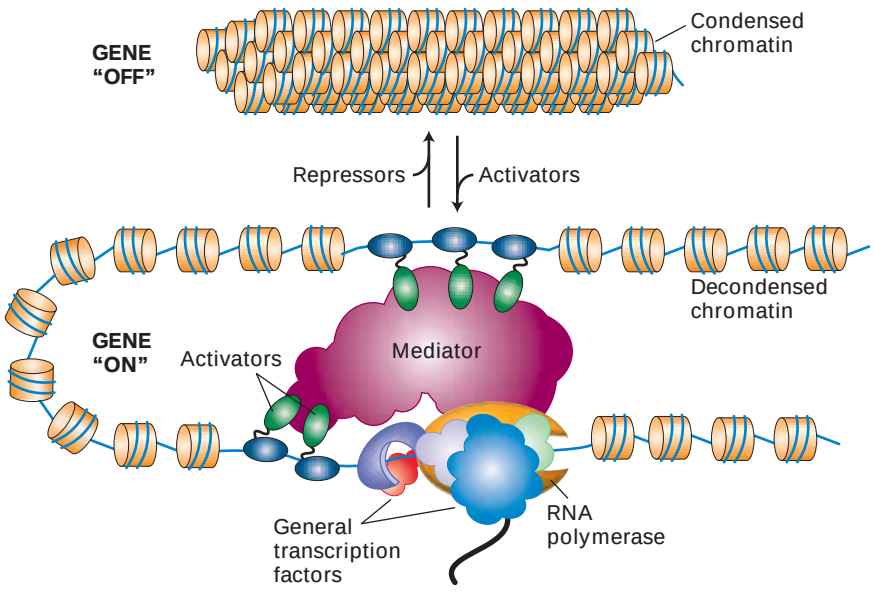
11.1 Overview of Eukaryotic Gene Control and RNA Polymerases

In bacteria, gene control serves mainly to allow a single cell to adjust to changes in its environment so that its growth and division can be optimized. In multicellular organisms, environmental changes also induce changes in gene expression. An example is the response to low oxygen (hypoxia) that is described in Chapter 15. However, the most characteristic and biologically far-reaching purpose of gene control in multicellular organisms is execution of the genetic program that underlies embryological development. Generation of the many different cell types that collectively form a multicellular organism depends on the right genes being activated in the right cells at the right time during the developmental period.

In most cases, once a developmental step has been taken by a cell, it is not reversed. Thus these decisions are fundamentally different from the reversible activation and repression of bacterial genes in response to environmental conditions. In executing their genetic programs, many differentiated cells (e.g., skin cells, red blood cells, and antibody-producing cells) march down a pathway to final cell death, leaving no progeny behind. The fixed patterns of gene control leading to differentiation serve the needs of the whole organism and not the survival of an individual cell. Despite the differences in the purposes of gene control in bacteria and eukaryotes, two key features of transcription control first discovered in bacteria and described in Chapter 4 also apply to eukaryotic cells. First, protein-binding regulatory DNA sequences, or control elements, are associated with genes. Second, specific proteins that bind to a gene's regulatory sequences determine where transcription will start, and either activate or repress its transcription. As represented in Figure 11-1, in multicellular eukaryotes, inactive genes are assembled into condensed chromatin, which inhibits the binding of RNA polymerases and general transcription factors required for transcription initiation. Activator proteins bind to control elements near the transcription start site of a gene as well as kilobases away and promote chromatin decondensation and binding of RNA polymerase to the promoter. Repressor proteins bind to alternative control elements, causing condensation of chromatin and inhibition of polymerase binding. In this chapter we consider how activators and repressors control chromatin structure and stimulate or inhibit transcription initiation by RNA polymerase.

Most Genes in Higher Eukaryotes Are Regulated by Controlling Their Transcription

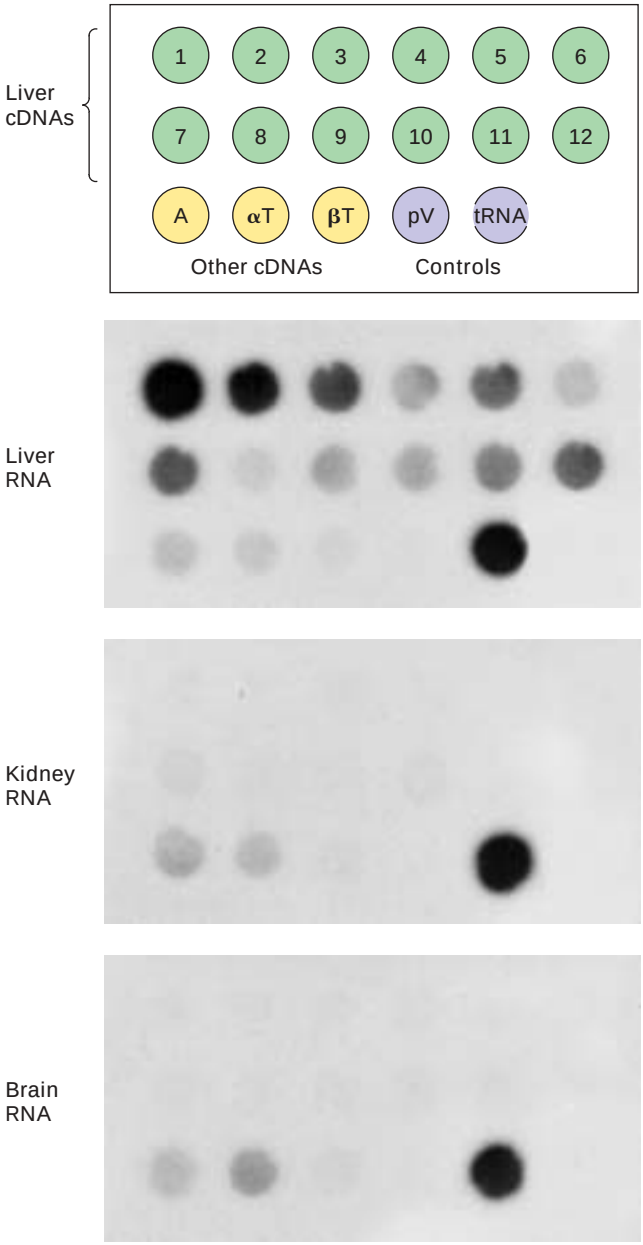
Direct measurements of the transcription rates of multiple genes in different cell types have shown that regulation of transcription initiation is the most widespread form of gene control in eukaryotes, as it is in bacteria. *Nascent-chain analysis* is a common method for determining the relative rates of transcription of different genes in cultured cells. In this method, also called *run-on transcription analysis*, isolated nuclei are incubated with ^{32}P-labeled ribonucleoside triphosphates for a brief time (e.g., 5 minutes or less). During



