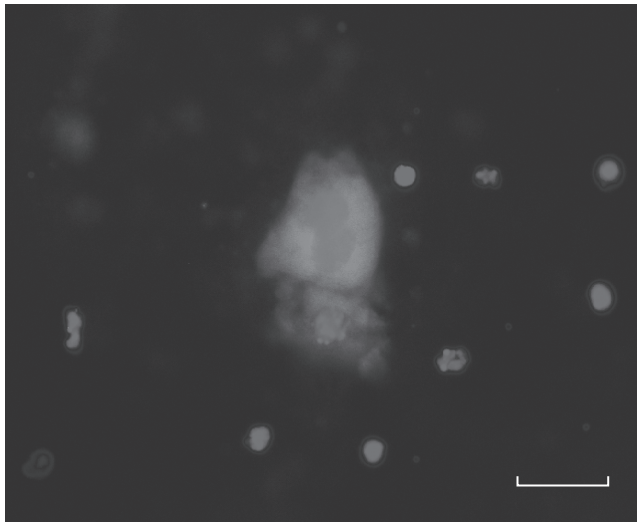# 2

REGULATION OF GENE EXPRESSION

Mobilization of albumin-positive hepatocyte-like cells into the blood in response to the upregulation of interleukin-6 in ischemic cardiac injury. The mobilized hepatocyte-like cells can be recruited to injured cardiac tissue, promoting the proliferation of cardiomyocytes in the zone of cardiac injury. Red: albumin. Blue: cell nuclei. Sclae: 10 μm. See color insert.

Gene expression is the transcription of mRNA (transfer of genetic information from DNA to mRNA) and the translation of protein from a specific mRNA (transfer of genetic information from mRNA to protein). Gene expression is regulated at several key levels, including the transcription of pre-mRNA, the conversion of pre-mRNA to mature mRNA, and the synthesis of protein. The regulation of gene expression involves basically two types of factors: the *cis*-acting regulatory elements and *trans*-acting regulatory factors. *Cis-acting regulatory elements* are DNA-sequences that are protein-binding sequences and are found near the initiation site of gene transcription. *Trans-acting regulatory factors* are proteins that bind to specific *cis*-acting elements and control gene transcription. In addition, as we see below, the components of chromatin also participate in the regulation of gene expression.

There are several common features for the regulation of gene expression:

1. *Trans*-acting factors can bind only to specific *cis*-acting elements.
2. The binding of *trans*-acting factors to *cis* elements can induce conformational changes in the chromatin, a critical process for regulating gene transcription.
3. The binding of *trans*-acting factors may induce positive or negative regulation of gene transcription. Positive regulation is a process by which the binding of *trans*-acting factors stimulates gene transcription, whereas negative regulation exerts an opposite effect.
4. A transcription regulatory signal can be effective for only a limited time. The signal is turned off after a mission is accomplished.

The principles of gene expression regulation are briefly discussed in this section.

BASIC DNA ELEMENTS FOR REGULATING GENE EXPRESSION [2.1]

The expression of genes is controlled at the level of mRNA transcription. For each gene, there are specialized short sequences, known as *cis*-acting regulatory elements, which mediate the attachment and activation of RNA polymerases, necessary processes for initiating mRNA transcription. In mammalian cells, *cis*-acting regulatory elements include promoters, promoter-proximal elements, and enhancers. Each *cis*-acting element can be recognized and activated by *trans*-acting regulatory factors. These factors can bind to the promoter and initiate the attachment of RNA polymerase II, inducing RNA transcription. A typical promoter is composed of TATA nucleotides, known as the *TATA box*. The TATA box is usually found at a site about 30 bp upstream the first base pair of the transcription site. On the action of *trans*-acting factors, a promoter mediates the initiation of RNA transcription in coordination with promoter-proximal elements and enhancers. Typical promoter-proximal elements, which often include the CCAAT box and GC-rich segments, are located about 100–200 bp away from the TATA box. Experiments with point mutation have demonstrated that a mutation in the TATA or the promoter-proximal elements induces a decrease in the rate of RNA transcription, whereas mutation in other regions exhibits little influence (Fig. 2.1). Each gene contains several sequence motifs of enhancers, which are located at a distance, some times up to 50 kb, from the TATA box. Different motifs serve as binding sits for specific *trans*-acting factors. The function of promoter-proximal elements and enhancers is to facilitate the activity of the promoters and enhance gene transcription.
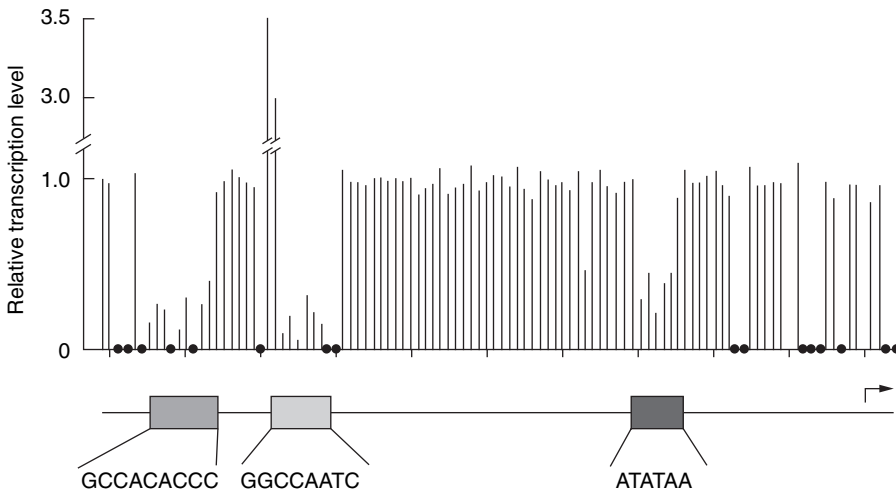
Figure 2.1. Identification of the promoter and promoter-proximal elements that control the expression of the β-globin gene. Point mutations were induced on most nucleotides through the promoter region of the β-globin gene, and the effect of each point mutation was tested. Mutations within the TATA promoter and the promoter-proximal elements caused a significant decrease in the level of transcription, suggesting that these *cis*-elements are critical to the transcription of the β-globin gene. (Reprinted by permission from Maniatis T, Goodbourn S, Fischer JA: *Science* 236:1237–45, 1987. Copyright 1987 AAAS.)

