REVIEW ARTICLE

Transcriptional control and the role of silencers in transcriptional regulation in eukaryotes

Steven OGBOURNE¹ and Toni M. ANTALIS

Queensland Cancer Fund Experimental Oncology Program, The Queensland Institute of Medical Research, Brisbane, 4029 Queensland, Australia

Mechanisms controlling transcription and its regulation are fundamental to our understanding of molecular biology and, ultimately, cellular biology. Our knowledge of transcription initiation and integral factors such as RNA polymerase is considerable, and more recently our understanding of the involvement of enhancers and complexes such as holoenzyme and mediator has increased dramatically. However, an understanding of transcriptional repression is also essential for a complete understanding of promoter structure and the regulation of gene expression. Transcriptional repression in eukaryotes is achieved through 'silencers', of which there are two types, namely 'silencer elements' and 'negative regulatory elements' (NREs). Silencer

elements are classical, position-independent elements that direct an active repression mechanism, and NREs are position-dependent elements that direct a passive repression mechanism. In addition, 'repressors' are DNA-binding transcription factors that interact directly with silencers. A review of the recent literature reveals that it is the silencer itself and its context within a given promoter, rather than the interacting repressor, that determines the mechanism of repression. Silencers form an intrinsic part of many eukaryotic promoters and, consequently, knowledge of their interactive role with enhancers and other transcriptional elements is essential for our understanding of gene regulation in eukaryotes.

INTRODUCTION

The phenotypic differences that characterize the multitudes of cells that we observe in higher eukaryotes are largely due to differential gene expression within those cells. Hence, precise mechanisms which govern the required transcriptional regimes must exist. Some mechanisms control the constitutive expression of 'housekeeping genes', while others determine a rapid increase in expression as a consequence of external stimuli; others still are responsible for the complete inactivation of gene expression in specific tissues. Research into the field of gene regulation is fast unravelling the secrets of transcription; however, it is just as quickly realizing its seemingly boundless complexities.

A relatively new concept in this field is the recognition that, in addition to the 'switching on' of gene expression through enhancer elements, gene expression may be actively repressed through 'negative regulatory' genetic elements known as silencer elements or negative regulatory elements (NREs). In the present review we will examine the growing body of literature implicating eukaryotic silencers in the regulation of gene transcription, with an emphasis on the molecular interactions involved in silencer function. In order to put these data into perspective, we will first briefly review the various factors and transcriptional apparatus which contribute to the control of gene expression at the basal level, but the reader will be referred to more comprehensive reviews throughout this summary.

THE CONTROL OF GENE EXPRESSION

There are several key points at which gene expression is controlled in eukaryotic cells, including activation of gene structure, transcription initiation, termination of elongation, nuclear RNA processing, mRNA transport, mRNA translation, and mRNA stability. Although protein processing, targeting and stability are also integral to the overall regulation of protein production within eukaryotic cells, their precise roles are beyond the scope of the present review and will not be discussed here.

Activation by chromatin priming and DNA methylation

The highly organized and densely packed structure known as chromatin, which physically inhibits responses to initiation factors and transcription of DNA [1], must be activated at an early stage if transcription initiation is to occur (reviewed in [2–4]). The unravelling or priming of chromatin may be regulated by acetylation or methylation of the free amino groups of lysine residues at the N-terminal end of histone molecules [5,6], resulting in a decreased net positive charge and a subsequent lower affinity for the DNA phosphate backbone (reviewed in [7]). Demethylation of DNA contained in the promoter region of the target gene has also been reported to be involved in chromatin priming [8,9].

Recent work has identified specific mechanisms which regulate transcription via DNA methylation. For example, methylation

Abbreviations used: RNA pol I, II and III, RNA polymerase I, II and III; CTD, RNA pol II C-terminal domain; GTF, general transcription factor; NRE, negative regulatory element; GTF, general transcription factor; UTR, untranslated region; TBP, TATA-binding protein; TAFs, TBP-associated factors; TFIIA, TFIIB etc., transcription factor IIA, IIB etc.; NRSF, neuron restrictive silencer factor (also called 'REST'); NRSE, neuron restrictive silencer element [also called repressor element-1 ('RE-1')]; hTSH β , human thyrotropin β gene; MAR, matrix-attachment region; EMSA, electrophoretic-mobility-shift assay; PAI-2, plasminogen activator inhibitor type-2; CAT, chloramphenicol acetyltransferase; DSP1, dorsal switch protein 1; YY1, nuclear factor Yin-Yang 1; HPV16, human papillomavirus type 16; SV40, simian virus 40; Pc-G, polycomb group; hb, hunchback; TPBF, TBP-promoter-binding factor; SMRT, silencing mediator for the retinoid and thyroid-hormone receptors; N-CoR, nuclear-receptor co-repressor; IFN- γ , interferon- γ ; HDAC, histone deacetylase.

¹To whom correspondence should be addressed (e-mail toniA@qimr.edu.au).

of cytosine residues at cytosine–guanine (CpG) sites has been shown to provide a binding site for MeCP2, an abundant mammalian protein capable of transcriptional repression [10]. Where methylation exceeds a threshold level [11], MeCP1 has been shown to form a complex with DNA, and it seems likely that this complex results in transcriptional repression [12]. Finally, histone H1 and methylated DNA-binding protein H1 have also been suggested as candidates for generalized methyl-CpG-binding proteins [10].

Initiation of transcription

Factors controlling RNA synthesis, structure and stability are all clearly important in the regulation of gene expression. It is generally accepted, however, that the majority of regulatory events that control gene expression occur at initiation, that is, the processes which orchestrate the assembly of the transcription apparatus and more specifically, the positioning of RNA polymerase II (RNA pol II) at the promoter. These events and their relevance to silencer function are examined in more detail later in the present review.

