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Review

Transcriptional repression in eukaryotes: repressors and repression mechanisms

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Abstract. For many, if not most genes, the initiation of transcription is the principle point at which their expression is regulated. Transcription factors, some of which bind to specific DNA sequences, generally either activate or repress promoter activity and thereby control transcription initiation. Recent work has revealed in molecular detail some of the mechanisms used by transcription factors to bring about transcriptional repression. Some transcriptional repressor proteins counteract the activity of positively acting transcription factors. Other repressors inhibit the basal transcription machinery. In addition, the repression of transcription is often intimately associated with chromatin re-organisation. Many transcriptional repressor proteins interact either directly or indirectly with proteins that remodel chromatin or can themselves influence chromatin structure. This review discusses the mechanisms by which transcriptional repression is achieved and the role that chromatin re-organisation plays in this process.

Key words. Transcription; repression; gene expression; chromatin; histone; gene silencing; RNA polymerase.

Introduction

When transcriptional repression mechanisms in eukaryotes were first described, gene-specific repression was often thought to be either direct or indirect. Indirect or passive repression mechanisms were thought to be of two kinds: repression by competition between an activator and a repressor for a common binding site, or repression by the sequestration of an activator by a repressor into an inactive complex. In contrast to passive repression, direct or active repression was thought to involve direct inhibitory contacts between the repressor and components of the basal transcription machinery. However, over recent years, there has been a huge increase in the number of characterised repression mechanisms and few of them can easily be placed into these two categories. Any classification of repression mechanisms is complicated by the complexity and diversity of protein-protein interactions involved in the regulation of transcription. Furthermore, a great many repressors are now known to act via several mechanisms and the mechanism used by a particular repressor is often promoter dependent. For example, the mammalian cell cycle regulator and tumour supressor protein Rb uses multiple mechanisms to repress transcription and represses different promoters using different combinations of mechanisms [1–8]. In this review, we will first outline how transcription is regulated and describe the various classes of repressor proteins. We will then attempt to divide the myriad of repression mechanisms that have been identified into one of three categories: inhibition of the basal transcription machinery, ablation of activator function and remodelling of chromatin. We will describe the repression mechanisms that have been studied in detail and we suggest that this analysis of repressors and their mechanisms of ac-

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tion leads to a more coherent understanding of repressor function.

The regulation of transcription

In higher eukaryotes, transcription initiation at promoters recognised by RNA polymerase II is brought about by the concerted action of general transcription factors (GTFs) and the RNA polymerase core enzyme, an assembly of around ten RNA polymerase core subunits. The GTFs are recruited to promoters in an ordered fashion in vitro and aid the binding of RNA polymerase to promoter DNA (fig. 1 A). The first GTF to bind in vitro is TFIID, a complex containing the TATA box-binding protein (TBP) and a number of TBP-associated factors (TAFs). The assembly of GTFs on naked DNA templates forms the pre-initiation complex (PIC) and in the presence of NTPs, the PIC can initiate transcription and begin transcription elongation. The low level of transcription directed by these proteins is known as basal transcription [reviewed in ref. 9]. In cells, RNA polymerase II is in fact loosely associated with a variety of proteins and these complexes are referred to as the RNA polymerase II holoenzyme. Holoenzyme complexes may be recruited to promoters without the prior ordered assembly of GTFs (fig. 1 B) [10]. In yeast and mammalian cells, one set of factors that are associated with RNA polymerase II are the Srb/Med proteins. These proteins allow RNA polymerase to respond to both activators and repressors. In mammalian cells, in addition to Srb/Med proteins, a number of proteins that alter the structure of chromatin (chromatin remodelling factors) are components of holoenzyme complexes [reviewed in refs 9, 11].

Activators of transcription are often defined as sequence-specific DNA-binding proteins that stimulate transcription initiation or elongation. Many activators interact with proteins known as co-activators; these proteins do not have DNA-binding activity but they help the activator to perform its function. One set of co-activators are the TAF proteins present in TFIID, although these proteins can also be regarded as GTFs [reviewed in ref. 12]. Most activators and many co-activators act by stimulating the formation of the PIC (fig. 1 C). This is often brought about by direct interactions between these proteins and various components of the PIC. In addition, both activators and co-activators can activate transcription by promoting the alteration of chromatin structure in the vicinity of the promoter (fig. 1 D). Three classes of protein associated with the RNA polymerase II holoenzyme are involved in this remodelling of chromatin: histone-modifying enzymes, chromatin-binding proteins, and ATP-dependent nucleosome-remodelling proteins [reviewed in ref. 9]. Activators and co-activators can recruit one or more of these proteins to a promoter and the resulting chromatin remodelling can alter histone-DNA interactions, nucleosome-nucleosome interactions, and/or reposition nucleosomes relative to transcription factor-binding sites [reviewed in ref. 13]. These changes in chromatin structure regulate transcription by altering the local accessibility of the DNA to transcription factors, RNA polymerase II, and other components of the PIC.

Repression

Transcriptional repression is of two types: general or global repression and gene-specific repression. General repression occurs when a repressor protein or complex either sequesters or modifies a central component of the PIC or a component of RNA polymerase II, so that it is unavailable for transcription. Thus general repression will down-regulate the expression of all the genes transcribed by this RNA polymerase. For example, phosphorylation of a core subunit of RNA polymerase II by the herpes virus (HSV-1) proteins ICP22 and UL13 kinase generates a non-functional form of polymerase resulting in general repression [14]. Nucleosomes also generally repress promoter activity by sequestering promoter DNA into chromatin [reviewed in ref. 15]. In contrast, gene-specific repression occurs when the transcription of a particular gene or set of genes is controlled by the activity of a gene-specific repressor or co-repressor. Repressors can bring about gene-specific repression by decreasing the concentration of a functional activator/co-activator at the promoter or by counteracting the stimulatory effect of these proteins on transcription. In addition, some repressors inhibit transcription by interacting in a promoter-specific fashion with components of the PIC or by recruiting chromatin-remodelling proteins.

Gene-specific repressor proteins often bind either directly or indirectly to DNA and they can regulate transcription from binding sites proximal to, or at a distance from, the promoter [reviewed in ref. 16]. Repression that is effected by distally located repressor proteins is often known as ‘long-range’ repression and these proteins are sometimes known as ‘long-range’ repressors. Distally bound repressors may repress promoter activity by remodelling chromatin at or near the promoter. Alternatively, they may contact transcription activators and/or components of the PIC by looping out the intervening DNA [17, and references therein]. In contrast to long-range repression, ‘short-range’ repression occurs when a repressor protein acts locally. In this case, the repressor down-regulates the activity of nearby activator proteins or components of the PIC but does not affect the activity of distally located activators. Proteins that bring about this ‘short-range’ repression are sometimes referred to as ‘short-range’ repressors. However, these two types of repression are not necessarily mutually exclusive and a re-