▶ FIGURE 11-1 **Overview of transcription control in multicellular eukaryotes.** Activator proteins bind to specific DNA control elements in chromatin and interact with multiprotein coactivator machines, such as mediator, to decondense chromatin and assemble RNA polymerase and general transcription factors on promoters. Inactive genes are assembled into regions of condensed chromatin that inhibit RNA polymerases and their associated general transcription factors (GTFs) from interacting with promoters. Alternatively, repressor proteins bind to other control elements to inhibit initiation by RNA polymerase and interact with multiprotein corepressor complexes to condense chromatin.

this incubation period, RNA polymerase molecules that were actively transcribing genes when the nuclei were isolated incorporate several hundred labeled nucleotides into each nascent (growing) RNA chain, but little initiation of new chains occurs. The total radioactive label incorporated into RNA is a measure of the overall transcription rate. The fraction of the total labeled RNA produced by transcription of a particular gene—that is, its relative transcription rate—is determined by hybridizing the labeled RNA to the cloned DNA of that gene attached to a membrane.

The results of run-on transcription analyses illustrated in Figure 11-2 show that transcription of genes encoding proteins expressed specifically in hepatocytes (the major cell type in mammalian liver) is readily detected in nuclei prepared from liver, but not in nuclei from brain or kidney. Since the run-on transcription assay measures RNA synthesis, these results indicate that differential synthesis of liver-specific proteins is regulated by controlling transcription of the corresponding genes in different tissues. Similar results have been obtained in run-on transcription experiments with other cell types and a wide variety of tissue-specific proteins, indicating that transcriptional control is the primary mechanism of gene control in complex organisms.



Regulatory Elements in Eukaryotic DNA Often Are Many Kilobases from Start Sites

In eukaryotes, as in bacteria, a DNA sequence that specifies where RNA polymerase binds and initiates transcription of a gene is called a **promoter.** Transcription from a particular promoter is controlled by DNA-binding proteins, termed **transcription factors,** that are equivalent to bacterial *repressors* and *activators*. However, the DNA control elements in eukaryotic genomes that bind transcription factors often are located much farther from the promoter they regulate than is the case in prokaryotic genomes. In some cases, transcription factors that regulate expression of protein-coding genes in higher eukaryotes bind at regulatory sites tens of thousands of base pairs either **upstream** (opposite to the direction of transcription) or **downstream** (in the same direction as transcription) from the promoter. As a result of this arrangement, transcription from a single promoter may be regulated by binding of multiple transcription factors to alternative control elements, permitting complex control of gene expression.

For example, alternative transcription-control elements regulate expression of the mammalian gene that encodes transthyretin (*TTR*), which transports thyroid hormone in blood and the cerebrospinal fluid that surrounds the brain

◀ EXPERIMENTAL FIGURE 11-2 **Measurement of RNA production in various tissues demonstrates that transcription of a given gene generally occurs only in the cell types in which it is expressed.** Nuclei from mouse liver, kidney, and brain cells were exposed to ^{32}P-UTP. The three resulting labeled RNA samples were hybridized to separate nitrocellulose membranes; on each membrane was fixed an identical pattern of various cDNAs (*top*). The cDNAs labeled 1–12 (green) encode proteins synthesized actively in liver (e.g., albumin, transferrin) but not in most other tissues. The other cDNAs tested were actin (A) and α- and β-tubulin (αT, βT) (yellow), which are proteins found in almost all cell types. A gene encoding methionine tRNA and the plasmid vector DNA (pV) in which the cDNAs were cloned were included as controls (purple). After removal of unhybridized RNAs, the labeled RNAs complementary to the cDNAs were revealed by autoradiography. RNA from the liver sample hybridized extensively with the cDNAs. Little hybridization is seen for the kidney and brain samples, indicating that genes encoding proteins found in hepatocytes are transcribed in liver cells but not in the cells of the other tissues. [See E. Derman et al., 1981, *Cell* **23**:731, and D. J. Powell et al., 1984, *J. Mol. Biol.* **197**:21]