TRANS-ACTING REGULATORY FACTORS [2.1]

Trans-acting regulatory factors are proteins that bind to and activate target genes and regulate RNA transcription. In mammalian cells, there exist a large number of *trans*-acting factors. These factors, while in their inactive state, are present in the cytoplasm. Once activated in response to the stimulation of signaling molecules (see Chapter 5), a *trans*-acting factor can be translocated from the cytoplasm to the nucleus and bind to specific *cis*-acting elements, or promoters and enhancers, of target genes. A *trans*-acting factor, once bound to a *cis*-acting element, can form a complex with RNA polymerase II and other necessary regulatory factors (See Table 2.1), assisting the polymerase to initiate mRNA transcription. Each *trans*-acting factor contains two domains. The first domain is responsible for binding to *cis*-acting elements, whereas the second is for activating mRNA transcription. A typical example of *trans*-acting factors is the complex of *t*ranscription *f*actors for RNA polymerase *II*, or TFII. Each TFII is composed of a TATA box-binding protein (TBP) and several associated proteins (Fig. 2.2). These proteins are referred to as *basal trans-acting factors*. Examples of other *trans*-acting factors include activator protein 1 and nuclear factor κ B. The functions of these factors are discussed in Chapter 5.

REGULATION OF GENE TRANSCRIPTION

At the transcription level, several mechanisms are involved in the regulation of gene expression, including the activation of *trans*-acting factors, modification of the chromatin structure, and DNA modulation. These mechanisms are briefly described as follows.
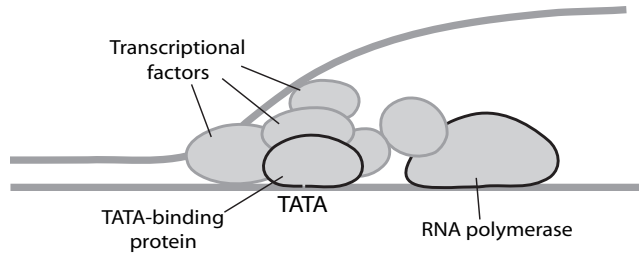
Figure 2.2. Schematic representation of controlling gene transcription by *trans*-acting factors (based on bibliography 2.1).

Control of the Activity of *Trans*-Acting factors [2.2]

In eukaryotes, there are two types of *trans*-acting factor: transcription-activating proteins (transcriptional activators) and transcription-inhibiting proteins (transcriptional repressors). The two types of factor regulate gene transcription via controlling the function of the RNA polymerase holoenzyme and the structure of chromatin. These factors dynamically counterbalance each other, ensuring an appropriate level of gene expression.

    Trans-acting factors interact with DNA transcription apparatus, which is composed of *cis*-acting elements and protein coactivators. While *trans*-acting proteins act on specific *cis* elements, protein coactivators enhance the regulation of gene transcription, which is independent of specific *cis* elements. The binding of *trans*-acting factors to *cis*-acting DNA elements induces activation and promotes reorientation of coactivator proteins, which are necessary conditions for the initiation of gene transcription.

    The activity of *trans*-acting proteins is controlled by three basic approaches: modification of preexisting proteins, de novo synthesis of proteins, and targeted degradation. These processes may coordinate in a time- and space-dependent manner, providing synergistic inputs for the regulation of gene transcription. Among these regulatory processes, the modification of preexisting proteins is the most important process that causes immediate activation of signaling events, resulting in prompt cellular activities. Common types of modification include phosphorylation and dimerization of proteins. Protein *phosphorylation* occurs primarily on the serine, threonine, and tyrosine residues, and is regulated by protein kinases and protein phosphatases, which phosphorylate and dephosphorylate target proteins, respectively. The phosphorylation of signaling molecules may lead to activation or deactivation of *trans*-acting factors, depending on the nature of specific signaling pathways. In most cases, phosphorylation induces activation of *trans*-acting factors. Such a process enhances the interaction of *trans*-acting factors with nuclear export machineries, which facilitate the translocation of the *trans*-acting factors from the cytoplasm to the nucleus. In addition, the phosphorylation of the DNA-binding domain of a *trans*-acting factor mediates the binding of the trans-acting factor to the target DNA. The involvement of protein phosphorylation in specific signaling pathways is described on Chapter 5.

    Dimerization is another form of modification of transcriptional factors. Most *trans*-acting factors bind to DNA in the form of homodimer or heterodimer. The dimerization of *trans*-acting factors enhances their binding to target DNA and provides a mechanism for the specificity and selectivity of gene transcription. For instance, the members of the activating protein-1 family, including Jun, Fos, and activating transcription factor (ATF), form homodimers (Jun-Jun, Fos-Fos, ATF-ATF) and heterodimers (Fos-Jun, Jun-ATF,

TABLE 2.1. Characteristics of Selected Gene Transcription Regulators*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
RNA polymerase II, subunit 1	RNA polymerase II 14.5-kDa subunit, polymerase RNA II DNA-directed polypeptide I 14.5kDa, DNA-directed RNA polymerase II polypeptide I	125	14	Ubiquitous	Forming a complex with subunits 2 and 3 and regulating mRNA synthesis in eukaryotes
RNA polymerase II, subunit 2	RNA polymerase II subunit B, RNA polymerase II 140-kDa subunit, DNA-directed RNA polymerase II 140-kDa polypeptide	1174	134	Ubiquitous	Forming a complex with subunits 1 and 3 and regulating mRNA synthesis
RNA polymerase II, subunit 3	RNA polymerase II subunit C, DNA-directed RNA polymerase II 33-kDa polypeptide	275	31	Ubiquitous	Forming a complex with subunits 1 and 2 and regulating mRNA synthesis
TATA box-binding protein	GTF2D, SCA17, spinocerebellar ataxia 17	339	38	Ubiquitous	Together with TATA box-binding protein-associated factors, forming transcription factor IID (TFIID), which positions the RNA polymerase II to a target gene promoter and regulates mRNA transcription

*Based on bibliography 2.1.