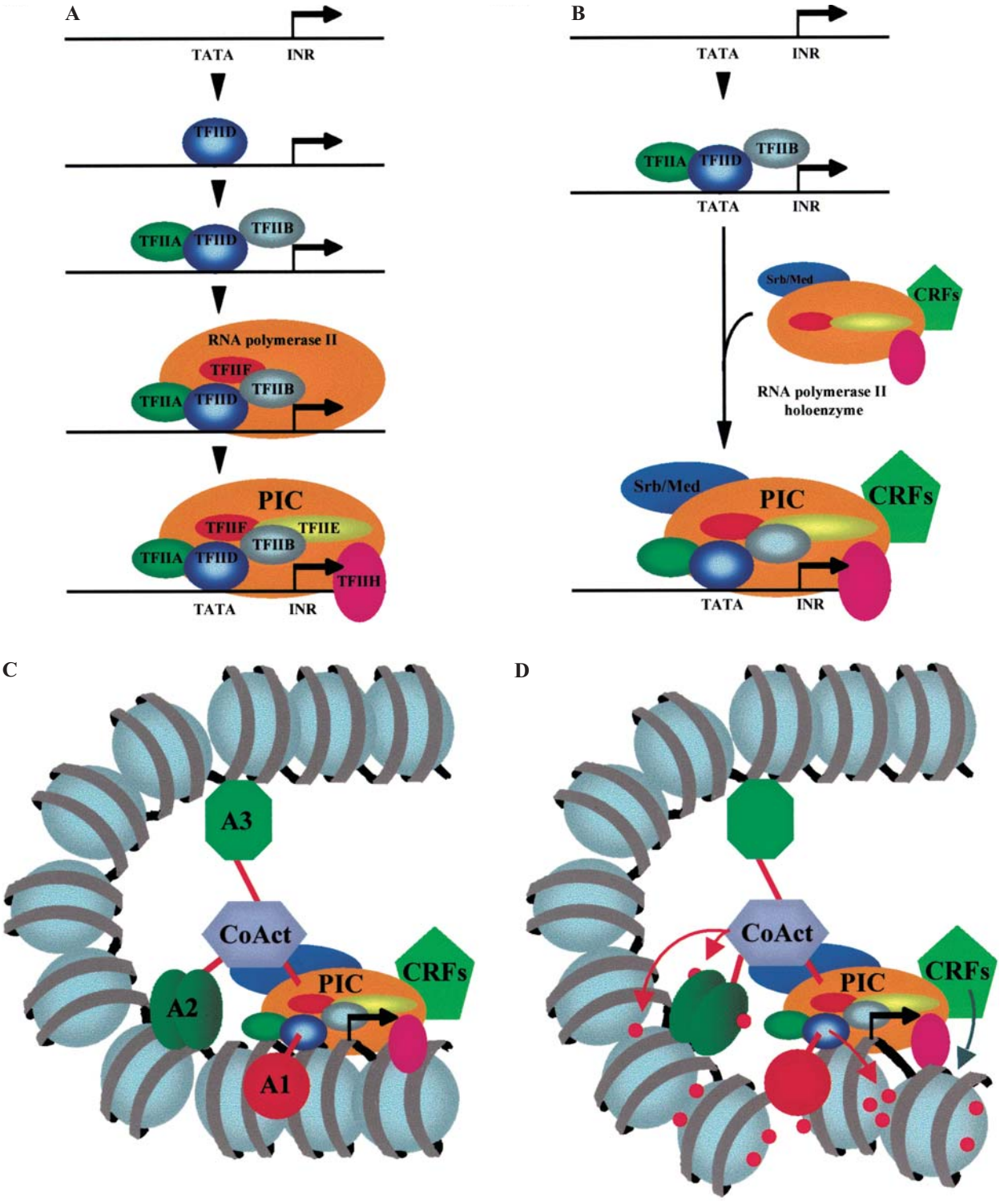

Figure 1. Pre-initiation complex formation and the activation of transcription. (*A*) GTFs bind in an ordered fashion to form the preinitiation complex (PIC). The TATA box and initiator element are indicated by TATA and INR, respectively. The transcription start site is represented by the bent arrow. (*B*) PIC formation may involve the recruitment of an RNA polymerase II holoenzyme. The holoenzyme comprises RNA polymerase, GTFs, Srb/Mediator proteins (Srb/Med), and chromatin-remodelling factors (CRFs). (*C*) The activation of transcription in the context of chromatin. Activators often stimulate the formation of the PIC by making direct contacts (red lines) with GTFs (A1). Activators bound at promoter-proximal locations (A2) or at promoter distal locations (A3) can recruit co-activators (CoAct). (*D*) Several co-activators as well as some components of the TFIID complex have histone acetyltransferase activity (red arrows) and the acetylation of histones (red dots) brings about changes in chromatin structure. Activators and co-activators can also bring about changes in chromatin structure by recruiting a variety of CRFs.

pressor may bring about both long- and short-range repression depending on the context of its binding site and its exact mode of action [18, 19]. For example, the repressor protein Hairy can bring about both long- and short-range repression [20, 21]. Furthermore, one should note that the terms long and short range do not refer to any particular molecular mechanisms that bring about repression.

Repressor proteins

Gene-specific repressor proteins are a large group of diverse proteins that negatively regulate transcription, and they are not easily defined. Repressor proteins have been categorised in a number of ways. They have been divided into proteins that bring about short- or long-range repression [19]. Repressors have also been divided into groups that can or cannot recruit histone deacetylases to promoters. In addition, they have been categorised as either sequence-specific DNA-binding repressors or non-DNA-binding co-repressors. There are, however, many examples of repressors that do not fit into any of these categories. In addition, there are a rapidly expanding number of 'context-dependent' transcription factors that bind DNA and are capable of positively or negatively regulating transcription depending on the context of their binding sites, the complement of protein interactions they can make and other environmental cues.

To categorise the different kinds of repressor proteins we have defined three main classes (table 1). However, we would like to point out that the members of each class can repress via multiple mechanisms and that some repressors fall into more than one category. Class I repressors are DNA-binding proteins that negatively regulate the transcription of specific genes. These repressor proteins are of two types: sequence-specific DNA-binding proteins (class IA) and proteins that bind to methylated DNA (class IB). Examples of the former are the *Drosophila* zinc-finger protein Krüppel [22, 23] and homeodomain protein Engrailed (En) [24]. Examples of the latter are MeCP2 [25] and MBD2 [26, 27]. In contrast to class I repressors, class II repressors are proteins that do not bind DNA directly. Instead, they are recruited to promoters by other proteins. Class II repressors can be divided into two subclasses. Class IIA repressors interact with 'dedicated' repressor proteins and can be considered to be true co-repressors. Examples of this type of repressor are Dnmt3 and MBD3; these proteins repress transcription by interacting with the class I repressors RP58 and MBD2, respectively [28, 29]. Unlike the class IIA repressors, class IIB repressors interact with both 'dedicated' class I repressors and 'context-dependent' transcription factors, proteins that will in other situations activate transcription. Class IIB repressors include the SMRT [30] and NCoR [31] proteins and members of the Groucho/Tup1 and CtBP families [18, 19, 32]. Class IIB repressors also include proteins such as the GAL80 repressor protein from yeast and the human MDM2 tumour supressor protein which bring about repression by protein-protein interactions with the activator proteins GAL4 and p53, respectively [33, 34]. Subunits from a number of repressive chromatin-remodelling complexes can also be considered as class II repressors. For example, the Sin3 and MBD3 proteins present in the SIN and NuRD chromatin-remodelling complexes can also be considered to be class II repressor proteins, because they can bind directly to DNA-binding proteins such as Ikaros and MBD2, respectively [35, 36].

Class III repressors are proteins that do not necessarily bind to DNA directly or indirectly. These repressors often target activators, co-activators or components of the PIC and usually reduce the amount of functional protein available to regulate transcription. Class IIIA repressors often

Table 1. Classes of Repressor Proteins.

Class	Defining feature	Examples
Class I: DNA-binding proteins		
A	sequence-specific DNA binding	PRH, Eve, Krüppel, TGIF, Mad, IRF-2, RP58, E2F-6
B	methyl-CpG-binding proteins	MeCP2, MBD2
Class II: bind to DNA-binding proteins		
A	proteins (co-repressors) that bind to dedicated repressors	Dnmt3, MBD3, Tup1, Groucho
B	proteins that bind to context-dependent transcription factors	Groucho, CtBP, TGIF, NcoR, Rb, MDM2
Class III: other repressors		
A	bind to activators, co-activators, or PIC	IκB, Mot1, FIR, GAL80, E1A243R, Rb, PHO80,
B	post-translationally modify activators, co-activators or PIC	OGT, PHO80, CARM-1, Srb10, CCK-II

sequester these proteins into inactive complexes, or alter their stability or nuclear localisation without post-translational modification. An example of this group is the IκB protein which sequesters the activator NFκB activator protein in the cytosol [37]. Other examples of this group are the adenovirus E1A243R (12S) repressor protein and the Mot1 repressor protein. These proteins interact with TBP and dissociate TBP bound to TATA boxes [38, 39]. Class III B repressors post-translationally modify their targets. Examples are the PHO80/85 kinase cyclin complex from yeast that phosphorylates and inactivates the activator PHO4 [40] and the mammalian CARM-1 protein which methylates the co-activator CBP [41].

As pointed out earlier, some repressors fall into more than one of the categories described above. PHO80, for example, can be considered a class III A repressor as it binds to PHO4, a DNA-binding activator protein, and 'masks' the PHO4 activation domain [42]. However, the PHO80/PHO85 complex can phosphorylate PHO4, and in this context, PHO80 acts as a class III B repressor [40]. Similarly, the homeodomain protein TGIF is a class I A repressor protein that binds to the retinoid X receptor (RXR) response element [43]. However, TGIF can also function as a class II B repressor protein at promoters that are regulated by the SMAD proteins [44].

Repression mechanisms

There are three major routes through which repressor proteins can down-regulate specific genes: inhibition of the basal transcription machinery, ablation of activator function, and remodelling/compaction of chromatin [45]. The details for each route to transcriptional repression can vary in a promoter- and repressor-specific fashion. Below, we describe some of the best-understood repression mechanisms.

Repression via the basal transcription machinery

Although targeting of the basal transcription machinery might be expected to result in a global shutdown of transcription, there are a number of repressor proteins that act in a gene-specific fashion by interacting with one or more of the GTFs or core subunits. This can result in the inhibition of basal and activated transcription at specific promoters and is often known as 'active' or 'direct' repression.

Modifying the RNA polymerase II large subunit

The RNA polymerase II large subunit C-terminal domain (CTD) is a target for direct repression (fig. 2 A). The CTD of RNA polymerase is glycosylated and dephosphorylated during transcriptional initiation, whereas it is deglycosylated and phosphorylated during elongation [46, 47]. Altering the extent or the timing of these modifications on the CTD brings about transcriptional repression. As mentioned earlier, the RNA polymerase II holoenzyme in yeast contains several 'suppressors of RNA polymerase B/Mediator' (Srb/Med) components that mediate the response of the holoenzyme to many transcriptional activators and repressors. One protein present in this complex is the kinase Srb10. Srb10 represses the transcription of a set of genes involved in cell type specificity, meiosis and sugar utilisation by phosphorylating the CTD before the holoenzyme associates with promoter DNA. Phosphorylation by Srb10 thus inhibits formation of the PIC and hence transcription initiation [48]. How the activity of Srb10 is regulated so that can repress only specific sets of genes is not yet known, however, one possibility is that Srb10 activity is regulated by Tup1. The yeast Tup1 protein is a member of the Groucho family of class II repressors. Genetic and biochemical experiments have demonstrated that Srb10 and Tup1 interact and that the kinase activity of Srb10 is important for repression by Tup1 [49, 50]. Furthermore, Tup1 prevents the association of the RNA polymerase II holoenzyme with promoters [50]. One mechanism through which Tup1 represses transcription could be by enhancing the activity of the Srb10 kinase [50].