Termination of elongation

Once initiation is complete, nRNA synthesis occurs via one of three RNA polymerases, and finally elongation of the nRNA transcript is terminated. Termination of elongation with RNA pol I occurs at a discrete 18 bp recognition sequence found between 100 and 4000 bp downstream of the mature 3' end [13,14], while RNA pol III terminates at the second, third or fourth uridine in a run of four uridine bases surrounded by GC pairs [15,16]. The mode of termination for RNA pol II is less clear. In fact, precise termination signals within a 'termination region' are yet to be defined. Fortunately, it is not the termination of elongation, but cleavage of the primary transcript, which is the significant event in determination of the 3' end. The precise cleavage point is signalled by the consensus sequence 5'-AAUAAA-3' (reviewed in [17,18]), and being located 11–30 bp upstream of the future polyadenylation sequence, the consensus also determines the start point of the poly(A) tail [13].

Post-transcriptional regulation

Maturation of nRNA to mRNA requires splicing of non-coding intronic sequences, 3' polyadenylation, and 5' capping. The 5' end is 'capped' by the addition of a methylated guanine residue after approx. 30 nucleotides have been synthesised [19,20]. The 5' cap is an important signal for the selection of the start AUG by the ribosome complex during initiation of translation, and also appears to protect the RNA molecule from 5'–3' degradation [21,22]. The 3' end, which is modified by the addition of 100–200 adenosine residues [18,23], aids in the export of the RNA molecule to the cytoplasm.

Splicing of introns occurs at highly conserved sequences located at both the 5' and 3' ends of introns. These consensus sequences, termed the donor site or 5' splice site [5' (C/A)AGGU(A/G)AGU 3'] and acceptor site or 3' splice site [5' (U/C)11N-(U/C)AG(G/A) 3'], signal the precise location for excision by the spliceosome and subsequent removal of an intron [24,25]. The two splice sites overlap the intron/exon boundaries. The first bases found within the intron of the 5' splice site, and the last two intronic bases of 3' splice sites, are underlined.

Mechanisms controlling intron splicing are integral in controlling the final structure of the protein molecule and are implicated as potential points of transcriptional regulation [26,27]. Indeed, transport of RNA to the cytoplasm will not occur

until intron splicing is complete. For example, if the spliceosome is still attached to the RNA, it will not be recognized by the receptor proteins within the nuclear-pore complex [28] that facilitate their transport to the cytoplasm. Translational control mechanisms also regulate overall gene expression; however, like mRNA transport, they do not govern transcriptional regimes and are beyond the scope of the present review.

THE CONTROL OF TRANSCRIPTION INITIATION

RNA polymerase

As noted above, transcription initiation is a key control point of gene transcription, the key enzyme to which is RNA polymerase. RNA polymerases are large proteins, being complexes of 8–14 subunits and of molecular mass 500 kDa or more. In eukaryotic cells, transcription is carried out by one of three RNA polymerases, each with different specificities. (a) rRNA is transcribed by RNA pol I, is localized to the nucleolus and is responsible for approx. 50–70 % of the total RNA polymerase activity; (b) RNA pol II synthesizes heteronuclear RNA; and (c) RNA pol III synthesizes tRNA, 5 S RNA and small nuclear RNA. RNA pol II and III are both localized in the nucleoplasm and account for approx. 20–40 % and 10 % of the total RNA polymerase activity respectively.

Assembly of the general transcription factors (GTFs)

RNA pol II, which is responsible for differential gene expression in eukaryotic organisms, is completely dependent on auxiliary transcription factors (TFs) to allow it to initiate transcription. The complex of RNA pol II and auxiliary TFs are known as the basal transcription apparatus or GTFs. The individual components of the GTFs congregate at the proximal promoter in a highly regulated and defined order [29–31], forming a complex of molecular mass greater than 2500 kDa [32], which provides a platform for the recruitment of RNA pol II that subsequently initiates transcription at a precise site known as the transcription initiation site (Figure 1).

Eukaryotic promoter sequences contain a core promoter which is sufficient to signal transcription initiation, and in humans it is located approx. –45 bp to –20 bp relative to the transcription initiation site. In most eukaryotic core promoters an element known as the TATA box is located approx. 25 bp upstream of the transcription initiation site. This element is an 8 bp consensus sequence (5' TATAAAA 3'), which is surrounded by GC-rich sequences and is recognized by TATA-binding protein (TBP) [33].

TBP, the only GTF to make sequence-specific contacts with the DNA, begins GTF assembly by binding to the TATA box. A group of proteins known collectively as TBP-associated factors (TAFs) [34] then bind to TBP, together making up the complex known as transcription factor IID (TFIID) [34–36]. The recently identified GAGA factor has been shown to bind to the proximal region of the *hsp70* promoter [37] and may be capable of priming the chromatin to facilitate TFIID binding [38]. Interestingly, TAFII250 contains histone acetyltransferase activity [39] and, as a result, recruitment of TFIID may be sufficient to complete chromatin priming in the vicinity of the proximal promoter.

