Keynote

Chromosome regions that are transcriptionally active have looser DNA–histone structures than do chromosome regions that are transcriptionally inactive, resulting in sensitivity of the DNA to digestion by DNase I. The promoter regions of active genes typically have an even looser DNA–histone structure, resulting in hypersensitivity to DNase I. In other words, the chromatin structure at the core promoter of a nonexpressed gene is repressive to transcription. Remodeling of the chromatin in this region is necessary to activate transcription and is brought about by the binding of activators to enhancers. The activators recruit chromatin remodeling complexes, either a type that acetylates nucleosomes, thereby loosening their association with the DNA, or a type that moves or restructures nucleosomes, allowing the transcription machinery to access the promoter.

Gene Silencing and Genomic Imprinting

To this point, we have discussed the regulation of transcription at the individual gene level. That is, we saw how specific activators and repressors regulate gene transcription and how chromatin structure plays a role in regulating the initiation of transcription. By contrast, **gene silencing** is a phenomenon whereby a gene is transcriptionally silent due to its location, not because of the action of a specific repressor. Gene silencing is an example of an *epigenetic* phenomenon, that is, a heritable change in gene expression which occurs without a change in DNA sequence; see Chapter 12, p. 349. Commonly, gene silencing is a property of heterochromatin, which is highly condensed (see Chapter 2, p. 27), and, therefore, may involve large sections of DNA and many genes. Heterochromatic regions of chromosomes are found, for example, at telomeres and centromeres, as well as dispersed throughout the genome. Some regions of heterochromatin—constitutive heterochromatin—are present in all cells at identical positions on both homologous chromosomes of a pair (e.g., telomeres and centromeres), whereas other regions—facultative heterochromatin—vary in state in different cell types, at different developmental stages, and sometimes from one homologous chromosome to the other. Typically, other proteins bind to heterochromatin that directly or indirectly prevent transcription initiation at genes in the heterochromatic region.

Gene Silencing at a Telomere

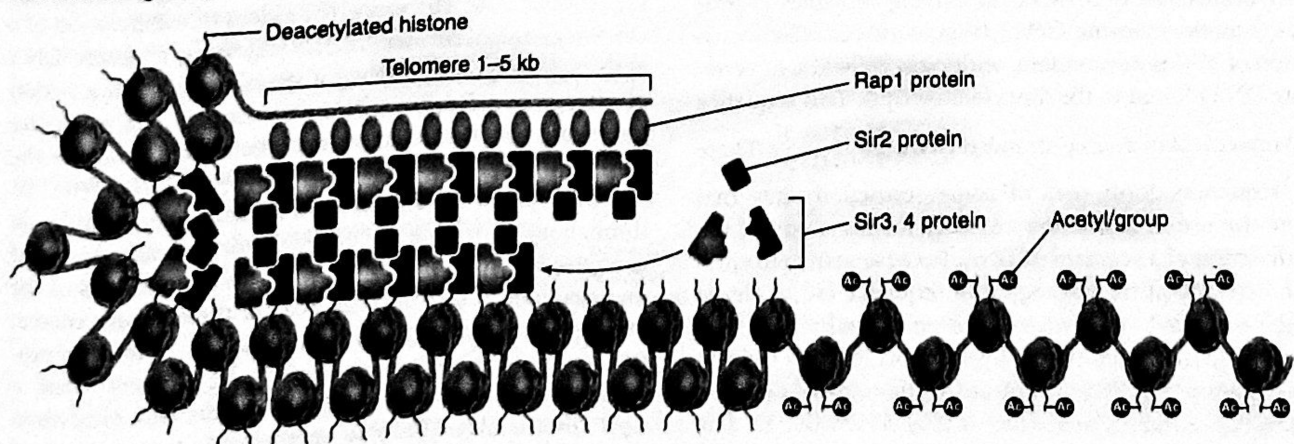
Let us consider gene silencing at a telomere. In yeast, the last 1–1.5 kb of the chromosome consists of telomere repeat sequences folded into a complex hairpin structure (Figure 18.11). Normally, no protein-coding genes are found at telomeres. However, when active genes are moved to a telomere region, those genes are silenced, a phenomenon called *telomere position effect*. This effect is associated with a physical grouping of the telomeres into four or five bouquets physically bound to the nuclear envelope.

A gene moved to a telomere region that becomes silenced can be used to search for yeast mutants that relieve silencing. Such mutants define the silent information regulation genes, *SIR2*, *SIR3*, and *SIR4*, for the Sir2p, Sir3p, and Sir4p proteins, respectively. Another protein, Rap1p (product of the repressor–activator protein gene, *RAP1*), binds to telomere repeat sequences. Once bound, Rap1p recruits the Sir silencing complex consisting of Sir2p, Sir3p, and Sir4p. The Sir complex also contacts the histones, and Sir2p, a histone deacetylase, catalyzes the local removal of acetyl groups from histone tails. Deacetylated histones are now recognized directly by the silencing complex, causing a wave of binding and deacetylation to spread along the chromosome for a limited distance and generating the highly condensed heterochromatin structure.

Gene Silencing by DNA Methylation

Transcription can also be silenced by the methylation of particular DNA sequences. This type of silencing is

Figure 18.11
Gene silencing at a yeast telomere.

