CHAPTER

27

Transcription Factors in the Central Nervous System

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OUTLINE

The functioning of the CNS requires regulated communication between neurons that are in contact with one another. Generally, this can be viewed as presynaptic neurons modulating the activity of postsynaptic neurons. While the propagation of action potentials is the most rapid form of neuron–neuron communication, there is a slower form of communication where the neurotransmitters and neuromodulators released into the synaptic cleft from presynaptic axons bind to postsynaptic neuronal membranes, inducing the postsynaptic cell to respond. This postsynaptic response can be a cascade of events, often resulting in regulation of the production of mRNA and proteins within cells. This direct cell–cell communication can be modulated by factors which diffuse over long distances and, consequently, do not require specific secretion into the synaptic cleft, yet these factors can also function in selected cell types to again alter transcription and post-transcriptional events. In this chapter, the regulation of transcription in neurons will be discussed first by presenting some basic background concerning transcription and then by highlighting two important classes of modulators of transcription, namely, those associated with glucocorticoid and cAMP regulation of transcription. Finally, given the complexities of transcriptional regulation, a brief discussion of possible sites for pharmacological intervention in the transcriptional process will be presented.

THE TRANSCRIPTIONAL PROCESS

To understand regulation of transcription, it is necessary to categorize the molecules involved. Transcription is the process by which RNA is made from genomic DNA. DNA is a linear polymer composed of four nucleotides,

deoxyadenosine, deoxyguanosine, deoxycytosine and thymidine, which are linked in a linear sequence via phosphate linkages. Genomic DNA is double-stranded, oriented in an anti-parallel arrangement such that base pairing of adenosine with thymidine and of guanosine with cytosine will stabilize the structure. The linear sequence of the nucleotides contains sequences known as genes that are the regions of genomic DNA that can be copied into RNA. This arrangement suggests that the functional structure of the DNA sequences giving rise to RNAs is modular, with an RNA-encoding region as well as regulatory regions of sequence both 5′ and 3′ to the transcription unit (Fig. 27-1). The transcription process giving rise to mRNA requires the binding of proteins responsible for RNA synthesis to the DNA template immediately 5′ to the start of transcription. Indeed, the recent sequencing of multiple genomes has revealed that the transcriptional machinery is largely conserved between different species ranging from yeast to human, reflecting the fundamental nature of the transcriptional process. These studies have revealed good sequence conservation of over 100 proteins known to be involved in the transcriptional process.

The protein that synthesizes mRNA is known as RNA polymerase II. RNA polymerase II is a large protein complex composed of multiple subunits. This protein binds to a DNA sequence, which is known as the TATA box because of the linear arrangement of the DNA sequence and its proximity to the start of transcription. However, RNA polymerase II does not bind to the TATA box without the prior association of several other proteins, including transcription factors TFIID, TFIIA and others, with this DNA region (Hori & Carey, 1994). These proteins interact with one another, forming a complex to which RNA polymerase II can bind. This scaffolding of protein interactions at the TATA box forms the transcriptional apparatus. This basic transcriptional complex is likely the same for all genes in all cells of an organism, yet it is clear that transcription of selective genes can be on or off, as well as differentially regulated, in distinct cell types to yield different amounts of mRNA. This process of transcriptional regulation is multifaceted and involves the association of several additional proteins in particular arrangements with the transcriptional complex. The arrangement and identity of transcriptional accessory proteins, also called transcription factors, can be unique for individual genes.

Finally, and perhaps most importantly, transcription is the starting point for a series of biological amplifications necessary for cellular functioning. To illustrate this point, a single gene can be transcribed into multiple mRNAs, in some cases, giving rise to thousands of mRNA molecules (Fig. 27-2). Next, each of these mRNAs may be translated into hundreds of protein molecules, hence providing the quantities required for cellular viability. Cells could not function without this amplification of genetic information. Transcription of selective genes in selective cell types, and the resultant cell-specific abundances of the transcribed RNAs, provide the opportunity for other biological processes to modulate protein production. The involvement of transcription factors in regulating the first biological amplification step is the subject of the remainder of this chapter.

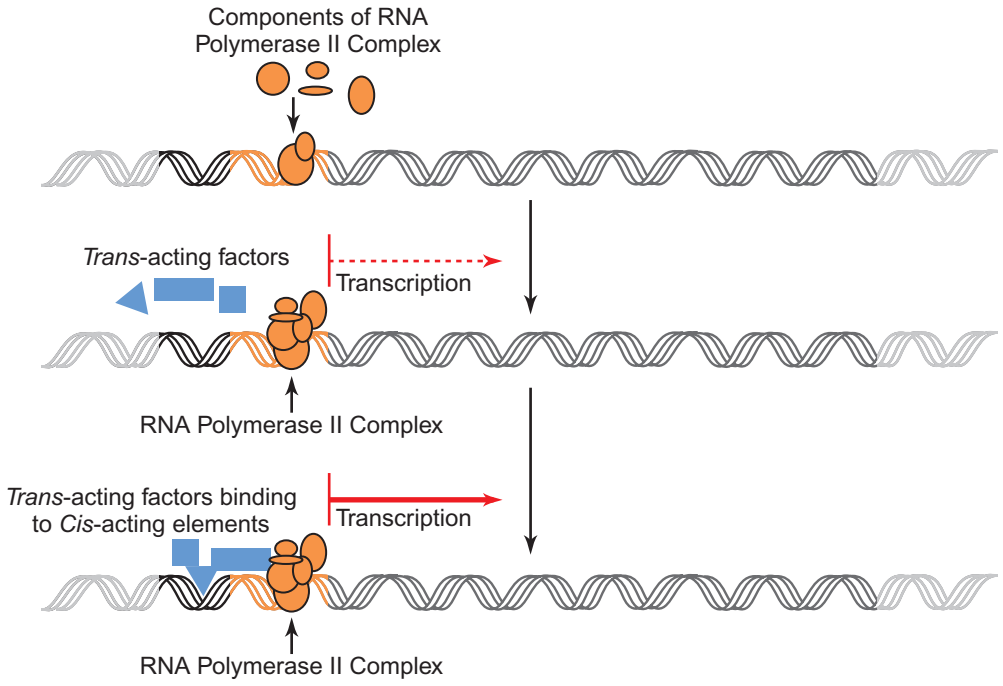

FIGURE 27-1 **Formation and regulation of the transcriptional complex.** The transcribed region of a gene is indicated in *black*. Immediately to the **right** of this box, on the 5′-side, is an *orange helix*, indicating the TATA box sequence. These are *cis*-regulatory sequences. The transcription complex binds to the TATA box and can initiate transcription when stimulated. Transcription can be regulated by the binding of various *trans*-acting factors to the enhancers *(blue shapes)* adjacent to the TATA box. The positioning of these sequences permits a direct interaction between *trans*-acting factors and the RNA polymerase II complex.

Co-regulators of transcription—modulation of chromatin structure

An important feature of a gene's transcriptional response that has been slow to be appreciated is the chromatin structure of the gene. Co-regulators of transcription fall into two classes, co-activators and co-repressors, and are usually proteins that modulate RNA polymerase activity, often at the level of modulating chromatin structure (Natoli, 2004). Eukaryotic DNA exists in the nucleus as a long, double-stranded molecule that wraps around a core protein complex composed of histone proteins. This structure is called a nucleosome. Nucleosomes are packed into a tight bundle that forms the chromatin structure of the gene and genome. Transcription occurs at genes where the promoter is accessible such that RNA polymerase and the modulatory transcription factors can bind. The polymerase moves from the promoter across the gene, making RNA as it progresses; this occurs through the activity of the core transcriptional complex displacing the DNA from the nucleosome such that it can be copied into RNA. While the nucleosome doesn't necessarily hinder RNA elongating, the ability of the nucleosome to block RNA polymerase binding to the promoter is now well documented. The blocking activity of the nucleosome is mediated in large part through the regulated activity of the histone proteins.

The nucleosome is composed of two molecules of each of four core histone proteins: H2A, H2B, H3 and H4. A fifth histone protein, H1, acts as a linker histone that binds to nucleosome core particles near the wrapping point of DNA and acts to facilitate chromatin folding. An accessible part of the histone, called the histone tail, is a common target for modifications that include acetylation, methylation, ubiquitination and phosphorylation. Addition of these marks, sometimes referred to as epigenetic marks, provides a means of modifying the genome without affecting the nucleotide sequence. These marks may be either transient or longer lasting, and provide an additional level of transcriptional regulation.

Histone acetylation

The tail regions of histones 3 and 4 (H3 and H4,) tend to be phosphorylated in actively transcribed regions. Histone acetylases (HATs) transfer acetyl groups from acetyl-COA to the amino group of the lysine residues within a histone. Multiple sites of potential modification are present on each tail; for example, in H3, the main acetylation sites are lysines 9, 14, 18 and 12. Acetylation neutralizes the positive charge of histone proteins, producing a more open chromatin structure (Fig 27-3). Some acetyltransferases have been found to be tightly associated with the RNA polymerase II holoenzyme, or with nuclear receptors, allowing targeted opening of gene structure.

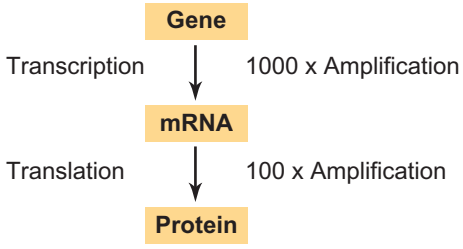

FIGURE 27-2 Gene expression as a biological amplifier. Transcription is an amplification step, giving rise to thousands of mRNA molecules from a single gene. Translation is a second amplification process, producing hundreds of protein molecules from a single mRNA molecule.

Acetylation is a reversible process. The functional antagonists of HATs are histone deacetylases (HDACs). HDACs remove acetyl groups, prevent acetylation and maintain a closed chromatin configuration to prevent transcription factor access. HDACs have recently emerged as potential therapeutic targets for the treatment of cancer and neurological and psychiatric disorders; however, as HDACs have a very wide range of intracellular targets, determining the entirety of their functions and interactions is a challenging task. Therefore, the lack of specificity of their actions may limit their suitability as therapeutic targets.

Acetylation has been linked to regulation by the MAPK superfamily (see Ch. 25). In the hippocampus, serotonin regulates glucocorticoid receptor transcription via binding of NGFI-A to a site on the exon 1_7 glucocorticoid receptor (GR) promoter. ERK activates mitogen and stress-related kinase (MSK), which acts to phosphorylate CREB. Phosphorylated CREB recruits CREB binding protein (CBP), which functions both as a HAT and a scaffolding protein. CBP associates with the exon 1_7 promoter, enhancing the ability of NGFI-A to bind to activate gene expression. The binding of NGFI-A to the exon 1_7 promoter can be regulated by behavior. For example, a mother rat exhibits different amounts of maternal care, such as grooming or nursing her pup. Pups of mother rats that have experienced high levels of licking/grooming have increased NGFI-A binding to the GR promoter, and will also show higher levels of binding when they become adults. Furthermore, as adults, these animals show fewer stress- and fear-related behaviors compared to rats from mothers who showed fewer maternal behaviors (Zhang et al., 2010) (see Fig 27-4). Thus, these molecular changes have a behaviorally relevant outcome.