Recruitment of the enzyme O-GlcNac transferase (OGT) to promoters by the mammalian class II repressor protein Sin3a has been suggested to be another mechanism for the direct repression of transcription. This suggestion is based on the fact that Sin3a can interact with OGT and that directly tethering OGT to promoter DNA as a Gal4-OGT fusion protein results in the repression of transcription. Furthermore, proteins bound to Sin3-dependent promoters are more heavily glycosylated when the promoter is repressed [51]. Since the RNA polymerase CTD is known to be glycosylated, gene-specific repression might be brought about by recruiting OGT to Sin3-regulated promoters. Glycosylation or hyperglycosylation of the CTD might inhibit elongation or the recycling of RNA polymerase between transcription elongation and initiation [51].

Inhibiting the binding of TBP to DNA

An important mechanism of gene-specific repression is interference with the binding of TBP and hence TFIID to the TATA box (fig. 2 B). This can be gene specific for three reasons. First, repressors that act in this way can be targeted to individual promoters either through direct or indirect binding to specific DNA sequences. Second, many genes transcribed by RNA polymerase II do not contain TATA box sequences and are thus probably not sensitive to this type of repression; this is particularly true

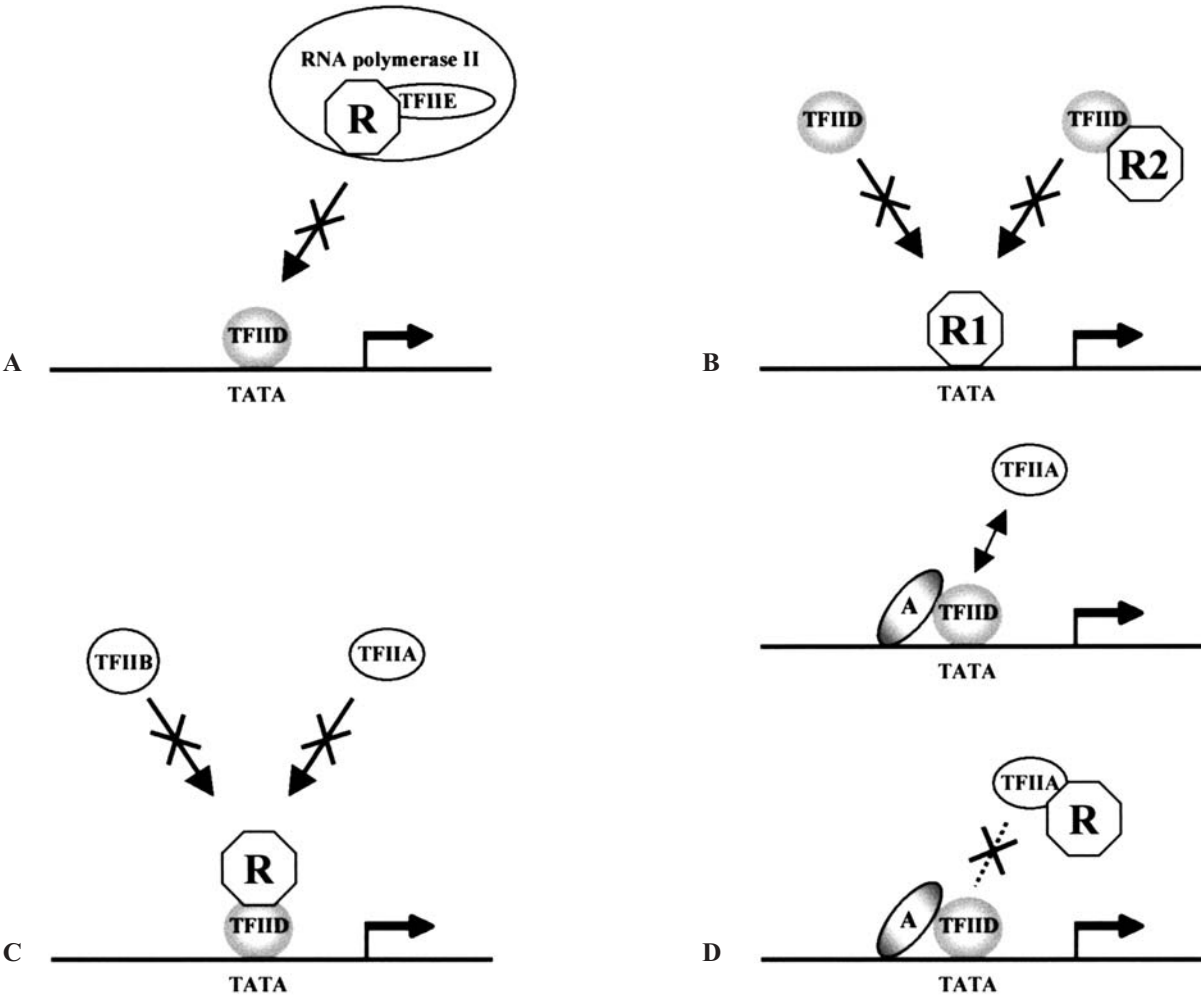

Figure 2. Repression via the basal machinery. (*A*) Repressors bind to and/or modify RNA polymerase or GTFs and block binding to the promoter. (*B*) Repressors block the binding of TFIID to the TATA element either by competing for the TATA element (R1) or by binding to TFIID (R2). (*C*) Repressors block interactions between GTFs. (*D*) Repressors block activator-dependent interactions between GTFs.

of housekeeping genes. Third, at least some of the genes transcribed by RNA polymerase II are regulated by a PIC that does not contain TBP [52]. Several class I repressor proteins have been shown to bind TBP including the *Drosophila* homeodomain protein Even-skipped (Eve) [53]. In vitro, the Eve repression domain together with the Eve homeodomain bind directly to TBP and this interaction can block the binding of TBP/TFIID to the TATA box and thus prevent assembly of the PIC [54]. Evidence to support this mechanism of repression comes from the fact that phosphorylation of Eve prevents the Eve-TBP interaction and results in the loss of repression [55]. The adenovirus E1A243R (12S) protein also represses transcription by making direct contacts with TBP that interfere with formation of the TBP-TATA complex. Intriguingly, a pre-formed TBP-TATA box complex can be dissociated by the E1A N-terminal repression domain [38]. The N-terminal repression domain of E1A also binds to the co-activator CBP and a mutational analysis has demonstrated that the repression domain in E1A is not separable from the CBP interaction domain. This has led to a model for repression where E1A243R interacts with CBP at CBP-regulated promoters and then dissociates/interferes with formation of the TBP-TATA complex [56].

Mot1 is a repressor protein that can dissociate a TBP-TATA complex in an ATP-dependent manner. Mot1 is a member of the SWI/SNF family of ATP-dependent nucleosome-remodelling proteins [see below and ref. 57]. Mot1 removes TBP from DNA by interacting with TBP [39]. Interestingly, Mot1 competes with TFIIA for binding to TBP as they bind to the same surface of TBP. Since TFIIA stabilises the TFIID/DNA complex, Mot1 counteracts the stabilising activity of TFIIA. Although Mot1 was originally thought to function as a general regulator of basal transcription, it has recently been demonstrated to repress (and activate) the transcription of specific sets of genes [58]. One possibility is that Mot1 is recruited to

promoters in a gene-specific fashion by interaction with DNA-binding proteins [59].

The inhibition of TBP-DNA binding does not necessarily involve protein-protein interactions between the repressor and the TBP. Several repressor proteins can bind to TATA box sequences and sterically hinder the binding of TBP. For example, En is a class I repressor that can bind to A/T-rich sequences and compete with TFIID for binding to TATA box sequences [60]. Whether En uses this mechanism to repress transcription in vivo is not known, but studies in haematopoietic cells suggest that the vertebrate homeodomain protein PRH (also known as Hex) might repress transcription in this way. Like En, PRH can bind to TATA box sequences in vitro; furthermore, mutations in the PRH homeodomain that block binding to these sequences abolish homeodomain-dependent transcriptional repression [61]. In vitro studies with Eve suggest that when this protein is bound to high-affinity binding sites upstream of the promoter it can recruit further Eve proteins to low-affinity sites around the TATA box. The binding of Eve to these low-affinity sites is thought to prevent TBP binding and hence inhibit PIC formation. This has been termed 'cooperative blocking' of the promoter [62] and may also occur when the papillomavirus E2 protein binds cooperatively to its four binding sites within the papillomavirus genome and represses viral gene expression [63].