GTF assembly continues with the recruitment of TFIIA to form the transcription factor IIA (TFIIA)–TFIID complex. TFIIA–TFIID must at this stage undergo a conformational change or isomerization to allow binding of TFIIB and the remaining GTFs [40,41]. Binding of TFIIB then facilitates association of the pre-assembled TFIIF–RNA pol IIa (non-phosphorylated form) [42] with the growing complex. TFIIE

Figure 1 GTF assembly at a typical eukaryotic promoter

A general eukaryotic promoter is shown, containing a TATA box (TATA), an initiator motif (INR), which overlaps the transcription initiation site (arrow), and two GAGA elements (GAGA, not coloured). One or more GAGA factors may interact with one or more GAGA elements, allowing interaction of the TBP (pink). Several TAFs (pale pink) then join the complex followed by TFIIA (TFs not coloured). The TFIIA–TFIID complex must then isomerize to allow further stepwise addition of TFIIB, RNA pol IIa (red)—TFIIF dimer, TFIIE, TFIIH and the mediator (dark pink) complex. Note that the frequency of initiation can be increased by interaction with the pre-formed RNA pol II holoenzyme. DNA melting and CTD phosphorylation, which together stimulate promoter clearance, must occur to allow elongation to take place. Recycling of various parts of the spent GTFs (broken arrows) provide mechanisms to further increase the rate of re-initiation.

then interacts directly with RNA pol IIa and subsequently recruits TFIIB to the C-terminal domain (CTD) of RNA pol IIa [42]. A large aggregate of proteins known as mediator, consisting of 20 or so proteins, including several suppressors of RNA polymerase B (II) proteins ('SRBs') [43], then assemble at the CTD of RNA pol IIa [43–45].

Although initiation of transcription is effectively complete at this stage, the CTD of RNA pol IIa must be phosphorylated in order for the transition from initiation to elongation to take place [46,47]. Phosphorylation of the CTD allows the removal of TFIIB, TFIIE and TFIIH (promoter clearance) [30,48,49], which in turn permits RNA pol IIo (phosphorylated form of RNA pol II) [49,50] to commence elongation [42]. Once elongation has been terminated, RNA pol IIo detaches from the DNA and, before it can re-enter initiation, the CTD must be dephosphorylated [47,51]. Assembly of the GTFs has been extensively reviewed in [30,49,52].

More recently a large multisubunit complex known as RNA pol II holoenzyme, containing RNA pol II, TFIIB, TFIIB and mediator [53,54] has been identified that can assemble independently of a promoter [44,45]. The identification of RNA pol II holoenzyme that is capable of responding to enhancers [43] suggests a different model for transcription-apparatus assembly. In this model, transcription enhancers first initiate the interaction of TFIID with the TATA box [34,55]. The same or other enhancers then interact with RNA pol II holoenzyme to facilitate its association with the TFIID–TFIIA promoter complex [53]. If we assume that stepwise and holoenzyme assembly occur simultaneously *in vivo*, it is likely that the frequency of initiation may be increased by enhancers through RNA pol II holoenzyme recruitment. Furthermore, promoter clearance leaves TFIID, TFIIA and the TAFs pre-assembled at the TATA box, providing another point at which stepwise assembly can be truncated.

Non-structural functions of the GTFs

Although many of the factors involved in basal-transcription-apparatus assembly have only been shown to possess structural roles, some are known to perform enzymic functions that contribute to the initiation of transcription. For example, TFIIE facilitates the recruitment of TFIIB, but sequence analysis has revealed structural similarities to a bacterial DNA repair protein UvrA [56]. TFIIB has two subunits, one of which has an ATP-dependant helicase activity which may be involved in melting the DNA at initiation [54,57,58]. TFIIB has a kinase activity which can phosphorylate the CTD [57], and it appears that mediator confers a 30–50-fold stimulation of this TFIIB-dependent phosphorylation [44,59]. Finally, RNA pol IIo dephosphorylation has been attributed to the holoenzyme complex [47,51,60].

It should be noted that assembly of the GTFs and initiation of transcription differs with each RNA polymerase and is likely to change with different promoters [61]. Furthermore, many genes have now been identified which possess TATA-less promoters [62]. These appear to rely on upstream TFs such as Sp1 [63], an element known as the *ETS* motif (GGCTTCCTGTCT) [64,65] or the pyrimidine-rich initiator motif (CTCANTCT) [66,67], to initiate transcription (reviewed in [68]).

REGULATION OF TRANSCRIPTION INITIATION

The presence of a core promoter sequence and assembly of the basal transcription apparatus is all that is required to initiate transcription of any given gene. However, the levels of tran-

scription that are achieved at such a promoter are minimal, and upstream factors are required to modify transcriptional rates. Early ideas suggested that transcription would only occur if the given gene was actively 'switched on' as a result of TFs interacting with enhancers. It now seems clear that genes will be constitutively expressed at a minimal rate determined by its core promoter unless the rate of transcription is increased by an enhancer or is 'switched off' by the actions of a silencer (reviewed in [69]).

Enhancers

Because the concept of the enhancer and its role in promoter function was realized far earlier than that of the silencer, our knowledge of enhancers is considerable in comparison with that of silencers. Many enhancers and their corresponding proteins have been identified, and it is now clear that several are common to the majority of promoters. For example, the CCAAT box, named after its 9 bp consensus of 5' GGCCAATCT 3', is an enhancer generally located around 75 bp 5' of the transcription initiation site and is recognized by CTF/NF1 [70,71]. The GC box element is a 6 bp consensus sequence (5' GGGCGG 3'), which is present in the majority of eukaryotic promoters. It is generally located within the first 100 bp upstream of the transcription initiation site and is recognized by Sp1 [72].

Elements such as CCAAT and GC boxes are obviously paramount in increasing transcriptional rates to significant levels. However, a large number of other motifs, such as the 'response elements' (e.g. glucocorticoid response element, 'GRE' [73,74]) have also been identified which respond to external stimuli and alter rates of gene expression. These motifs are usually located further upstream than GC and CCAAT boxes, but in general they exert their effects on transcriptional rates in the same manner.