In addition to acetylation, histones can also be methylated and phosphorylated, although these processes are not well understood. Histone phosphorylation has been directly correlated with the induction of immediate early gene expression. In addition, both histone acetylation and histone phosphorylation are regulated in the hippocampus during memory formation and consolidation (see Ch. 56). The underlying mechanism by which H3 phosphorylation affects gene expression is not known, although it has been suggested that the addition of negatively charged phosphate groups may generate a more open configuration of chromatin by disrupting electrostatic interactions.

Unlike acetylation and phosphorylation, the methylation state of histones is not known to directly affect chromatin configuration. Lysine and arginine residues on histone 3 and 4 can be doubly and triply methylated with trimethylation of histone 3, lysine 4 (H3K4) being found in actively transcribed chromatin regions. However, other specific methylation sites on H3 and H4 are closely linked to transcriptional repression, such as di- or trimethylation of histone 3, lysine 9 (H3K9). Turnover of lysine methylation is a slow process compared to acetylation or phosphorylation.

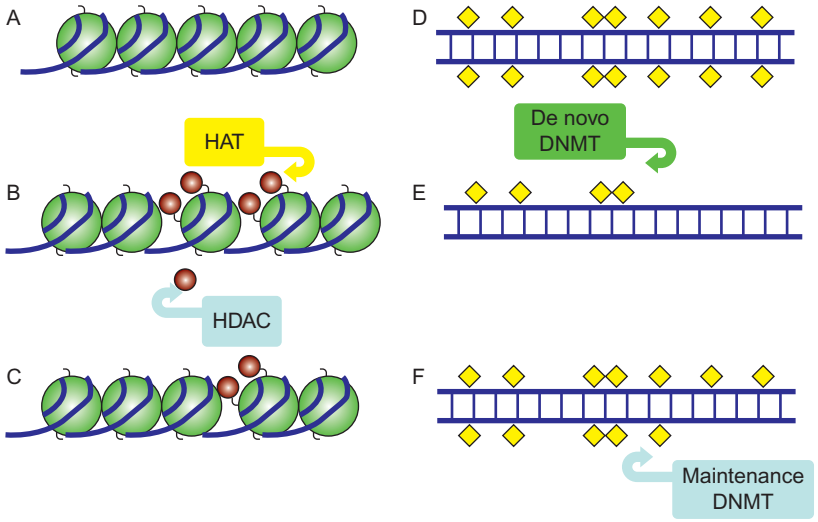

FIGURE 27-3 **Epigenetic modifications.** **A.** Histones (green) are wrapped with DNA (purple) to form a structure that resembles beads on a string. Histone tails (black) are available sites for modification. **B.** Acetylation (red) by HATs (histone acetyltransferases) generates a more open chromatin conformation. **C.** Histone deacetylation by HDACs (histone deacetylases) generates a more closed chromatin conformation. **D.** Methylation marks (yellow) are present on CpG sites on DNA (purple.) **E.** De novo methyltransferases add methyl groups to sites on a single strand of DNA where the sites have no corresponding methylated marks on the complementary strand. **F.** Maintenance methyltransferases add methyl groups when methylation is present on the complimentary strand.

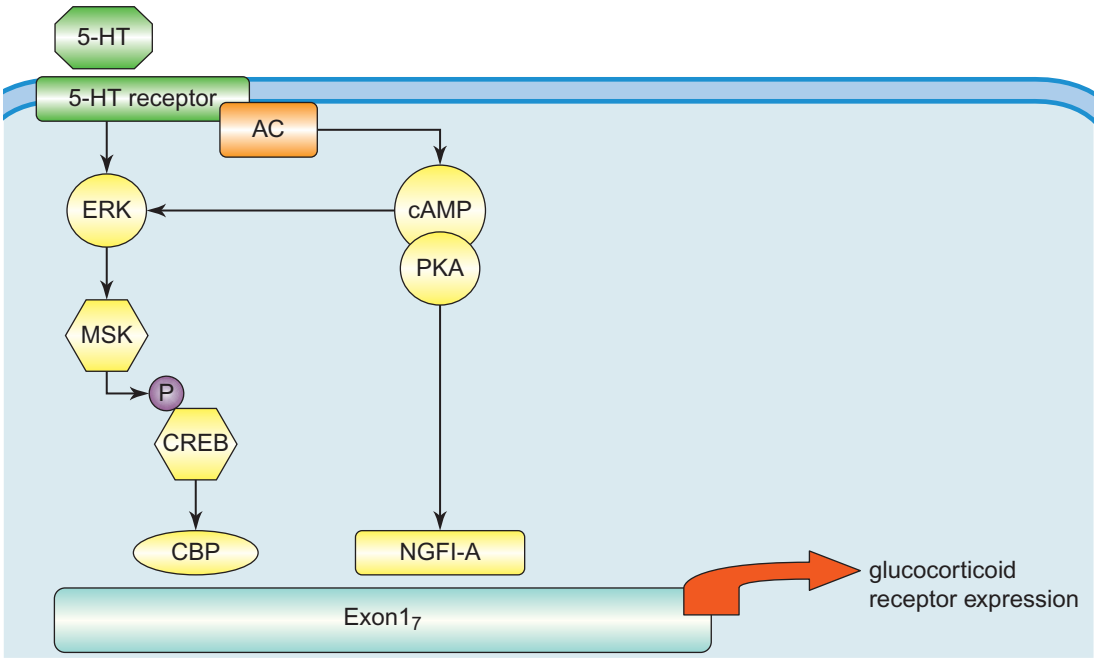

FIGURE 27-4 **Serotonergic signaling can modulate glucocorticoid receptor (GR) expression.** ERK activation occurs through serotonin binding. MSK phosphorylates CREB. Phosphorylated CREB recruits CBP, which associates with the exon 1_7 promoter of GR gene. cAMP and PKA are released via adenylate cyclase coupled to the serotonin receptor, resulting in NGFI-A expression. NGFI-A binding to the glucocorticoid receptor gene is enhanced by the CBP binding, resulting in enhanced GR expression.

Histone and DNA methylation

Methylation marks are also present on DNA, and play a part in transcriptional regulation. DNA methylation is found specifically at cytosine nucleotides followed directly by a guanine (CpG). DNA methyltransferases (DNMTs) add methyl groups to cytosines located at the fifth carbon position of the pyramidal ring. Genomic CpG methylation is found throughout the genome, with the exception of specific unmethylated regions known as CpG islands. CpG islands occur in gene promoters, and presumptively serve to regulate expression of target genes. For example, changes in DNA methylation of

the genes for brain-derived neurotrophic factor (BDNF), early growth response factor 1(Egr), reelin and calcineurin have been shown in the hippocampus during memory formation. These changes are transient, and levels of methylation return to baseline following 1 day of learning (Miller et al., 2010). Dysregulation of DNA methylation has also been implicated in a number of diseases including but not limited to schizophrenia, depression and fragile X syndrome (see further in Chs. 58–60).

There are two forms of DNMTs; the de novo methyltransferases such as DNMT3a and 3b, which methylate previously unmethylated CpG sites in DNA where no methylation is present on either strand, and a second set of maintenance DNMTs that add methyl groups to CpGs when the complementary DNA strand is already methylated. Stretches of methylation of promoters are linked to stable gene silencing. Studies have shown the role of methylation is more complex, however. For example, CpG methylation has long been implicated in X chromosome inactivation, as early evidence showed hypermethylation of promoters on the inactive chromosome. However, more recent findings indicate that the inactive chromosome is less methylated than the active chromosome, particularly over gene sequences. Intriguingly, methylated cytosine residues have been shown to guide histone acetylation to specific chromatin domains, and methylation-dependent silencing may depend on histone acetylation. Clearly, the structural regulation of chromatin is a critical element in the regulation of transcription, yet the histone code has not been fully deciphered.

REGULATION OF TRANSCRIPTION BY TRANSCRIPTION FACTORS

Transcription factors are co-activators or co-repressors that are categorized as *trans*-acting factors because they are regulatory agents that are not part of the regulated gene(s). These *trans*-acting factors regulate gene transcription by binding directly or through an intermediate protein to the gene at a particular DNA sequence, called a *cis*-regulatory region. This *cis*-regulatory region is usually located in the 5′-flanking promoter region of the gene and is composed of a specific nucleotide sequence, for example, the cAMP response element with the consensus sequence TGACGTCA. There is also a class of *cis*-regulatory elements called enhancers, which can be positioned anywhere in a gene (and, consequently, are not restricted to the promoter region), which bind, for example, the glucocorticoid receptor. Binding of the *trans*-acting factor to the *cis*-regulatory region alters the initiation of transcription, probably through a direct interaction of the *trans*-acting factor with the RNA polymerase complex. This binding likely occurs through the secondary and tertiary structures of the genomic DNA–protein complexes (see Box 27-1.) Usually, transcription factors bind as dimers to the DNA, suggesting that there are dimerization sites on transcription factors, as well as DNA-binding sites. There are several types of transcription factors, which are grouped by virtue of sequence similarities in their protein interaction and/or DNA-binding domains (Fig. 27-5). Similarities in protein–protein interaction sites suggest that monomers of different transcription factors can interact to form heterodimers. An individual heterodimer may bind to multiple *cis*-acting elements or, alternatively, interact with differing affinity for the same *cis*-acting element as compared to the homodimer. Indeed, these types of protein interactions do occur and provide a major source of regulatory complexity.

A particular *cis*-acting element may be present in multiple genes so that activation of a single transcription factor has potential for altering the expression of multiple target genes. Furthermore, an individual transcription factor may increase transcription of one gene while decreasing the transcription of another gene. This difference is partly due to the positioning of the *cis*-acting element relative to the start of transcription as well as to the identity of the protein partners in the heterodimer complex. It is important to note that, with the increase in sequence information being generated by the Human Genome Project, *cis*-acting elements can be found in genes, suggesting potential gene regulatory mechanisms without any biological information. While *cis*-acting element sequence identification in a gene is indeed predictive and necessary, this is often not sufficient to fully characterize the transcriptional regulation of that gene. *cis*-acting elements for a particular gene are commonly nonfunctional in all tissues; even more often, they are silent in one tissue and active in another due to the differential distribution of transcription factors and their binding factors, as described later in this chapter.