Inhibiting interactions between the GTFs

A number of repressors inhibit GTF-GTF interactions and thereby repress transcription (fig. 2C). For example, the NC2 (Dr1-Drap or Bur6-Ydr1) heterodimer [64–66] binds to TBP in vitro and blocks interactions between TBP and TFIIA. The NC2- and TFIIB-binding sites on TBP are adjacent and the binding of NC2 possibly directly impedes the binding of TFIIB. However, the NC2- and TFIIA-binding sites on TBP are on opposing surfaces, suggesting that the interaction of NC2 with TBP alters the conformation of TBP so that it can no longer interact with TFIIA [65–67, reviewed in ref. 57]. Although NC2 has been characterised as a general repressor of transcription, evidence suggests that it can also function in a gene-specific fashion as both an activator and a repressor of transcription [68]. The class I repressor proteins Krüppel and the unliganded thyroid receptor (TR) are also thought to repress transcription by altering interactions between GTFs. Whilst monomeric Krüppel is an activator that binds to TFIIB, dimeric Krüppel binds to the TFIIEβ subunit and inhibits formation of the PIC [69]. Similarly, in the presence of ligand, TR is an activator that interacts with TFIIB. However, in the absence of ligand, TR can interact with TBP and inhibit the formation of a functional PIC. In vitro transcription studies have shown that TR binds directly to TBP and interferes with the formation of TBP-TFIIA or TBP-TFIIA-TFIIB complexes [70–72]. The class II repressor proteins MDM2 and N-CoR also interact directly with GTFs. MDM2 binds to TFIIE and TBP and directly interferes with basal transcription [73]. Similarly N-CoR, a protein that mediates repression by unliganded nuclear hormone receptor proteins such as TR, can interact with TAF32, TFIIB and TAFII70 simultaneously and inhibit the functional interaction of TFIIB and TAF32 [74]. This suggests that N-CoR, like the repressors described above, makes interactions with the GTFs that lock the PIC into a non-functional state or conformation.

In all of the examples described so far, the repressors block activator-independent GTF-GTF contacts. In contrast, RBP/CBF1 is a class I repressor that is able to disrupt activator-dependent GTF-GTF interactions (fig. 2D) [75]. Sp1-activated transcription can be repressed by RBP. Sp1 interacts with *Drosophila* TAF110 (dTAF110) in vitro and dTAF110 associates with TFIIA resulting in transcription activation. RBP binds to TFIIA and prevents the Sp1-induced interaction of TFIIA and dTAF110. RBP does not repress transcription if the Sp1-dTAF110-TFIIA complex is preformed and RBP does not prevent the interaction of Sp1 with dTAF110. Thus RBP does not inhibit the interaction of the activator with a GTF but, rather, inhibits activator-dependent interactions between GTFs [75].

Finally, one should note that many proteins involved in chromatin remodelling can also repress transcription via direct contacts with GTFs. The Polycomb group (PcG) proteins, for example, are chromatin-binding proteins that are recruited to specific promoters by interaction with class I and class II repressors. PcG proteins can repress transcription by interacting directly with a number of GTFs [76, 77]. Similarly, MeCP2, a methyl-CpG-binding protein involved in transcriptional silencing, interacts with TFIIB to inhibit basal transcription and also can organise DNA into large nucleoprotein complexes [78].

Repression via the ablation of activator function

Many transcriptional repressor proteins regulate the activity or location of a transcription activator/co-activator (fig. 3). This can be achieved by regulating the turnover and hence levels of an activator, regulating its intracellular localisation, inhibiting its DNA-binding activity, or by inhibiting any of the protein-protein interactions that the activator makes with the transcription machinery.

Regulating activator/co-activator turnover

Proteins that regulate the stability and turnover of an activator can indirectly regulate transcription. The MDM2 oncoprotein is an example of a repressor that ubiquiti-

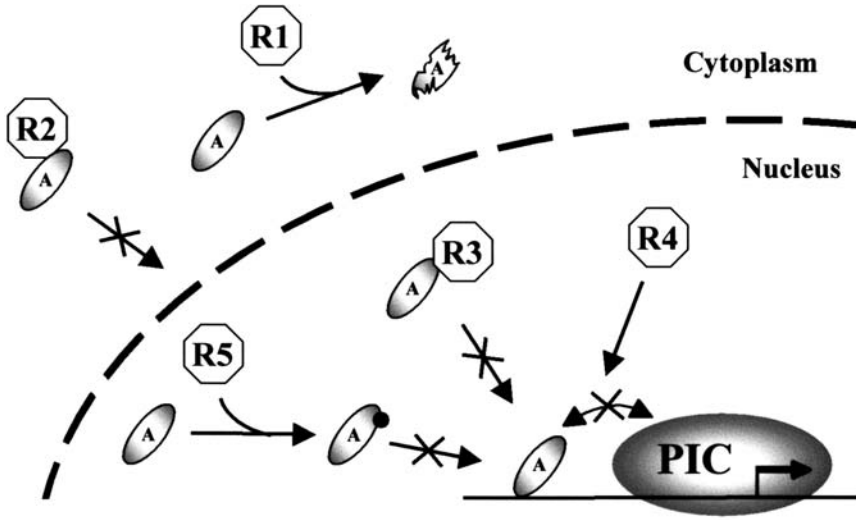

Figure 3. Repression by the ablation of activator function. Repressors often target activators or co-activators for degradation (R1) or hold activators in non-productive complexes (R2 and R3). Some repressors block activator-PIC contacts (R4). Other repressors post-translationally modify activators (R5). Note that R1 and R5 need not necessarily act only in the cytoplasm and nucleus, respectively.

nates an activator and thereby promotes its degradation by the proteasome. Ubiquitination of p53 by MDM2 triggers the nuclear export of p53 and its degradation [79, 80]. Another example of a repressor that promotes the degradation of an activator is the kinase Srb10. As mentioned earlier, Srb10 can repress specific subsets of yeast genes by phosphorylating the RNA polymerase CTD. Srb10 can also repress genes activated by the transcription factor GCN4 [81]. In this case, however, Srb10 acts by phosphorylating GCN4. The phosphorylation of GCN4 by Srb10 results in the recruitment of a complex that ubiquitinates GCN4 and marks it for degradation by the proteasome. Thus, Srb10 ultimately represses GCN4-dependent genes by promoting the degradation of their activator [82]. Although the promotion of degradation/turnover of an activator by a repressor is generally mediated by the proteasome, there is at least one case where a repressor is thought to directly cleave proteins involved in transcription. The class I repressor protein AEBP1 has carboxypeptidase activity and this activity is required for its repressor function [83]. Whether activators or components of the PIC are cleaved by this protein remains unclear.

Promoting the degradation of a co-activator can also bring about the repression of transcription. The LIM homeodomain proteins (LIM-HDs) are involved in cell lineage determination and the regulation of differentiation [84]. When associated with CLIM proteins (co-factor of LIM-HD), the LIM-HDs activate transcription. However, R-LIM proteins inhibit the activity of LIM-HD-CLIM complexes in several ways. They compete with CLIM proteins for binding to LIM-HD, they recruit histone-modifying complexes and, uniquely, they can also ubiquitinate CLIM factors bound to LIM-HDs whilst they are bound to DNA [84, 85]. Ubiquitination results in the degradation of CLIM proteins and thus R-LIM represses LIH-HD activity by regulating the turnover of CLIM co-activator proteins [84].

Regulating the intracellular localisation of an activator

A well-known transcription factor that functions by preventing nuclear localisation of the activator is the repressor IκB. IκB blocks the nuclear import of NFκB, a heterodimer comprised of p50 and p65, two members of the Rel family of transcription activators [86]. The interaction of NFκB with IκB 'masks' the nuclear localisation signal of NFκB and thereby prevents its nuclear import and, hence, the activation of NFκB-dependent genes [37]. The interaction of IκB with NFκB is subject to extensive regulation by kinases and ubiquitinylating enzymes that target the repressor for degradation [reviewed in ref. 87]. Post-translational modification of an activator can also inhibit nuclear localisation. The PHO80 repressor protein from *Saccharomyces cerevisiae* is a kinase that interacts with a cyclin-like partner protein PHO85. Under conditions where phosphate is in excess, the PHO80/85 heterodimer phosphorylates the activator protein PHO4 [40]. Phosphorylated PHO4 becomes localised to the cytosol resulting in the repression of genes required for phosphate uptake [88].

Inhibiting activator-DNA interactions

One of the first mechanisms of repression to be studied in detail was that arising as a consequence of competition between two different transcription factors for a

common binding site. This type of repression is analogous to that observed when proteins compete with TBP for binding to the TATA box. Engrailed competes with the activator Fushi-taratzu (Ftz) for a common binding site and can thereby repress transcription [89]. Similarly, the GC-rich sequence-binding factor is able to repress transcription of several genes by competing with the transcription activator Sp1 for binding sites [90]. This repression mechanism allows transcription factors that normally activate transcription to down-regulate gene expression. The transcription factor AP1 provides an example of this situation. AP1 is usually an activator, however, it represses basal and retinoic acid-induced transcription of the osteocalcin gene by competing with the retinoic acid receptor protein for overlapping DNA-binding sites [91]. Some repressors which bind to single-stranded DNA sequences have also been proposed to repress transcription by competing with activator proteins that bind to the same sequences when they are in double-stranded DNA [92, 93].

