

Multiple Mechanisms of Transcriptional Repression in Eukaryotes

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Abstract The selective transcription of eukaryotic genes is regulated by both positive and negative inputs from sequence-specific DNA-binding factors. These proteins provide the information essential for correct temporal and spatial control of transcription. The activities of transcriptional repressors have been characterized by a variety of methods, but in many cases the physiological relevance of proposed mechanisms has not been established. This chapter reviews pathways of repression, critically evaluates criteria by which repression mechanisms can be analyzed, and discusses recent progress in identifying the functional relevance of multiple repression activities of transcriptional repressors.

Keywords Transcription · Repression · Corepressor · Chromatin

1

What Is Transcriptional Repression?

A broad view of transcriptional repression includes cellular processes that interfere with the transcription of genes on a global or local level (Fig. 1). Processes that degrade, sequester, covalently modify, or remove from the

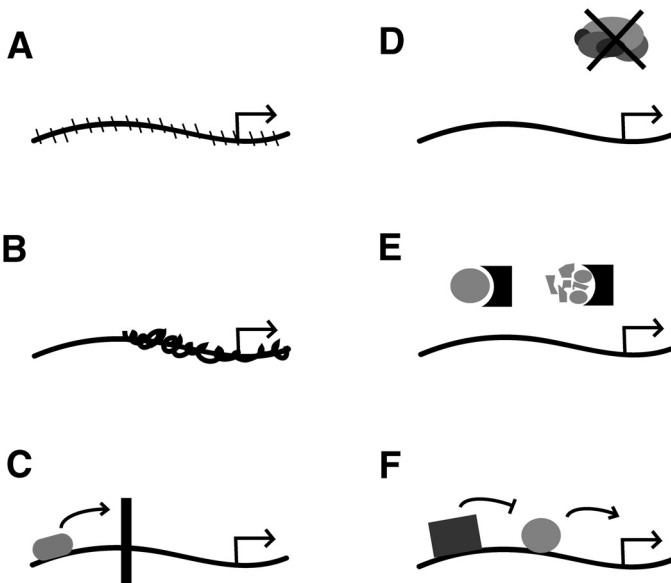


Fig. 1A–F General models of transcriptional repression. **A** Genome- or chromosome-wide effects of chromatin structure (*hatch marks*). **B** Directed assembly of localized chromatin structures under the control of silencers. **C** Local inhibition of transcriptional enhancers (*gray oval*) by boundary elements or insulators (*black bar*). **D** Covalent modifications to components of the general transcriptional machinery. **E** Sequestration, modification, or degradation of positively acting sequence-specific transcriptional activators (*gray circle*) by inhibitory proteins or proteolytic machinery (*black shape*). **F** Gene-specific repression by DNA-binding proteins (*dark gray rectangle*) and their associated corepressors through interactions with activators, basal machinery, and chromatin

nucleus positively acting transcription factors can cause transcriptional repression of genes by eliminating an activating signal. Similarly, global gene regulation can be effected by modification of the general transcriptional machinery, i.e., RNA polymerases and basal factors used at most promoters. In these cases, negatively acting factors need not directly interact with genes to inhibit transcription. Many forms of transcriptional repression, however, involve the activity of DNA-binding proteins that do directly contact genes. The binding of repressors can be relatively nonsequence-specific. For example, large-scale modifications of chromatin influence expression of genes on a chromosomal scale. These effects are seen with specialized chromatin structures present at telomeres and centromeres, in loci controlled by mating type loci in yeast, and with sex-linked dosage compensation systems (Akhtar 2003; Huang 2002; Henikoff 2000). In other cases, genes are negatively regulated by boundary elements, which modulate interactions between regulatory regions and transcriptional start sites (Gerasimova and Corces 2001).

These pathways of repression are of documented importance, but it is clear that many repressors act primarily at a local level, targeting specific genes by means of sequence-specific DNA-binding domains. This chapter is focused on DNA-binding repressors and their cofactors because of their importance in controlling gene switches critical for a variety of biological processes. In addition, many such candidate transcription factors, identified in sequencing projects by virtue of their characteristic DNA-binding motifs, remain to be functionally characterized. This chapter describes approaches currently used to characterize repressors and lays out some of the limitations of these techniques. The discussion centers on repression of protein coding genes, which are transcribed by RNA polymerase II. The regulation of RNA polymerase I and III genes are discussed in recent reviews (Grummt 2003; White 2004).

Consistent with the variety of inhibitory processes involved in repression, the effects of repression are also heterogeneous. A single gene can be transiently repressed by the action of factors binding directly to that gene, or whole classes of genes can be regulated by modification of general transcription machinery or chromatin. Repression can be limited to the time when negative regulatory factors are found at a gene, or repression can leave an 'imprint' so that transcription is blocked for the life of an organism. In general, more is understood mechanistically about how a repressed state is established; less well studied but equally important is how repression is reversed.

2 What Is a Transcriptional Repressor?

2.1 Functional Complexity

Some proteins appear to have dedicated roles as transcriptional repressors or activators; that is, in any context in which they have been studied, they have only one activity. In many other cases, however, regulatory proteins can work in a context-dependent manner, either facilitating or inhibiting transcription depending on the regulatory element, signals from signal transduction cascades, responding basal promoter, or levels of cofactors (Barolo and Posakony 2002; Rogatsky et al. 2002). Such physiological diversity is complicated by the heterogeneous assays used to measure factor activity. In many cases, the description of a particular factor as a repressor comes from limited analysis that does not necessarily correlate with true activities. By testing repressors in dechromatinized systems, overexpressing them at high levels, and tethering them directly to a basal promoter as a fusion protein, potential repressors may be induced to exhibit novel activities. Thus, the panoply of mechanisms for repressors comprehensively reviewed in recent articles (Roberts 2000; Gaston and Jayaraman 2003) must be taken as a picture of possible mechanisms involved in gene regulation, rather than as proof that a particular protein actually uses such mechanisms to effect gene control.

2.2 Use of Phylogeny to Illuminate Function

The human genome project and other genomic efforts have identified thousands of presumptive transcriptional regulatory factors, mainly based on sequence conservation especially in the DNA-binding domain. In many cases, homologous factors are able to mediate similar activities in a heterologous organism, indicating functional conservation (Malicki et al. 1992; Halder et al. 1995). However, sequence conservation alone does not guarantee that functional activity is conserved. In some cases, obvious transcription factor homologs, often identified by means of highly conserved DNA-binding domains, have functionally distinct activities in divergent species. For example, the SMAD pathway inhibitor TGIF plays a negative role in vertebrates, but appears to activate in *Drosophila* (Hyman et al. 2003). Evolutionary studies have revealed in some cases particular changes in proteins that correlate with change in function from a dedicated activator to a facultative repressor (Galant and Carroll 2002; Ronshaugen et al. 2002). The strict conservation of residues critical for recruiting corepressors provides compelling evidence for function, but in most cases the rapid evolution of regions outside the

DNA-binding domains makes it difficult to predict whether homologs retain function as positive or negative acting factors.

3 **Cis-Elements Regulated by Transcriptional Repressors**

3.1 **Initial Studies of Transcriptional Switches and Terminology**

In prokaryotes, work with the paradigmatic Lac repressor led to an initial view that most genes were controlled by repression. Later studies demonstrated the importance of both positive and negative elements of gene switches, including the *lac* operon (Muller-Hill 1996). In eukaryotes, early studies of transcriptional regulation were based on viral gene regulation. A number of well-characterized viral regulatory elements function as dedicated transcriptional activation elements, leading to a focus on transcriptional activation. The term ‘transcriptional enhancer’ was coined to describe a *cis*-regulatory element that functioned to activate transcription in an orientation and distance independent manner (Banerji et al. 1981). Early biochemical studies of eukaryotic regulatory factors also focused on transcriptional activation, because technically it is easier to monitor enhanced, rather than reduced, transcriptional initiation. However, later studies of cellular enhancers determined that these elements are often more complex, mediating repression as well as activation functions (Davidson 2001). In most cases, repressor-binding sites are found within regions that also contain binding sites for activators, producing a *cis*-regulatory element with multiple potential activities. In some cases, dedicated negatively acting elements termed ‘silencers’ have been identified. This term has been applied both to elements that work in a nondirectional manner and to elements that mediate directional gene silencing in yeast (Busturia et al. 1997; Henikoff 2000). In recent work, some authors have chosen to adopt the general term ‘*cis*-regulatory element’ rather than ‘enhancer,’ to more accurately reflect possible positive and negative aspects of control (Carroll et al. 2001). The related term ‘promoter’ is firmly embedded in the literature, however. Although the word connotes a strictly positive effect, it can refer to the entire set of *cis*-regulatory elements that are required for expression of a gene, including positive and negative elements. Alternatively, the term ‘promoter’ may refer to just the minimal region surrounding the initiation site containing sequences necessary for binding of the basal machinery (Arnosti 2003; Smale and Kadonaga 2003).

3.2

Cis-Element Design

Eukaryotic *cis*-regulatory elements rarely consist of single binding sites for a transcription factor. Instead, they generally comprise short segments of DNA to which a number of proteins bind in a sequence-specific manner (Fig. 2). Such elements form nucleoprotein complexes that can have varying degrees of higher order structure (Merika and Thanos 2001; Struhl 2001; Kulkarni and Arnosti 2003). In some cases, the distribution of binding sites for activators and repressors represents a carefully selected design that reflects highly cooperative interactions between the factors, while in others, binding site locations are relatively flexible, permitting binding sites to drift over the course of evolutionary time (Ludwig et al. 2000). Depending on the range of action, the repression can be local, limited to the enhancer in which the repressor binds, or long-range, which results in inhibition of multiple enhancers. Repressor sites may be located close to the transcriptional initiation site, a fact that may be important if repressors compete with components of the basal machinery for binding to DNA. In other cases, repressors

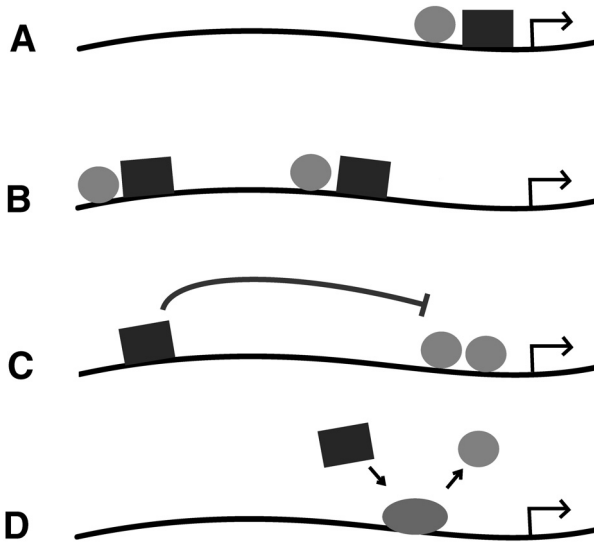


Fig. 2A–D Design of *cis*-regulatory elements. **A** Binding sites for sequence-specific activators and repressors (*circles* and *rectangles*, respectively) may be closely linked to the basal promoter. **B** Alternatively, sites may be distributed at some distance from the basal promoter, 5' or 3' of the start of transcription, in modules termed enhancers. **C** Dedicated negatively acting elements may be located at some distance from the transcriptional start site; such repression may involve chromatin remodeling or insulator function. **D** Some DNA-binding proteins (*gray oval*) can act as either activators or repressors, depending on association with a coactivator (*circle*) or corepressor (*rectangle*)

are located many kilobases from the transcriptional start site, and most likely function by interfering with neighboring activators. A conserved architecture has been identified for some *cis*-regulatory elements responding to a number of signal transduction pathways, such as the Wnt and Hedgehog pathways. *Cis*-elements targeted by these pathways can contain binding sites for bifunctional proteins that act as default repressors, which are converted to activators in response to signaling activity. As these activators are apparently often rather weak, the *cis*-elements contain binding sites for constitutively active activators that boost signal output and provide an additional level of cell type specific control (Barolo and Posakony 2002).