Technology that has hastened the study of transcription

Promoter characterization by filter-binding assays, UV crosslinking and various genetic assays, such as animal and cellular models with knockout and regulated overexpression of target genes, have been invaluable in assessing translational regulatory mechanisms. However, the cellular complexity of the CNS makes use of these standard biochemical analyses problematic. Newer methodologies have been developed that facilitate a more specific analysis of gene transcriptional mechanisms in the CNS and other tissues.

The chromatin immunoprecipitation assay (ChIP) is a procedure that is employed to determine which genes are being transcriptionally regulated by a particular transcription factor, co-activator or co-repressor or any protein that affects gene activity. Biochemical analysis of transcription requires the isolation of cellular lysates; however, when a cell is lysed, charged proteins, like many transcription factors, often bind indiscriminately to DNA. The ChIP assay has the advantage of providing *in vivo* targets for analysis (Fig. 27-6). The assay works by first cross-linking DNA-binding proteins to chromatin DNA, *in vivo,* using formaldehyde. This cross-linking freezes the protein of interest to the DNA to which it is bound *in vivo*. The chromatin is then isolated and sheared to small fragments. Antibodies to the protein of interest (e.g., transcription factor) are used to immunoprecipitate the specific protein and any DNA that would be cross-linked to the protein. The immunoprecipitated protein-bound DNA is then PCR amplified and sequenced to identify the genes to which the protein was bound. The disadvantage is that the

TRANSCRIPTIONAL DYSREGULATION IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited neurological disorder that presents in adulthood (see Chaps. 48, 49). It is characterized by involuntary movements known as chorea, as well as cognitive impairments, personality changes, depression and behavioral disturbances. These changes are elicited in response to neurodegeneration of the caudate and putamen, beginning with the medium spiny neurons, as well as of specific regions of the cortex during later stages of the disease. HD is caused by a CAG expansion in the first coding exon of the huntingtin gene, resulting in a polyglutamine expansion in the huntingtin protein (Htt). Polyglutamine expansions are linked to striatal atrophy and the aggregation of huntingtin into inclusion bodies. The number of expansions influences both presence of the disease and the age of onset, with more expansions leading to a more severe phenotype. The striatal cell death is believed to be linked to widespread dysregulation of transcriptional regulation.

Microarray studies have shown changes in the expression profile of brain tissue of HD patients that are apparent before neurological symptoms present. A number of pathways have been implicated in HD that involve transcriptional regulation either directly by mutant Htt, or by altering the activity of transcriptional regulatory proteins interacting with the mutant protein. Htt can directly bind DNA or co-repressor complexes. Transcriptional regulators known to interact with Htt include specificity protein 1 (Sp1), TATA-box-binding protein-associated factor II, 130kDa(TAFII130), cAMP-response–element binding protein (CREB) and repressor element 1-silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) (Buckley, N. J., et al. 2010).

The interaction of Htt with these transcriptional regulators has been examined for clues as to their potential importance in HD. Mutant Htt can interact with CBP (CREB binding protein) that has histone acetyltransferase activity, suggesting that histone modifications may be implicated in gene expression changes occurring in HD. The transcription factor Sp1 has been shown to interact with mutant Htt with a greater affinity than wild type Htt. Although levels of Sp1 are not decreased in HD, the association of Sp1 binding with its target genes is interrupted by mutant Htt (Cha, J. H., 2007).

Htt binds to two intermediate proteins that interact with the transcriptional repressor REST, which causes REST to sequester in the cytoplasm. Mutant Htt disrupts this process, and allows REST to enter the nucleus, where it represses BDNF expression. One hypothesis of the cause of striatal cell death in HD is due to the loss of trophic support provided by the growth factor brain derived neurotrophic factor (BDNF). Wild-type Htt protein can activate BDNF gene transcription, whereas mutant Htt represses BNDF gene transcription. Microarray studies of gene expression changes show a substantial number of REST target genes are altered in HD brains. REST acts to silence gene expression by recruiting histone deacetylases such as HDAC1 and HDAC2 as well as histone demethylases, histone methyltransferases and chromatin remodelling factors. Thus, REST has a large number of potential neuronal targets, and may exert a large influence on the neuronal transcriptome.

REST is also a regulator of noncoding mRNAs, including micro RNAs (miRNAs). Micro RNAs are short, noncoding RNAs that play a major role in post-transcriptional regulation. The expression of several miRNAs is decreased in HD, and in both mouse models of HD and in human tissue samples, predicted REST target miRNAs are dysregulated. Interestingly, their expression profiles show regional variation, suggesting regulation is differentially modulated across cell types, which may be related to the observation that Htt toxicity is localized, whereas Htt is expressed in many cell types. Htt protein stabilizes the interaction of Argonaut with processing bodies, two key players in the microRNA silencing pathway. Argonaut proteins sequester target mRNAs to cytoplasmic structures. In the presence of mutant Htt, microRNA silencing was shown to be greatly impaired (Savas, J. N., et al. 2008). The loss of miRNA silencing has been linked to degeneration of Purkinje neurons, and may suggest another means by which neurodegeneration occurs in HD. Further, microRNA dysregulation has been linked to a number of other disorders, including cancer, neurodegeneration and psychiatric disorders.

References

Buckley, N. J., Johnson, R., Zuccato, C., Bithell, A., & Cattaneo, E. (2010). The role of REST in transcriptional and epigenetic dysregulation in Huntington's disease. *Neurobiology of Disease*, *39*, 28–39.

Cha, J. H. (2007). Transcriptional signatures in Huntington's disease. *Progress in Neurobiology*, *83*, 228–248.

Savas, J. N., Makusky, A., Ottosen, S., Baillat, D., Then, F., Krainc, D., Shiekhattar, R., Markey, S. P., & Tanese, N. (2008). Huntington's disease protein contributes to RNA-mediated gene silencing through association with Argonaute and P bodies. *Proceedings of the National Academy of Sciences*, *105*(31), 10820–10825.

specificity of the reaction can be limited by the number of proteins cross-linked to a particular protein-DNA complex. Nonspecific results can easily be generated, so great attention must be paid to secondary screens to confirm the ChIP-generated data.

Traditionally the analysis of genes regulated by particular transcription factors has proceeded one candidate gene at a time. With the advent of microarrays it is now possible to assess the abundances of thousands of mRNAs simultaneously. Microarrays have been used to determine the target genes for various transcription factors through the specific activation or repression of the biological activity of the transcription factor, followed by RNA isolation, probe generation and microarray screening (Fig. 27-7). Since transcription can be a very rapid event, effort is made to isolate the RNA quickly after experimental manipulation of the system under study. While such data can be quite useful, various cautionary notes must be kept in mind when assessing these data. The static RNA abundances assessed using this procedure reflect a balance between the transcriptional activity of the gene and the degradation of the RNA product. Little is known about the regulation of RNA degradation, but presuming that it

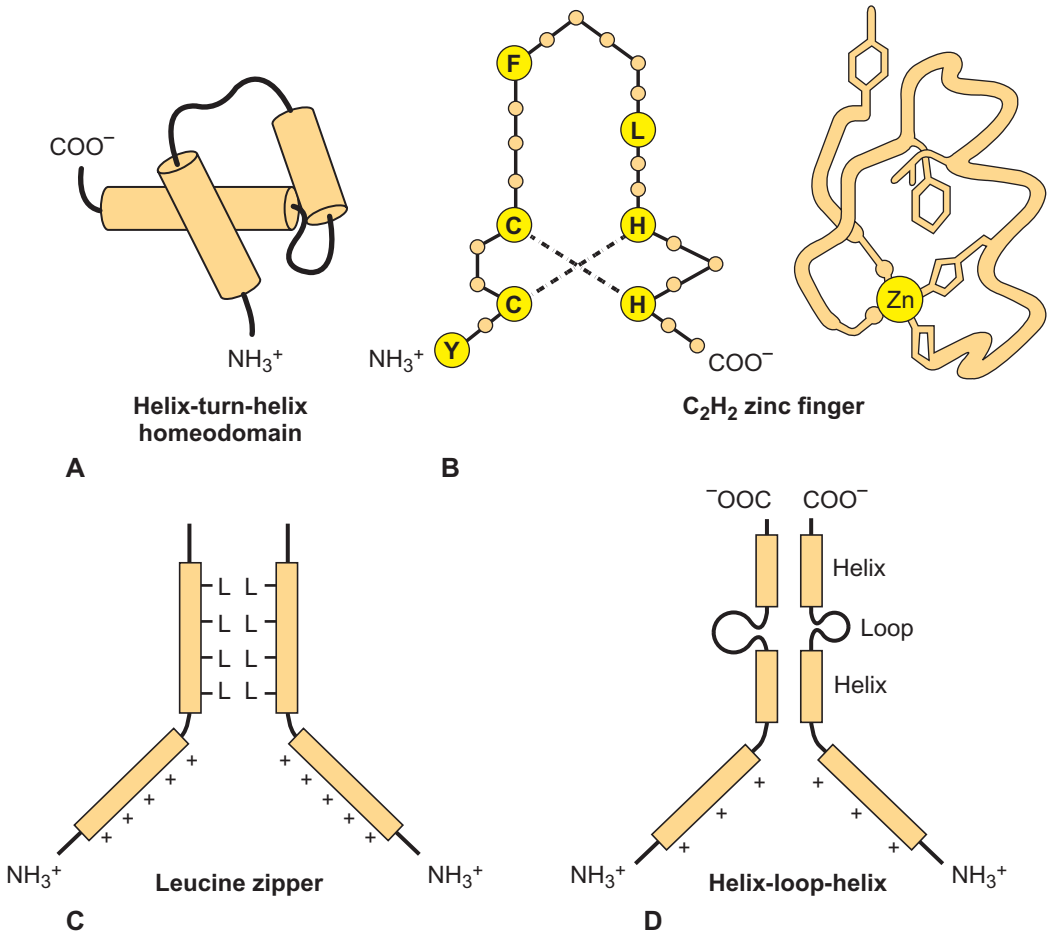

FIGURE 27-5 **Structure of transcription factors from different families.**

can be a regulated and rapid event, the microarray data will inform the investigator of genes that are regulated by transcription and degradation. Large-scale microarray analysis has been referred to as genomics and the resultant data as the transcriptome, i.e., the transcribed genes in a particular cell or tissue. The regulated transcriptome for any particular type of stimulation would produce a subset of regulated genes that are good candidates for being associated with the function of particular transcription factors.

NextGen sequencing to assess the cellular transcriptome

While microarray analysis has been and continues to be useful, it is a biased approach to gene expression analysis as particular genes, splice forms of these genes and regions (usually the 3′-end of the gene) are specifically selected for analysis. The ideal output for analysis of the transcriptional responsiveness of a particular cell type would be identification and quantification of every RNA made in a cell, including all alternatively spliced forms of the mRNAs and the noncoding RNAs.