Fos-ATF). Each type of dimer targets specific genes, thus increasing the specificity and also the capacity of transcriptional regulation. repress

In contrast to gene activation by *trans*-acting proteins, gene transcription can be inhibited by transcriptional protein repressors. The mechanisms of transcriptional repression are diverse. Some repressors can bind to transcription activators, reducing their activities. The TATA-box-binding protein (TBP) is a major target of transcription repressors. For instance, the binding of the repressor NC2 to the TATA-box-binding protein prevents the assembly of the RNA polymerase II holoenzyme into the initiation complex of gene transcription, thus repressing gene transcription. Another repression mechanism involves the competition of transcriptional repressors with transcriptional activators for DNA binding sites. The outcome, activation, or repression of gene transcription is determined on the basis of the relative activity of transcriptional activators and repressors as well as their affinity to target DNA binding sites.

In addition to the modification of existing *trans*-acting factors, de novo synthesis of *trans*-acting proteins contributes to the regulation of gene transcription. The synthesis of a *trans*-acting factor is induced by upregulating the expression of a specific gene that encodes the *trans*-acting factor. An increase in the level of a *trans*-acting factor enhances gene transcription. However, this approach is relatively slow and is not as effective as the modification of existing *trans*-acting factors. A *trans*-acting factor is usually degraded by enzymes when its task is accomplished. The degradation of *trans*-acting factors is an effective approach ensuring that gene transcription can be stopped promptly.

Chromatin Modification [2.3]

In addition to the regulation of gene transcription by transcriptional activators and repressors, dynamic changes in chromatin components contribute significantly to the regulation of gene transcription. Chromatin is composed of nucleosomes, the basic chromatin units. In each nucleosome, DNA is wrapped around a cylinder-like histone core. The nucleosomes are further arranged into three-dimensional structures. The structure of chromatin components can be modified via different processes, including histone phosphorylation on the serine and threonine residues, histone ubiquitination, histone acetylation on the lysine residue, and histone methylation on the lysine and arginine residues. These processes significantly influence the activities of gene transcription.

Histone phosphorylation can be induced by certain members of the mitogen-activated protein kinase family. Such a process is often a result of growth factor action and facilitates the transcription of mitogenic genes. *Histone ubiquitination* is a process that regulates gene transcription via degrading histone (see Chapter 5). Such a process influences the stability of chromatin and thus the activity of gene transcription. A typical example is the ubiquitination of histone H1. This type of histone is involved in the packaging of chromatin nucleosomes into a higher-ordered structure and is referred to as a *linker histone*. The formation of a higher-order chromatin structure usually results in the repression of gene transcription. The degradation of histone H1 thus promotes activation of gene transcription.

The *acetylation of histone* occurs primarily on the lysine residues at the *N*-terminus. This process is catalyzed by the histone acetyl transferase and can be reversed by the histone deacetylase. The acetylation of histones is thought to weaken the interaction of histone cores with DNA, increase the mobility of DNA and accessibility of *trans*-acting factors to *cis*-acting elements, and thus facilitate transcriptional activation. Furthermore,
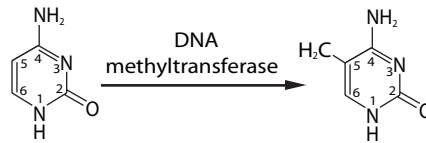
Figure 2.3. Methylation of cytosine, catalyzed by the DNA methyltransferase (DNMTs). Based on bibliography 2.4.

histone acetylation may enhance the association of *trans*-acting factors and *cis*-acting elements and facilitates gene transcription. Another means of nucleosome modification is *histone methylation*, which occurs usually on the lysine and arginine residues. A well-characterized example is the methylation of histone H3. In this type of histone, methylation is catalyzed on lysine 4 (the 4th lysine residue) and lysine 9. The outcome of methylation is dependent on the location of methylation. Methylation on the 4th lysine residue of histone H3 is associated with transcriptional activation, whereas methylation on lysine 9 suppresses gene transcription.

DNA Modification [2.4]

In addition to the modification of histones, the structure of DNA can be modified, which influences gene transcription. A well-characterized form of modification is *DNA methylation* at cytosines located prior to guanosines, which are referred to as the *CpG dinucleotides*. These dinucleotides are rare, but can be found in certain regions of a gene in the form of cluster. A cluster of CpGs is termed a *CpG island*, which is often found in the promoter region of a gene. DNA methylation is found in mammalian cells and is catalyzed by an enzyme known as *DNA methyltransferase* (DNMT), which transfers a methyl group from the donor molecule *S*-adenosyl-methionine to a cytosine base (Fig. 2.3). DNA methylation is often associated with inactive genes, whereas active genes are relatively demethylated, suggesting that DNA methylation suppresses gene transcription. In particular, DNA methylation in a promoter region causes gene silencing. While the mechanisms remain poorly understood, it has been hypothesized that DNA methylation may enhance the binding of methyl-CpG-binding proteins (MBPs), which are capable of recognizing and binding to methylated cytosine–guanosine groups. These methyl-CpG-binding proteins have been found to recruit histone deacetylase to chromatin, enhancing histone deacetylation. As a result, the accessibility of *trans*-acting factors to *cis*-acting elements is reduced and gene transcription is repressed. A physiological aspect of DNA methylation is that DNA methylation may help maintain certain genes in an inactive state. Such a process is highly selective and physiologically active genes may not be the target of DNA methylation.