4 Categorizing Transcriptional Repressors

4.1 Repressors and Corepressors

Transcriptional repressors have been categorized by a number of criteria, including the presence or absence of DNA-binding activity, the types of amino acids present in 'repression domains,' and functional aspects of repression. Proteins that are able to recognize DNA in a sequence-specific manner are termed repressors, while those that are recruited to a gene via protein-protein interactions with a repressor are commonly termed corepressors. Confusingly, in some systems, non-DNA-binding factors, such as the yeast Gal80 protein, are also termed repressors. Some authors have termed Gal80 an 'inhibitor' to differentiate its mechanism of action, masking of the Gal4 activation domain, from autonomous repression activity (Ansari et al. 1998). The distinction between repressor and corepressor does underscore the importance in many cases of a particular protein's role in targeting repression to specific loci, but the nomenclature is to some extent arbitrary, because some proteins can interact with genes either by directly contacting the DNA or via protein-protein interactions. For example, nuclear hormone receptors are able to mediate activation or repression via specific DNA-binding sites, but also inhibit gene expression through 'composite' elements that bind to AP-1 and NF- κ B transcriptional activators (Nissen and Yamamoto 2000). The CTIP corepressor facilitates COUP-TF repression as a corepressor, and also binds to separate DNA elements independently to repress (Senawong et al. 2003). As a further indication of the similarity of repressors and corepressors, many corepressors such as C-terminal-binding protein (CtBP), Groucho/TLE and NCoR exhibit repression activity when fused to a heterologous DNA-binding domain (Wen et al. 2000; Flores-Saaib and Courey 2000; Ryu et al. 2001).

4.2

Transcriptional Repression Domains

By deletion analysis, portions of repressor proteins have been identified as critical and sufficient for repression, leading to their identification as ‘repression domains’ (often without regard to the notion of a domain in structural terms as an autonomously folding portion of a protein). Because of their diversity, repression domains have not been successfully classified by the overall amino acid content, although some repressors have been found to contain alanine-rich segments (Hanna-Rose and Hansen 1996). In many cases, repression domains contain structural motifs that interact with distinct classes of corepressors. These motifs can be short and degenerate, but they are often conserved between homologous genes, facilitating their identification. For example, members of the Hairy/E(spl) family, from insects to vertebrates, all contain a WRPW-like motif at or near the C terminus important for contacting Groucho/TLE corepressors, and repressors that interact with the CtBP cofactor contain PXDLS related motifs (Chen and Courey 2000; Davis and Turner 2001; Turner and Crossley 2001).

4.3

Types of Activities

DNA-binding repressors can be functionally categorized as ‘passive’ (direct competition for a factor-binding site) and ‘direct’ or ‘active’ (all other mechanisms) (Jaynes and O’Farrell 1991; Gaston and Jayaraman 2003). An alternate classification distinguishes ‘short-range’ (able to interfere with positively-acting elements within about 100 bp) from ‘long-range’ (active over more than 1 kbp) repressors (Gray and Levine 1996; Courey and Jia 2001). The ability to repress locally or over a larger distance can be exploited to provide unique forms of gene regulation; for example, short-range repression is indispensable for selective repression of an individual module of a complex regulatory locus. These distinctions have some utility in the interpretation of particular repression assays, but repressors can in some cases fall into more than one category, depending on the gene context (Nibu et al. 2001). As molecular mechanisms of repression are better understood, these simple categories will likely be superseded.

5 Methods Used to Characterize Repressors

5.1 Measuring Transcriptional Repression—Biochemical Approaches

Assays used to study transcriptional repression include techniques from biochemistry, genetics, and molecular biology, although in practice these categories overlap to a great extent (Table 1). Biochemical assays for tran-

Table 1 Experimental approaches to characterizing transcriptional regulatory proteins

| Approach | Advantages | Disadvantages |
|---|---|---|
| Biochemical | | |
| In vitro transcription | Obtain mechanistic details of transcription mechanisms Determine effects of molecular modifications to transcription factors | Repression may be artifactual May lack chromatin Difficult to replicate long-range or multi-enhancer regulation |
| Genetic | | |
| Mutational analysis, gene array studies | Endogenous genes studied Can study even large, complex genes Native chromatin environment. | Difficult to differentiate direct from indirect effects (footprinting or chromatin immunoprecipitation used to establish direct effects) |
| Molecular biological | | |
| Transient transfections | Rapid In vivo setting with endogenous cofactors often present | DNA not integrated in chromosome Chromatin structure not well defined Factors often overexpressed at nonphysiological levels |
| Stable transfections | Reporter genes integrated in chromosome Regulated expression of transcriptional effectors with IPTG, Tet, Dex and other small molecule inducers | Establishment of cell lines time-consuming Gene often integrates as multiple, tandem copies Difficult to analyze large regulatory regions |
| Transgenic organisms | Reporter genes integrated in chromosome Present in all cell types, developmental regulation recapitulated By using transgenesis to misexpress repressor, effects on endogenous target genes can be observed | Can be tedious, especially with vertebrate systems Can be difficult to analyze large regulatory regions Gene expression patterns and levels can be influenced by position effects i.e., site of integration on chromosome |

scriptional repression rely on reconstituted *in vitro* systems, where fractionated or highly purified transcription factors are allowed to interact with a genetic regulatory region, usually in the absence of chromatin. Transcription is measured in the presence or absence of a DNA-binding repressor by quantification of RNA produced *in vitro*. This method has the advantage of providing a well-characterized setting for repression, and it has been possible to identify putative biochemical mechanisms of repression, e.g., to measure directly by DNA footprinting the effects of a repressor on the binding of basal machinery (Ross et al. 1999). With *in vitro* assays, it is critical to differentiate nonspecific repressive effects from specific ones, because *in vitro* transcription is readily inhibited by addition of salt, denaturants, or large molar excesses of DNA-binding proteins. In addition, previous *in vitro* studies have suffered from the limitation that they have been generally carried out in the absence of chromatin, and recent work indicates that chromatin is critical for the activity of many repressors (see below). Long-distance repression by elements located far from the transcriptional initiation site has also been difficult to simulate *in vitro*. Thus, the results from *in vitro* assays must be tested using a complementary approach that more closely approximates an *in vivo* situation.

5.2

Genetic Approaches

Genetic studies have focused on gene expression in mutants with defects in genes for repressors or corepressors. Genetic approaches have been extensively utilized in model organisms such as yeast or *Drosophila* to characterize the activity of the transcriptional apparatus, including repressors. In many cases, the physiological roles of transcriptional repressors and their placement in genetic networks were established before details of their molecular modes of action were elaborated. In vertebrates, by contrast, many proteins have been identified first as putative transcription factors through biochemical or molecular biological approaches, and in many cases their activities in a physiological setting are still poorly understood.

Genetic screens allow for an unbiased search for putative repressor targets or cofactors. In addition, genetic characterization of repression has the advantage that the genes regulated by repressors are in their natural chromatinized state. In all cases, however, a challenge faced in genetic characterizations is interpreting and differentiating direct from indirect effects. In one case, a genetic screen for cofactors of the Knirps repressor instead identified cell cycle regulatory genes; mutations in these genes slow the cell cycle sufficiently to permit expression of a related gene, *knirps-related*, which rescued the original phenotype (Ruden and Jackle 1995). Even in more directed experiments, where responses of target genes are measured to the presence or absence of a putative repressor, similar questions arise. For example, in

gene array experiments that measure the effects of a repressor, cellular steady state levels of mRNAs are measured in the presence or absence of the repressor (DeRisi et al. 1997; Holstege et al. 1998). mRNA species whose levels increase in the absence of a transcriptional regulator may be true, direct transcriptional targets of a repressor, but the regulation may also be indirect, effected by means of an activator that is itself controlled by the repressor. In conditional knockout experiments, indirect effects can be minimized by measuring gene response within a short period of time after inactivation of the repressor. For a temperature-sensitive allele, the temperature is shifted up or down and gene responses are measured within a short period of time, minimizing the possibility of additional layers of regulation (Moqtaderi et al. 1996). The use of temperature-sensitive alleles is largely restricted to microbial systems, unless a temperature-sensitive allele is fortuitously available. In some cases, direct and indirect effects can be distinguished by carrying out the experiment in the presence of cycloheximide to inhibit translation, but blocking translation can itself introduce a variety of nonspecific effects. Direct effects can also be established if the repressor in question can be shown to bind to the target gene *in vivo*. One widely used method involves chromatin immunoprecipitation (ChIP) assays (Kuo and Allis 1999). Cells are treated with formaldehyde to crosslink proteins to a gene, then chromatin is prepared from the cells, after which antibodies to the protein being investigated are used to precipitate the gene fragments in question. The fragments are characterized by PCR or by hybridization to a gene array. These assays can establish the presence of a protein somewhere within approximately 300 bp of a given regulatory sequence. More precise binding information can come from *in vitro* DNase I footprinting or *in vivo* footprinting (Dai et al. 2000).

5.3

Molecular Biological Approaches

Molecular biological characterization of repressors includes cell-based assays, where target genes are transfected into cells in the presence or absence of a repressor, and whole organism assays, where the target gene and/or the repressor in question is reintroduced into the organism by transgenesis, either in the intact state or modified state. Cell culture studies allow rapid characterization of particular repressors and target genes, but suffer from a number of drawbacks. In the case of transiently transfected reporter genes, the chromatin state of the reporter gene may differ from that found for chromosomally located genes, which may influence transcriptional regulation. Transient expression of repressors usually involves overexpression at non-physiological levels, which might also introduce artifacts, just as with *in vitro* assays. These limitations can be overcome by analyzing repression of endogenous genes in cell culture, or by stably integrating reporter genes. Ex-

pression of repressors can be titrated in regulated systems, using for example the Tet system (see the chapter by Bohl and Heard, this volume).