Mechanisms of enhancer mediated regulation

Initially it was thought that enhancers carried out their function through physical interactions with RNA pol II [75,76]. Now it is believed that their effects are indirect, involving intermediary factors that convey signals from activators to the transcription proteins [59]. Mediator was identified as one such intermediary factor by experiments which mutated the CTD, such that mediator could no longer bind, in turn resulting in the abolition of effects seen by activator proteins [77]. Sp1 was found to be the sole exception to this observation and, considering that Sp1 interacts with TAFs [34,35,78], TAF proteins were nominated as a second intermediary factor. Upstream stimulatory activity ('USA') [79] is currently under investigation as a third intermediary factor, and others are likely to exist.

Early summaries of promoter structure and function suggested that upstream factors and response elements were restricted to well-defined areas of the gene and directed gene expression in well-defined patterns. Recent studies, however, have shown that both silencers and enhancers can be located 5' or 3' of the transcription initiation site, in introns, exons or even on the transcribed RNA itself. Intuitively, the majority of upstream elements must rely on nucleosome condensing, DNA looping and protein flexibility in order for them to interact with intermediary factors or *cis*-elements, and ultimately alter the frequency of initiation and gene expression.

SILENCERS AND TRANSCRIPTIONAL REPRESSION

A brief survey of the recently published literature on transcriptional repression will quickly reveal a lack of consistency in term definition. In the present review we will summarize the

recent literature on newly identified silencers, revealing that, although several mechanistically separable subclasses of silencer exist, two definitive functional types encompass all of these subclasses. In so doing we will show that it is the DNA sequences themselves and their context within a given promoter, rather than the DNA-binding proteins, that determines the repressor mechanism at that given promoter. We go on to propose that a classical, position-independent element that directs an active repression mechanism (generally by interfering with GTF assembly) should be referred to as a 'silencer element', and that a non-classical, position-dependent element that directs a passive repression mechanism (generally by interfering with upstream elements) be referred to as an 'NRE', and that the associated TF be known as a 'repressor'.

Silencers were initially defined as sequence elements which are capable of repressing promoter activity in an orientation- and position-independent fashion, in the context of a native or a heterologous promoter [80]. However, in practice, a silencer is now generally considered to be a short, specific sequence of nucleotides which are located in the 5' upstream promoter region of a given gene. These sequences are capable of recruiting TF proteins to the promoter, which in turn carry out specific functions. More recently it has become clear that many different types of silencers exist that are capable of affecting many aspects of gene regulation, such as cytoplasmic retention of TFs, activity of positive acting TFs, chromatin structure, intron splicing, 3' upstream untranslated signal recognition [81], as well as GTF assembly, to ultimately down-regulate gene expression.

In the remainder of the present review we will present the different subclasses of silencers by reviewing recent literature on newly identified silencers. Secondly, several broad categories of repressors will be summarized, as well as some recent contributions to overall repressor function. Using this information, suggested modifications to the currently standing nomenclature will then be presented. Finally, a detailed review of the neuron restrictive silencer factor/neuron restrictive silencer element (NRSE/NRSF) repressor system, which is perhaps the most comprehensively understood to date, will be presented.

Classical silencers (silencer elements)

Since their definition during the mid-1980s, a large number of silencers have been identified and characterized to various extents. Recently, several reports clearly describe elements with the properties of classical silencers, but only a small number of these present a detailed functional mechanism for their corresponding repressor(s).

A good example of a classical silencer is found in the human thyrotropin- β gene (hTSH β) promoter. hTSH β expression has been shown to be restricted to thyrotrophs by transcriptional silencing [82]. The silencing activity was localized to an AT-rich octamer-binding domain (Oct-1) located approx. -140 bp from the transcription initiation site and was active in an orientation- and position-independent fashion.

AT-rich sequences have been shown to possess strong nucleotide-unpairing properties [83], which may be involved in unzipping the DNA double helix during transcription initiation [84,85]. During eukaryotic interphase, chromosomes are organized into looped domains which are anchored to the nuclear matrix through DNA regions called matrix-attachment regions (MARs) [19,86]. MAR-binding proteins generally recognize and bind to the minor groove or the intrinsic bend of AT-rich DNA [87], and subsequently may function as important transcriptional control proteins during interphase. It is conceivable that Oct-1

functions as a MAR-binding nuclear matrix protein [82]. Alternatively, Oct-1 could silence transcription by interfering with TFIID directed helicase activity at the AT-rich site.

Preliminary identification and localization of functional regulatory elements within a promoter generally involves transient transfection assays using reporter-gene deletion constructs. Two recently published papers [88,89] present examples of this type of approach, and although the identity of the respective TFs have not been realized, the two elements may turn out to bind the same repressor.

Haecker and co-workers [89] identified a NRE within the chicken ovalbumin 5' promoter which represses transcription in the absence of steroids. Transient transfection assays localized the silencer to a region between nucleotides -239 and -220, and showed that the element demonstrated the properties of a classical silencer. It was also shown that the silencer repressed expression of the heterologous thymidine kinase promoter by 60-80%, while electrophoretic-mobility-shift assays (EMSA) detected two proteins from chicken oviduct nuclear extracts which bound to the silencer element [89].

A similar approach has identified a classical silencer element in the plasminogen-activator-inhibitor type-2 (PAI-2) gene promoter [88]. This silencer (5' CTCTCTAGAGAG 3') is located approx. -1800 bp from the transcription initiation site and represses chloramphenicol acetyltransferase (CAT) reporter activity by 98% in the monocytic-like U937 cell. Furthermore, a single DNA-binding protein was identified using EMSA with HeLa and U937 cell nuclear extracts. Database searches using the PAI-2 or the chicken ovalbumin 5' promoter silencer elements have revealed the presence of several similar binding sites in other genes. Consequently, a consensus sequence, 5'-ANCC-TCTCT-3', was defined which may represent a universal silencer element [89].