REGULATION OF PRE-mRNA CONVERSION TO MATURE mRNA

Gene transcription is a process that produces a primary transcript known as pre-mRNA. This form of mRNA cannot be directly processed for protein translation. To translate a protein, it is necessary to convert the pre-mRNA to mature mRNA. Such a conversion is accomplished via several post-transcriptional processes, including capping at the 5′ terminus, polyadenylation at the 3′ terminus, pre-mRNA splicing, and pre-mRNA transport.

5′-Terminal Capping and Decapping of pre-mRNA [2.5]

5′-Terminal capping is a process by which a 7-methyl guanosine is linked to the first nucleoside of the transcript with a 5′-triphosphate (Fig. 2.4). This process is necessary for efficient splicing and export of pre-mRNA as well as for the initiation of protein translation. Several enzymes are required for 5′-terminal capping, including RNA triphosphatase, RNA guanylyltransferase, and RNA methyltransferase. The RNA triphosphatase catalyzes the formation of a diphosphate from a triphosphate at the 5′-terminus of the mRNA transcript. The RNA guanylyltransferase induces the capping of the 5′-diphosphate with a GMP. The RNA methyltransferase catalyzes the methylation at the N7 position of the 5′-guanine base. These three steps are necessary for the 5′-terminal capping and take place during transcription when the transcript is about 25–30 nucleotides in length. The capping structure is required for the arrangement of mRNA in the ribosome for protein translation. The 5′ capping also stabilizes the structure of mRNA and protects mRNA from degradation by 5′-exoribonucleases.

The 5′-terminal cap of mRNA can be removed by decapping enzymes, a process leading to mRNA degradation. There are two types of decapping enzyme, including the
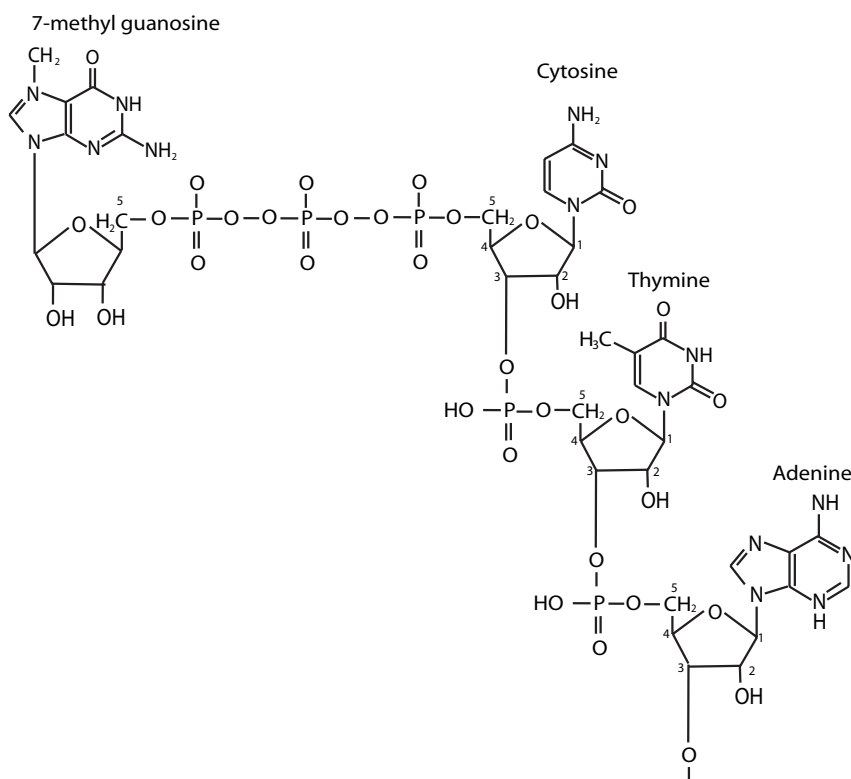

Figure 2.4. Chemical structure of the mRNA cap. The cap consists of N7-methyl guanosine linked by an inverted 5′-5′ triphosphate bridge to the first nucleoside of the mRNA chain (base N can be adenine, guanine, cytosine, or uracil). (Reprinted by permission from Gu M, Lima CD: Processing the message: Structural insights into capping and decapping mRNA, *Curr Opin Struct Biol* 15:99–106, 2005.)

Dcp1/Dcp2 decapping complex and DcpS. The Dcp1/Dcp2 complex regulates mRNA cap degradation in the 5′→3′ mRNA decay pathway. Within the Dcp1/Dcp2 complex, Dcp2 can catalyze the hydrolysis of the 5′-cap, resulting in the release of GDP from the mRNA, and Dcp1 serves as a regulator that stimulates the activity of Dcp2. In contrast, DcpS regulates mRNA cap degradation in the 3′→5′ mRNA decay pathway and catalyzes residual mRNA cap hydrolysis.

Polyadenylation [2.6]