Many putative transcriptional repressors have been characterized solely when overexpressed in transfections of heterologous cells, in the context of multimerized binding sites for the factor introduced adjacent to a basal promoter element. Unless proper controls are carried out to verify that the repressive effects are gene specific, i.e., dependent on the binding sites, and also are not simply effects of steric hindrance of a bulky protein blocking access to the promoter, the labeling of factors as repressors based solely on such assays must be viewed with caution. In some cases, the activity of repressors has been analyzed in parallel in cell culture and in whole organisms; here, the similarities of the activities suggests that at least some of the repressor function can be recapitulated on transiently introduced target genes (Ryu and Arnosti 2003). In other cases, activities of repressor proteins or domains have been identified in transfection assays that have subsequently not been confirmed from *in vivo* analysis (Tolkunova et al. 1998).

To capture some of the features of repression that transient assays lack, more labor-intensive approaches have been used, involving stable transformation of intact organisms to introduce reporter genes or modified versions of repressors. Such analysis is readily done in yeast, as well as model organisms such as *Drosophila* or *Arabidopsis*. Chromosomal integration of reporter genes is usually preferable, to avoid artifacts arising from high copy number plasmids. Reporter gene expression is also affected by the site of chromosomal integration, an effect that can be reduced in some cases by surrounding the reporter gene with boundary elements. Such analyses are less common in vertebrate systems, reflecting the greater difficulty and expense of working with transgenic mice and fish. However, only through such approaches are the contributions of transcriptional repression to developmental timing and patterning apparent, as well as more subtle responses of regulatory elements to repressors.

5.4

Criteria for Identifying the Physiological Activity of a Repressor

In general, transfection assays have been most successful in identifying *potential* activities of factors, such as their ability to repress, possible cofactors, and characterization of structural features of the protein, whereas more physiological assays are required to determine how the repressor functions on endogenous regulatory elements. The best understood function of transcriptional repressors on true target genes comes from studies in which three criteria have been satisfied: (1) the repressor has been shown genetically to affect expression of the target gene; (2) the repressor has been shown to interact directly with the gene *in vitro* by footprinting or gel-shift

assays, or in vivo by ChIP; and (3) the specific mutation of the repressor binding sites disrupts repression.

6 Mechanisms of Transcriptional Repression

6.1 Covalent Modification of Transcriptional Activators

As discussed above, modification, degradation or sequestration of transcriptional activators can lead to repression of genes. Examples of such modifications are indicated in Table 2. In many cases, the modification can occur when the factor is not binding to a gene and/or is not located within the nucleus, or alternatively, modification can also occur to DNA-bound transcriptional activators. The proteins that carry out such modifications generally

Table 2 Modifications of transcriptional regulators that contribute to transcriptional repression

| Modification | Effect | Example |
|---|---|---|
| Deacetylation | Reduction in DNA-binding affinity | E2F1 deacetylation mediated by Rb associated HDACs Martinez-Balbas et al. 2000 |
| Glycosylation | Blocking coactivator contact | Sp1 glycosylation by O-GlcNAc transferase Yang et al. 2001 |
| Ubiquitylation | Loss of nuclear localization or protein degradation | p53 nuclear export and turnover Brooks and Gu 2003 |
| Sumoylation | Unknown | Sumoylation of glucocorticoid nuclear hormone receptor inhibits activation Verger et al. 2003 |
| Arginine methylation | Prevention of activator binding by coactivator | CBP coactivator methylation by CARM-1 prevents binding to CREB transcription factor Xu et al. 2001 |
| Heterodimerization with non-DNA-binding partner | Inhibition of DNA binding by basic helix loop helix factors | Id interaction with E2A family members E12/E47 Ruzinova and Benezra 2003 |
| Binding of cytoplasmic inhibitor | Prevention of nuclear import | I- κ B binding to NF- κ B (rel family) proteins Karin and Ben-Neriah 2000 |
| Phosphorylation | Inhibition of cofactor binding | Interaction with E2F transcription factors inhibited by Rb corepressor phosphorylation Taya 1997 |
| Allosteric inhibition of DNA-binding domain | Inhibition of DNA binding | Ets-1 autoinhibitory domain Pufall and Graves 2002 |

are not DNA-binding proteins themselves, but are targeted to activators via protein–protein interactions. Constitutive pathways that remove covalent modifications can provide a level of negative regulation. The SMAD proteins, downstream effectors of transforming growth factor (TGF)- β pathway signaling, are phosphorylated in response to pathway activation, and dephosphorylation returns them to an inactive state (Inman et al. 2002).

Major pathways for DNA-bound repressors and corepressors are: (1) direct competition between a repressor and an activator for the same or overlapping binding sites; (2) interactions with the basal machinery, including RNA polymerase II and associated factors, to inhibit initiation or elongation; (3) direct interaction between (co)-repressors and activators, resulting in inhibition of activator function, sometimes termed ‘masking’; and (4) chromatin alterations, involving the change of DNA/nucleosomal structure, or covalent modification of chromatin or DNA (Fig. 3). Additional, less understood mechanisms have also been suggested, including intranuclear targeting of repressed genes to heterochromatic sites, ‘repulsion’ of coactivators, or possible direct proteolysis by transcriptional repressors (He et al. 1995; Brown et al. 1997; Senger et al. 2000).

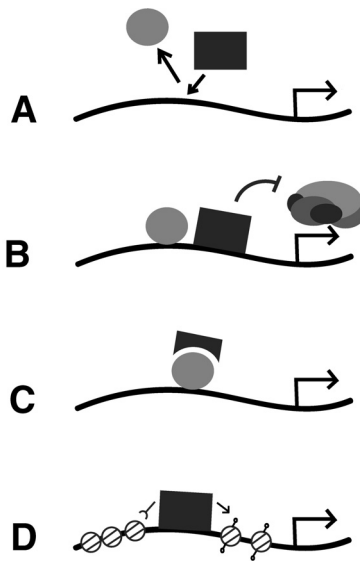


Fig. 3A–D Modes of repression by DNA-associated transcriptional repressors and corepressors. **A** Direct displacement of an activator (*circle*) by the competitive binding of a DNA-binding repressor (*rectangle*). **B** Direct interactions between the transcriptional repressor and the basal transcriptional machinery. **C** ‘Masking’ of a transcriptional activation domain by a corepressor. **D** Chromatin remodeling, either by nucleosome repositioning or covalent modification of histones. Additional levels of regulation not shown include controlled proteolysis of transcriptional activators, subnuclear targeting, and covalent modifications of activators (see Table 2)

6.2

Direct Competition

Some transcriptional repressors have been documented to inhibit gene expression by displacing activators from identical or overlapping sites. In most instances this activity has been noted under conditions when the repressor has been overexpressed, usually in cell culture assays, or in vitro, where arbitrarily large amounts of repressor can be added. For instance, the chicken ovalbumin upstream promoter transcription factors (COUP-TFI and II, also known as Ear 2 and 3) are widely conserved orphan nuclear hormone receptors that have binding specificity similar to that of RAR, VDR, and SF-1 receptors (Pereira et al. 2000). Support for a physiological role as transcriptional repressors comes from the complementary expression patterns between COUP-TF factors and putative target genes (Shibata et al. 2003a). When COUP TF factors are overexpressed in transfection assays, activation by SF-1 of the vertebrate *CYP17* promoter is inhibited (Shibata et al. 2003b). In vitro, COUP-TFII can bind to and displace SF-1 from the DNA (Bakke and Lund 1995). Similar effects are noted with overexpression experiments involving other nuclear hormone receptors, such as RXR, RAR, HNF-4, and the *Drosophila* EcR (Zelhof et al. 1995). However, from these experiments, it has not been possible to establish whether competition at the level of DNA binding is a significant factor under physiological conditions. Other work suggests that COUP TF factors operate via noncompetitive modes. For example in repressing the *MHC I* promoter, COUP TFII antagonizes the activity of NF- κ B from nonoverlapping sites, suggesting a mode of repression that is independent of competition (Zhao et al. 2003). Further substantiating the notion of active repression, portions of COUP TFII can mediate repression when bound to the heterologous Gal4 DNA-binding domain, apparently by recruiting the histone deacetylases (HDACs) or the N-CoR corepressor (Smirnov et al. 2000).

A second example of competition comes from studies of the *Drosophila* Knirps transcriptional repressor. This protein binds to regulatory elements of the *Krüppel* and *even-skipped* (*eve*) genes, countering the activity of the Bicoid activator (Small et al. 1991). In cell culture studies, overexpression of Knirps inhibited activation by Bicoid from small element derived from the *Krüppel* promoter, and Knirps blocked Bicoid from binding to a 16-bp target sequence derived from this element in vitro, suggesting a repression mechanism that involved competition (Hoch et al. 1992). However, later work showed that Knirps contains active repression domains that interact with the CtBP corepressor and other factors, enabling it to act from nonoverlapping sequences (Arnosti et al. 1996b; Nibu et al. 1998; Keller et al. 2000). In the embryo, the Knirps DNA-binding domain alone is unable to mediate repression of *eve*, indicating that competition does not play a significant role (Struffi et al. 2004). Finally, a recent study of the *Krüppel* repressor indicated

that competition can be observed in vivo if the binding sites are directly overlapping Dorsal and Twist activator sites, but bioinformatics analysis of native regulatory elements indicates that Kruppel tends to be located ~15 bp from Bicoid activator sites, suggesting that competition is not a preferred mode of regulation (Nibu et al. 2003; Makeev et al. 2003).

6.3

Direct Interaction with Basal Machinery

Transcriptional repressors have also been suggested to act by direct interactions with the basal transcriptional machinery. Targets within the basal machinery that have been identified in functional studies include TFIIA, TFIIB, TFIID, RNA polymerase, and holoenzyme subunits Srb10/11. Comprehensive summaries of potential targets are reviewed elsewhere (Gaston and Jayaraman 2003), and are addressed here to present the limits of our knowledge. In vertebrates, the transcriptional effector of the conserved Notch signaling pathway is the DNA-binding CBF-1 protein (also known as RBP-J κ , CSL, and LMP-2), with which the intracellular portion of Notch interacts in the nucleus to activate target genes. In the absence of Notch signaling, CBF-1 acts as a repressor. To identify the mechanism of repression, the adenovirus pI promoter was studied; this simple promoter contains a CBF-1 site positioned between a single Sp1 activator site and the TATA box (Olave et al. 1998). One strength of this study is that a direct comparison was made between the activity of CBF in vivo (by transfection of reporter genes) and in vitro on a variety of reporter genes. CBF-1 repressed only when positioned between the Sp1 site and the TATA box, and in a highly purified transcription system, repression activity correlated with CBF-1 contacts on TFIIA and TFIID (specifically the TAFII110 subunit). Preassembling the activator complex, or moving the repressor site 5' of the activator, prevented repression. This specific promoter appears to take advantage of direct protein-protein interactions between the repressor and basal machinery, but in general it appears that CBF-1 proteins use other mechanisms to repress cellular promoters. The *Drosophila* CBF-1 homolog, Su(H), represses in the absence of Notch signaling, and genetic and biochemical studies clearly indicate that repression is dependent on the activity of the CtBP and Groucho cofactors that interact with HDAC. Vertebrate CBF-1 has also been found to interact with HDAC-recruiting corepressors, namely SMRT and NCoR (Lai 2002). Furthermore, the binding sites of Su(H) are effective at mediating repression from a variety of locations, not just between an activator and the TATA box, suggesting that the very stringent positioning seen with the pI promoter is an exception (Furriols and Bray 2001). Whether CBF-1 interactions with basal machinery plays a role on other promoters is unclear, but from the extreme level of context dependence identified previously, it is unlikely that the same protein-protein interactions are generally utilized.