Several other classical silencers have been identified in recent times, and in some cases the nature of the DNA-protein interaction and hence silencing mechanism has been partially determined. For example, Liu and co-workers localized a NRE (actually a silencer element by the definitions given in the present review) from the platelet-derived-growth-factor A-chain promoter to nucleotides -1418 and -1388 [90]. EMSA revealed several DNA-binding complexes that displayed unique preferences for binding to the sense, antisense and double-stranded forms of the element, and subsequent work revealed two inverted repeats in the regions flanking the NRE that appeared to stabilize a loop in the DNA. As a result it was hypothesized that formation of a DNA triplex such as H-DNA or other single-stranded-DNA structures in the silencer region may serve to bring the repressor into correct orientation with downstream positive regulatory elements [90]. It is well known that TFs often bind to bent DNA or generate DNA bending upon binding [91-93], and this example provides a detailed mechanism as to how DNA bending may occur.

Perhaps a more elegant example involves the previously identified dorsal switch protein (DSP1) [94]. DSP1 has been shown to repress the *Drosophila zerknullt* (*zen*) promoter (as well as a heterologous promoter) in a position-independent manner [94], by directly binding to TBP. As a result, DSP1 displaces TFIID, which inhibits recruitment of TFIID-RNA pol II to the promoter. TFIID also blocks access of other repressors to TBP [95,96], and therefore DSP1-mediated repression may be more complicated than is reported so far. Several other repressors, such as Dr1 [97] and Dr2 [98], interact with TBP to inhibit its association with TFIID and TFIIB respectively, while others still have been shown to interact with TFIIB (unliganded thyroid hormone receptor [99,100]) and TFIIE (*Kruppel* gene [101]).

Position-dependent silencers (NREs)

Classical silencers form the largest group of silencers that have been identified and reported so far. However, passive silencers or position-dependent silencers (NREs) are now recognized in a large number of gene promoters, as well as in introns, exons and various flanking sequences. NREs physically inhibit the interaction of TFs with their specific DNA-binding sites, or interfere with specific signals which control various transcriptional events, such as splicing sites, 5' polyadenylation signals, ATG translational start sites or by affecting transcriptional elongation. Recently, several groups have identified silencers which provide examples of some passive mechanisms.

An NRE was identified in the 5' untranslated region of the first exon of the human α 1-chimaerin gene [102]. Deletion of a 70 bp region containing this NRE increased promoter activity by 5–6-fold, and also suppressed the heterologous TK promoter in an orientation-independent but position-dependent manner. These observations led to the hypothesis that the silencer affects transcriptional elongation by premature termination [102], as has been identified in the *c-myc* [103], *c-myb* [104], HIV-1 and HIV-2 promoters [105] or by pausing RNA pol II as occurs in the *Drosophila* heat-shock protein 70 (*HSP70*) gene [106]. However, further evidence is required to substantiate this hypothesis.

The mechanics of repression at an overlapping pair or set of binding sites of a typical NRE is simple to visualize. However, results following the identification of an 8 bp silencer element (–97 to –90 bp) (referred to a NRE in accordance with the above definition) in the 5' promoter of the human Pi Class glutathione S-transferase (*GSTP1*) gene [107] reveals a slight complexity that may at first be overlooked. The NRE was shown to suppress *GSTP1* expression in MCF7 cells, but not in the context of a heterologous promoter. It was also shown, however, that introduction of a half helical turn (5 bp in B-DNA) between the NRE and the overlapped element abrogated repressor activity, revealing that in some cases both binding sites must exist on the same side of the DNA helix to facilitate silencer function.

As a consequence of the overlapping nature of position-dependent silencers, NREs often present one of the simplest forms of silencers to characterize. They may simply flank important regulatory signals or promoter elements, while complicated interactions with basal transcription apparatus or upstream and downstream elements may not be involved in the observed repression. A good example of this type of silencer is the NRE of the *c-fos* promoter. The NRE is bound by the nuclear factor Yin-Yang 1 (YY1), which induces a bend in the promoter that blocks the interaction of the cAMP-response element with *cis*-elements in the *c-fos* promoter [108]. Further examples have been identified in the human ϵ -globin [109,110], human osteocalcin [111], chicken δ -1-crystallin [112], rat β -fibrinogen [113], human hypoxanthine phosphoribosyltransferase [114] and avian embryonic β -type globin genes [115].

Orientation-dependent silencers

A small number of silencers have been described as orientation-dependent, but at this stage their functional significance is unclear. The activity of various TF-binding sites depends heavily on the sequences directly adjacent to the binding motif [116] and, in a large number of orientation-dependent silencers, changes in silencer properties due to an antisense orientation is probably a result of this fact.

Despite this lack of understanding, it seems likely that a completely orientation-dependent silencer or enhancer functions by presenting its specific binding factor in a particular position or

direction relative to other regulatory sequences or factors. For example, DNA bending as a result of silencer complex binding has been shown to result in transcription silencing by physically hindering upstream elements [108]. Therefore, it is quite possible that inversion of the silencer could produce a bend in the opposite direction, thus preventing steric hindrance of the upstream enhancer elements. Otherwise, a given repressor complex may overlap an adjacent enhancer element when bound in one position, but may be significantly asymmetrical so that it does not carry out the same function when bound in the opposite direction. In the latter case it should be noted that the silencer would be both orientation- and position-dependent.

Promoter-dependent silencers

Orientation- and position-independent but promoter-dependent silencers, such as the rep27 silencer element in the rat PAP I gene [117], may in the future reveal a new form of functionally significant silencer. However, at this stage their possible mechanism of action and limited identification would suggest that they are rare. For example, a silencer which is position- and orientation-independent must specifically inhibit the GTFs or alter overall promoter structure in such a way as to decrease the frequency of initiation of transcription. A promoter-dependent silencer must have the additional requirement that it affects one of these processes via a promoter-specific factor, such as a promoter-specific TAF or mediator component. Therefore, promoter-dependent silencers, although a specialized version, are referred to as silencer elements according to the definitions given in the present review.