Polyadenylation is a process by which a poly-A sequence about 200 bases is added to a pre-mRNA molecule, a process necessary for the maturation of pre-mRNA. There are two basic steps for polyadenylation: cleavage of the nascent pre-mRNA transcript and addition of a poly-A sequence to the 3′ terminus. Several proteins are involved in the regulation of pre-mRNA cleavage and polyadenylation, including the cleavage and poly-adenylation stimulation factor (CPSF), cleavage stimulation factor (CstF), cleavage factor I (CFI), and cleavage factor II (CFII). These factors form a complex and bind to a pre-mRNA site that is downstream to a ubiquitous sequence AAUAAA and immediately upstream to a GU-rich sequence. The AAUAAA sequence serves as an initiation signal for the binding of the protein complex. A CPSF molecule, CPSF-160, is responsible for recognizing the AAUAAA signal. The binding of the regulatory factors induces the cleavage of the pre-mRNA sequence at the binding site. The resulting 3′-terminus by cleavage is immediately polyadenylated by a poly(A) polymerase (Fig. 2.5). The polyadenylation of pre-mRNA contributes to the stabilization of mRNA.
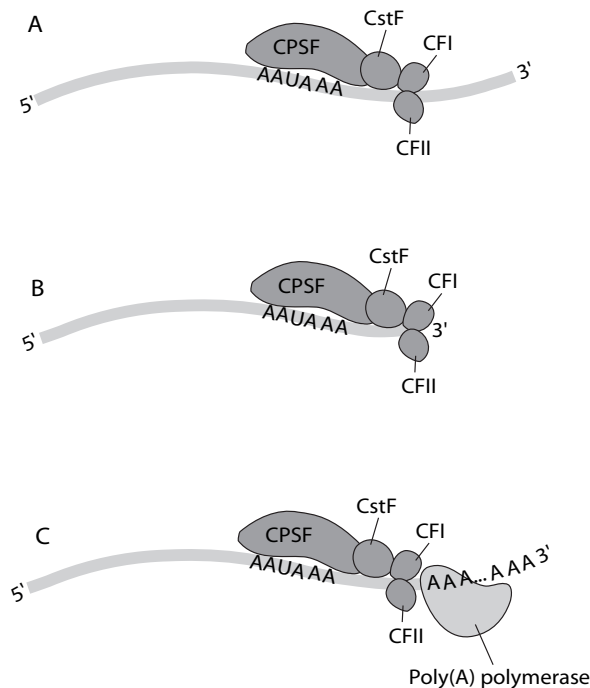

Figure 2.5. Schematic representation of polyadenylation of pre-mRNA (based on bibliography 2.6).

Pre-mRNA Splicing [2.7]

Pre-mRNA splicing is a process by which a transcribed pre-mRNA molecule is modified by removing the introns and rejoining the exons. This process is critical to the maturation of mRNA and the selection of various specific mRNA molecules from the same pre-mRNA. A pre-mRNA molecule contains protein-coding exons and noncoding introns. To generate mature mRNA, the introns must be removed and the exons must be rejoined in an appropriate order. Pre-mRNA splicing takes place in a structure known as *spliceosome*, which consists of a number of small nuclear ribonucleoproteins (SNRNPs) and several small nuclear RNA (snRNA) molecules (note that snRNAs are involved in not only RNA splicing, but also maintenance of the telomeres or the chromosome ends). These components are thought to mediate the cleaving and rejoining of nucleotides (Fig. 2.6). In the spliceosome, multiple mature mRNA molecules with different functions can be formed by alternative splicing and selective combination of exons, which are regulated by specific regulatory small nuclear ribonucleoproteins and snRNAs. This is a mechanism for producing cell- or tissue-specific proteins based on the same pre-mRNA molecule. The mechanisms of selective splicing remain poorly understood.

mRNA Transport [2.8]

mRNA transport is a process by which transcribed and processed mRNA is moved from the cell nucleus to the cytoplasm. The transcription of mRNA takes place in the cell nucleus, whereas protein translation occurs in the cytoplasm. Spliced mRNA must be transported to the cytoplasm and localized to designated sites for appropriate protein
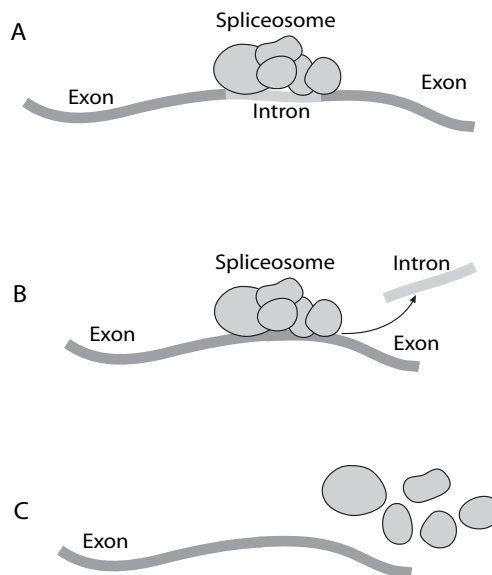

Figure 2.6. Schematic representation of pre-mRNA splicing (based on bibliography 2.7).

translation. The regulation of these processes involves specified RNA sequences, which are termed *cis-acting RNA elements*, and regulatory RNA-binding proteins, which are termed *trans-acting RNA-binding* factors. The *trans*-acting RNA-binding factors can recognize and interact with *cis*-acting RNA elements to form ribonucleotide protein (RNP) complexes. These complexes can move from the nucleus to the cytoplasm and interact with motor proteins, such as kinesin and dynein molecules, which further move the ribonucleotide protein complexes along the microtubules to the destination sites. The ribonucleotide protein complexes can also interact with myosin molecules, which move the complexes along the actin filaments. Once transported to destination sites, *trans*-acting RNA-anchoring proteins recognize and interact with the ribonucleotide protein complexes, anchoring the complexes to the specified sites. At these sites, the ribonucleotide protein complexes interact with ribosomes, initiating protein translation. It is interesting to note that unspliced mRNA cannot be transported. The exact mechanisms for selective mRNA transport remain poorly understood.

In addition to mRNA capping, splicing, and transport, the posttranscriptional stability of mRNA, which determines the concentration of mRNA, is also a critical factor for the regulation of gene expression. The mRNA concentration is controlled by the rates of RNA transcription, splicing, and transport as well as the rate of mRNA degradation. The stability of mRNA varies widely with half-life ranging from minutes to hours. The mRNAs of cytokines and growth factors, which are involved in short-term signaling events, are usually short-lived. In contrast, the mRNAs of constitutive proteins possess a relatively long-term lifespan. The stability of mRNAs is controlled by enzymes known as *nucleases*. These enzymes are often coupled to protein translation and can be activated when excessive proteins are produced, inducing degradation of specific mRNAs and reduction in protein translation.