Cell cycle and cancer studies have focused on the retinoblastoma (Rb) tumor suppressor and proteins functioning in this pathway, which are thought to play key roles in cell cycle regulation (see the chapter by Hauck and von Harsdorf, this volume). A number of cell cycle regulated genes are controlled by the E2F family of DNA-binding transcriptional activators and repressed by Rb family corepressors. Biochemical studies with purified transcriptional factors suggest that repression of E2F regulated promoters by the Rb may in some cases involve the prevention or displacement of contacts between the E2F activator and the basal transcriptional factors TBP and TFIID (Pearson and Greenblatt 1997). Notably, however, E2F cannot activate *in vitro* in the absence of TBP associated factors (TAFs). Further *in vitro* studies indicated that Rb may also prevent the recruitment of TFIID, perhaps by direct interactions with TAF1 (also known as TFIID250) (Ross et al. 1999). In the nonchromatinized *in vitro* E2F-activated system, Rb was suggested to act at the level of preventing the assembly of the preinitiation complex, but in a chromatinized *in vitro* system, in which Rb repressed not E2F but Sp1, repression was found to function at a step following recruitment of the preinitiation complex, apparently independently of HDAC activity (Ross et al. 2001). As discussed below, chromatin modifications have been linked in a variety of ways to Rb-mediated repression. It remains unknown how much, if at all, direct interactions with the basal machinery contribute to overall repression by Rb.

In the cases mentioned above, detailed biochemical information has been produced about direct interactions with the basal transcriptional machinery, but the *in vivo* relevance of such contacts still remains unresolved. In the case of the Tup1 corepressor in yeast, strong evidence links repression to activity of the basal machinery, but there is no agreement whether this is a direct effect. The Tup1 corepressor is recruited to a variety of promoters by contacting different classes of DNA-binding proteins (Smith and Johnson 2000). Genetic suppression screens linked the activity of the Tup1 corepressor to the activity of the Srb10/11 cyclin/cyclin dependent kinase subunits of the RNA Polymerase II holoenzyme, suggesting that this repressor might function by affecting the phosphorylation of the C terminus of RNA polymerase. One line of inquiry suggests that the Tup1 protein directly contacts the Srb10/11 proteins; alternatively, Tup1 has also been suggested to mediate nucleosome repositioning on affected genes (Li and Reese 2001; Schuller and Lehming 2003). In this latter view, the loss of Srb 10/11 function might reduce polymerase sensitivity to the changes in chromatin, providing an indirect route for suppression of Tup1 activity. Tup1 also appears to work by recruiting HDACs (Deckert and Struhl 2001; Davie et al. 2003). Thus, despite the excellent genetic and molecular biological tools available in yeast, there is still considerable uncertainty about how Tup1 acts at different promoters.

Chromatin immunoprecipitation assays were used to investigate the activity of glucocorticoid receptor (GR) in repressing genes activated by

NF- κ B proteins. In the case of the interleukin-8 and intracellular adhesion molecule type 1 genes, neither activator binding nor polymerase loading was prevented on promoters repressed by GR. Instead, repression is associated with failure of RNA polymerase II to become phosphorylated in the serine 2 position of the C-terminal domain (CTD) tail, a modification that is normally associated with promoter clearance (Nissen and Yamamoto 2000). In this case, it was not determined whether GR directly interacts with a CTD kinase protein to block its activity, but it was possible to rule out activator exclusion or prevention of preinitiation complex assembly as possible mechanisms.

6.4

Masking of Activation Domains

As opposed to displacing activators from the DNA, some inhibitory proteins bind to and block an activation domain. As discussed above, Rb binds to E2F proteins, thereby interfering with their interactions with the basal transcriptional machinery, although this is clearly only one facet of Rb activity. In yeast, repression of the *GAL1* promoter is effected in part by the Gal80 protein binding to and obstructing the activation domain of the Gal4 activator protein, preventing the recruitment of HAT complexes (Carrozza et al. 2002). Interestingly, Gal80 is not entirely displaced from the Gal4 activator when the *GAL1* promoter is activated, but rather is bound in an alternative conformation (Leuther and Johnston 1992; Sil et al. 1999). A similar 'masking' role has been ascribed to the Mdm2 repressor in inhibition of the p53 tumor suppressor protein, although the binding of this protein also leads to destabilization of the activator (reviewed in the chapter by Asker et al., this volume). In contrast to the examples of these corepressors, there are few documented cases where DNA bound transcriptional repressors directly contact the activation domain of an adjacent activator to block its action. Many repressors have been found to inhibit a wide variety of transcriptional activators in a nonselective manner, suggesting that they do so without having to make specific activator-repressor contacts. Such promiscuity has been demonstrated for a number of repressors, such as short-range repressors in *Drosophila* (Arnosti et al. 1996a).

6.5

Interaction with Chromatin-Modifying Factors

Earlier studies had established firm correlations between certain DNA and chromatin modifications and transcriptional activity of genes. Repressed genes were generally found to exhibit lower levels of histone acetylation and increased DNA methylation (generally 5-methyl C), and subsequent studies have gone on in many cases to show that a wide variety of DNA-binding

transcriptional repressors and corepressors interact with DNA and chromatin modifying activities. The best understood activities include: (1) chromatin remodeling complexes; (2) HDACs; (3) histone methyl transferases; and (4) DNA methyl transferases.

6.5.1

Chromatin Remodeling and Repression

As discussed in the chapter by Herrera et al. in this volume, chromatin remodeling complexes are ATP-driven multiprotein machines that can change the physical interaction between DNA and core nucleosomes (Peterson 2002; Narlikar et al. 2002). Genomic analyses have revealed that in most cases, these proteins are involved in gene activation, but a subset of cellular target genes in yeast are upregulated when *snf/swi* or *chd1* activity is removed from the cell (Tran et al. 2000; Sudarsanam et al. 2000). In animal cells, the activity of the Brg1 and Brm chromatin remodeling complexes have been linked to the Rb corepressor protein (Zhang and Dean 2001). Genetic evidence from *Drosophila* places Brm components in the Rb pathway, and Rb-mediated cell cycle arrest depends on the presence of these complexes, but it is not clear whether this involved a direct interaction of the complexes with Rb (Strobeck et al. 2000; Brumby et al. 2002). Recent reports have suggested that Brg/Brm dependence of Rb activity might be indirect, mediated through activity of the p21 promoter, thus influencing the phosphorylation status of Rb (Kang et al. 2004). Other studies have found Brg/Brm complexes bound directly to Rb regulated promoters (Strobeck et al. 2000; Wang et al. 2002).

Evidence for a direct role for Swi/Snf proteins in repression comes from chromatin immunoprecipitation studies that demonstrate that these proteins are present at the repressed *ser3* promoter in yeast, and that a physically distinct chromatin structure was present (reviewed in Martens and Winston 2003). It is not clear at this point how remodeling actually induces loss of gene expression. One idea suggests that remodeling of chromatin structure might permit assembly of a stable repressor complex that would involve the activity of other proteins, such as HDACs. An additional clue is provided by the phenotype of the *ddm1* mutant of *Arabidopsis*; this mutant was originally identified by a loss of DNA methylation, but turned out to encode a DNA remodeling enzyme (Bourc'his and Bestor 2002). Similar findings have subsequently been made in the mouse, suggesting that one general pathway for chromatin remodeling-mediated repression is by induction of a structure that provides access for DNA methylases, discussed below (Bourc'his and Bestor 2002).

6.5.2 HDACs

As discussed in the chapter by Herrera et al. (this volume), acetylation of lysine residues of the core histone proteins can facilitate transcription of genes, possibly by reducing the affinity of histone proteins for DNA or by allowing the association of activating proteins via acetyl-lysine binding bromodomains. Removal of these acetyl groups by HDACs therefore has an inhibitory effect on gene expression. There are three classes of HDAC, all of which have been linked to transcriptional repression: class I enzymes, homologous to the yeast Rpd3 protein, are found in the nucleus; class II enzymes, homologous to yeast Hda1 protein, are found both in cytoplasm and nucleus; the structurally distinct class III enzymes are homologous to yeast Sir2, an NAD-dependent deacetylase that functions in maintenance of stable repressed chromatin structure at telomeres and silent mating type loci (Thiagalingam et al. 2003). In a few cases, DNA-binding transcriptional repressors have been found to contact these enzymes directly, while in a number of other examples, these enzymes are recruited by corepressors, including CtBP, N-CoR, SMRT, Sin3, Rb, Groucho/TLE, CoREST, and others (Chen and Courey 2000; Ahlinger 2000; Jepsen and Rosenfeld 2002; Chinnadurai 2002; Lunyak et al. 2002). HDACs have been found generally in large protein complexes, including the Sin3 complex, and in the NuRD complex, which includes chromatin remodeling proteins. Because class I and II enzymes are sensitive to the inhibitor trichostatin A (TSA), many studies have sought to identify a potential role for HDACs in repression by comparing transcriptional signals with and without TSA. Care must be taken when interpreting results of such experiments, however, because this drug can cause nonspecific activation of gene expression. Therefore, it is important to verify that increases in reporter gene activity after treatment with TSA are specific to a reporter that is bound by the repressor.

Important targets of HDACs are presumably histone tail residues targeted by histone acetyltransferases, including histone H3 lysines 9 and 14, and H4 lysine 5 (discussed in the chapter by Herrera et al. in this volume). Although numerous repressors appear to recruit histone deacetylases, we have an incomplete understanding of the mechanisms by which HDACs interfere with transcription. Loss of histone acetylation has been proposed to interfere with transcriptional activation by several means, including the induction of a more compact chromatin structure due to restoration of DNA contacts with positively charged lysine tails, which would disfavor binding by activators or general transcription factors. This simple view is contradicted by findings that chromatin that has been deacetylated and is in an inactive conformation can still be accessed by components of the general transcription machinery, suggesting that deacetylation might have more subtle effects (Sekinger and Gross 2001; Saurin et al. 2001). An alternative consideration is

that acetyl-lysine residues provide recognition sites for bromodomain containing transcription factors, and binding of specific factors might be destabilized by deacetylation. Finally, although HDACs have demonstrated activity on histones, it is possible that important targets may include other proteins whose functional properties are modified by acetylation, such as the transcriptional activators E2F and p53 (Martinez-Balbas et al. 2000; Brooks and Gu 2003).

