requirements. *Trans*-acting factors bind to these enhancer sites and are thought to alter the tertiary structure or conformation of the DNA in a manner that facilitates the binding and assembly of the transcription-initiation complex at the promoter region, perhaps in some cases by forming a broad loop of DNA in the process. Biochemical modification of select promoter or enhancer sequences, such as methylation of cytosine-phosphate-guanine (CpG)–rich sequences, can also modulate transcription; methylation typically suppresses transcription. The terms *silencer* and *suppressor* elements refer to *cis*-acting nucleotide sequences that reduce or shut off gene transcription and do so through association with *trans*-acting factors that recognize these specific sequences.

Regulation of transcription is a complex process that occurs at several levels. The expression of many genes is regulated to maintain high basal levels; they are known as *housekeeping* or *constitutively expressed* genes. They typically yield protein products that are essential for normal cell function or survival and must be maintained at a specific steady-state concentration in all circumstances. Many other genes are not expressed or are only modestly expressed under basal conditions; however, with the imposition of some stress or exposure of the cell to an agonist that elicits a cellular response distinct from that of the basal state, expression of these genes is induced or enhanced. For example, the heat shock protein genes encoding *stress proteins* are rapidly induced in response to diverse pathophysiologic stimuli (e.g., oxidative stress, heavy metals, inflammation) in most cells and organisms. Increased heat shock protein expression is complementary to the basal level of heat shock proteins, which are molecular chaperones that play key roles during protein synthesis to prevent protein misfolding, increase protein translocation, and accelerate protein degradation. These adaptive responses often mediate changes in phenotype that are homeostatically protective to the cell or organism.

Micro-RNAs and Gene Regulation

Less is known about the determinants of translational regulation than is known about transcriptional regulation. The recent discovery and identification of small RNAs (21-mer to 24-mer clusters), called *micro-RNAs* (miRNAs), adds further complexity to the regulation of gene expression in the eukaryotic genome. First discovered in worms more than 15 years ago, miRNAs are conserved noncoding strands of RNA that bind by Watson-Crick base pairing to the 3′-untranslated regions of target mRNAs, enabling gene silencing of protein expression at the translational level. Gene-encoding miRNAs exhibit tissue-specific expression and are interspersed in regions of the genome unrelated to known genes.

Transcription of miRNAs proceeds in multiple steps from sites under the control of an mRNA promoter. RNA polymerase II transcribes the precursor miRNA, called *primary miRNA* (pri-miRNA), containing 5′ caps and 3′ poly-A tails. In the nucleus, the larger pri-miRNAs of 70 nucleotides form an internal hairpin loop, embedding the miRNA portion that undergoes recognition and subsequent excision by a double-stranded RNA–specific ribonuclease called *Drosha*. Gene expression is silenced by the effect of miRNA on nascent RNA molecules targeted for degradation.

Because translation occurs at a fairly invariant rate among all mRNA species, the stability or half-life of a specific mRNA also serves as another checkpoint for the regulation of gene expression. The 3′-untranslated region of mRNAs contains regions of sequence that dictate the susceptibility of the message to nuclease cleavage and degradation. Stability appears to be sequence specific, and in some cases, it depends on *trans*-acting factors that bind to the mRNA. The mature mRNA contains elements of untranslated sequence at the 5′ and 3′ ends that can regulate translation.

Beginning in the organism's early development, miRNAs may facilitate much more intricate ways for the regulation of gene expression, as have been shown for germline production, cell differentiation, proliferation, and organogenesis. Because studies have implicated the expression of miRNAs in brain development, cardiac organogenesis, skeletal muscle regeneration, colonic adenocarcinoma, and viral replication, this novel mechanism for gene silencing has potential therapeutic roles for congenital heart defects, viral disease, neurodegeneration, regenerative medicine, and cancer.

Chromatin Remodeling and Gene Regulation

The size and complexity of the human genome with 23 chromosomes ranging in size between 50 and 250 Mb pose formidable challenges for transcription factors to exert the specificity of DNA-binding properties in gene regulation. Control of gene expression takes place in diverse types of cells, often with exquisite temporal and spatial specificity throughout the lifespan of the organism. In eukaryotic cells, the genome is highly organized into densely packed nucleic acid DNA- and RNA-protein structures, called *chromatin*. The building blocks of chromatin are called *histones*, a family of small basic proteins that occupy one half of the mass of the chromosome. Histones derive their basic properties from the high content of basic amino acids, arginine, and lysine. Five major types of histones—H1, H2A, H2B, H3, and H4—have evolved to form complexes with genomic DNA. Two pairs each of the four types of histones form a protein core, the histone octomer, which is wrapped by 200 base pairs of DNA to form the nucleosome (Fig. 1-4). The core proteins within the nucleosomes have protruding amino-terminal ends, exposing critical lysine and arginine residues for covalent modification. Further DNA condensation is achieved as higher-order structure is imposed on the chromosomes. The nucleosomes are further compacted in layered stacks with a left-handed superhelix resulting in negative supercoils that provide the energy for DNA strand separation during replication.

Condensation of DNA in chromatin precludes the access of regulatory molecules such as transcription factors. Reversal of chromatin condensation typically occurs in response to environmental and other developmental signals in a tissue-dependent manner. Promoter sites undergoing active transcription and relaxation of chromatin structure that become susceptible to enzymatic cleavage by nonspecific DNAase I are called *hypersensitive sites*. Transcription factors on promoter sites may gain access by protein-protein interactions to enhancer elements containing tissue-specific proteins at remote sites (several thousand bases away), resulting in transcription activation or repression.