Recent advances in DNA sequencing chemistry have made this goal realistic. NextGen sequencing takes advantage of novel chemistry and microfluidics chambers to perform rapid, high-throughput sequencing (Schuster et al., 2008). The essential component of this novel chemistry is the ability to visualize fluorescent signal generation or alteration upon nucleotide addition to a growing cDNA chain. The throughput for NextGen sequencing is much faster than conventional sequencing, yielding 4 billion nucleotides in the same time frame as it takes conventional sequencing to produce 25,000 bases (Mortazavi et al., 2008). While still a nascent technology, it has identified various novel insights into the transcriptome including anti-sense RNA sequences from the 5′-end of genes that may play a role in modulating transcription, and the presence of retained introns in selected cytoplasmically localized RNAs. mRNAs transcribed from particular genes often are groups of mRNAs composed of alternatively spliced exons. Exon differences between these mRNAs can give rise to proteins with distinct functional protein motifs. Many of these detailed sequence differences between RNAs can be assessed by microarray analysis but it is quite labor intensive and requires foreknowledge of what to expect. NextGen sequencing does not require such foreknowledge and as such provides an unbiased transcriptome analysis. This is also true for ChIP-Seq, which is the NextGen sequencing alternative to ChIP-chip (Johnson et al., 2007). Currently transcript

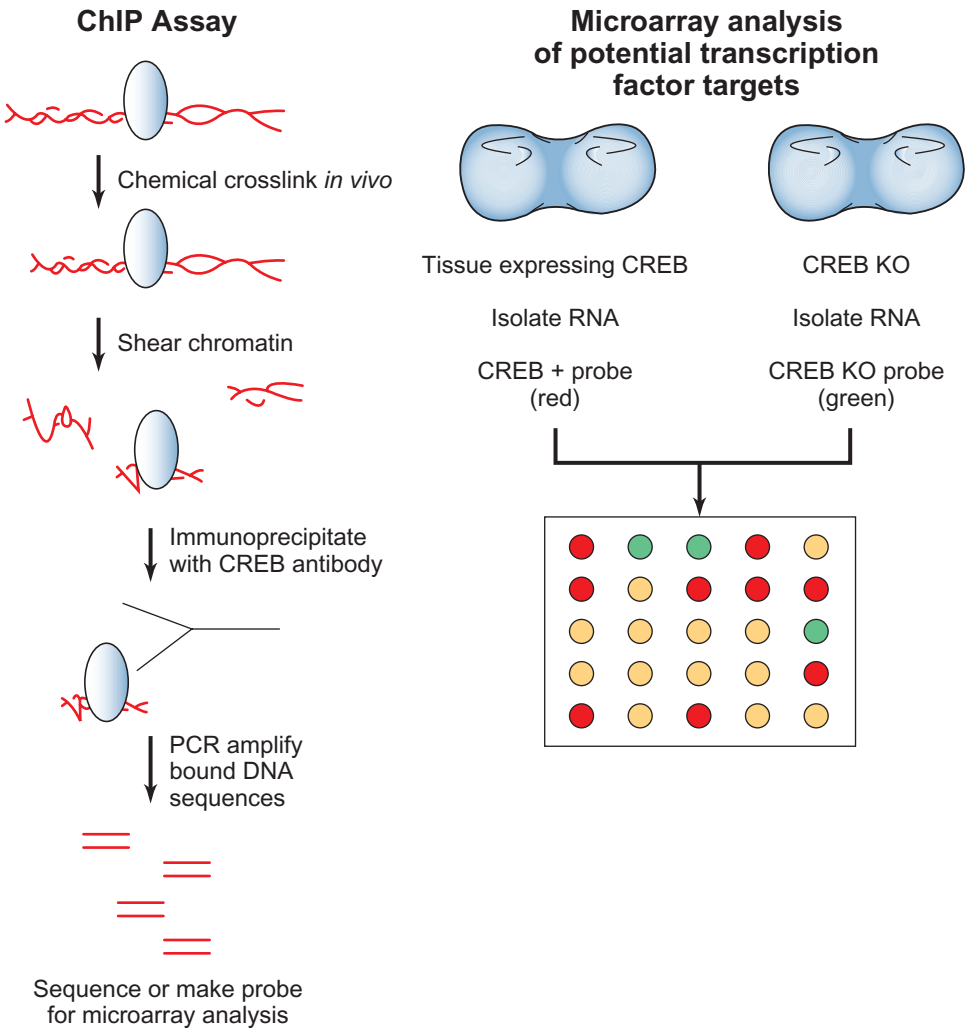

FIGURE 27-6 **ChIP and microarray analysis of transcription.** The chromatin immunoprecipitation assay is depicted on the left-hand portion of this figure. Each of the steps leading to characterization of the DNA sequence associated with selected transcription factors is illustrated for the CREB transcription factor. On the right-hand portion of this figure is a schematic of one method for selecting putative CREB responsive genes. Brain tissues from a normal mouse and a CREB knockout (KO) mouse are dissected, RNA is isolated and probes are made from each tissue with the CREB+probe giving rise to red fluorescence while the KO probe gives rise to green fluorescence. When simultaneously hybridized to an array containing thousands of immobilized clones, any RNA that is more abundant in CREB-containing tissue would be RED; any RNA that is less abundant in CREB containing tissue (knockout mouse) would be GREEN; and those RNAs that are present in nearly equal abundances appear as the YELLOW overlapped signal. The RED fluorescing clones correspond to candidate genes whose transcription is stimulated by CREB, while those that are GREEN represent candidate genes whose transcription is inhibited by CREB.

abundance determination by NextGen sequencing is more problematic than with microarray but this obstacle will certainly be overcome.

GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS AS TRANSCRIPTION FACTORS

The steroid hormones known as mineralocorticoids and glucocorticoids are synthesized in the adrenal cortex of mammals (Funder, 1987). The physiological mineralocorticoid is aldosterone, and it is involved in regulating unidirectional Na^+ transport across the epithelium. The physiological glucocorticoid in most mammalian species is cortisol; however, in rats and mice, it is corticosterone (CORT). Initially, glucocorticoids were characterized by their ability to stimulate glycogen deposition and by their release into the circulation in response to stress. Glucocorticoids regulate a wide range of responses, including aspects of immunosuppression and inflammation (Chaps. 33, 34, 55). Glucocorticoids are released in response to increases in circulating adrenocorticotrophic hormone (see further in Ch. 55).

Two distinct classes of mineralocorticoid-binding sites were first described in the rat kidney. High-affinity cytosolic aldosterone-binding sites are termed mineralocorticoid receptors (Arriza et al., 1987). Lower-affinity aldosterone-binding

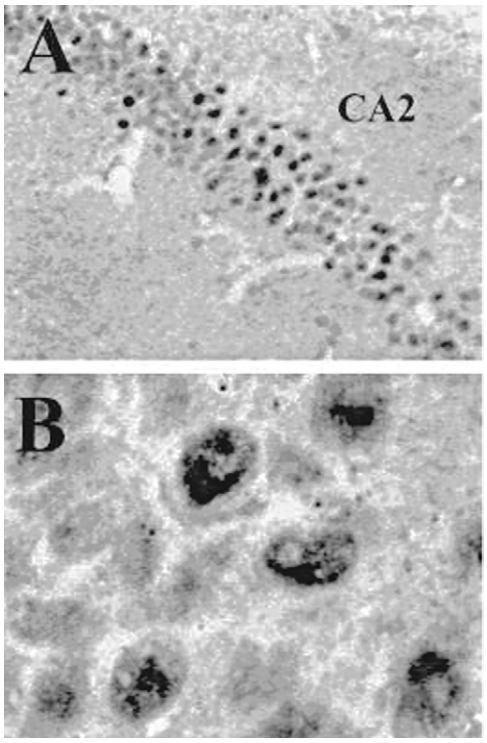

FIGURE 27-7 **Immunohistochemical localization of type I corticosteroid receptor (mineralocorticoid receptor) in the rat hippocampus.** **A:** Mineralocorticoid immunoreactivity is concentrated in pyramidal cell fields of the cornu ammons *(CA2)*. **B:** High-power photomicrograph shows that steroid-bound mineralocorticoid receptors are primarily localized to neuronal cell nuclei. (Photomicrographs courtesy of Dr. James P. Herman, Department of Anatomy and Neurobiology, University of Kentucky.)

sites are termed glucocorticoid receptors. Glucocorticoid receptors are the same as dexamethasone-binding receptors (Hollenberg et al., 1985).

While the affinity of mineralocorticoid receptors for aldosterone is higher than that for CORT, circulating concentrations of CORT are several orders of magnitude higher than those of aldosterone. CORT is effectively blocked from binding to the mineralocorticoid receptors by plasma proteins such as transcortin, which bind preferentially to CORT. Another mechanism that serves to alter the balance of CORT and aldosterone binding to receptors is the activity of 11β-hydroxysteroid dehydrogenase, which, in the rat kidney, rapidly oxidizes CORT to inactive metabolites. This facilitates binding of aldosterone to mineralocorticoid receptors in the presence of high concentrations of CORT (Reul & de Kloet, 1985).

Corticosteroid receptors regulate transcription in the nervous system

Intracellular binding sites selective for [^{3}H]-CORT were first identified in various brain regions, in particular the hippocampus, of adrenalectomized rats. A similar autoradiographic pattern was obtained using [^{3}H]-aldosterone as a ligand, suggesting that these receptors were mineralocorticoid receptors. The [^{3}H]-dexamethasone-binding pattern shows selective differences in the pattern of binding compared to the CORT and aldosterone patterns, suggesting the existence of multiple corticosteroid receptors. [^{3}H]-CORT binding performed in the presence of unlabeled dexamethasone or aldosterone reveals that CORT binds to at least two receptor types in the brain.

Two high-affinity intracellular corticosteroid receptors have been characterized and are distinguished on the basis of binding properties, amino acid sequence, neuroanatomical distribution and physiological function. The type I corticosteroid receptor, or mineralocorticoid receptor, is localized in various brain regions, including the septum and hippocampus (Arriza et al., 1988). The type I receptor binds CORT and aldosterone with high affinity, ~ 0.5 nM. The relative steroid binding affinity of the type I receptor for CORT is greater than or equal to that for aldosterone, which in turn is greater than that for dexamethasone. The type I receptor is present in all subregions of the hippocampus, namely, the CA1, CA2 and CA3 and the granular cells of the dentate gyrus (Fig. 27-8).