The extent of histone deacetylation at repressed genes has been mapped by ChIP assays. In some cases, deacetylation is restricted to a single nucleosome, as in the case of the Rb-repressed *cyclinE* gene and the Rpd3-repressed genes in yeast (within the limits of measurement for this technique) (Morrison et al. 2002; Deckert and Struhl 2002). In other cases, silenced regions affected by the Sir2 deacetylase in the *Saccharomyces cerevisiae* mating-type locus were found to be deacetylated over a range of kilobases (Braunstein et al. 1993, 1996). The limit of deacetylation is not necessarily a function of the deacetylase itself, as Rpd3 is associated with local deacetylation as well as long-range repression, but may rather reflect the assembly of repressor proteins that recruit this deacetylase.

6.5.3

Histone Methyltransferases

Lysine residues of histone proteins H3 (K4, 9, 27, 36, 79) and H4 (K20) have been identified as targets of cellular methylases. Arginine residues of histone tails are also methylated, but this modification appears to be associated exclusively with gene activation (Zhang and Reinberg 2001). As with histone acetylation, histone methylation patterns are specific for widespread areas, reflecting chromatin accessibility, and are also modified at the level of individual genes, influencing their individual expression. At a bulk level, methylation patterns can predict overall gene activity; for example, genomic surveys of the region around the silenced mating type locus in the yeast *Schizosaccharomyces pombe* revealed a strong correlation of H3 K4 methylation in active regions, and H3 K9 within inactive regions (Noma et al. 2001). The methylation associated with inactive genes is carried out by a specific class of methyltransferase first identified through genetic studies of heterochromatin function in *Drosophila*. The *Su(var)3-9* gene and its vertebrate homologues (SUV39H1 and 2) encode proteins containing a conserved SET domain with histone methyltransferase activity (reviewed in Kouzarides 2002; Sims et al. 2003). SUV39 methyltransferases are recruited by corepressors such as KAP1, CtBP, and Rb. Some 73 putative genes in the human genome encode SET domains, only a few of which have been characterized. SUV39 proteins are specific for histone H3 lysine 9, providing a specific binding motif recognized by the so-called chromodomain of the HP-1 protein. HP-1 is found to be enriched on heterochromatin, and also on specific repressed

genes located in euchromatic regions (Eissenberg and Elgin 2000). HP-1 is thought to contribute directly to repression by recruiting HDACs. The SET1 family protein EZH has been implicated in methylation of histone H3 lysine 27, a modification involved in binding by the Polycomb protein (discussed below).

Unlike histone acetylation, histone methylation does not appear to be readily reversible. No verified histone demethylases are known to function in transcription, but it has been proposed that histone methylation is reversed by unknown oxidative activities, by proteolysis of methylated tails, or by turnover of methylated histone subunits. Consistent with the relative stability of methylation, previous models suggested that HP-1 binding established a condensed, inactive locus. However, recent work demonstrates that this protein is not tightly bound, but is rather in dynamic equilibrium with associated loci, and that HP-1 at repressed E2F regulated promoters, for instance, is readily dissociated (Cheutin et al. 2003; Young and Longmore 2004).

6.5.4 DNA Methylation

In many, but not all, multicellular organisms, DNA methylation plays an important role in repression of gene expression (Attwood et al. 2002; Robertson 2002). The 5-methyl cytosine modification at CpG dinucleotides has been associated not with the rapid, reversible repression found at some promoters, but rather with stable repression. Thus, DNA methylation has been implicated in the repression of transposable elements and establishment of long-term transcriptionally repressed states, although recent research indicates that it may be important for more flexible regulation as well (Klose and Bird 2003). DNA methylation is catalyzed by DNA methyltransferases that transfer an activated methyl group from S-adenosyl methionine, either in *de novo* reactions (establishment of a new site of methylation) or in maintenance methylase reactions (for hemimethylated sites produced after DNA replication). As with histone modifications, a correlation with gene inactivity and DNA methylation was established long before the mechanism of action was known. The treatment of cells with 5-azacytidine, a nonmethylatable substrate, was sufficient to reactivate genes that had been silenced, suggesting that this modification played a causative role, but it was the identification of methyl-C-binding proteins that provided a link to repression pathways (Ballestar and Wolffe 2001; Jaenisch and Bird 2003). Methyl-C-binding proteins have been found to recruit HDAC, which may propagate the repressed state through chromatin effects. In addition to this influence of DNA methylation on chromatin structure, there are also clear indications that chromatin structure regulates DNA methylation. As indicated above, chromatin remodeling complexes have been found to regulate DNA methyl-

ation in plants, and the dim-5 histone methyltransferase has been shown to be important for DNA methylation in *Neurospora* (Tamaru and Selker 2001). Methyl-C-binding protein 2 has also been found to interact with histone methyltransferases, providing a cooperative link between these two forms of methylation (Fuks et al. 2003).

6.6 Polycomb Proteins and Epigenetic States— Combinations of Repression Mechanisms

The differentiation of cell types during development often involves the stable repression of genes. Such repression can involve epigenetic mechanisms, that is, stably inherited modifications to a gene that affects its expression but does not affect its DNA sequence (Jaenisch and Bird 2003). Most recent evidence suggests that such epigenetic marks include covalent modification of DNA and chromatin by methylases and deacetylases. The role of Polycomb group (PcG) proteins has been extensively analyzed in this regard. PcG genes were originally identified in screens of *Drosophila* for genes that affect the developmental fate of the adult structures (homeotic transformations), and vertebrate homologs have been found to be involved in various forms of cancer (reviewed in Simon and Tamkun 2002; Orlando 2003). In PcG mutants in *Drosophila*, initial repression of homeotic genes is observed, but later in development these genes are ectopically expressed. In *Drosophila*, PcG proteins have been found to function in complexes that can be localized to ~100 sites on polytene chromosomes of the salivary gland. In vertebrates and the fly, at least two distinct complexes containing PcG proteins have been identified. E(z) and Esc proteins (or their vertebrate homologs) are included in related, developmentally dynamic complexes of 600 kDa and greater that also possess HDAC and histone methyl transferase activities (Furuyama et al. 2003). A distinct 2-MDa PRC-1 complex includes the Polycomb protein itself (Shao et al. 1999). The PRC-1 complex has been demonstrated to antagonize chromatin remodeling activity by SWI/SNF complexes in vitro, although it is not known if a similar activity is used in vivo (King et al. 2002).

The Polycomb protein itself possesses a chromodomain, a motif also found in HP1. Whereas HP1 binds preferentially to methylated H3 lysine 9, Polycomb specificity is directed toward trimethylated histone H3 lysine 27 (Min et al. 2003). These observations have led to a model for Polycomb-mediated repression. Transient repression of target genes early in embryogenesis by means of repressors such as Krüppel and Hunchback leads to the recruitment of the E(z) complex proteins, which deacetylate the local region and add stable histone methylation signatures. Methylation of histone H3 lysine 27 provides a histone code that leads to association of the PRC-1 complex proteins, thereby blocking the activity of positively acting histone re-

modeling complexes. In addition, Polycomb proteins can associate with elements of the basal machinery, suggesting an additional level of targeting the general transcriptional machinery (Saurin et al. 2001). Polycomb proteins dissociate and reassociate with target genes during mitosis, and can be removed from repressed genes for brief periods of time, suggesting that a chromatin 'mark' provides the stable memory for continuous association of polycomb gene products (Beuchle et al. 2001). Additional specificity comes from interactions of PcG proteins with sequence specific-binding proteins such as Pleiohomeotic, Zeste and Trithoraxlike (GAGA factor). The importance of some of the activities associated with PcG proteins is not clear, but genetic studies suggest that chromatin modifying activities are necessary for complete and stable repression of target genes. In some cases, subsets of the PcG proteins, rather than one of these subcomplexes, may be utilized to effect transient repression at promoters. Rb has been found to interact with the vertebrate Polycomb homolog PC2, and the PcG protein Osa is found to work through SWI/SNF remodeling complexes to effect repression in *Drosophila* (Collins and Treisman 2000; Dahiya et al. 2001).

6.7

Context Influences the Activity of Putative Transcriptional Repressors

Relevant to the study of novel transcription factors for putative repression activity, it is worthwhile to examine factors that influence repressor activity, for in the absence of the proper context, a factor may not exhibit its physiological activity. Some of the factors known to affect the function of a repressor include: (1) the nature of the binding site that the protein contacts; (2) the nature of flanking binding sites that permit different factors to bind adjacent to the repressor; (3) the physiological state of the cell with respect to hormonal signaling, signal transduction pathways, and cofactor expression.

Relatively modest differences in DNA-binding sites can induce alternative corepressor complexes to form. For instance, the two DNA-binding moieties of the POU family Pit-1 factor can bind differently to cognate sites so that in one configuration it acts as a constitutive activator, while in the other as a repressor or activator (Scully et al. 2000). Pit-1 activates the growth hormone gene in rat pituitary somatotrope cells and the prolactin gene in lactotrope cells. The growth hormone promoter is not active in lactotrophs, despite the presence of the Pit-1 at the promoter, because the conformation adopted by Pit-1 at the binding site in this promoter allows Pit-1 to recruit the NCoR corepressor, in cooperation with additional factors. Substitution of the Pit-1 binding site with an alternative Pit-1 binding sequence allowed ectopic expression of the gene in lactotrophs.

Flanking DNA-binding sites can also be critical for repressor activity. The rel-family Dorsal protein activates a number of genes in the *Drosophila* embryo in cooperation with Twist transcriptional activators (Szymanski and

Levine 1995). On regulatory elements of the *zen* and *dpp* genes, however, Dorsal functions in a negative mode by recruiting the Groucho corepressor. This Dorsal repressor function is dependent on the binding of Cut and Dri proteins to sites immediately flanking Dorsal (Valentine et al. 1998). The positioning of Cut and Dri binding sites relative to Dorsal is intolerant of any changes, unlike the flexibility seen with disposition of Dorsal and Twist sites for activation (Cai et al. 1996). Flanking binding sites are also important for selection of particular E2F family members that function primarily as repressors or activators (Giangrande et al. 2003).

Modification of cellular factors through hormonal or signal transduction cascades are also critical for determining the potential activities of transcriptional repressors. Nuclear hormone receptors exchange corepressor complexes for coactivator complexes upon binding of ligand. For example, unliganded thyroid hormone receptor or retinoic acid receptors interact with the NCoR/SMRT corepressors to mediate active repression. Upon binding of ligand, these cofactors are replaced by coactivators (reviewed in Aranda and Pascual 2001; Jones and Shi 2003). In addition to the modulation of repressor activity by hormone binding, different availability of transcriptional cofactors can also modify activity. Tamoxifen and related drugs that bind to the estrogen receptor demonstrate tissue-specific effects; depending on the types of ER cofactors present, the drug acts as an antagonist in some tissues and as an agonist in others (Smith et al. 1997).

