mutants of yeast that fail to switch mating type fall into three complementation groups, SW1, SW2 and SW3. These mutants all have 100 fold decreased activity of the HO endonuclease required for the switch of one of the silent mating type cassettes with the expressed gene. Similarly there are three sets of yeast mutations that decrease sucrose utilization, SNF1, SNF5 and SNF6 (sucrose non-fermenting). Both sets of mutants reduce expression of large sets of genes and their gene products are believed to code for a large protein complex that is necessary for chromatin remodeling during transcription. The observation that SWI2 and SNF2 were identical loci suggested that all five proteins may work together in a complex to stimulate transcription at least a 100 fold.
Many genes stimulate their expression by recruiting the activities of this generalized transcription factor to the promoter site. Experiments with Drosophila genes bicoid and fushi tarazu as well as the mammalian steroid receptors shows that the proteins binding to the upstream activating region do attract this complex.
Two classes of second site revertants of the Switched phenotype were found and one class maps to the gene for histone 3. The mutations make expression independent of the need for the General activator complex. The second class of switched independent mutations occurs in a major non-histone chromosomal protein fraction known as HMG1/2 proteins (high mobility group for its electrophoretic behavior).

The structure of the nucleosome by Moudrianakis shows that all the SIN2 mutant alleles in H3 map to the lower region near the C-terminus and close to the cysteine groups on those proteins.

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