Post-translational modification of many activators can inhibit their ability to bind DNA (fig. 3). For example, during mitosis, phosphorylation of the Oct-1 homeodomain by protein kinase A inhibits Oct-1 DNA-binding activity and represses the transcription of Oct-1-dependent genes [94]. Similarly, acetylation of the C-terminal zinc-finger domain of YY1 decreases the DNA-binding activity of this bifunctional activator/repressor protein [95]. Many other repressors regulate transcription by preventing activators from binding DNA but do not post-translationally modify the activator. This type of repression usually involves protein-protein interactions between the repressor and the activator. Repressor proteins belonging to the helix-loop-helix (HLH) family and the basic-leucine zipper (B-LZ) family function by forming non-DNA-binding heterodimers with other family members that activate transcription. The class III A repressor protein, Id, for example is an HLH protein that lacks DNA-binding activity but which heterodimerises with the activators MyoD, E12 and E47 and inhibits their binding to DNA [96]. The class I repressor protein Mad is a member of the basic-helix-loop-helix-leucine-zipper (B-HLH-LZ) family of transcription factors and forms DNA-binding heterodimers with the B-HLH-LZ proteins Myc and Max [97]. Myc activates transcription but only as a heterodimer with Max. Heterodimerisation of Mad with Max competes with the formation of the Myc/Max complex and can thereby block transcription activation. In addition, the Mad/Max heterodimer can compete with Myc/Max for DNA sequences known as E-boxes. Thus, Mad competes with Myc for an interacting partner and the Mad/Max heterodimer competes with Myc/Max for a common binding site on DNA. In addition, the Mad/Max complex recruits proteins that alter chromatin structure [see below and ref. 98].

Inhibiting activator-target interactions

Many class II repressors bind to activators and prevent them from interacting with their targets. When the repressor binds to the activation domain, this type of repression is sometimes referred to as masking. Masking was first characterised in detail for the yeast repressor protein GAL80 which binds directly to the activator GAL4. The GAL4 activation domain interacts with the Srb4 subunit of the RNA polymerase II holoenzyme [99]. Since the binding site for the GAL80 protein on GAL4 partially overlaps with the GAL4 activation domain, the interaction of GAL80 with GAL4 can inhibit the interaction of GAL4 with the transcription machinery [33, 100]. Presumably, at least one of the interactions that is masked by GAL80 is the Srb4-GAL4 interaction. Interestingly, repression is augmented if there is more than one GAL4-binding site at the promoter and protein-protein interactions between GAL80 repressor molecules that are simultaneously bound to GAL4 activator proteins have been suggested to enhance masking of the GAL4 activation domain [101]. Masking of an activation domain has been suggested as a mechanism of repression for at least three other class II repressor proteins. The Rb protein 'masks' the activation domain of the activator E2F [1, 2]. Similarly, MDM2 'masks' the p53 activation domain [102, 103]. Masking of p53 by MDM2 involves an amphipathic α helix in p53 that is involved both in the binding of MDM2 and in transcription activation [104]. Finally, the yeast PHO80 repressor protein has been suggested to 'mask' the activation domain of PHO4. However, in this case, the PHO80 interaction region is adjacent rather than co-incident with the PHO4 activation domain [42].

Some repressors that inhibit activator-target interactions do not bind to activation domains. Fuse binding protein (FBP) binds to the c-myc promoter and activates transcription. The FBP-interacting repressor protein (FIR) binds to FBP and to its target GTF. FBP contacts TFIIH and stimulates the 3′-5′ helicase activity of the p89/XPB TFIIH subunit. The 3′-5′ helicase activity of this GTF is required for promoter melting, transcription initiation and promoter escape. FIR inhibits activated transcription by simultaneously interacting with FBP and p89/XPB and decreasing the 3′-5′ helicase activity of TFIIH [105, 106]. Repressors can also act by inhibiting co-activator-polymerase II/GTF contacts. The yeast Tup1 protein was recently proposed to repress transcription by inhibiting the interaction of Srb7, an essential component of the RNA polymerase II holoenzyme, with the co-activator Med6. Tup1 can compete with Med6 for binding to Srb7 and since the interaction of Srb7 with Med6 is essential for full activation by several activators, this leads to the repression of a subset of yeast genes [107].

When class I repressor proteins block interactions between a DNA-bound activator and one or more compo-

nents of the PIC, this is sometimes known as 'quenching'. YY1, is a mammalian Krüppel-like transcription factor [108, 109] that can quench transcription activated by AP-1 [110]. In this case, the repression mechanism is known: YY1 binds to the co-activator CBP and prevents the interaction of CBP and AP-1 [110]. However, quenching does not refer to a particular repression mechanism and other class I repressors can 'quench' in other ways. A novel mechanism of transcriptional repression has recently been described where activator-co-activator interactions are disrupted but where the disruption does not require direct interactions between the repressor and either the activator or the co-activator. The IRF-2 oncoprotein is a class I repressor that represses virus-induced transcription of the interferon (IFN)-β gene. IRF2 represses transcription by binding to the IFN-β enhancer and inhibiting the recruitment of the co-activator CBP by the activator IRF-1 and/or by destabilising the CBP-IRF-1 interaction. Remarkably, repression by IRF-2 does not rely on direct protein-protein interactions between the repressor and CBP. Instead, there is a small basic domain in IRF-2 that has been proposed to 'repel' incoming CBP (or alternatively to destabilise the interaction of CBP and activator proteins such as NFκB, IRF-1 and ATF-2/c-Jun present at the IFN-β enhancer) [111].

Post-translational modification of an activator or co-activator

We have already described how phosphorylation and acetylation can regulate the DNA-binding activity of activators. However, in many cases, repressors post-translationally modify activators and thereby prevent their interaction with other proteins. These modifications act as molecular switches that can rapidly regulate the transcription of a set of genes. An example of a repressor that acts in this fashion is the calcium/calmodulin-dependent kinase II (CCK-II). This class III B repressor phosphorylates the activator protein CREB. Phosphorylation of CREB inhibits the interaction of CREB with the co-activator CBP and results in the down-regulation of transcription [112]. CBP itself has been shown to acetylate the *Drosophila* activator protein TCF and in vitro this reduces the affinity of TCF for the co-activator β-catenin [113]. The glycosylation and deacetylation of transcription factors can also block activation. For example, the glycosylation of Sp1 by OGT inhibits the interaction between Sp1 and the co-activator dTAF110 as well as inhibiting the self-association of Sp1 [114]. Deacetylation of MyoD by HDAC1 has been proposed as a mechanism for regulating MyoD activity in undifferentiated cells. MyoD is a B-HLH protein that activates the expression of muscle-specific genes. However, MyoD can interact with HDAC-1 to repress transcription of these genes in undifferentiated cells. HDAC1 interacts directly with MyoD and can deacetylate MyoD in vitro [115]. Which of the interactions of MyoD with the transcription machinery is disrupted by its deacetylation is not yet known.

Post-translational modification of a co-activator protein can also act as a molecular switch that regulates a specific group of genes. For example, CARM1 (co-activator-associated arginine methyltransferase) represses transcription activated by CREB by methylating CBP and blocking the interaction of CBP and CREB. Methylated CBP is, however, still able to interact with transcription factors other than CREB and to co-activate transcription in other contexts [41]. Similarly, the co-activator protein ACTR functions together with a number of nuclear hormone receptor proteins, including the estrogen receptor (ER) to activate transcription, and the acetylation of ACTR by CBP results in the repression of ER-activated transcription [116].

Repression by the recruitment of chromatin-remodelling factors

The eukaryotic genome is packaged into a complex protein-DNA fibre known as chromatin (fig. 4). The DNA is first wrapped around histones H2A, H2B, H3 and H4 to form nucleosomes. The nucleosomes are then built into higher-order structures, the exact nature of which is still not completely understood. At a gross level, chromatin appears to be of two types: euchromatin and heterochromatin. Euchromatin contains most genes that are expressed in the cell and is sometimes called decompacted chromatin. In contrast, heterochromatin consists of regions of darkly staining, highly compacted chromatin that contains very few active genes. Heterochromatin is replicated late in S phase and is commonly located at telomeres and centromeres and at the silent (HM) mating loci in *S. cerevisiae*. The repression of transcription in or by heterochromatin is known as gene silencing. Chromatin condensation patterns in general are said to be epigenetic because they are stably inherited after mitosis but do not rely on specific DNA sequences. This means that a silenced allele and its transcriptionally active counterpart may be of identical sequence and yet their different transcriptional states are maintained over many cell divisions [117].