Signal transduction pathways also converge on numerous DNA-binding factors that act facultatively as repressors. For example, the Notch pathway effector Su(H)/CBF-1 protein complexes with HDAC complexes, as described above, in the absence of Notch signaling, or with a portion of Notch itself as coactivator when the pathway is activated. In this instance, as with the Wnt, TGF- β , and Hedgehog pathways, default repression activities have been suggested to provide especially tight gene regulation to amplify the differences between active and inactive states (Barolo and Posakony 2002). These sorts of contextual variables indicate that characterization of a novel protein as a repressor based only on its ability to depress gene expression from a simplified reporter gene in transfection assays is inadequate. Nonetheless, many fruitful studies of transcriptional repressors have initiated with such modest beginnings.

7

Multiple Activities of Transcriptional Repressors

As suggested by the discussion of the Polycomb protein complexes, transcriptional repression can involve multiple enzymatic activities to provide multiple levels of genetic programming, including establishment and maintenance of repression. Even at the level of a single transcriptional repressor

or corepressor with temporally limited effects, multiple repression activities are commonly identified. The *Drosophila* Knirps repressor has two repression activities, one dependent on the CtBP corepressor and the other CtBP-independent (Keller et al. 2000). The vertebrate nuclear hormone RIP140 co-repressor was recently reported to possess no fewer than four autonomous repression domains as defined in cotransfection assays (Christian et al. 2004). Corepressors such as NCoR and CtBP have each been found to associate with multiple activities. Three nonexclusive explanations for the utilization of multiple repression pathways and cofactors by individual repressors are: (1) qualitatively distinct repression—different repression activities are deployed to interfere with different activators or basal promoters; (2) quantitative effects—multiple activities are simultaneously deployed to provide sufficient inhibitory activity; (3) artifactual repression—some of the multiple activities ascribed to repressors do not play a meaningful role in physiological contexts.

7.1

Qualitative Effects

Activator or promoter specificity has been documented for individual activities of a number of metazoan repressors. Rb family corepressors repress early S phase promoters such as *cyclin E* via HDAC-dependent pathways, while promoters repressed into G₂ phase are repressed by other mechanisms, including the activity of histone methyltransferases, SWI/SNF remodeling complexes, and Polycomb protein (Strobeck et al. 2000; Dahiya et al. 2001; Nielsen et al. 2001). Similarly, the vertebrate REST/NRSF protein represses genes in non-neuronal tissues using HDAC specific mechanisms and other mechanisms, in a promoter specific manner (Lunyak et al. 2002). The *Drosophila* Brinker transcriptional repressor is an effector of the TGF- β pathway, and represses a variety of genes during development (Affolter et al. 2001). Brinker binds directly to the Groucho and CtBP corepressors, both of which are known to recruit HDAC to effect long- and short-range repression, respectively. A genetic assay of the cofactor requirement for Brinker activity on endogenous genes revealed that this protein requires either CtBP or Groucho for repression of genes at different points in development, and for some genes in the blastoderm embryo, neither cofactor (Hasson et al. 2001).

In all three of these cases, it is not known which particular attributes of the target genes contribute to the context-specific requirement for individual corepressors. In at least some cases, however, multiple repression activities are suggested to be directed at distinct activators. The zinc finger/homeodomain Zeb transcriptional repressor regulates lymphocyte and muscle differentiation. This protein was found to repress the MEF2C activator in a

CtBP-independent manner, and the Ets and Myb activators in a CtBP-dependent manner (Postigo and Dean 1999).

7.2

Quantitative Effects

As noted above, different repressor activities have been linked to distinct genes, but the molecular features of these target genes that dictate such specificity is largely unknown. In addition to possible activator specificity, repressors might use multiple repressor activities to achieve quantitatively adequate levels of activity. Knirps repression of distinct enhancers directing expression of the *even-skipped* gene in *Drosophila* was used as a test case for comparison of CtBP corepressor-dependent and -independent activities. *eve* is expressed in the early embryo in a series of seven transverse stripes under the control of stripe enhancers located 5' and 3' of the coding sequence. In the absence of CtBP, Knirps is unable to repress the *eve* stripe 4/6 enhancer, but can still repress the *eve* 3/7 enhancer by means of its CtBP-independent activity (Struffi et al. 2004). The structure of the stripe 3/7 enhancer suggests that it is more sensitive to Knirps due to the larger number and higher affinity of the binding sites (Clyde et al. 2003). The lower sensitivity *eve* stripe 4/6 enhancer can respond to the CtBP-independent activity of Knirps if levels of this protein are elevated. This result suggests that the activators driving the *eve* stripe 4/6 enhancer are not intrinsically resistant to the CtBP-independent activity, and that the requirement for CtBP-dependent activity is a quantitative effect. Consistent with this quantitative hypothesis, when tested independently, the CtBP-dependent and -independent domains of Knirps are sufficient to mediate repression of a sensitive reporter gene, but only when combined together are they able to mediate repression of a more robust reporter (Sutrias-Grau and Arnosti 2004).

7.3

Artifactual Repression?

Given the heterogeneity of assays used to measure repression, it is likely that some of the observed repression activities do not represent those used in physiological settings. Caution should be exercised when interpreting results of repression assays. The identification of closely overlapping repressor and activator binding sites within the *eve* stripe enhancers led to the suggestion that direct competition might be the basis of repression (Stanojevic et al. 1991). Indeed, evidence from transfection assays suggested that Knirps could compete with the Bicoid activator at directly overlapping binding sites in DNA (Hoch et al. 1992). However, subsequent work indicated that repressor sites could be moved some distance from activators without ablating repression, and overexpression of the Knirps DNA-binding domain alone did

not have a repressive effect (Arnosti et al. 1996b; Struffi et al. 2004). Thus, in this particular case, the purported competition for DNA binding is probably not physiologically relevant.

As discussed above, *in vitro* experiments can be susceptible to artifacts caused by addition of large amounts of DNA-binding proteins, which might compete for binding to the basal promoter element. Even in cases where specific contacts have been mapped between repressors and components of the basal transcriptional machinery, it is necessary to determine whether mutations that disrupt the interaction surfaces will also disrupt repression. Pharmacological treatments, such as the use of the HDAC inhibitor TSA have been used extensively in transfection assays to determine whether a particular repressor utilizes HDAC activity. This drug can nonspecifically stimulate a variety of promoters, and so suitable controls (i.e., testing for TSA responses in the absence of the repressor) are required.

In many cases, putative corepressors have been identified by yeast two-hybrid screening or by co-immunoprecipitation assays. In the latter case, when two proteins are demonstrated to co-immunoprecipitate after having been coexpressed in cell culture, it is important to establish whether the co-immunoprecipitation reflects direct protein contacts. In some cases, it has not been possible to distinguish whether protein association is merely mediated by fragments of DNA. Strong support for initially characterized repressor–corepressor interactions comes from the mapping of contact surfaces in the factors, which when mutated, lead to loss of repression. In other cases, subsequent genetic analysis has strongly supported these findings, in that mutation of the putative cofactor in question impaired the repression activity of the partner.

In summary, modern genetic and genomic approaches have greatly expanded our abilities to identify and characterize transcriptional repressors. The complexity of these regulatory proteins, and the contextual factors that affect their activities, demand that the investigator use well planned and complementary approaches to fully understand their biological functions.

References

- Affolter M, Marty T, Vigano MA, Jazwinska A (2001) Nuclear interpretation of Dpp signaling in *Drosophila*. *EMBO J* 13:3298–3305
- Ahringer J (2000) NuRD and SIN3 histone deacetylase complexes in development. *Trends Genet* 16:351–356
- Akhtar A (2003) Dosage compensation: an intertwined world of RNA and chromatin remodelling. *Curr Opin Genet Dev* 13:161–169
- Ansari AZ, Reece RJ, Ptashne M (1998) A transcriptional activating region with two contrasting modes of protein interaction. *Proc Natl Acad Sci USA* 95:13543–13548
- Aranda A, Pascual A (2001) Nuclear hormone receptors and gene expression. *Physiol Rev* 81:1269–1304

- Arnosti DN (2003) Analysis and function of transcriptional regulatory elements: insights from *Drosophila*. *Annu Rev Entomol* 48: 579–602
- Arnosti DN, Barolo S, Levine M, Small S (1996a) The eve stripe 2 enhancer employs multiple modes of transcriptional synergy. *Development* 1:205–214
- Arnosti DN, Gray S, Barolo S, Zhou J, Levine M (1996b) The gap protein knirps mediates both quenching and direct repression in the *Drosophila* embryo. *EMBO J* 14:3659–3666
- Attwood JT, Yung RL, Richardson BC (2002) DNA methylation and the regulation of gene transcription. *Cell Mol Life Sci* 59:241–257
- Bakke M, Lund J (1995) Mutually exclusive interactions of two nuclear orphan receptors determine activity of a cyclic adenosine 3',5'-monophosphate-responsive sequence in the bovine CYP17 gene. *Mol Endocrinol* 9:327–339
- Ballestar E, Wolffe AP (2001) Methyl-CpG-binding proteins. Targeting specific gene repression. *Eur J Biochem* 268:1–6
- Banerji J, Rusconi S, Schaffner W (1981) Expression of a beta-globin gene is enhanced by remote SV40 DNA sequences. *Cell* 2:299–308
- Barolo S, Posakony JW (2002) Three habits of highly effective signaling pathways: principles of transcriptional control by developmental cell signaling. *Genes Dev* 16:1167–1181
- Beuchle D, Struhl G, Muller J (2001) Polycomb group proteins and heritable silencing of *Drosophila* Hox genes. *Development* 6:993–1004
- Bourc'his D, Bestor TH (2002) Helicase homologues maintain cytosine methylation in plants and mammals. *BioEssays* 24:297–299
- Braunstein M, Rose AB, Holmes SG, Allis CD, Broach JR (1993) Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev* 7:592–604
- Braunstein M, Sobel RE, Allis CD, Turner BM, Broach JR (1996) Efficient transcriptional silencing in *Saccharomyces cerevisiae* requires a heterochromatin histone acetylation pattern. *Mol Cell Biol* 16:4349–4356
- Brooks CL, Gu W (2003) Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr Opin Cell Biol* 15:164–171
- Brown KE, Guest SS, Smale ST, Hahm K, Merckenschlager M, Fisher AG (1997) Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. *Cell* 91:845–854
- Brumby AM, Zrally CB, Horsfield JA, Secombe J, Saint R, Dingwall AK, Richardson H (2002) *Drosophila* cyclin E interacts with components of the Brahma complex. *EMBO J* 21:3377–3389
- Busturia A, Wightman CD, Sakonju S (1997) A silencer is required for maintenance of transcriptional repression throughout *Drosophila* development. *Development* 21:4343–4350
- Cai HN, Arnosti DN, Levine M (1996) Long-range repression in the *Drosophila* embryo. *Proc Natl Acad Sci USA* 18:9309–9314
- Carroll SB, Grenier JK, Weatherbee SD, Grenier J, Wetherbee S (2001) From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design. Blackwell Scientific, Malden
- Carrozza MJ, John S, Sil AK, Hopper JE, Workman JL (2002) Gal80 confers specificity on HAT complex interactions with activators. *J Biol Chem* 277:24648–24652
- Chen G, Courey AJ (2000) Groucho/TLE family proteins and transcriptional repression. *Gene* 1/2:1–16
- Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, Misteli T (2003) Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* 299:721–725