The type II corticosteroid receptor, also called the glucocorticoid receptor, is widely distributed in the brain and exhibits high-affinity binding to dexamethasone. The type II receptor binds with 10-fold lower affinity to corticosterone, 5 nM, as compared with the type I receptor. The relative binding affinities for steroid interaction with the glucocorticoid receptor are such that dexamethasone binding is greater than corticosterone binding, which in turn is much greater than aldosterone binding. Glucocorticoids are present at high concentrations in all regions of the hippocampus, with the exception of the CA3 region, where concentrations are exceedingly low (Chao et al., 1989).

Mineralocorticoid and glucocorticoid receptors can coexist in the same neurons as evident from their demonstrated colocalization in the CA1 region of the hippocampus (deKloet et al., 1975). These CA1 neurons are exquisitely sensitive to the whole range of glucocorticoid concentrations. Because of their high affinity for corticosteroids, the type I receptors are approximately 80% occupied, suggesting that the number of type I receptors is likely to be the rate-limiting factor in mineralocorticoid receptor functioning. However, glucocorticoid receptor functions are most dependent on the extent of glucocorticoid receptor occupancy and are likely to be regulated by the concentration of the available steroid. The presence of multiple binding sites for corticosteroids as well as their differing concentrations in different cell types form the molecular basis for their differential actions in the rat brain (Herman et al., 1989).

The mechanisms of corticosteroid receptor regulation of transcription have been elucidated

Both type I and type II corticosteroid receptors are members of a superfamily of ligand-activated transcription factors defined by protein sequence similarity. Included in this superfamily are various other steroid receptors, such as the estrogen receptor, as well as members of the retinoic acid receptor family and thyroid hormone receptors. The ligand-activated transcription factor superfamily is estimated to

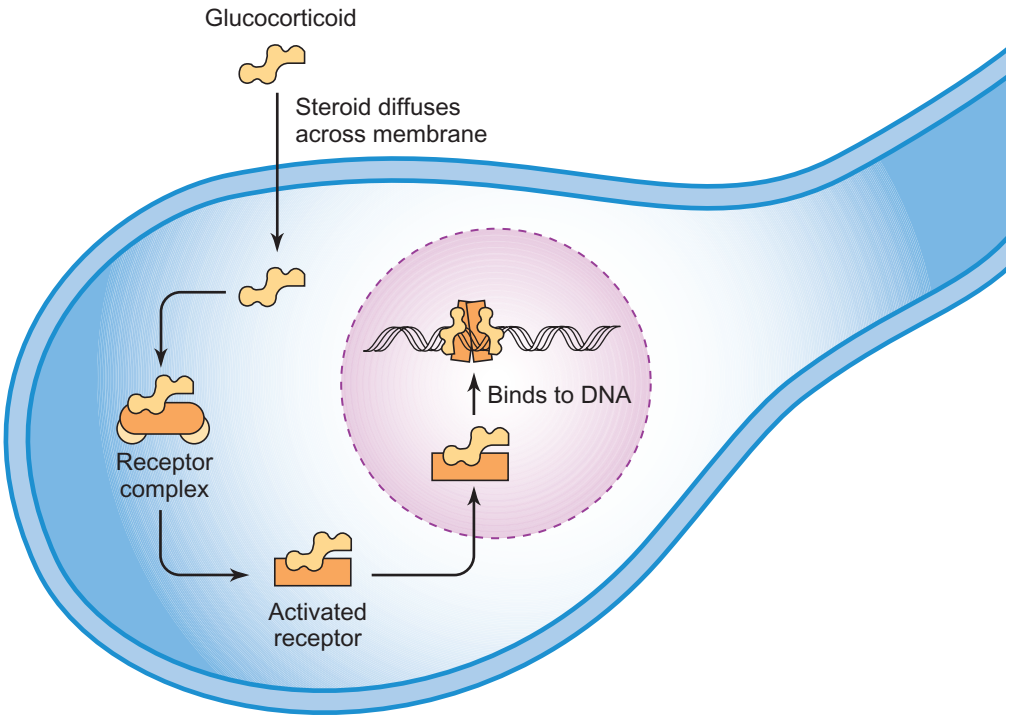

FIGURE 27-8 **Activation of glucocorticoid receptors.** Glucocorticoids (*red*) ( ) diffuse across the plasma membrane and bind to the glucocorticoid receptor (*dark orange*). Upon glucocorticoid receptor binding to the steroid, the receptor undergoes a conformational change, which permits it to dissociate from the chaperone heat shock proteins *(light orange circles)*. The activated glucocorticoid receptor translocates across the nuclear membrane and binds as a homo- or heterodimer to glucocorticoid response element sequences to regulate gene transcription.

contain several hundred members. Mineralocorticoid and glucocorticoid receptors are each composed of four distinct protein regions, including an N-terminal region, a DNA-binding domain, a nuclear localization signal and a C-terminal hormone-binding region. The N-terminal region is associated with activation of transcription through an as yet unknown mechanism. The DNA-binding domain is a charged protein sequence, known as a zinc finger. In this case, there are two fingers, each composed of the sequence Cys-X2-Cys-X13-Cys-X2-Cys. The C-terminal region binds glucocorticoids, which, when bound, initiate the conformational change of the receptor that facilitates its translocation.

In the cytoplasm, the mineralocorticoid and glucocorticoid receptors are associated with a large multiprotein complex that contains the heat shock proteins (HSP) HSP70 and HSP90 (Fig. 27-6). This complex maintains unbound corticosteroid receptors in a ligand-accessible but inactive protein conformation. Binding of ligand causes dissociation of receptors from the heat shock proteins, followed by translocation of the activated receptors to the nucleus. In the nucleus, ligand-bound corticosteroid receptors bind to a *cis*-acting element called glucocorticoid response element (GRE). The GRE is a 15-base sequence, TGGTACAAATGTTCT, and is also called an enhancer element because it generally functions to enhance transcription. The GRE can be positioned anywhere within the gene, not just in the promoter region. Modulation of corticosteroid-responsive genes occurs through interactions of the type I or II receptors bound to the enhancer sequence, with the transcriptional complex.

Ligand-bound corticosteroid receptors have been shown to interact to form heterodimers with other transcription factors, such as the jun protein. Such interactions are responsible for transactivation of the *cis*-regulatory sites known as AP-1 sites and for the glucocorticoid-mediated suppression of transcription, such as that seen in the pro-opiomelanocortin gene. A number of such specific protein interactions have been reported; these interactions and their locations relative to other transcription factors transform a ubiquitous steroid hormone signal into a tissue-specific, graded cellular response.

More recently, the protein–protein co-immunoprecipitation assay coupled with the ChIP assay have highlighted particular genes whose promoters bind to steroid receptors and whose transcription can be modulated by these receptor-associated proteins (Nagaich et al., 2004; McKenna & O'Malley, 2002). Among these co-activators are various acetyltransferases such as CBP and p300 and the ATP-coupled chromatin remodelling SWI/SNF complex as well as the protein methylase CARM-1 (Glass & Rosenfeld, 2000). Each of these proteins has the capacity to modulate chromatin structure through post-translational modification of the chromatin. Co-repressors of steroid receptor–mediated gene transcription have also been characterized using similar methodologies (Habener, 1990). At least some steroid receptor co-repressors can function through chromatin deacetylation through the mediation of Sin3 and histone deacetylase. The characterization of co-regulators of gene expression promises to highlight mechanisms for the coordinate regulation of multiple genes by a specific modulator as well as mechanisms for cell-specific responses to regulatory signals.

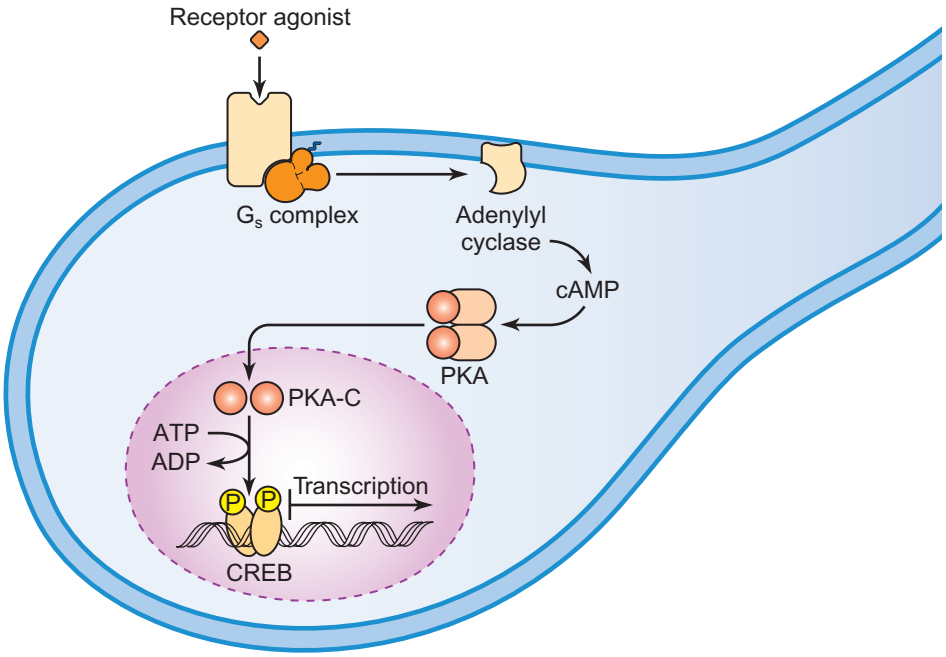

FIGURE 27-9 Activation of cAMP-regulated genes. When agonists (◆) bind to G protein-coupled receptors that activate dissociation of G_s from the G_s complex, G_s will interact with the enzyme adenylyl cyclase, resulting in synthesis and increase of intracellular cAMP. cAMP binds to the regulatory subunits *(orange ovals)* of protein kinase A *(PKA)*. This binding facilitates the dissociation of the catalytic subunits *(dark orange spheres)* from the PKA complex. The catalytic subunits then translocate across the nuclear membrane and phosphorylate cAMP response element–binding protein *(CREB)* and CREB-related proteins. Phosphorylated CREB increases gene transcription *(right arrow)*.