Intron-located silencers

As our knowledge of silencer elements increases, it is becoming clear that a distinction between position-dependent and independent silencer elements does exist. As it is unclear at this stage whether intron, exon or 3' untranslated region (UTR)-located silencers also form defined and practically separable groups, they will be dealt with separately in the present review.

Silencers which reside in gene introns could repress transcription in a number of ways. A repressor could bind to its recognition sequence and physically block transcriptional elongation, it could prevent recognition of intronic splice sites, or just as simply abrogate basal transcription apparatus assembly as might a classical 5'-promoter-located silencer. Recently a considerable number of genes have been identified which contain intronic silencers. However, it remains unclear whether intronic silencers inhibit specific aspects of transcription.

The mechanism controlling the ability of an intron-located silencer to inhibit intronic splicing in a position-dependent or -independent fashion is a simple concept. Physical blockage of a 3' or 5' splice site or alteration of secondary RNA structure by formation of a DNA bend which inhibits RNA processing are two possibilities. An example of this type of NRE was located upstream of the human papillomavirus type 16 (HPV16) late polyadenylation site, which extends downstream of four weak 5' splice sites [27]. Previous work strongly implicated splice site 2 in repressor activity [26], but deletion of sites 1 and 2 failed to de-repress CAT activity [27]. Interestingly, the majority of silencer activity was finally attributed to a GU-rich region located downstream of site 2, which contrasts with observations made on the bovine papillomavirus-1 gene, where virtually all silencer activity was attributed to a single 5'-splice-site consensus sequence [26].

Although the precise mechanism for silencing in the *HPV16* gene is far from certain, several results have raised a number of

possibilities. UV-cross-linking experiments have shown that a 65 kDa protein binds to a GU-rich element [27] that has considerable homology with the B2P2 sequence known as the U2AF⁶⁵ binding site [118]. Although, a role for the splicing auxiliary factor U2AF⁶⁵ in inhibition is yet to be described, its interactions with U1 small nuclear ribonucleoprotein [119] and its potential to change RNA secondary structure [120] have been considered as possible mechanisms of U2AF⁶⁵-associated silencer activity [27,121].

Several other groups have described similarly located silencers, but few have progressed sufficiently to be able to determine the underlying mechanisms of repression. For example, a 24 bp element within the third intron of the human collagen type IV gene was identified. This element, termed COL4, suppresses the heterologous TK promoter and binds a collagen-type-IV-expressing cell-specific factor (SILBF), which at this stage has not been characterized [122]. A pair of hepatic-cell-specific silencers, which appear to bind a similar factor, were localized to the first intron of the human apolipoprotein A-II gene [123], and a 14 bp silencer which suppresses the heterologous simian-virus-40 (SV40) promoter in the sense orientation only, was found in the *c-Fes* gene [124].

Functional studies of silencer elements *in vivo* are rare, but an elegant set of experiments which identify a CD4-specific silencer [125] provide a further example of an intron-located silencer and also reveal a potential downfall of *in vitro*-based studies. A 190 bp fragment from the first intron of the human CD4 gene was identified which represses promoter activity in CD4 cells, but not in CD8 cells, of transgenic mice. This fragment is capable of repression *in vivo* when cloned into a truncated CD4 construct, but, interestingly, the silencer was less effective *in vitro* [125]. This observation may be attributable to an incompatibility between the TATA-less CD4 transcription machinery and the TATA-containing cytomegalovirus promoter [125].

Exon- and 3'-UTR-located silencers

Until now, only a very small number of groups report identification of 3' UTR [126]-or exon [127–129]-located silencers. It appears that these silencers may behave similarly to 5'-promoter-located silencers, be they strictly classical or position-dependent. However, it has been clearly demonstrated that silencers located within an exon have the unique ability to carry out their activity prior to transcription or as an RNA-based element [127].

An exon-located NRE was identified between nucleotides +39 and +104 in the rat osteocalcin gene, and is capable of suppressing promoter activity in proliferating osteoblasts [127]. The NRE was active when placed 3' of the RNA-coding region of the osteocalcin–CAT fusion gene, but was not active when placed 5' or in the context of heterologous SV40 early promoter [127]. These results strongly suggest that there is an interaction between the transcribed silencer and specific osteocalcin elements, but it is unclear as to the precise mechanism of silencer activity. In contrast, the silencer identified in the mouse *Hsp70.3* coding region repressed the SV40 early promoter independently of orientation and position [129].

RNA-based silencers may not form a large or functionally significant group, but their presence reveals several other areas where gene expression may be controlled. For example, repressors could bind to RNA and prevent export to the cytoplasm, or cytoplasmic factors could interact with mRNA to prevent ribosomal binding or extension. Furthermore, introduction of abnormal secondary structure into an RNA molecule due to RNA bending may inhibit translation or several other post-transcriptional mechanisms.

REPRESSORS

The present review has purposefully concentrated on the role of DNA elements in transcriptional repression. However, the role of repressors and repressor complexes are obviously paramount in this area. Therefore we will now briefly describe several groups of repressor complexes and how they regulate gene expression, as well as detailing several examples of TFs which can behave as activators or repressors.

NRSF/NRSE

Although research on silencers has increased rapidly in the last 10 years, our knowledge of silencer function still remains limited when compared with other areas of transcriptional control and regulation. Accordingly, well-understood systems in which silencers and their interacting repressors have been identified and characterized are still a long way off. NRSF (or 'REST') and NRSE [or 'repressor element-1' ('RE-1')], which play a key role in directing neuron-specific expression, represent one of the more understood systems.