- Chinnadurai G (2002) CtBP, an unconventional transcriptional corepressor in development and oncogenesis. *Mol Cell* 2:213–224
- Christian M, Tullet JM, Parker MG (2004) Characterisation of four autonomous repression domains in the corepressor RIP140. *J Biol Chem* (in press)
- Clyde DE, Corado MS, Wu X, Pare A, Papatsenko D, Small S (2003) A self-organizing system of repressor gradients establishes segmental complexity in *Drosophila*. *Nature* 426:849–853
- Collins RT, Treisman JE (2000) Osa-containing Brahma chromatin remodeling complexes are required for the repression of wingless target genes. *Genes Dev* 24:3140–3152
- Courey AJ, Jia S (2001) Transcriptional repression: the long and the short of it. *Genes Dev* 15:2786–2796
- Dahiya A, Wong S, Gonzalo S, Gavin M, Dean DC (2001) Linking the Rb and polycomb pathways. *Mol Cell* 8:557–569
- Dai SM, Chen HH, Chang C, Riggs AD, Flanagan SD (2000) Ligation-mediated PCR for quantitative in vivo footprinting. *Nat Biotechnol* 18:1108–1111
- Davidson EH (2001) *Genomic Regulatory Systems: Development and Evolution*
- Davie JK, Edmondson DG, Coco CB, Dent SY (2003) Tup1-Ssn6 interacts with multiple class I histone deacetylases in vivo. *J Biol Chem* 278:50158–50162
- Davis RL, Turner DL (2001) Vertebrate hairy and Enhancer of split related proteins: transcriptional repressors regulating cellular differentiation and embryonic patterning. *Oncogene* 58:8342–8357
- Deckert J, Struhl K (2001) Histone acetylation at promoters is differentially affected by specific activators and repressors. *Mol Cell Biol* 8:2726–2735
- Deckert J, Struhl K (2002) Targeted recruitment of Rpd3 histone deacetylase represses transcription by inhibiting recruitment of Swi/Snf, SAGA, and TATA binding protein. *Mol Cell Biol* 18:6458–6470
- DeRisi JL, Iyer VR, Brown PO (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–686
- Eissenberg JC, Elgin SC (2000) The HP1 protein family: getting a grip on chromatin. *Curr Opin Genet Dev* 10:204–210
- Flores-Saaib RD, Courey AJ (2000) Analysis of Groucho-histone interactions suggests mechanistic similarities between Groucho- and Tup1-mediated repression. *Nucl Acids Res* 21:4189–4196
- Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T (2003) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J Biol Chem* 278:4035–4040
- Furriols M, Bray S (2001) A model Notch response element detects Suppressor of Hairless-dependent molecular switch. *Curr Biol* 11:60–64
- Furuyama T, Tie F, Harte PJ (2003) Polycomb group proteins ESC and E(Z) are present in multiple distinct complexes that undergo dynamic changes during development. *Genesis* 35:114–124
- Galant R, Carroll SB (2002) Evolution of a transcriptional repression domain in an insect Hox protein. *Nature* 6874:910–913
- Gaston K, Jayaraman PS (2003) Transcriptional repression in eukaryotes: repressors and repression mechanisms. *Cell Mol Life Sci* 60:721–741
- Gerasimova TI, Corces VG (2001) Chromatin insulators and boundaries: effects on transcription and nuclear organization. *Annu Rev Genet* 35:193–208
- Giangrande PH, Hallstrom TC, Tunyaplin C, Calame K, Nevins JR (2003) Identification of E-box factor TFE3 as a functional partner for the E2F3 transcription factor. *Mol Cell Biol* 23:3707–3720

- Gray S, Levine M (1996) Transcriptional repression in development. *Curr Opin Cell Biol* 3:358–364
- Grumt I (2003) Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus. *Genes Dev* 17:1691–1702
- Halder G, Callaerts P, Gehring WJ (1995) Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. *Science* 268:1788–1792
- Hanna-Rose W, Hansen U (1996) Active repression mechanisms of eukaryotic transcription repressors. *Trends Genet* 12:229–234
- Hasson P, Muller B, Basler K, Paroush Z (2001) Brinker requires two corepressors for maximal and versatile repression in Dpp signalling. *EMBO J* 20:5725–5736
- He GP, Muise A, Li AW, Ro HS (1995) A eukaryotic transcriptional repressor with carboxypeptidase activity. *Nature* 378:92–96
- Henikoff S (2000) Heterochromatin function in complex genomes. *Biochim Biophys Acta* 1470:O1–O8
- Hoch M, Gerwin N, Taubert H, Jackle H (1992) Competition for overlapping sites in the regulatory region of the *Drosophila* gene *Kruppel*. *Science* 255:94–97
- Holstege FC, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, Young RA (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* 94:717–728
- Huang Y (2002) Transcriptional silencing in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Nucl Acids Res* 30:1465–1482
- Hyman CA, Bartholin L, Newfeld SJ, Wotton D (2003) *Drosophila* TGIF proteins are transcriptional activators. *Mol Cell Biol* 23:9262–9274
- Inman GJ, Nicolas FJ, Hill CS (2002) Nucleocytoplasmic shuttling of Smads 2, 3, and 4 permits sensing of TGF- β receptor activity. *Mol Cell* 10:283–294
- Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33 Suppl:245–254
- Jaynes JB, O'Farrell PH (1991) Active repression of transcription by the engrailed homeodomain protein. *EMBO J* 6:1427–1433
- Jepsen K, Rosenfeld MG (2002) Biological roles and mechanistic actions of co-repressor complexes. *J Cell Sci* 115:689–698
- Jones PL, Shi YB (2003) N-CoR-HDAC corepressor complexes: roles in transcriptional regulation by nuclear hormone receptors. *Curr Top Microbiol Immunol* 274:237–268
- Kang H, Cui K, Zhao K (2004) BRG1 controls the activity of the retinoblastoma protein via regulation of p21CIP1/WAF1/SDI. *Mol Cell Biol* 24:1188–1199
- Karin M, Ben-Neriah Y (2000) Phosphorylation meets ubiquitination: the control of NF- κ B activity. *Annu Rev Immunol* 18:621–663
- Keller SA, Mao Y, Struffi P, Margulies C, Yurk CE, Anderson AR, Amey RL, Moore S, Ebels JM, Foley K, Corado M, Arnosti DN (2000) dCtBP-dependent and -independent repression activities of the *Drosophila* Knirps protein. *Mol Cell Biol* 19:7247–7258
- King IE, Francis NJ, Kingston RE (2002) Native and recombinant polycomb group complexes establish a selective block to template accessibility to repress transcription in vitro. *Mol Cell Biol* 22:7919–7928
- Klose R, Bird A (2003) Molecular biology. MeCP2 repression goes nonglobal. *Science* 302:793–795
- Kouzarides T (2002) Histone methylation in transcriptional control. *Curr Opin Genet Dev* 12:198–209
- Kulkarni MM, Arnosti DN (2003) Information display by transcriptional enhancers. *Development* 130:6569–6575

- Kuo MH, Allis CD (1999) In vivo cross-linking and immunoprecipitation for studying dynamic Protein:DNA associations in a chromatin environment. *Methods* 19:425–433
- Lai EC (2002) Keeping a good pathway down: transcriptional repression of Notch pathway target genes by CSL proteins. *EMBO Rep* 9:840–845
- Leuther KK, Johnston SA (1992) Nondissociation of GAL4 and GAL80 in vivo after galactose induction. *Science* 256:1333–1335
- Li B, Reese JC (2001) Ssn6-Tup1 regulates RNR3 by positioning nucleosomes and affecting the chromatin structure at the upstream repression sequence. *J Biol Chem* 276:33788–33797
- Ludwig MZ, Bergman C, Patel NH, Kreitman M (2000) Evidence for stabilizing selection in a eukaryotic enhancer element. *Nature* 679:564–567
- Lunyak VV, Burgess R, Prefontaine GG, Nelson C, Sze SH, Chenoweth J, Schwartz P, Pevzner PA, Glass C, Mandel G, Rosenfeld MG (2002) Corepressor-dependent silencing of chromosomal regions encoding neuronal genes. *Science* 559:1747–1752
- Makeev VJ, Lifanov AP, Nazina AG, Papatsenko DA (2003) Distance preferences in the arrangement of binding motifs and hierarchical levels in organization of transcription regulatory information. *Nucl Acids Res* 31: 6016–6026
- Malicki J, Cianetti LC, Peschle C, McGinnis W (1992) A human HOX4B regulatory element provides head-specific expression in *Drosophila* embryos. *Nature* 358:345–347
- Martens JA, Winston F (2003) Recent advances in understanding chromatin remodeling by Swi/Snf complexes. *Curr Opin Genet Dev* 13:136–142
- Martinez-Balbas MA, Bauer UM, Nielsen SJ, Brehm A, Kouzarides T (2000) Regulation of E2F1 activity by acetylation. *EMBO J* 19:662–671
- Merika M, Thanos D (2001) Enhanceosomes. *Curr Opin Genet Dev* 11:205–208
- Min J, Zhang Y, Xu RM (2003) Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. *Genes Dev* 17:1823–1828
- Moqtaderi Z, Bai Y, Poon D, Weil PA, Struhl K (1996) TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature* 659:188–191
- Morrison AJ, Sardet C, Herrera RE (2002) Retinoblastoma protein transcriptional repression through histone deacetylation of a single nucleosome. *Mol Cell Biol* 22:856–865
- Muller-Hill B (1996) The Lac Operon: A Short History of a Genetic Paradigm.
- Narlikar GJ, Fan HY, Kingston RE (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell* 4:475–487
- Nibu Y, Senger K, Levine M (2003) CtBP-independent repression in the *Drosophila* embryo. *Mol Cell Biol* 23:3990–3999
- Nibu Y, Zhang H, Bajor E, Barolo S, Small S, Levine M (1998) dCtBP mediates transcriptional repression by Knirps, Kruppel and Snail in the *Drosophila* embryo. *EMBO J* 23:7009–7020
- Nibu Y, Zhang H, Levine M (2001) Local action of long-range repressors in the *Drosophila* embryo. *EMBO J* 9:2246–2253
- Nielsen SJ, Schneider R, Bauer UM, Bannister AJ, Morrison A, O'Carroll D, Firestein R, Cleary M, Jenuwein T, Herrera RE, Kouzarides T (2001) Rb targets histone H3 methylation and HP1 to promoters. *Nature* 412:561–565
- Nissen RM, Yamamoto KR (2000) The glucocorticoid receptor inhibits NFkappaB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev* 18:2314–2329
- Noma K, Allis CD, Grewal SI (2001) Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. *Science* 293:1150–1155