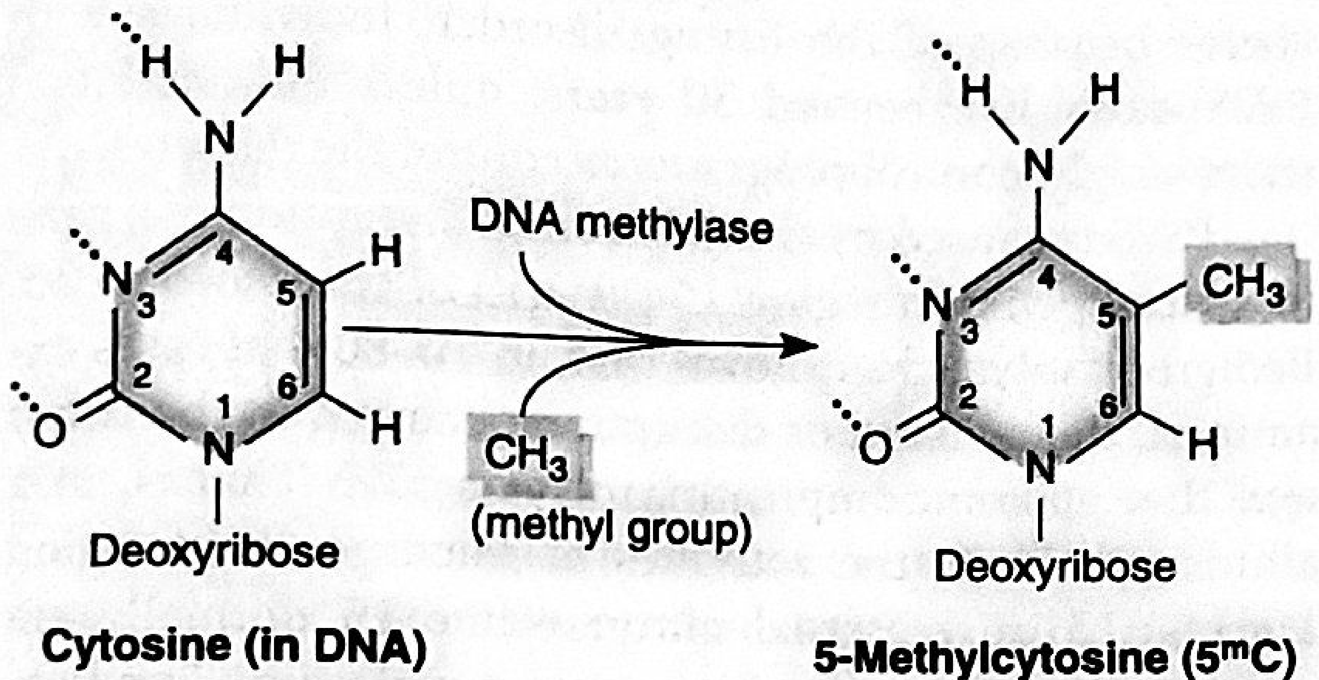


common in many eukaryotes, particularly vertebrates, but is not found in yeast. DNA methylation involves DNA methyltransferases (DNMTs) modifying cytosines to produce 5-methylcytosine (5^mC) (Figure 18.12). The distribution of 5^mC is nonrandom, with most (60–90% in vertebrate DNA) found in the dinucleotide CpG. This sequence is symmetrical in double-stranded DNA: $\begin{matrix}5'\text{-CG-}3'\\3'\text{-GC-}5'\end{matrix}$. These CG sequences form part of some restriction sites that allow the use of restriction enzymes for the study of the methylation of a segment of DNA, because many enzymes with cytosine in their recognition sequence fail to cleave double-stranded DNA when cytosine is methylated. The enzyme *Hpa*II ("hepa-two"), for example, cleaves DNA at the sequence 5′-CCGG-3′ but not if the internal cytosine of the two is methylated (i.e., if it is 5′-C^mCGG-3′. The enzyme *Msp*I ("M-S-P-one") also cleaves the same CCGG sequence, but, unlike *Hpa*II, it *will* cleave the methylated sequence C^mCGG. Therefore, the extent of methylation of a DNA region can be analyzed by digesting genomic DNA with each enzyme and using a specific probe for a region of interest in a Southern blot experiment. The particular fragments that are detected, and their sizes, indicate the pattern of methylation in the region.

CpG dinucleotides are not distributed randomly throughout vertebrate genomes. Rather, some regions of genomes have CpG-rich segments with many copies of the dinucleotides, called CpG **islands.** In the human genome, many protein-coding genes have CpG islands in their promoters. These CpG islands usually are unmethylated, a state that facilitates transcription initiation. However, when CpG dinucleotides become methylated, transcription is

Figure 18.12

Production of 5-methylcytosine from cytosine in DNA by the action of the enzyme DNA methylase.



repressed. Repression in this way involves histone modifications of the kind already discussed. That is, specific proteins recognize and bind to methylated CpG and then recruit HDACs (histone deacetylases). Recall that histone deacetylases cause chromatin remodeling, in this case in the direction toward a conformation in which promoters are not accessible.

An example of methylation affecting gene expression is found in the development of fragile X syndrome (OMIM 309550; see Chapter 16, pp. 475–476), which is the leading cause of inherited mental retardation. The syndrome develops after expansion (a significant increase in the number of copies) of a triplet repeat (a repeated 3 base-pair sequence) in the *FMR-1* gene and abnormal methylation of the gene to the point that transcription of the *FMR-1* gene is silenced.