REGULATION OF PROTEIN TRANSLATION [2.9]

In addition to the regulation of transcription and conversion of pre-mRNA to mature mRNA, gene expression is also controlled at the protein translation level. There are two known regulatory mechanisms at this level: modification at the 5′-terminus of mRNA and modification of initiation factors. The modification of mRNA at the 5′-terminus is usually a negative mechanism of translation regulation by RNA-binding proteins. These proteins serve as translation repressors, bind to mRNA at the 5′-terminus, and block the assembly of initiation factors and the $40S$ subunit of ribosomes. Such a process results in the suppression of protein translation. The modification of translation initiation factors is another powerful means for the regulation of protein translation. A major form of modification is phosphorylation, which controls the activity of translation initiation factors and thus regulates the rate of protein translation. For example, eukaryotic initiation factor 2 (eIF2) is a protein translation initiation factor. The α subunit of this protein can be phosphorylated on the Ser 51 residue by a serine/threonine protein kinase known as *RNA-dependent protein kinase* (PKR), which can be activated in response to the stimulation of various factors, such as viruses and double-stranded RNA (dsRNA). The phosphorylation of the eukaryotic initiation factor 2 reduces its activity and thus results in the suppression of protein synthesis. In the case of virus infection, the suppression of protein synthesis helps to reduce viral propagation.

BIBLIOGRAPHY

2.1. DNA Regulatory Elements and Transcriptional Factors

Cramer P, Bushnell DA, Fu J, Gnatt AL, Maier-Davis B et al: Architecture of RNA polymerase II and implications for the transcription mechanism, *Science* 288:640–8, 2000.

Acker J, Mattei MG, Wintzerith M, Roeckel N, Depetris D et al: Chromosomal localization of human RNA polymerase II subunit genes, *Genomics* 20:496–9, 1994.

Fanciulli M, Bruno T, Di Padova M, De Angelis R, Lovari S et al: The interacting RNA polymerase II subunits, hRPB11 and hRPB3, are coordinately expressed in adult human tissues and down-regulated by doxorubicin, *FEBS Lett* 427:236–40, 1998.

Pati UK, Weissman SM: The amino acid sequence of the human RNA polymerase II 33-kDa subunit hRPB 33 is highly conserved among eukaryotes, *J Biol Chem* 265:8400–3, 1990.

Hobbs NK, Bondareva AA, Barnett S, Capecchi MR et al: Removing the vertebrate-specific TBP N terminus disrupts placental beta-2M-dependent interactions with the maternal immune system, *Cell* 110:43–54, 2002.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

Imbert G, Trottier Y, Beckmann J, Mandel JL: The gene for the TATA binding protein (TBP) that contains a highly polymorphic protein coding CAG repeat maps to 6q27, *Genomics* 21:667–8, 1994.

Kao CC, Lieberman PM, Schmidt MC, Zhou Q, Pei R et al: Cloning of a transcriptionally active human TATA binding factor, *Science* 248:1646–50, 1990.

Martianov I, Viville S, Davidson I: RNA polymerase II transcription in murine cells lacking the TATA binding protein, *Science* 298:1036–9, 2002.

Nikolov DB, Hu SH, Lin J, Gasch A, Hoffmann A et al: Crystal structure of TFIID TATA-box binding protein, *Nature* 360:40–6, 1992.

Nikolov DB, Chen H, Halay ED, Hoffman A, Roeder RG et al: Crystal structure of a human TATA box-binding protein/TATA element complex, *Proc Natl Acad Sci USA*, 93(10):4862–7, May 14, 1996.

Peterson MG, Tanese N, Pugh BF, Tjian R: Functional domains and upstream activation properties of cloned human TATA binding protein, *Science* 248:1625–30, 1990.

Rosen DR, Trofatter JA, Brown RH, Jr: Mapping of the human TATA-binding protein gene (TBP) to chromosome 6qter, *Cytogenet Cell Genet* 69:279–80, 1995.

Veenstra GJC, Weeks DL, Wolffe AP: Distinct roles for TBP and TBP-like factor in early embryonic gene transcription in Xenopus, *Science* 290:2312–4, 2000.

2.2. Control of the Activity of *Trans*-Acting Factors

Holmberg CI, Tran SE, Eriksson JE, Sistonen L: Multisite phosphorylation provides sophisticated regulation of transcription factors, *Trends Biochem Sci* 27:619–27, 2002.

Firulli AB: A HANDful of questions: The molecular biology of the heart and neural crest derivatives (HAND)-subclass of basic helix-loop-helix transcription factors, *Gene* 312:27–40, 2003.

Falke D, Juliano RL: Selective gene regulation with designed transcription factors: Implications for therapy, *Curr Opin Mol Ther* 5:161–6, 2003.

Roeder RG: Transcriptional regulation and the role of diverse coactivators in animal cells, *FEBS Lett* 579:909–15, 2005.

McDonnell DP, Norris JD: Connections and regulation of the human estrogen receptor, *Science* 296:1642–4, 2002.

Della Fazia MA, Servillo G, Sassone-Corsi P: Cyclic AMP signalling and cellular proliferation: Regulation of CREB and CREM, *FEBS Lett* 410:22–4, 1997.

Sassone-Corsi P: Transcription factors responsive to cAMP, *Annu Rev Cell Dev Biol* 11:355–77, 1995.

2.3. Chromatin Modification

van Attikum H, Gasser SM: The histone code at DNA breaks: a guide to repair? *Nature Rev Mol Cell Biol* 6:757–65, 2005.

Herman JG, Baylin SB: Gene silencing in cancer in association with promoter hypermethylation, *N Engl J Med* 349:2042–54, 2003.

Thiriet C, Hayes JJ: Chromatin in need of a fix: Phosphorylation of H2AX connects chromatin to DNA repair, *Mol Cell* 18:617–22, 2005.