- Olave I, Reinberg D, Vales LD (1998) The mammalian transcriptional repressor RBP (CBF1) targets TFIID and TFIIA to prevent activated transcription. *Genes Dev* 11:1621–1637
- Orlando V (2003) Polycomb, epigenomes, and control of cell identity. *Cell* 112:599–606
- Pearson A, Greenblatt J (1997) Modular organization of the E2F1 activation domain and its interaction with general transcription factors TBP and TFIIF. *Oncogene* 15:2643–2658
- Pereira FA, Tsai MJ, Tsai SY (2000) COUP-TF orphan nuclear receptors in development and differentiation. *Cell Mol Life Sci* 57:1388–1398
- Peterson CL (2002) Chromatin remodeling: nucleosomes bulging at the seams. *Curr Biol* 12:R245–R247
- Postigo AA, Dean DC (1999) Independent repressor domains in ZEB regulate muscle and T-cell differentiation. *Mol Cell Biol* 12:7961–7971
- Pufall MA, Graves BJ (2002) Autoinhibitory domains: modular effectors of cellular regulation. *Annu Rev Cell Dev Biol* 18:421–462
- Roberts SG (2000) Mechanisms of action of transcription activation and repression domains. *Cell Mol Life Sci* 57:1149–1160
- Robertson KD (2002) DNA methylation and chromatin—unraveling the tangled web. *Oncogene* 21:5361–5379
- Rogatsky I, Luecke HF, Leitman DC, Yamamoto KR (2002) Alternate surfaces of transcriptional coregulator GRIP1 function in different glucocorticoid receptor activation and repression contexts. *Proc Natl Acad Sci USA* 26:16701–16706
- Ronshaugen M, McGinnis N, McGinnis W (2002) Hox protein mutation and macroevolution of the insect body plan. *Nature* 6874:914–917
- Ross JF, Liu X, Dynlacht BD (1999) Mechanism of transcriptional repression of E2F by the retinoblastoma tumor suppressor protein. *Mol Cell* 3:195–205
- Ross JF, Naar A, Cam H, Gregory R, Dynlacht BD (2001) Active repression and E2F inhibition by pRB are biochemically distinguishable. *Genes Dev* 15:392–397
- Ruden DM, Jackle H (1995) Mitotic delay dependent survival identifies components of cell cycle control in the *Drosophila* blastoderm. *Development* 1:63–73
- Ruzinova MB, Benezra R (2003) Id proteins in development, cell cycle and cancer. *Trends Cell Biol* 13:410–418
- Ryu JR, Arnosti DN (2003) Functional similarity of Knirps CtBP-dependent and CtBP-independent transcriptional repressor activities. *Nucl Acids Res* 31:4654–4662
- Ryu JR, Olson LK, Arnosti DN (2001) Cell-type specificity of short-range transcriptional repressors. *Proc Natl Acad Sci USA* 23:12960–12965
- Saurin AJ, Shao Z, Erdjument-Bromage H, Tempst P, Kingston RE (2001) A *Drosophila* Polycomb group complex includes Zeste and dTAFII proteins. *Nature* 6847:655–660
- Schuller J, Lehming N (2003) The cyclin in the RNA polymerase holoenzyme is a target for the transcriptional repressor Tup1p in *Saccharomyces cerevisiae*. *J Mol Microbiol Biotechnol* 5:199–205
- Scully KM, Jacobson EM, Jepsen K, Lunyak V, Viadiu H, Carriere C, Rose DW, Hooshmand F, Aggarwal AK, Rosenfeld MG (2000) Allosteric effects of Pit-1 DNA sites on long-term repression in cell type specification. *Science* 5494:1127–1131
- Sekinger EA, Gross DS (2001) Silenced chromatin is permissive to activator binding and PIC recruitment. *Cell* 105:403–414
- Senawong T, Peterson VJ, Avram D, Shepherd DM, Frye RA, Minucci S, Leid M (2003) Involvement of the histone deacetylase SIRT1 in chicken ovalbumin upstream promoter transcription factor (COUP-TF)-interacting protein 2-mediated transcriptional repression. *J Biol Chem* 278:43041–43050

- Senger K, Merika M, Agalioti T, Yie J, Escalante CR, Chen G, Aggarwal AK, Thanos D (2000) Gene repression by coactivator repulsion. *Mol Cell* 6:931–937
- Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, Kingston RE (1999) Stabilization of chromatin structure by PRC1, a Polycomb complex. *Cell* 1: 37–46
- Shibata H, Kobayashi S, Kurihara I, Saito I, Saruta T (2003a) Nuclear receptors and co-regulators in adrenal tumors. *Horm Res* 59 Suppl 1:85–93
- Shibata H, Kurihara I, Kobayashi S, Yokota K, Suda N, Saito I, Saruta T (2003b) Regulation of differential COUP-TF-coregulator interactions in adrenal cortical steroidogenesis. *J. Steroid Biochem. Mol Biol* 85:449–456
- Sil AK, Alam S, Xin P, Ma L, Morgan M, Lebo CM, Woods MP, Hopper JE (1999) The Gal3p-Gal80p-Gal4p transcription switch of yeast: Gal3p destabilizes the Gal80p-Gal4p complex in response to galactose and ATP. *Mol Cell Biol* 19:7828–7840
- Simon JA, Tamkun JW (2002) Programming off and on states in chromatin: mechanisms of Polycomb and trithorax group complexes. *Curr Opin Genet Dev* 12:210–218
- Sims RJr, Nishioka K, Reinberg D (2003) Histone lysine methylation: a signature for chromatin function. *Trends Genet* 19:629–639
- Smale ST, Kadonaga JT (2003) The RNA polymerase II core promoter. *Annu Rev Biochem* 72:449–479
- Small S, Kraut R, Hoey T, Warrior R, Levine M (1991) Transcriptional regulation of a pair-rule stripe in *Drosophila*. *Genes Dev* 5:827–839
- Smirnov DA, Hou S, Ricciardi RP (2000) Association of histone deacetylase with COUP-TF in tumorigenic Ad12-transformed cells and its potential role in shut-off of MHC class I transcription. *Virology* 268:319–328
- Smith CL, Nawaz Z, O'Malley BW (1997) Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. *Mol Endocrinol* 11:657–666
- Smith RL, Johnson AD (2000) Turning genes off by Ssn6-Tup1: a conserved system of transcriptional repression in eukaryotes. *Trends Biochem Sci* 25:325–330
- Stanojevic D, Small S, Levine M (1991) Regulation of a segmentation stripe by overlapping activators and repressors in the *Drosophila* embryo. *Science* 253:1385–1387
- Strobeck MW, Knudsen KE, Fribourg AF, DeCristofaro MF, Weissman BE, Imbalzano AN, Knudsen ES (2000) BRG-1 is required for RB-mediated cell cycle arrest. *Proc Natl Acad Sci USA* 97:7748–7753
- Struhl K (2001) Gene regulation. A paradigm for precision. *Science* 292:1054–1055
- Struffi P, Corado M, Kulkarni M, Arnosti DN (2004) Quantitative contributions of CtBP-dependent and -independent repression activities of Knirps. *Development* 131:2419–2429
- Sudarsanam P, Iyer VR, Brown PO, Winston F (2000) Whole-genome expression analysis of *snf/swi* mutants of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 97:3364–3369
- Sutrias-Grau M, Arnosti DN (2004) CtBP contributes quantitatively to Knirps repression activity in an NAD binding dependent manner. *Mol Cell Biol* (in press)
- Szymanski P, Levine M (1995) Multiple modes of dorsal-bHLH transcriptional synergy in the *Drosophila* embryo. *EMBO J* 10:2229–2238
- Tamaru H, Selker EU (2001) A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* 414:277–283
- Taya Y (1997) RB kinases and RB-binding proteins: new points of view. *Trends Biochem Sci* 22:14–17

- Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF (2003) Histone deacetylases: unique players in shaping the epigenetic histone code. *Ann NY Acad Sci* 983:84–100
- Tolkunova EN, Fujioka M, Kobayashi M, Dekka D, Jaynes JB (1998) Two distinct types of repression domain in engrailed: one interacts with the groucho corepressor and is preferentially active on integrated target genes. *Mol Cell Biol* 5:2804–2814
- Tran HG, Steger DJ, Iyer VR, Johnson AD (2000) The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. *EMBO J* 19:2323–2331
- Turner J, Crossley M (2001) The CtBP family: enigmatic and enzymatic transcriptional co-repressors. *BioEssays* 8:683–690
- Valentine SA, Chen G, Shandala T, Fernandez J, Mische S, Saint R, Courey AJ (1998) Dorsal-mediated repression requires the formation of a multiprotein repression complex at the ventral silencer. *Mol Cell Biol* 11:6584–6594
- Wang S, Zhang B, Faller DV (2002) Prohibitin requires Brg-1 and Brm for the repression of E2F and cell growth. *EMBO J* 21:3019–3028
- Wen YD, Perissi V, Staszewski LM, Yang WM, Kronen A, Glass CK, Rosenfeld MG, Seto E (2000) The histone deacetylase-3 complex contains nuclear receptor corepressors. *Proc Natl Acad Sci USA* 13:7202–7207
- White RJ (2004) RNA polymerase III transcription—a battleground for tumour suppressors and oncogenes. *Eur J Cancer* 40:21–27
- Xu W, Chen H, Du K, Asahara H, Tini M, Emerson BM, Montminy M, Evans RM (2001) A transcriptional switch mediated by cofactor methylation. *Science* 294:2507–2511
- Young AP, Longmore GD (2004) Differences in stability of repressor complexes at promoters underlie distinct roles for Rb family members. *Oncogene* 23:814–823
- Zelhof AC, Yao TP, Chen JD, Evans RM, McKeown M (1995) Seven-up inhibits ultraspiracle-based signaling pathways in vitro and in vivo. *Mol Cell Biol* 15:6736–6745
- Zhang HS, Dean DC (2001) Rb-mediated chromatin structure regulation and transcriptional repression. *Oncogene* 20:3134–3138
- Zhang Y, Reinberg D (2001) Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes Dev* 18:2343–2360
- Zhao B, Hou S, Ricciardi RP (2003) Chromatin repression by COUP-TFII and HDAC dominates activation by NF- κ B in regulating major histocompatibility complex class I transcription in adenovirus tumorigenic cells. *Virology* 306: 68–76