NRSF was one of the first transcriptional regulators to be implicated in negative gene regulation during vertebrate neuronal development. NRSF/REST was originally identified as a silencer-binding protein for the neuron-specific sodium channel gene (*SCG10*) [130], which binds to a conserved element known as NRSE [130,131] and was simultaneously cloned in separate studies [132,133]. NRSF/REST is known to be a 116 kDa protein [133] containing eight non-canonical zinc fingers [132], only one of which has been shown to be crucial for repression of the type II sodium channel gene [134]. Interestingly, several research groups have now identified NRSE-directed silencer activity in a variety of neuronal as well as non-neuronal genes [130–132,135–141].

EMSA, RNase protection assays and transient transfection assays with reporter-gene deletion constructs of promoters from the rat m4 muscarinic acetylcholine receptor gene (*mAChR*) [138,142], the human synapsin I gene [139] and the rat dopamine β -hydroxylase gene [137] have recently revealed the presence of cell-specific silencers that were identified as NRSEs by sequence. In all three systems, NRSE was shown to silence promoter expression in several non-neuronal cells, but not in neuronal cells.

Using a sequence derived from several NRSEs, the GenBank[®] DNA sequence database was searched which identified 22 more genes containing NRSE-like sequences [140]. Of these genes, 17 were expressed primarily in neuronal tissues, and two represented neuronal TFs, suggesting that NRSF/REST may repress neuronal differentiation both directly and indirectly [140]. EMSAs determined that 15 out of 16 neuronal genes, and five out of eight non-neuronal genes, bound NRSF/REST. These experiments also revealed that silencers in the Na⁺/K⁺-ATPase $\alpha 3$ and the dopamine β -hydroxylase genes may not be NRSEs.

Early data implied that NRSF was expressed in most non-neuronal cells as well as undifferentiated neural precursors [132,133], suggesting that it may prevent precocious expression of the neural phenotype during neurogenesis [140] and that it was likely to function as a neurogenesis-specific master negative regulator [132]. However, more recent work has discovered low-level expression of NRSF in neuronal cells, as well as a dual functionality of NRSF depending on its context within the promoter [143]. For example, when located within 50 bp of the TATA box or in the 5' UTR, as occurs in 10 out of 17 neuronal genes previously mentioned [140], NRSF enhances transcription in neuronal cells, while always acting as a silencer in non-

neuronal cells [143]. Consequently, the following model for NRSF function has been proposed. At low NRSF concentrations (e.g. in neurons), NRSF cannot repress distant NRSE-containing promoters, but enhances proximal NRSE-containing promoters. At high NRSF concentration (e.g. non-neuronal cells), NRSF elicits repression regardless of promoter context [143].

Finally, NRSF has been shown to be absent from the insulinoma cell line INS-1 as well as from three other insulin- and glucagon-producing cell lines [141]. Consequently, NRSF/REST appears to be a key factor in the control of neuronal markers in β -cells as well as neuronal cells [141].

Polycomb group proteins

In general, TFs play a transient role in transcription regulation, and as such are required in continuous supply to carry out their function in gene regulation. However, the identification of the polycomb group (Pc-G) of repressors from *Drosophila*, and the functionally homologous yeast Rme1p [144], *Xenopus* XPc [145] and human HPH1 [146,147] proteins has revealed a mechanism which overcomes this requirement [148].

Pc-G proteins have been shown to keep homoeotic genes stably and heritably silenced during *Drosophila* development [148]. These proteins show no sequence-specific binding [149–151], suggesting that they are anchored to the DNA by other pre-bound proteins [148]. In fact, several Pc-G proteins have been shown to act as transcriptional repressors when tethered to DNA-binding domains [148,152]. The following simple model based on previous *Drosophila* promoter analysis has been proposed. The gap protein hunchback (hb) binds regulatory sequences of the homoeotic gene *Ultrabithorax* (*Ubx*) [153,154] to form a negative transcription complex. This complex then promotes occupancy of Pc-G response elements, which further promotes the recruitment of secondary Pc-G proteins. As a result, a stable silencing complex is assembled, which later becomes independent of hb.

Insulators

As a consequence of the capacity of enhancers to function over several kilobases to regulate gene expression, it is obvious that mechanisms must exist which prevent activation of neighbouring transcriptional units. If an enhancer can activate expression of a given gene from several kilobases of the targeted initiation site, why does it not activate expression of closely linked genes?

Short-range repressors that function over 50–100 bp have been shown to inhibit or quench closely linked enhancers, thereby allowing independent function of enhancers within a modular promoter [155,156]. Similarly, a group of proteins known as insulators, while not strictly being repressors, play an important role in overall repressor function.

Insulators act as dominant repressors that function over long distances to block interactions between *cis*-elements and inappropriate promoters [157]. For example, a 340 bp insulator DNA within the *Drosophila* gypsy retrotransposon disrupts gene activity by blocking the interactions of distal enhancers with target promoters [158]. It is noteworthy that reduction of *mod(modg4)+* (an accessory factor required for gypsy insulator function), causes the insulator to act as a promoter-specific repressor [158].

Scs and scs', which flank the *Drosophila* *hsp70* locus, are perhaps the best characterized and most understood authentic insulators [157] and are mechanistically indistinguishable from the gypsy insulator [158]. It is believed that insulators might block distal enhancers via DNA bending, mimicking the structural changes mediated by TFIID binding to the promoter

[159,160]. Alternatively, the mechanism of 'insulation' may involve chromatin looping to separate genes into distinct nuclear compartments [161]. Insulators appear to possess some regulatory specificity, as they selectively block enhancers but not repressors [158].