Martin C, Zhang Y: The diverse functions of histone lysine methylation, *Nature Rev Mol Cell Biol* 6:838–49, 2005.

Henikoff S, Ahmad K: Assembly of variant histones into chromatin, *Annu Rev Cell Dev Biol* 21:133–53, 2005.

2.4. DNA Modification

Baylin SB: DNA methylation and gene silencing in cancer, *Nat Clin Pract Oncol* 2 (Suppl 1):S4–11, 2005.

Herman JG, Baylin SB: Gene silencing in cancer in association with promoter hypermethylation, *N Engl J Med* 349:2042–54, 2003.

Jones PA, Laird PW: Cancer epigenetics comes of age, *Nature Genet* 21:163–7, 1999.

Jones PA, Baylin SB: The fundamental role of epigenetic events in cancer, *Nature Rev Genet* 3:415–28, 2002.

Herman JG: Hypermethylation of tumor suppressor genes in cancer, *Semin Cancer Biol* 9:359–67, 1999.

Holliday R: Epigenetic inheritance based on DNA methylation, *EXS* 64:452–68, 1993.

2.5. 5′-Terminal Capping of Pre-mRNA

Parker R, Song H: The enzymes and control of eukaryotic mRNA turnover, *Nat Struct Mol Biol* 11:121–7, 2004.

Dunckley T, Parker R: The DCP2 protein is required for mRNA decapping in Saccharomyces cerevisiae and contains a functional MutT motif, *EMBO J* 18:5411–22, 1999.

Wang Z, Jiao X, Carr-Schmid A, Kiledjian M: The hDcp2 protein is a mammalian mRNA decapping enzyme, *Proc Natl Acad Sci USA* 99:12663–8, 2002.

Lykke-Andersen J: Identification of a human decapping complex associated with hUpf proteins in nonsense-mediated decay, *Mol Cell Biol* 22:8114–21, 2002.

van Dijk E, Cougot N, Meyer S, Babajko S, Wahle E et al: Seraphin, Human Dcp2: A catalytically active mRNA decapping enzyme located in specific cytoplasmic structures, *EMBO J* 21:6915–24, 2002.

Wang Z, Kiledjian M: Functional link between the mammalian exosome and mRNA decapping, *Cell* 107:751–62, 2001.

Liu H, Rodgers ND, Jiao X, Kiledjian M: The scavenger mRNA decapping enzyme DcpS is a member of the HIT family of pyrophosphatases, *EMBO J* 21:4699–708, 2002.

Lima CD, Wang LK, Shuman S: Structure and mechanism of yeast RNA triphosphatase: An essential component of the mRNA capping apparatus, *Cell* 99:533–43, 1999.

Changela A, Ho CK, Martins A, Shuman S, Mondragon A: Structure and mechanism of the RNA triphosphatase component of mammalian mRNA capping enzyme, *EMBO J* 20:2575–86, 2001.

Håkansson K, Doherty AJ, Shuman S, Wigley DB: X-ray crystallography reveals a large conformational change during guanyl transfer by mRNA capping enzymes, *Cell* 89:545–53, 1997.

Shuman S, Lima CD: The polynucleotide ligase/RNA capping enzyme superfamily of covalent nucleotidyltransferases, *Curr Opin Struct Biol* 14:757–64, 2004.

Håkansson K, Wigley DB: Structure of a complex between a cap analogue and mRNA guanylyl transferase demonstrates the structural chemistry of RNA capping, *Proc Natl Acad Sci USA* 95:1505–10, 1998.

Ho CK, Sriskanda V, McCracken S, Bentley D, Schwer B et al: The gaunylyltransferase domain of mammalian mRNA capping enzyme binds to the phosphorylated carboxy-terminal domain of RNA polymerase II, *J Biol Chem* 273:9577–85, 1998.

Bentley D: The mRNA assembly line: transcription and processing machines in the same factory, *Curr Opin Cell Biol* 14:336–42, 2002.

She M, Decker CJ, Sundramurthy K, Liu Y, Chen N et al: Crystal structure of Dcp1p and its functional implications in mRNA decapping, *Nat Struct Mol Biol* 11:249–56, 2004.

van Dijk E, Le Hir H, Seraphin B: DcpS can act in the 5′-3′ mRNA decay pathway in addition to the 3′-5′ pathway, *Proc Natl Acad Sci USA* 100:12081–6, 2003.

Gu M, Lima CD: Processing the message: Structural insights into capping and decapping mRNA, *Curr Opin Struct Biol* 15:99–106, 2005.

2.6. Polyadenylation

Colgan DF, Manley JL: Mechanism and regulation of mRNA polyadenylation, *Genes Dev* 11:2755–66, 1997.

Zhao J, Hyman L, Moore C: Formation of mRNA 3′ ends in eukaryotes: Mechanism, regulation, and interrelationships with other steps in mRNA synthesis, *Microbiol Mol Biol Rev* 63:405–45, 1999.

Shatkin AJ, Manley JL: The ends of the affair: Capping and polyadenylation, *Nat Struct Biol* 7:838–42, 2000.

de Vries H, Ruegsegger U, Hubner W, Friedlein A, Langen H et al: Human pre-mRNA cleavage factor IIm contains homologs of yeast proteins and bridges two other cleavage factors, *EMBO J* 19:5895–904, 2000.

Dichtl B, Keller W: Recognition of polyadenylation sites in yeast pre-mRNAs by cleavage and polyadenylation factor, *EMBO J* 20:3197–209, 2001.

Zhao J, Hyman L, Moore C: Formation of mRNA 3′ ends in eukaryotes: Mechanism, regulation, and interrelationships with other steps in mRNA synthesis, *Microbiol Mol Biol Rev* 63:405–45, 1999.

Buratowski S: Connections between mRNA 3′ end processing and transcription termination, *Curr Opin Cell Biol* 17:257–61, 2005.