A compendium of genes that are good candidates for glucocorticoid regulation has been generated using microarray technology. For example, the glucocorticoid-regulated abundances of RNAs encoded by various genes have been characterized in lymphoid cells during apoptosis. In support of the selectivity of the glucocorticoid responsiveness of these genes, data were generated showing that many of these genes are nonresponsive to glucocorticoids when glucocorticoid receptors are blocked by a glucocorticoid receptor antagonist. Data such as these highlighting potential multiple targets of steroid hormone receptor regulation may provide therapeutic targets to modulate unwanted side effects of glucocorticoid receptor therapeutics.

cAMP REGULATION OF TRANSCRIPTION

Activation of a transcription factor known as the cAMP response element binding protein (CREB) is the final step in a signal transduction pathway, which is initiated by the binding of a specific class of cell-surface receptors (Fig. 27-9) (Montminy et al., 1990). Binding of ligand to a G_s protein-coupled receptor, such as the D1 dopamine receptor or the 5-hydroxytryptamine-1 receptor, liberates G_s protein from the G-protein complex (Chap. 21). Subsequently, G_s activates adenylyl cyclase, which in turn stimulates cAMP production. The increase in intracellular cAMP induces the dissociation of protein kinase A (PKA) catalytic subunits from their regulatory subunits (Chap. 22). The catalytic subunits move into the nucleus, where they phosphorylate a number of proteins, including the CREB transcription factor. This phosphorylation event activates CREB protein, and transcription is stimulated (Yamamoto et al., 1988). The genes, which are activated by CREB, are those that have the *cis*-acting palindromic CRE consensus sequence (TGACGTCA) in their promoter region. This is distinct from the enhancer activity of the previously discussed GREs, which can function at sites distinct from the promoter region of a given gene. CREB protein appears to be ubiquitously expressed in the brain, with high concentrations in the hippocampus (Fig. 27-10) and cortex. As seen in Figure 27-10, it is apparent that much of the CREB immunoreactivity is present in the nucleus of CA1 pyramidal cells.

The critical feature of CREB protein activation is phosphorylation, which is required for CREB-mediated stimulation of transcription (Bannister & Kouzarides, 1996). PKA phosphorylates CREB on a serine positioned at amino acid 133 in the CREB protein sequence (Ch. 25). How phosphorylation activates CREB-mediated transcription is unclear. Some investigators believe that phospho-CREB has a slightly higher affinity for CRE binding, and others believe that transcription factor interaction with the transcriptional complex is stimulated by phosphorylation. The time course for CREB stimulation of transcription is relatively rapid, peaking at approximately 30 min after cAMP stimulation, followed by a gradual decrease to basal levels of activation over the course of several hours. This decrease in activation occurs through the activity of protein phosphatase 1 and other phosphatases, which remove the phosphate group from CREB protein. It is important to note that there is increasing evidence that CREB

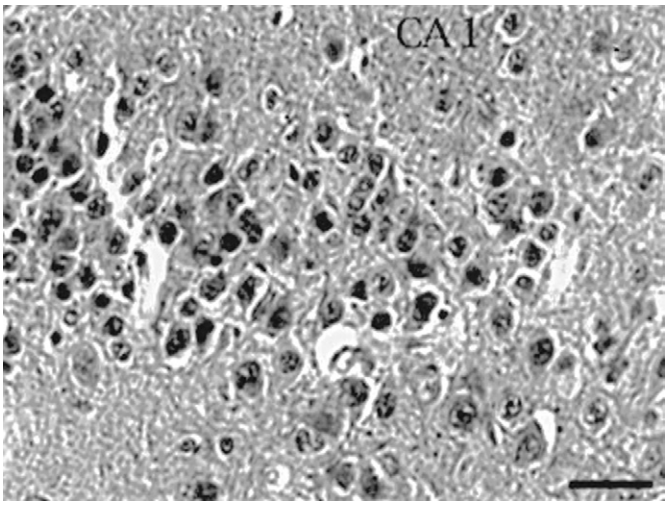

FIGURE 27-10 **Immunohistochemical localization of cAMP response element binding protein** *(CREB)* **in rat hippocampal neurons.** Using a polyclonal antibody that recognizes both CREB and phospho-CREB protein, it is apparent that CREB protein is enriched in the nucleus of pyramidal cells of the CA1 region of the hippocampus. Scale bar is 30μm. (Photomicrograph courtesy of Dr. Stephen Ginsberg, Department of Pathology, University of Pennsylvania.)

phosphorylation can be induced by factors other than an increase in intracellular cAMP concentrations. The relative contribution of these other stimulators, including an increase in intracellular Ca^{2+}, to the transcriptional activation exhibited by phospho-CREB is under active investigation.

Numerous studies have shown that once CREB is phosphorylated it, recruits the histone acetyltransferase CBP (CREB-binding protein) to activate gene transcription and this activation can be ameliorated through the activity of histone deacetylase (Fass et al., 2003). While this consensus model does appear to account for the bulk of CREB mediated transcription, more recent data using a constitutively active CREB mutant and microarray analysis of neuronal gene expression has highlighted the plethora of CREB-regulated genes (Cha-Molstad et al., 2004; deGroot & Sassone-Corsi, 1993). Examination of a subset of these genes upon CREB activation in the presence of a histone deacetylase inhibitor has show that not only are the RNA abundances for some of the CREB-responsive genes enhanced, but a subset of the genes is also inhibited by this treatment. ChIP assays have shown that the differential roles of histone deacetylases in CREB-mediated transcription are evident at the level of pre-initiation complex recruitment. These data suggest a complexity of CREB-responsive gene expression that likely involves several co-regulators beyond the simple acetyltransferase mediators.

In large part, the modulation of CREB-regulated genes represents the intracellular balance of active kinases and phosphatases, which is a reflection of cellular activation (see further in Ch. 25). This is one of the major differences between CREB- and glucocorticoid receptor–regulated transcription: CREB is an integrator of intracellular homeostasis, while the glucocorticoid receptor integrates whole-body homeostasis.

The cAMP response element–binding protein is a member of a family containing interacting proteins

After the mRNA encoding CREB protein was cloned and sequenced, several other members of the CREB family of transcription factors were identified. Using homology screens, the polymerase chain reaction and interaction screening assays, a protein called cAMP response element modulator (CREM) was isolated. CREM can both stimulate and inhibit cAMP-mediated gene transcription. Transcriptional inhibition can occur as a result of generation of modified CREM proteins because of alternative splicing of the CREM mRNA (Fig. 27-11). Among these different proteins are the α, β and γ forms of CREM, which lack the glutamine-rich region present in the activator forms of both CREM and CREB that is required for stimulation of transcription (Foulkes & Sassone-Corsi, 1992). Inhibition occurs due to heterodimer formation between this inactivating form of CREM and either CREM or CREB (De Cesare et al., 1999). The alternatively spliced forms of CREM as well as those recently discovered for CREB, which can also act as transcriptional inhibitors, occur in a tissue- and cell type–specific manner. Additionally, recent data have shown that CREB can interact with other leucine zipper–containing transcription factors to produce heterodimers that can interact with *cis*-acting elements distinct from the CRE, as previously discussed with regard to the corticosteroid receptors (Hai & Curran, 1991). These findings provide a mechanism for generation of tissue- and cell-type differences in cAMP responsiveness (Tully et al., 1994).

The function of the cAMP response element–binding protein has been modeled in transgenic organisms

The observation that serotonin stimulates learning and memory in the *Aplysia* system with the subsequent discovery that cAMP will mimic this effect suggested that cAMP was involved in this physiological process (see Chap. 56). Recently, direct evidence for the involvement of CREB in learning and memory was presented in the *Drosophila* system (see below). *Drosophila* as an experimental system has several advantages over mammalian systems, including the ease of generation of mutant as well as transgenic flies.

The role of CREB in learning and memory was first anticipated by screening *Drosophila* mutants in a learning and memory task in which flies were behaviorally tested in an odorant-association task, where an odorant was paired with an electric shock. Flies with "normal" phenotype learned that the odorant is associated with an electric shock and will learn to avoid the odorant. Two fly mutants were discovered that could not learn this association: *dunce* and *rutabaga*. The *dunce* mutant was deficient in cAMP phosphodiesterase, which breaks down cAMP, and the *rutabaga* mutant had a mutation in adenylyl cyclase, which synthesizes cAMP. These data combined with the prior *Aplysia* work suggested that CREB was involved in acquisition of learning and memory (Yin et al., 1995).

More recently, with the same odorant-association behavioral test, the specific role of CREB in learning and memory was assessed by using a dominant-negative CREB (dCREB) protein

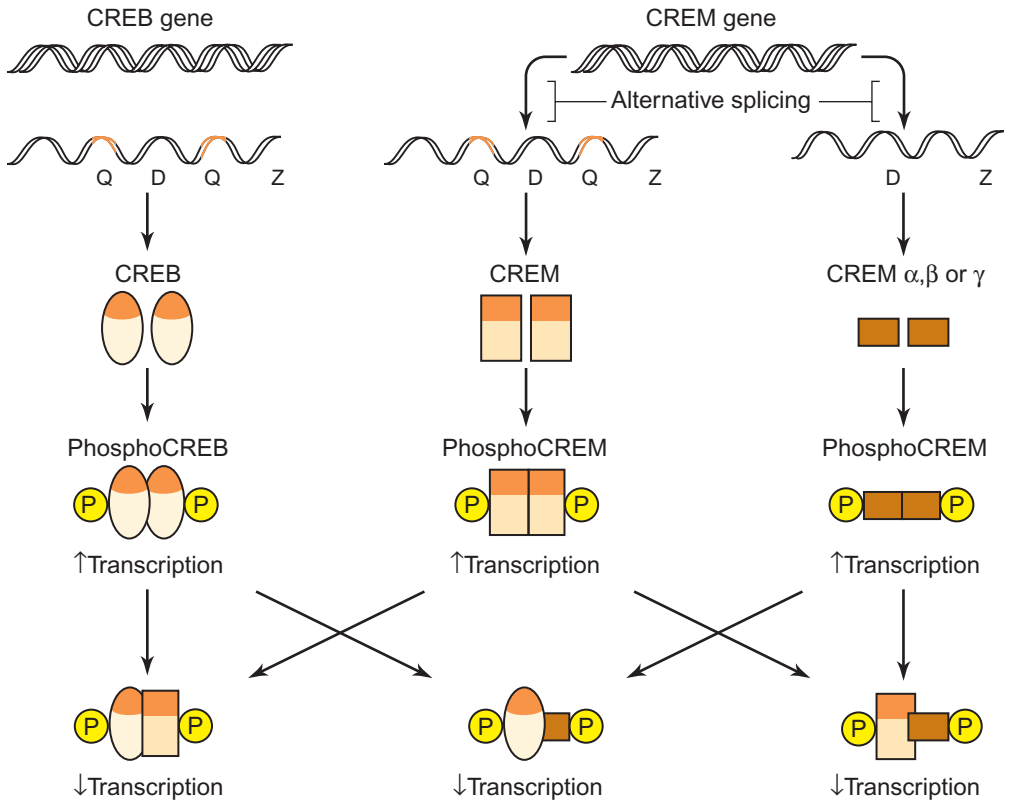

FIGURE 27-11 Mechanism for generation of activators and repressors of cAMP-stimulated transcription. The cAMP response element binding protein *(CREB)* and the cAMP response element modulator *(CREM)* genes can give rise to alternatively spliced mRNAs, which give rise to distinct protein products. While CREB and CREM can stimulate CRE-mediated gene expression, generation of the α, β or γ forms of CREM will produce proteins that can heterodimerize with activated CREB and CREM subunits inhibiting their stimulatory capacity. The symbols used to highlight functional regions of CREB and CREM protein are *Q* for the polyglutamine tract, which stimulates transcription; *D* for the DNA-binding region; and *Z* for the leucine-zipper region, which is involved in dimerization.

expressed in transgenic flies (Yin et al., 1994; Perrazzona et al., 2004). This dCREB complexes with normal CREB through a functional leucine-zipper region, yet inhibits the transcriptional activation mediated by normal CREB protein. The experiment was designed so that dCREB was controlled by a heat shock promoter and could be activated in the adult animal. This experimental paradigm allows for the assessment of the role of CREB in the adult animal, by using heat shock to induce dCREB expression, rather than by examining the behavioral consequences of CREB deficiency during development. Heat shock, in and of itself, does not alter learning and memory, although it can affect viability of the flies. (Yin et al., 1994; Perrazzona et al., 2004). Data from these experiments show that the form of memory requiring protein synthesis, called long-term memory (Chap. 56), was inhibited by expression of dCREB.