The alteration of chromatin structure into a repressive state can involve changes in nucleosome-DNA contacts and/or inter-nucleosomal contacts. One way in which these changes can be effected is by modifying histones. There are several different kinds of histone-modifying enzymes including histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs) and histone kinases, as well as enzymes that ubiquitinate histones [118]. Transcriptional activation is generally associated with the acetylation of histones, whereas transcriptional repression is generally associated

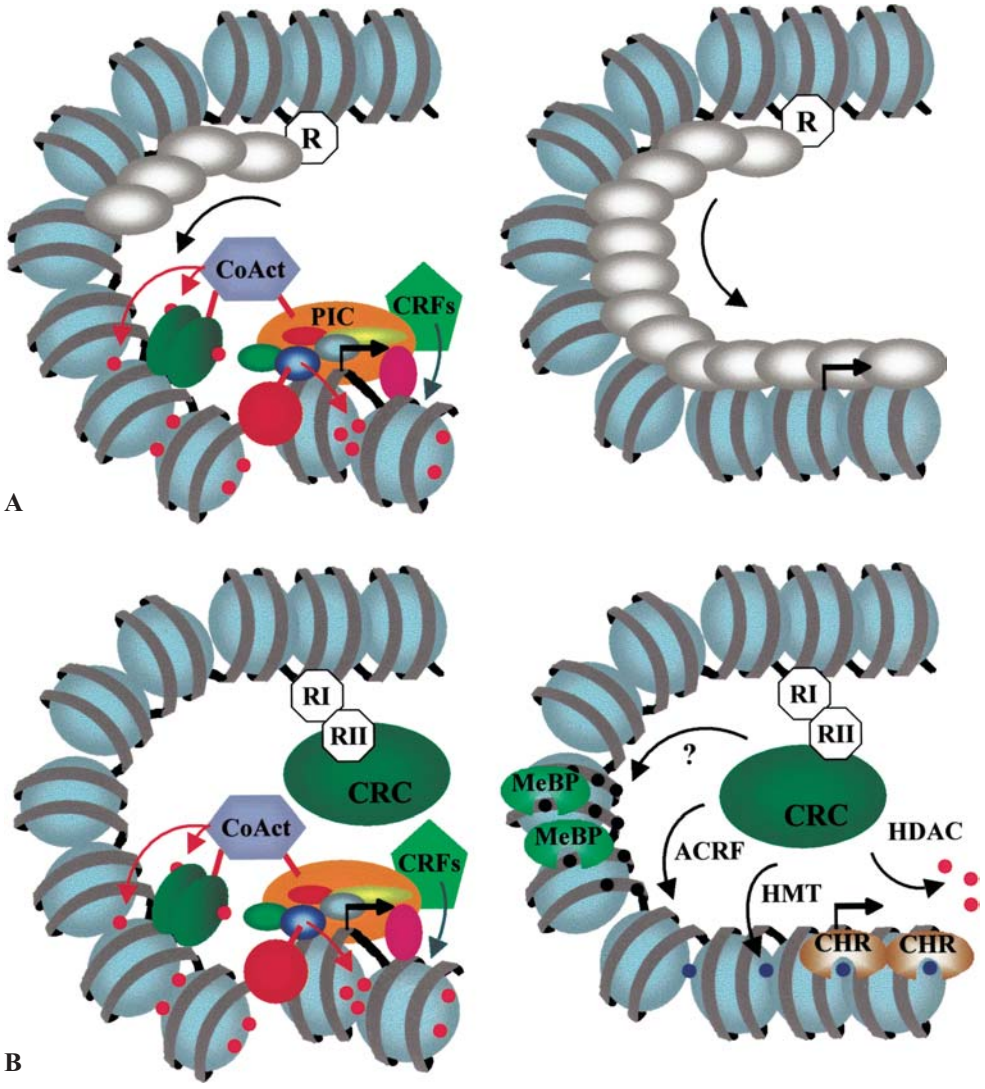

Figure 4. Repression by the recruitment of chromatin-remodelling factors. (*A*) A model that shows how repressors (R) might recruit chromatin-binding proteins (grey ovals) that oligomerise and spread along the chromatin fibre. (*B*) A model that shows how class I repressors (RI) can recruit class II repressors (RII) that are components of chromatin-remodelling complexes (CRCs). CRCs bring about histone deacetylation and histone methylation (blue dots) using HDAC and HMT activity, respectively. Histone methylation brings about the binding of chromodomain-containing proteins (CHR). CRCs can also remodel chromatin via their ATP-dependent chromatin-remodelling activity (ACRF). CRCs might bring about CpG methylation (black dots) allowing the binding of methyl-CpG-binding proteins (MeBP), although this is speculative.

with their deacetylation [119]. Thus, inhibitors of HDACs derepress many genes and silenced heterochromatin is generally deacetylated [120]. Histone modifications such as acetylation may directly change chromatin structure by altering the amount of charge on the N-terminal tails of histone proteins. Alternatively, the effects of histone modifications on chromatin structure may be mediated by chromatin-binding proteins (see below). Changes in chromatin structure can also be effected by proteins that alter nucleosome-DNA contacts or inter-nucleosomal contacts without modifying histones. For example, the SWI/SNF ATP-dependent remodelling complex [121, 122], originally isolated from *S. cerevisiae* but subsequently found to be conserved across all eukaryotes, is involved in the repression and activation of transcription [123] and there are many such ATP-dependent remodelling complexes in yeast and higher eukaryotes [reviewed in ref. 9]. Evidence from restriction site accessibility studies suggests that the ATP-dependent SWI/SNF remodelling subunits from the SWI/SNF complexes continuously generate multiple alternative DNA conformations. This provides multiple opportunities for the binding of regulatory factors to DNA to activate or repress transcription [124].

In higher eukaryotes, cytosine bases within the dinucleotide sequence CpG are often methylated and this also

plays an important role in the regulation of chromatin structure and the control of gene expression. Whilst heterochromatin is generally heavily methylated, CpG methylation patterns in euchromatin are complex, with areas of heavy methylation and areas of unmethylated CpG sequences, sometimes referred to as unmethylated CpG islands. DNA methylation is carried out by DNA methyltransferases (DMTs) and CpG sequences are methylated on both strands. DNA replication results in hemi-methylated DNA and this is a substrate for maintenance DMTs. Consequently, CpG methylation patterns are stably inherited and, indeed, these patterns play a key role in epigenetic phenomena such as dosage compensation and imprinting [reviewed in ref. 125]. Many observations link DNA methylation, histone modification and the repression of transcription. CpG methylation can often directly block the binding of transcription activators and thereby bring about repression [126]. In addition, CpG methylation can also block the binding of transcription factors indirectly by changing the conformation of chromatin and/or by recruiting methyl-CpG-binding proteins. Several methyl-CpG-binding proteins have been described and shown to compete with transcription factors for binding to CpG-methylated DNA. In addition, these methyl-CpG-binding proteins can bring about changes in chromatin structure in part at least by recruiting HDACs [127–129]. More recently, in the filamentous fungus *Neurospora crassa*, HMT activity has been shown to be required for CpG methylation, and thus in this organism, DNA methylation appears to be dependent upon histone methylation [130]. Furthermore, several repressor proteins recruit DMTs [28, 29, 131]. The class I repressor protein RP58, for example, binds to Dnmt3. However, the enzymatic activity of Dnmt3 does not appear to be required for RP58-mediated repression. Instead, Dnmt3 interacts with HDAC1 to bring about repression [28]. Similarly, Rb forms a complex with Dnmt1 and HDAC1 that can repress E2F responsive promoters and, again, the DMT activity of Dnmt1 does not appear to be required for repression [131]. Thus, although DMT proteins function as co-repressors, their ability to methylate DNA does not appear to be required, at least for short-term repression. Of course, long-term transcriptional repression might be a different matter and the ability to methylate DNA might be important in the maintenance of the repressed state through multiple rounds of cell division [reviewed in ref. 132].

Lessons from gene silencing

Three groups of chromatin-binding proteins that negatively regulate transcription have been characterised in detail: the Sir proteins, the HP1 family and the PcG proteins [133, 134]. The Sir proteins are required for telomeric silencing and the silencing of mating type in *S. cerevisiae*. These proteins can homo- and heterodimerise and they are thought to form a highly stable transcriptionally repressed state that spreads along the chromatin fibre. At telomeres, the DNA-binding protein Rap1p [135] recruits Sir3p and Sir4p [136] and the yeast telomere end-binding complex Ku70/80 helps in this process [137, 138]. At the mating type loci, the DNA-binding proteins Rap1p, ORC and Abf1 are together responsible for the recruitment of Sir proteins. Sir1p binds to ORC and can recruit Sir4p [139]. Both Sir4p and Sir3p bind directly to the N-terminal tails of histones [140] and Sir3p has been found to propagate along nucleosomes [141]. Sir4p is thought to recruit Sir2, an NAD-dependent histone deacetylase [142] and histone deacetylation is in turn thought to favour the assembly of Sir complexes.