Proudfoot N: New perspectives on connecting messenger RNA 3′ end formation to transcription, *Curr Opin Cell Biol* 16:272–8, 2004.

Proudfoot N, O'Sullivan J: Polyadenylation: A tail of two complexes, *Curr Biol* 12:R855–7, 2002.

2.7. Pre-mRNA Splicing

Black DL: Mechanisms of alternative pre-messenger RNA splicing, *Annu Rev Biochem* 72:291–336, 2003.

Chan SP, Kao DI, Tsai WY, Cheng SC: The Prp19p-associated complex in spliceosome activation, *Science* 302:279–82, 2003.

Fairbrother WG, Yeh RF, Sharp PA, Burge CB: Predictive identification of exonic splicing enhancers in human genes, *Science* 297:1007–13, 2002.

Konarska MM, Query CC: Insights into the mechanisms of splicing: More lessons from the ribosome, *Genes Dev* 19:2255–60, 2005.

Makarov EM, Makarova OV, Urlaub H, Gentzel M, Will CL et al: Small nuclear ribonucleoprotein remodeling during catalytic activation of the spliceosome, *Science* 298:2205–8, 2002.

Maniatis T, Tasic B: Alternative pre-mRNA splicing and proteome expansion in metazoans, *Nature* 418:236–43, 2002.

Moore MJ, Sharp PA: Evidence for two active sites in the spliceosome provided by stereochemistry of pre-mRNA splicing, *Nature* 365:364–8, 1993.

Nelson KK, Green MR: Mechanism for cryptic splice site activation during pre-mRNA splicing, *Proc Natl Acad Sci* 87:6253–7, 1990.

Ogle JM, Ramakrishnan V: Structural insights into translational fidelity, *Annu Rev Biochem* 74:129–77, 2005.

Zhou Z, Licklider LJ, Gygi SP, Reed R: Comprehensive proteomic analysis of the human spliceosome, *Nature* 419:182–5, 2002.

Jurica MS, Moore MJ: Pre-mRNA splicing: Awash in a sea of proteins, *Mol Cell* 12:5–14, 2003.

Staley JP, Guthrie C: Mechanical devices of the spliceosome: Motors, clocks, springs, and things, *Cell* 92:315–26, 1998.

2.8. mRNA Transport

Kindler S, Wang H, Richter D, Tiedge H: RNA transport and local control of translation, *Annu Rev Cell Dev Biol* 21:223–45, 2005.

Ainger K, Avossa D, Diana AS, Barry C, Barbarese E et al: Transport and localization elements in myelin basic protein mRNA, *J Cell Biol* 138:1077–87, 1997.

Chartrand P, Singer RH, Long RM: RNP localization and transport in yeast, *Annu Rev Cell Dev Biol* 17:297–310, 2001.

Chennathukuzhi V, Morales CR, El-Alfy M, Hecht NB: The kinesin KIF17b and RNA-binding protein TB-RBP transport specific cAMP-responsive element modulator-regulated mRNAs in male germ cells, *Proc Natl Acad Sci USA* 100:15566–71, 2003.

Farina KL, Singer RH: The nuclear connection in RNA transport and localization, *Trends Cell Biol* 12:466–72, 2002.

Goldstein LS, Yang Z: Microtubule-based transport systems in neurons: The roles of kinesins and dyneins, *Annu Rev Neurosci* 23:39–71, 2000.

Hachet O, Ephrussi A: Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization, *Nature* 428:959–63, 2004.

Han JR, Yiu GK, Hecht NB: Testis/brain RNA-binding protein attaches translationally repressed and transported mRNAs to microtubules, *Proc Natl Acad Sci USA* 92:9550–54, 1995.

Job C, Eberwine J: Localization and translation of mRNA in dendrites and axons, *Nature Rev Neurosci* 2:889–98, 2001.

Johnstone O, Lasko P: Translational regulation and RNA localization in Drosophila oocytes and embryos, *Annu Rev Genet* 35:365–406, 2001.

Kapp LD, Lorsch JR: The molecular mechanics of eukaryotic translation, *Annu Rev Biochem* 73:657–704, 2004.

Kloc M, Zearfoss NR, Etkin LD: Mechanisms of subcellular mRNA localization, *Cell* 108:533–44, 2002.

Kress TL, Yoon YJ, Mowry KL: Nuclear RNP complex assembly initiates cytoplasmic RNA localization, *J Cell Biol* 165:203–11, 2004.

2.9. Regulation of Protein Translation

Dean KA, Aggarwal AK, Wharton RP: Translational repressors in Drosophila, *Trends Genet* 18:572–7, 2002.

Gray NK, Wickens M: Control of translation initiation in animals, *Annu Rev Cell Dev Biol* 14:399–458, 1998.

Dey M, Cao C, Dar AC, Tamura T, Ozato K et al: Mechanistic link between PKR dimerization, autophosphorylation, and eIF2alpha substrate recognition, *Cell* 122:901–13, 2005.

Johnstone O, Lasko P: Translational regulation and RNA localization in Drosophila oocytes and embryos, *Annu Rev Genet* 35:365–406, 2001.

Okabe M et al: Translational repression determines a neuronal potential in Drosophila asymmetric cell division, *Nature* 411:94–8, 2001.

Hershey JWB: Translational control in mammalian cells, *Annu Rev Biochem* 60:717–55, 1991

Holcik M, Sonenberg N: Translational control in stress and apoptosis, *Nat Rev Mol Cell Biol* 6:318–27, 2005.

Dar AC, Dever TE, Sicheri F: Higher-order substrate recognition of eIF2alpha by the RNA-dependent protein kinase PKR, *Cell* 122:887–900, 2005.

Su Q, Wang S, Baltzis D, Qu LK, Wong AH et al: Tyrosine phosphorylation acts as a molecular switch to full-scale activation of the eIF2 alpha RNA-dependent protein kinase, *Proc Natl Acad Sci USA* 103:63–8, 2006.