The dissection of CREB involvement in various physiological or behavioral paradigms in the mammalian system has been much slower than in *Drosophila.* In the mammalian system, targeted gene disruption has been used in attempts to inhibit CREB function. In one set of experiments, the exon of the gene containing the initiator methionine was deleted in hopes of blocking CREB production. However, the targeting of this region facilitated an alternative splicing event so that a previously undiscovered spliced variant mRNA of the CREB gene was produced, and was, in fact, upregulated. In the other targeted disruption experiments, the DNA-binding region was disrupted and, indeed, CREB protein production was inhibited. Unfortunately, this disruption resulted in perinatal lethality, so no adult animals could be generated. The problem with the design of these experiments is that targeted disruption of CREB production would result in a developmental deficit, making it difficult to attribute any particular modification of behavior in the adult to a direct deficiency of CREB.

What is needed to directly assess the involvement of CREB in adult mammalian neurons is the production of a conditional knockout mouse in which the knockout could be activated in the adult animal in a tissue-specific manner. This mutant could be similar to the production of a dominant-negative mutant, as was done in the *Drosophila* system, or potentially through induction of genetic recombination in adult neurons using an inducible system similar to that being used to produce conditional knockouts for other genes (see Chap. 41). Such a model system would be very useful in understanding the potential involvement of CREB in long-term memory in the mammalian system as well as in other physiological conditions associated with CREB functioning, such as opiate tolerance and withdrawal (Chap. 61) or aspects of behavioral aggressiveness (Chap. 60).

THE ROLE OF TRANSCRIPTION FACTORS IN CELLULAR PHENOTYPE

Transcription factors navigate the roadmap of cellular maturation

Transcription factors occupy an important niche in cellular homeostasis, and are responsible for initiating cellular programs. One of the most remarkable examples demonstrating the power of transcription factors are cell fate decisions that take place during cellular differentiation and specialization. Cellular specialization in the brain is of particular importance for proper neurological function and behavior. Here, transcription factors act to guide neural progenitor cells toward terminal differentiation into mature cells. During this process, the neural progenitor cells commit to differentiating into a certain lineage, for instance, neural or glial. At the molecular level, the decision-making is often complex and involves an array of transcription factors and environmental cues that combine to trigger a commitment in one direction or another. To ensure proper neurogenesis, cellular programs present the cell with a number of choices or checkpoints at which the presence or absence of certain transcription factors are critical for the next decision. For instance, in the spinal cord of the developing chick, self-renewing progenitors express the transcription factor Sox2 (Graham et al., 2003). In general, if Sox2 expression in progenitor cells ceases or is inhibited, the cells exit the cell cycle and differentiate into neuroblasts. In contrast, forced Sox2 expression in progenitor cells prevents differentiation and maintains the cells in a progenitor-like state. These actions of Sox2 are carried out in collaboration with other transcription factors with specific spatio-temporal patterns. Thus, transcription factors guide the differentiation of a pool of spinal cord progenitors into lineage-restricted neuroblasts.

The Sox family is a group of transcription factors that are expressed by multiple types of neural progenitor and stem cells. In general, Sox proteins function to regulate cellular differentiation and specialization. Structurally, Sox proteins are characterized by a conserved 79-amino-acid–long high mobility group (HMG) domain that binds a short stretch of seven base pairs of DNA (e.g., 5′-CTTTGTT-3′). Sox proteins function either as transcriptional activators or suppressors, but have no function on their own. Sox binding to DNA introduces a sharp bend of the DNA double helix, which may either facilitate or prevent adjacent binding of cofactors or binding partners. Additionally, Sox proteins are often capable of recruiting DNA binding partners through protein–protein interactions independent of the HMG domain, and activate transcription in cooperation with these other DNA binding partners. In addition, the binding of these partners adjacent to Sox2 stabilizes the Sox2-DNA complex and synergistically enhances the effect of Sox2. The identity of Sox2 partners varies and depends upon the type and state of the cell (Wilson & Koopman, 2002). Therefore, the spatio-temporal expression pattern of Sox2 is not the only determinant for Sox2 activity, and therefore Sox2 activity has to be interpreted in conjunction with its partners. Because each cofactor binds a DNA motif that differs from Sox2, the combination of Sox2 and several different cofactors expands the repertoire of Sox2 gene targets and enables Sox2 to regulate transcription more specifically in different cell types. This example demonstrates how a single transcription factor can increase its functional specialization by cooperatively working with cofactors in executing its effect. An example of a Sox2 partner is the transcription factor Oct3/Oct4, which act on the Oct-Sox enhancer in embryonic stem (ES) cells (Fig 27-12) (Remenyi et al., 2003). Oct3/Oct4 bind adjacent to Sox2 and together they drive expression of Fgf4, UTF1, and Nanog as well as expression of Sox2 and Oct3/Oct4 themselves, and serve a crucial role in ES development. However, in the lens, Sox2 partners with another transcriptional factor, Pax6. Both Sox2 and Pax6 bind the DF5 enhancer that is located within an intron of delta-Crystallin, and cooperatively they regulate transcription of delta-Crystallin, which is important for lens differentiation (Fig 27-12). These examples show that Sox2 function is dependent upon the presence and activity of binding partners.

Ectopic expression of transcription factors can reprogram differentiated cells to induce "stemness"

The powerful potential of Sox2 to maintain the stem cell potential (totipotentiality) has proven advantageous in

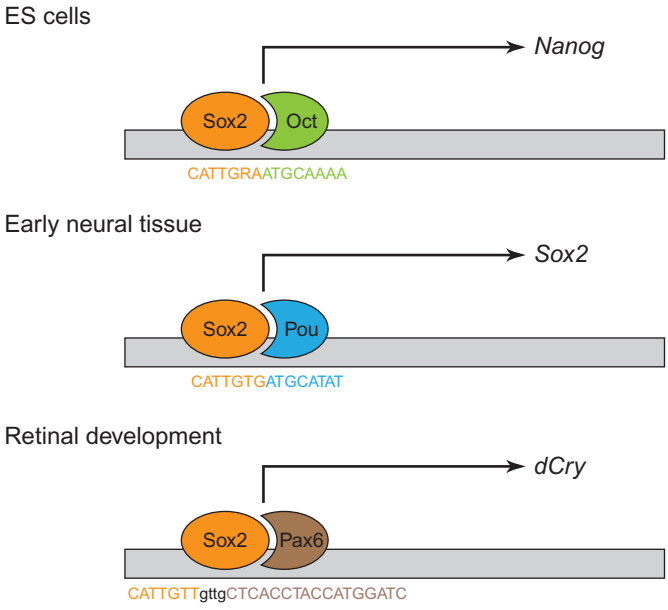

FIGURE 27-12 Sox2 activity is regulated by cell- or tissue-specific binding partners. The binding of cofactors or binding partners significantly augments transcriptional activation by Sox2. The binding partner dimerizes with Sox2 and both of them bind the DNA. Each partner binds a unique DNA sequence, while the sequence specificity for Sox2 remains the same regardless of the binding partner. In this way the specificity and dynamic range of Sox2 activity is increased, such that, in different tissues Sox2 may activate transcription of different genes, as shown in the cartoon. In ES cells, Sox2 may dimerize with Oct to drive expression of *Nanog*. In contrast, in early neural tissue, Sox2 may dimerize with Pou to drive expression of *Sox2* itself, while in retinal tissue Sox2 may dimerize with Pax6 to drive expression of *dCry*.

cellular reprogramming. By overexpressing Sox2 in somatic cells in combination with other transcription factors (for instance, c-Myc, Oct3/4 and Klf4), researchers have been able to artificially reprogram already differentiated cells to obtain cells with characteristics of stem cells or "stemness." These cells, called induced pluripotent stem (iPS) cells, harbor the potential to differentiate into virtually any cell type depending on the specificity of their exposure to environmental cues (see also Ch. 30). The first example of iPS cells was published by Shinya Yamanaka and colleagues (Takahashi et al., 2007; Takahashi & Yamanaka, 2006), who reprogrammed differentiated mouse fibroblasts into pluripotent cells. By transfecting mouse fibroblasts with expression vectors for Sox2, c-Myc, Oct3/4 and Klf4, they showed that these cells could be reprogrammed to express stem cell markers. The gold-standard test for "stemness" is the competence for producing germline chimeras, in which the cells are capable of renewing. Since this first report, several other groups have independently reported successful iPS cell production from both murine and human tissues using different sets of transcription factors.

Normally, pluripotent progenitor or embryonic stem (ES) cells are derived from embryonic tissues, and then differentiated in culture using an array of well-characterized mitogenic factors. The use of ES cells is an important reagent in basic research and drug development and potentially also in cell-replacement therapies such as cell transplantation in spinal cord injuries. Because ES cells can be differentiated into mature cells it is possible to use these for testing drugs on human material prior to clinical trials. Drug responsiveness can also be tested as a function of genetic factors or diseases using ES cells (Nishikawa et al., 2008). However, the use of ES cells presents legal and ethical issues that may be avoided by reprogramming somatic cells using the iPS cell technology. Although the iPS technology is still quite new, it holds an enormous potential as an ES-cell–free alternative to cell-based replacement therapies. Furthermore, the iPS technology is advantageous over ES cell lines, since the donor cell can be obtained from the same patient that the tissue will eventually be transplanted into, thus eliminating potential harmful and lethal immunogenic responses.