The mechanisms that bring about pericentric centromeric silencing in the fission yeast *Schizosaccharomyces pombe* have been elucidated in some detail. Chromatin immunoprecipitation assays have identified a number of proteins that are localised to different regions of the *S. pombe* centromeres including Mis6, Chp1 and Swi6. Swi6 is a member of the HP1 family of chromatin-binding proteins [143] that also includes the *Drosophila* protein SU(VAR)2-5 [144] and several mammalian HP1 proteins including HP1α, Hp1β and Hp1γ [145] (reviewed in [146]). These proteins are essential for the silencing of pericentric heterochromatin in *Drosophila* and mammalian cells. HP1 proteins are involved in a phenomenon known as position effect variegation (PEV). This refers to the silencing of genes that are placed adjacent to regions of pericentric heterochromatin (reviewed in [147]). Swi6/HP1 can coat and mediate the silencing of large noncentromeric DNA inserts within centromeric DNA [148]. The HP1 proteins bind to histones [149] and bind with high affinity to methylated histones using a domain known as a chromodomain [150, 151]. They can also self-associate using a domain known as a chromoshadow domain [149]. In *S. pombe*, the HDACs Clr6 and Clr3 are thought to remove acetyl groups from the N-terminal tail of histone H3 at lysine 9 and lysine 14, respectively. The HMT Clr4 can then methylate histone H3 on lysine 9 creating a high-affinity binding site for Swi6/HP1 [152]. Since Swi6/HP1 proteins can self-associate, bind to histones, and silence genes placed within centromeric DNA and adjacent to pericentric chromatin, it seems likely that these proteins silence by 'spreading' along the chromatin fibre in a manner analogous to the Sir proteins. Recent experiments suggest that RNA interference is involved in gene silencing in *S. pombe* [153–155]. In cells, double-stranded RNAs are processed into small interfering RNAs (siRNAs) that target mRNA transcripts with the same sequence for degradation. *S. pombe* mutants defective in siRNA processing also show a loss of methylation at histone H3 lysine 9 and loss of binding of Swi6 [155]. Proteins involved in siRNA pathways will likely also play

an important role in the establishment and maintenance of heterochromatin in other species.

Like the HP1 proteins, PcG proteins contain chromodomains suggesting that these proteins also bind to methylated histones [146, 156]. PcG proteins are found as homo- and hetero-oligomers in complexes that are associated with the maintenance of transcriptionally inactive homeotic gene complexes in *Drosophila* [157–159] and implicated in the control of the cell cycle and oncogenesis in humans [160, 161]. The PcG complexes appear to retain a molecular memory of the early state of activity of a gene complex such that once a gene has been silenced, it remains silent even after many rounds of cell division. PcG proteins use several mechanisms to repress transcription [reviewed in refs 162, 163]. In vitro studies with purified hPRC1 demonstrate that this human PcG complex inhibits chromatin remodelling. hPRC1 somehow alters the regulated locus such that it excludes SWI/SNF remodelling complexes. However, the DNA is still open to attack by micrococcal nuclease, suggesting that although PcG proteins exclude remodelling factors, the regulated locus is still accessible to other proteins [158, 164]. This finding is broadly in agreement with the fact that PcG complexes and GTFs can co-occupy promoters and that PcG complexes can also repress transcription by interacting with the basal transcription machinery [76, 77]. PcG complexes could also bring about the modification of histones, as some PcG proteins can recruit HDACs [165]. Alternatively, or in addition, the ability of PcG proteins to homo- and hetero-oligomerise suggests that PcG proteins could form 'spreading' complexes [149].

In the examples given above, chromatin-binding proteins from the SIR and PcG complexes act at specific sites because they are able to associate with DNA-binding proteins. The Sir proteins are recruited to DNA by DNA-binding complexes that include the Rap proteins. Several gene-specific repressors are thought to repress transcription by recruiting the PcG chromatin-binding proteins (fig. 4A). Repressors that recruit PcG complexes include RYBP, a class I repressor, and CtBP, a class II repressor. Repression by PcG proteins involves interactions between distal control elements (PREs) and promoters [166, 167]. RYBP binds to both YY1 and PcG proteins and the interaction between RYBP and YY1 is thought to be instrumental in targeting PcG complexes formed at distal PREs to RYBP-dependent promoters to bring about promoter-specific repression [168]. Like RYBP, the class II repressor CtBP can recruit PcG proteins. CtBP recruits HPC2 at some promoters [169] and is found in PcG complexes with the Rb protein [8]. In contrast to these gene-specific repressor proteins that recruit chromatin-binding proteins, some repressor proteins are themselves chromatin-binding proteins. For example, the class II repressor Tup1 can position nucleosomes at the STE6 gene [170]. Tup1 is recruited to promoters by several class I repressor proteins. The interaction between Tup1 and the class I repressor proteins is not direct, but is mediated by the Tup1-interacting protein Ssn6 [reviewed in ref. 171]. Tup1 and Groucho can bind directly to deacetylated histones H3 and H4 [172–174]. Tup1 has been suggested to repress transcription by interacting with hypoacetylated histones located close to the promoter [172]. Several observations have led to the proposal that Tup1/Groucho proteins bring about long-range repression by spreading along the chromatin fibre and recruiting HDACs in a manner similar to that observed for the Sir silencing complexes [reviewed in refs 18, 19]. First, members of the Groucho family of proteins allow the repression of promoters in a distance- and orientation-independent manner (long-range repression) [16, 19]. Second, chromatin immunoprecipitation assays with Tup1 at the yeast STE6 locus have shown that Tup1 is associated with the entire genomic STE6 coding region [175]. Third, Groucho and human TLE/Groucho proteins assemble into large oligomeric structures [173, 176, 177]. Finally, both Tup1 and Groucho proteins interact with histone deacetylases [178–180] (see below). The formation of spreading complexes by Tup1 is, however, still the subject of some controversy [180].

Chromatin-remodelling complexes

Many proteins involved in chromatin remodelling appear to be components of large multi-functional complexes, such as the NuRD and SIN complexes. Recruitment of any of the components of these complexes by a repressor is likely to result in the recruitment of all the proteins in either complex to the promoter (fig. 4B). The mammalian SIN complex contains chromatin-binding proteins, histone-modifying enzymes, and the Sin3 class II repressor protein. Similarly, the mammalian NuRD chromatin-remodelling complex contains chromatin-binding proteins, histone-modifying enzymes and ATP-dependent remodelling activities, and MBD3, a protein that contains a methyl-CpG-binding domain [129]. MBD3 does not bind directly to CpG-methylated DNA but instead associates with two components of NuRD, HDAC-1 and MTA2 [181] and also interacts with the methyl-CpG-binding protein MBD2 [29]. Recently, the MeCP1 complex was purified and found to contain both MBD2 and NuRD; this complex preferentially binds, remodels and deacetylates methylated nucleosomes [182]. Thus the NuRD complex also has the potential to bind methylated DNA.

A number of class I repressor proteins recruit NuRD and SIN to promoters by binding to class II repressors associated with these complexes. Examples of repressor proteins that recruit the SIN complex are the Mad/Max heterodimer, which requires the class II repressor proteins PML and c-ski for recruitment of the complex [183], and the unliganded nuclear hormone receptors, which require

the presence of the class II repressor protein NcoR/SMRT [reviewed in ref. 184]. Similarly, NuRD can be recruited by the class I repressors Hunchback, Ikaros, Aiolos and Tramtrack 69 (Tk69). These repressors interact directly with Mi-2, the ATP-dependent remodelling subunit of NuRD, which also contains chromatin-binding motifs [185–187].

As might be expected from our knowledge of the formation of silenced heterochromatin, repressor proteins that function within euchromatin also appear to recruit both HMTs and HDACs to bring about repression. For example, Rb interacts directly with both HDAC1 and the HMT Su(Var)39H1 and both histone deacetylation and histone methylation contribute to Rb-mediated repression [3, 4, 6]. Histone methylation by Su(Var)39H1 results in the formation of a high-affinity binding site for HP1. As yet, the precise function of HP1 in repression by Rb in euchromatin is not known [5]; however, it is likely to be involved in the initiation and propagation of inactive chromatin. In contrast, E2F-6 is a class I repressor protein that represses transcription, independently of Rb, by recruiting a complex that contains HMT activity but which does not appear to contain HDAC activity [188]. This could be an example of a repressive complex where the formation of inactive chromatin occurs in the absence of histone deacetylation. E2F-6 is a member of the E2F family of transcription factors but lacks the Rb-interacting domain and transcription activation regions present in the other family members. A complex known as E2F-6.com is present at the promoters of E2F-dependent genes when there is no requirement for transcription, for example, when cells are in the quiescent state. E2F-6.com contains chromatin-binding proteins and HMT activity, as well as DP-1 (the heterodimeric binding partner for E2F) and two other DNA-binding proteins, Mga and Max. Presumably, the local methylation of histones by the HMT in E2F6.com allows the binding of chromodomain proteins such as HP1 and PcG proteins, and this nucleates the formation of complexes that propagate inactive chromatin [188].

Somewhat surprisingly, relatively few class I repressors appear to interact directly with HDACs. Two class I repressors that do are YY1 and MeCP2. YY1 can both activate and repress transcription and this protein interacts directly with HDAC1 [189]. Interestingly, YY1 is related to Pleiohomeotic, a PcG protein from *Drosophila* [190]. MeCP2 is a methyl-CpG-binding protein that can interact directly with HDAC-1 [127, 128]. In contrast with class I repressors, most, if not all class II repressor proteins interact with HDACs directly and recruit them to the promoter. Rb [3, 4], TGIF [44], CtBP [191, 192] and Tup1/Groucho [178, 179], for example, all interact with HDAC s from the Rpd3 (HDAB/class I) group of HDACs. Both Tup1/Groucho and CtBP also interact with HDACs from the HDAA/class II group. Tup1 interacts with HDA1 [180] and CtBP with HDAC5 [193]. Not yet clear is what differences with regard to transcriptional repression result from recruiting members of these different groups of deacetylases. However, recruitment of HDA1 (HDAA group) by Tup1 has been demonstrated to lead to the specific deacetylation of histones H3 and H2AB within the ENA1 promoter [180]. In contrast, recruitment of Rpd3 (HDAB group) by Tup1 at the ENA1 locus leads to deacetylation of all four histones located within the ENA1 coding sequence. This suggests that Rpd3 could be important for a more global deacetylation of a chromatin domain [180].