THE TRANSCRIPTOME DICTATES CELLULAR PHENOTYPE

The ability to alter cellular phenotype through use of selective transcription factors as illustrated by the iPS data suggests a pleiotropy to cellular phenotype that is manifest at the level of the transcriptome. Indeed another phenotype remodeling approach is based solely upon the transcriptome and is called Transcriptome Induced Phenotype Remodeling (TIPeR) (Kim & Eberwine, 2010). With the TIPeR procedure the transcriptome of one cell type is isolated and transferred into another host cell. When this host cell is a distinct cell type, the host cell phenotype is altered to that of the donor cell type. In this process, it is important to note that the transplanted transcriptome contains RNAs that encode transcription factors, HDAC enzymes, kinases, phosphatases, etc. the composite of which produces a particular cellular phenotype. This transcriptome is not solely the presence or absence of particular RNAs, but also the relative abundances or the expression profile that has been used as an identifier of cellular phenotype and disease state. The expression profile is a reflection of both gene transcription and RNA degradation, with the activity of both producing the steady state levels of gene expression present in a particular cell, which has been shown to be diagnostic of phenotype. That transcriptome transfer can cause long-term phenotypic changes shows that the expression profile is the major indicator of cellular phenotype and as such is a significant factor in eliciting cellular drug responsiveness (Kim & Eberwine, 2010).

The use of TIPeR principals to create cells of predicted phenotype suggests that drug screens may become easier to perform and more reliable. Further important insight into second messenger–system function and other cellular pathways may be dissected through transfer of multiple RNAs with each component RNA species at a specified abundance thereby, enabling a detailed analysis of these components and the cellular pathways upon cellular function.

TRANSCRIPTION AS A TARGET FOR DRUG DEVELOPMENT

It should be clear from this brief discussion of transcription factors that disruption of transcription factor function may have dire consequences for neuronal functioning. Because of their pivotal role in the regulation of multiple genes, they are potential targets for pharmacological intervention in the control of disease processes (Darnell, 2002). Indeed, the multigenic regulatory role of transcription factors suggests one mechanism by which multigenic diseases can exhibit multiple physiological characteristics. The combinatorial nature of transcription factor activity implies that should the activity of a transcription factor be modulated, multiple downstream genes will likewise be regulated. This suggests that the problem with development of drugs targeted to transcription factors is that there are several hundred estimated *trans*-acting factors in the nucleus, many of which can heterodimerize to produce distinct transcriptional responses. The strategies being investigated to modulate transcription factor function focus on antisense knockout of expression and modulators targeted toward post-translational modifiers of transcription factors. Such modifiers include protein kinases and phosphatases.

Often, it is desirable to block expression of particular genes that are activated by transcription factors. The antisense knockout experiments are directed toward this end and require the addition of an antisense oligonucleotide, which will anneal to the *cis*-acting regulatory element for a particular transcription factor in a specific gene. The hope is that a triple helical structure will form around this oligonucleotide-binding site, inhibiting the expression of the downstream gene.

This same strategy can be attempted at the mRNA level to inhibit the translation of individual mRNAs. These experiments are almost never 100% efficient; hence, the problem becomes how much neutralization is necessary to elicit the desired effect. If the antisense oligonucleotide is targeted toward the mRNA for a particular transcription factor or splice form of a transcription factor, then it may be possible to knock down or reduce the expression of that particular protein. The

result of such a knockdown will be a new balance of transcription factor subunits in the cell, likely resulting in alteration of the relative amounts of particular homo- and heterodimers.

Finally, development of drugs targeted toward modification of kinases and phosphatases required for activation or inhibition of particular transcription factors is a promising therapeutic approach. The problem with this paradigm is the specificity of the kinases and phosphatases, since they will often act enzymatically on multiple proteins (Chs. 25 and 26). Furthermore, such drugs often lack specificity and may interact with multiple kinases or phosphatases.

Clearly, transcription is a critical regulatory nexus in neuronal function. More information is being generated about the biology of transcription factors, including how many transcription factor genes exist, which proteins dimerize, identification of the *cis*-acting elements to which they bind and how transcription is modulated by these proteins. From these studies, significant insight into the mechanisms of transcriptional responses to the local environment and to pharmacological agents in the normal and abnormal nervous system will be gained.

References

Arriza, J., Simerly, R., Swanson, L., & Evans, R. (1988). The neuronal mineralocorticoid receptor as mediator of glucocorticoid response. *Neuron*, *1*, 887–896.

Arriza, J. L., Weinberger, C., Cerelli, G., Glaser, T. M., Handelin, B. L., Housman, D. E. et al, (1987). Cloning of the human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. *Science*, *237*, 268–275.

Bannister, A. J., & Kouzarides, T. (1996). The CBP co-activator is a histone acetyltransferase. *Nature*, *384*, 641–643.

Cha-Molstad, H., Keller, D., Yochum, G., Impey, S., & Goodman, R. H. (2004). Cell-type-specific binding of the transcription factor CREB to the cAMO-response element. *Proceedings of the National Academy of Sciences United States of America*, *101*, 13572–13577.

Chao, H., Choo, P., & McEwen, B. (1989). Glucocorticoid and mineralocorticoid receptor mRNA expression in rat brain. *Neuroendocrinology*, *50*, 365–371.

Darnell, J. (2002). Transcription factors as targets for cancer therapy. *Nature Reviews Cancer*, *2*, 740–749.

De Cesare, D., Fimia, G., & Sassone-Corsi, P. (1999). Signaling routes to CREM and CREB: Plasticity in transcriptional activation. *Trends in Biochemical Sciences*, *7*, 281–285.

deGroot, R., & Sassone-Corsi, P. (1993). Hormonal control of gene expression: Multiplicity and versatility of cyclic adenosine 3′,5′-monophosphate-responsive nuclear regulators. *Molecular Endocrinology*, *8*, 145–153.

deKloet, E., Wallach, G., & McEwen, B. (1975). Differences in corticosterone and dexamethasone binding in rat brain and pituitary. *Endocrinology*, *96*, 598–609.

Fass, D., Butler, J., & Goodman, R. (2003). Deacetylase activity is required for camp activation of a subset of CREB target genes. *The Journal of Biological Chemistry*, *278*, 43014–43019.

Foulkes, N., & Sassone-Corsi, P. (1992). More is better: Activators and repressors from the same gene. *Cell*, *68*, 411–414.

Funder, J. (1987). Adrenal steroids: New answers, new questions. *Science*, *237*, 236–237.

Glass, C., & Rosenfeld, M. (2000). The coregulator exchange in transcriptional functions of nuclear receptors. *Genes and Development*, *14*, 121–141.

Graham, V., Khudyakov, J., Ellis, P., & Penvy, L. (2003). SOX2 functions to maintain neural progenitor identity. *Neuron*, *39*, 749–765.

Habener, J. (1990). Cyclic AMP response element binding proteins: A cornucopia of transcription factors. *Molecular Endocrinology*, *4*, 1087–1094.

Hai, T., & Curran, T. (1991). Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. *Proceedings of the National Academy of Sciences United States of America*, *88*, 3720–3724.

Herman, J., Patel, P., Akil, H., & Watson, S. (1989). Localization and regulation of glucocorticoid and mineralocorticoid receptor messenger RNAs in the hippocampal formation of the rat. *Molecular Endocrinology*, *3*, 1886–1894.

Hollenberg, S., Weinberger, C., Ong, E. S., Cerelli, G., Oro, A., Lebo, R. et al., (1985). Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature*, *318*, 635–641.

Hori, R., & Carey, M. (1994). The role of activators in assembly of RNA polymerase II transcription complexes. *Current Opinion in Genetics & Development*, *4*, 236–244.

Johnson, D. S., Mortazavi, A., Myers, R. M., & Wold., B. (2007). Genome-wide mapping of *in vivo*-protein DNA interactions. *Science*, *316*, 1497–1502.

Kim, J., & Eberwine, J. (2010). RNA: state memory and mediator of cellular phenotype. *Trends in Cell Biology*, *20*, 311–318.

Mardis, E. R. (2008). Next-generation DNA sequencing methods. *Annual Review of Human Genetics*, *9*, 387–402.

McKenna, N., & O'Malley, B. (2002). Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell*, *108*, 465–474.

Miller, C. A., Gavin, C. F., White, J. A., Parrish, R. R., Honasoge, A., Yancey, C. R. et al., (2010). Cortical DNA methylation maintains remote memory. *Nature Neuroscience*, *13*, 664–666.

Montminy, M., Gonzalex, G., & Yamamoto, K. (1990). Regulation of cAMP-inducible genes by CREB. *Trends in Neurosciences*, *13*, 184–188.

Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., & Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods*, *5*, 621–628.

Nagaich, D., Walker, R., Wolford, , & Hager, G. (2004). Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodelling. *Molecular Cell*, *14*, 163–174.

Natoli, G. (2004). Little things that count in transcriptional regulation. *Cell*, *118*, 406–408.

Nishikawa, S., Goldstein, R. A., & Nierras, C. R. (2008). The promise of human-induced pluripotent stem cells for research and therapy. *Nature Reviews Molecular Cell Biology*, *9*, 725–729.

Perrazzona, B., Isabel, G., Preat, T., & Davis, R. (2004). The role of cAMP response element–binding protein in *Drosophila* long-term memory. *Journal of Neuroscience*, *24*, 8823–8828.

Remenyi, A., Lins, K., Nissen, L. J., Reinbold, R., Scholer, H. R., & Wilmanns, M. T. (2003). Crystal structure of a POU/HMG/DNA ternary complex suggests differential assembly of Oct4 and Sox2 on two enhancers. *Genes and Development*, *17*, 2048–2059.

Reul, J., & deKloet, E. (1985). Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology*, *117*, 2505–2511.

Schuster, S. C. (2008). Next-generation sequencing transforms today's biology. *Nature Methods*, *5*, 16–18.

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, *126*, 663–676.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, *131*, 861–872.

Tully, T., Preat, T., Boynton, S., & Del Vecchio, M. (1994). Genetic dissection of consolidated memory in *Drosophila*. *Cell*, *79*, 59–67.

Wilson, M., & Koopman, P. (2002). Matching SOX: partner proteins and co-factors of the SOX family of transcriptional regulators. *Current Opinion and Genetics and Development*, *12*(4), 441–446.

Yamamoto, K., Gonzalez, G., Biggs, W., & Montminy., M. (1988). Phosphorylation-induced binding and transcriptional efficacy of nuclear factor CREB. *Nature*, *334*, 494–498.

Yin, J., Del Vecchio, M., Zhou, H., & Tully, T. (1995). CREB as a memory modulator: Induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. *Cell*, *81*, 107–115.

Yin, J., Wallach, J., Del Vecchio, M., Wilder, E. L., Zhou, H., Quinn, W. G. (1994). Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell*, *79*, 49–58.

Zhang, T. Y., Hellstrom, I. C., Bagot, R. C., Wen, X., Dioro, J., & Meaney, M. J. (2010). Maternal care and DNA methylation of a glutamic acid decarboxylase 1 promoter in rat hippocampus. *Journal of Neuroscience*, *30*, 13130–13137.