There are a number of examples where gene-specific transcriptional repression is brought about by the interaction of activator proteins with chromatin-remodelling factors. For example, the direct interaction of NFκB with HDAC1 and the indirect interaction of NFκB with HDAC2 through HDAC1 can bring about the repression of NFκB-regulated genes. This is believed to be important because although most NFκB is held in the cytoplasm by IκB, some NFκB is present in the nucleus even when the transcription of NFκB-activated genes is not required. Inhibition of HDAC enzymatic activity using the inhibitor Trichostatin A (TSA) prevents repression by NFκB and leads to the acetylation of histones near the regulated promoter. Repression in this case is proposed to have two components. First, the interactions between the activator and the HDACs cause a local chromatin organisation that decreases basal transcription. Second, the interaction of deacetylases with the activator 'passively' prevents protein-protein interactions between the activator and chromatin re-organising proteins that have a positive role in transcription [194]. Similarly, under some conditions, repression of promoters activated by the SMAD2/SMAD4/SMAD3 complex and the Fast activator can be brought about by recruitment of HDAC1 to the promoter. In this case, the interaction of HDAC-1 with the activator is indirect and occurs via the class II repressor TGIF [35, 44].

Finally, and as mentioned earlier, some repressors recruit ATP-dependent chromatin-remodelling proteins. Rb, for example, interacts with hBrm/BRG1, the ATP-dependent remodelling subunit from the human SWI/SNF complex. Although the human SWI/SNF complex is generally associated with the activation of transcription, Rb can interact with hBrm and simultaneously with HDAC1, to repress transcription of the G1 phase-transcribed cyclin E gene. The Rb-hBrm complex is also able to repress transcription of the S phase-transcribed cyclin A and cdc2 genes apparently in the absence of HDAC1, suggesting that in this case, ATP-dependent chromatin remodelling may be sufficient for repression [7]. Recently, CtBP and the PcG proteins HPC2 and Ring1 were also demonstrated to be present in the complex containing Rb at the cyclin A promoter. This complex, in association with hSWI/SNF activity, is required for the repression of cy-

clin A promoter activity [8]. Thus, this appears to be a case where ATP-dependent nucleosome-remodelling proteins and PcG proteins co-operate to repress transcription, possibly in the absence of histone-modifying enzymes. The transcription factor Ikaros [195] co-localises with HP1 in heterochromatin [196] and can also interact with Mi-2, the ATP-dependent remodelling subunit of NuRD, HDACs [186], CtBP [192] and with other proteins in the Ikaros family [197]. Interestingly, in cells, Ikaros-repressed genes are physically located with centromeres in a heterochromatin environment. However, when these genes are transcribed they are located within euchromatin [196, 198]. Ikaros-binding sites are found both at the promoters of repressed genes and within pericentric heterochromatin. Thus Ikaros is thought to bring about repression by moving genes that are to be silenced near to regions of heterochromatin and/or by recruiting silencing complexes to the promoter [198]. Thus, in this case, nuclear organisation appears to be important for the regulation of transcription [199].

Nuclear compartmentalisation

Nuclear organisation or compartmentalisation appears to regulate gene expression and hence transcription [reviewed in ref. 200]. The nuclear periphery of budding yeast is a compartment associated with gene silencing. The silenced telomeres of yeast are often localised to the nuclear periphery and are clustered into foci [201]. Moreover, tethering of genes to the nuclear membrane leads to silencing [202]. However, there are also compartments present in the nuclear periphery which are associated with inhibiting the spread of repressive heterochromatin. These compartments contain proteins that bind a central protein of the nuclear pore complex, Nup2p, and that can also bind DNA elements within boundary/barrier regions (regions of DNA that block the spread of heterochromatin). When these proteins are tethered to specific DNA elements within the boundary region and to the nuclear pore, the spread of repressive heterochromatin is blocked [203]. Another nuclear compartment associated with the regulation of transcription is the scaffold/matrix attachment region (S/MARs). MARs are chromatin regions that bind the nuclear matrix. The attachment of DNA to the nuclear matrix via MARs and interaction with MAR-binding proteins have been shown to alter chromatin conformation, promote an extended domain of histone acetylation, and thus influence transcription [204, 205]. One way that MARs may be able to direct the acetylation of an extended chromatin domain is by the recruitment of the co-activator CBP/p300. CBP/p300 binds to the MAR-binding protein scaffold attachment factor A (SAF-A), a major constituent of the nuclear matrix. Both p300 and SAF-A bind to MAR elements in the transiently silent topoisomerase I gene before its activation at G1 during the cell cycle [206]. This binding is accompanied by the acetylation of nucleosomes. Thus, the binding of MAR sequences by SAF-A and p300 likely results in activation via the initiation of acetylation within a chromatin domain [206]. Proteins that interfere in this process would be expected to bring about repression. The Bright transcription activator and nuclear matrix protein binds to MARs flanking the immunoglobulin heavy chain intronic enhancer (Emu). The Cux/CDP homeodomain protein is a repressor that competes with Bright for binding to these MAR sequences. In this way, Cux/CDP can eliminate the activatory effect of the MAR on transcription [207]. In summary, nuclear compartmentalisation is an important factor influencing gene regulation. The proteins that alter gene activity in this context ultimately appear to exert their effects on transcription by influencing chromatin organisation.

Conclusions

The rich variety of repression mechanisms that have been documented suggest that the proteins that repress transcription may be structurally equally diverse. Indeed, identifying a single feature common to all repressor proteins is impossible. Rather, similar repression motifs will more likely be found amongst proteins that share a common mechanism. For example, many repressors that inhibit basal transcription by interaction with the GTFs appear to contain alanine/proline-rich motifs, and hydrophobicity is thought to be an important characteristic for this type of repressor. Similarly, repressors that function by binding to class II repressor proteins such as Groucho contain specific protein-protein interaction motifs. As more repressors are characterised in detail, these motifs should become increasingly obvious and the mode of action of each repressor should be more easily predicted.

In this review we have considered enzymes that post-translationally modify DNA-binding proteins, chromatin-binding proteins or components of the PIC as repressors. Clearly, many of these proteins are also the end players of signal transduction pathways. The post-translational modification of proteins involved in gene regulation allows the rapid switching of transcriptional states. This is particularly important during short-term changes in gene expression such as those seen in response to growth factor stimulation. The modification of histones and other proteins involved in gene regulation may be ideally suited to this type of gene regulation. However, long-term gene regulation, such as the repression of genes during cell differentiation, may call for a different approach that includes histone and DNA modifications and the association of chromatin-binding proteins. The association of Rb, CtBP and the PcG proteins at some

E2F-dependent promoters has been suggested as a link between the long-term inhibition of cell proliferation and the differentiation events that lead to embryonic pattern formation [8]. Moreover, complexes containing both HP1 and PcG proteins are found at E2F-dependent promoters in cells that are quiescent.

There are many future challenges in understanding gene regulation and transcriptional repression. However, understanding the biochemical nature of chromatin fibres and their role in the control of gene expression must represent a key objective. The role that DNA methylation plays in the structure of heterochromatin and a detailed understanding of the biochemical nature of heterochromatin and euchromatin is still lacking. Furthermore, although short-range repression is relatively well understood, for example when a repressor makes direct contact with a specific activator or its target, how long-range repression mediated by large assemblies of chromatin-binding proteins is brought about is still unclear. For example, we do not yet know why the recruitment of Groucho tends to bring about long-range repression whereas the recruitment of CtBP tends to bring about short-range repression, even though both proteins recruit HDACs. One possibility is that Groucho oligomerises and spreads along the chromatin fibre causing the expansion of a repressive domain similar to that seen in silenced heterochromatin (fig. 4B). Although CtBP recruits PcG proteins, perhaps these proteins form complexes that do not so closely resemble silenced heterochromatin, thus resulting in the repression of smaller domains or in repression that is more easily reversed. Or perhaps PcG complexes repress transcription in these situations predominantly by contacting the basal machinery. Lessons learned from the study of gene silencing and position effect variegation, areas of research once regarded by some as esoteric, are sure to continue to throw light upon these questions.

A question that is related to an understanding of the nature of chromatin is whether genes travel to specific areas in the nucleus to be repressed or whether they need to be excluded from some areas in order to remain active. Recent work has shown that in yeast cells, the tethering of genes to the nuclear pore complex prevents the spreading of heterochromatin and the consequent gene silencing [203]. Conversely, genes repressed by Ikaros appear to be localised near to heterochromatin. Repression factories, analogous to the transcription factories that are believed to contain groups of transcriptionally active genes, may exist or most genes may be repressed in situ. The main advantage of a repression factory would be that repressors, and perhaps more importantly co-repressors, could stay with the RNA polymerase and repress incoming genes as required. The advantage of repressing genes individually could be that stray RNA polymerases would be unable to transcribe genes that should be repressed. A deeper understanding of the nature of transcription factories and the ultrastructure of the nucleus itself should help to establish the relative importance of these modes of repression.

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