Dual functionality of TFs

In the present review we have attempted to show that transcriptional repression, although ultimately carried out by repressor proteins, is actually controlled by promoter elements which direct repressor function. The main piece of evidence which supports this view is that many TFs can have dual functionality; that is, they can act as both repressors and activators.

YY1 probably presents the most widely known example of a TF with dual functionality, and although a large number of TFs have been identified solely as repressors, it is probably only a matter of time before they are shown to act as activators in other promoter systems.

YY1 has been shown to repress transcription by physically interacting with the GTFs and various activators, as well as displacing activators with overlapping binding sites (reviewed in [162,163]). On the other hand, YY1 has been shown to act as an enhancer, as well as an initiator, of transcription (reviewed in [162,163]). Furthermore, YY1 can simultaneously activate and repress different genes in the same cell [164,165]. Oct-1, another well-documented TF, has been shown to repress and activate transcription in different promoters (see [166] for a recent example), and Sp3, a relatively recently identified TF, has also been shown to repress and activate transcription [167].

Although mechanisms of dual functionality are varied [168], concentration-dependence is one mechanism by which TFs can function as repressors or enhancers. For example, transcription of the *Acanthamoeba* TBP is regulated by TBP-promoter-binding factor (TPBF). TPBF has two binding sites within the promoter: one higher-affinity binding site upstream of the TATA box (TPE) and another lower-affinity binding site between the TATA box and transcription initiation site (nTPE). At low concentrations TPBF binds the upstream element and enhances transcription [169], while at higher concentrations TPBF binds both sites, resulting in the disruption of TBP induced promoter bending [170] and subsequent repression [171]. YY1 has also been shown to act in this way in the context of the adenovirus p5 promoter [172,173], as has the phage repressor ' λ repressor' [174].

Finally, the precise nature of the binding sequence and flanking sequences can alter TF function. The POU domain of Oct-1 can adopt different conformations depending on what sequence it binds to, subsequently resulting in different cofactors being recruited to Oct-1 [175].

Co-repressors

Much of the published work on repressor mechanisms has revolved around the DNA-binding protein itself. Recently, however, several groups have identified secondary repressor complexes, named CoRs, which appear to elicit repression from several previously identified repression systems.

The repression activity of unliganded thyroid-hormone and retinoic acid receptors is dependent on a conserved motif in the ligand-binding domain [176,177] known as the CoR box [178]. The CoR box has since been shown to bind to the 270 kDa protein nuclear-receptor co-repressor (N-CoR) [178,179], which functions via conveyance of a signal to the GTFs [180]. Silencing

mediator for the retinoid and thyroid-hormone receptors (SMRT) contains several domains which are highly homologous with N-CoR [178,181,182] and function in a similar fashion to N-CoR [181].

The CoR complex that contains N-CoR has also been shown to contain members of the Sin3 family [183], as well as the histone deacetylase, RPD3 [184,185]. It is thought that this complex binds to unliganded hormone receptors and affects local histone acetylation, producing alterations in chromatin structure that block transcription [186]. Upon ligand binding, the CoR complex disassociates and is replaced by a co-activating complex which contains the cAMP-response-element-binding protein, p300, and/or p/CAF [187], each of which possess histone acetyltransferase activity [188–190].

Although a lot of the early findings on the N-CoR complex have centred around hormone receptors, it is thought that zinc-finger domains and motifs in the N-terminal domain of the repressor NRSF may interact with similar corepressors [134]. N-CoR and SMRT have also been shown to interact with COUP TF1 [191] and the viral oncogene RevErb A [182], while YY1 repression may be mediated by direct interaction with another mammalian histone deacetylase, HDAC2 [188]. Finally, the Mad-Max or Mxi-Max heterodimers have been shown to repress E-box-containing promoters by recruiting mSin3A/B and HDAC1/2 (mammalian members of the Sin3 family) [192–195].

Histones

Although histones are not strictly repressors or CoRs, they are capable of repressing transcription *in vitro* and *in vivo*, and recently histones have been implicated as the effector molecules in a number of systems (reviewed in [196,197]).

Nucleosomes, which fold chromosomal DNA, contain two molecules of each of the core histones H2A, H2B, H3 and H4. The N-termini of the histone molecules extend from the core and can be acetylated, methylated and phosphorylated, causing considerable changes to their overall charge and function. For example, acetylation neutralizes the positively charged lysine residues of the histone molecules, decreasing their affinity for DNA. As a consequence, DNA can unfold, providing better access to transcription factors and, ultimately, the GTFs [198,199].

As the present review has indicated, our understanding of transcription initiation and GTF assembly is rapidly increasing. However, it has long been a mystery as to how activators function and what role chromatin plays in their function. It is understood how transcription factors bind to DNA in the context of chromatin, and that the positioning of nucleosomes can bring distant regulatory elements into close proximity, but it is unclear as to how chromatin disruption occurs [197]. Several key proteins, including p300, pCAF, TAFII250, HDAC1, HDAC2, GCN5, ADA2, ADA3 and RPD3, have now been identified which have provided insights into just such questions. These proteins, and undoubtedly many others, have intrinsic acetyl- or deacetyltransferase activity, which has been linked to transcriptional activation and repression. Interestingly, many of these proteins have been associated with previously identified repressor and enhancer systems such as the Mad-Max-induced tethering of mSin3/HDAC to E-box-related motifs, resulting in histone deacetylation and transcriptional repression (Figure 2).

CONCLUDING REMARKS

A large number of silencers have now been identified, but for relatively few has a specific and defined binding sequence been determined. Table 1 provides a list of several genes which have

Figure 2 Mad-Max/N-CoR-mediated repression

(a) De-acetylation. Mad-Max interacts with an E-box-related motif which further interacts with N-CoR, possibly via an unknown factor (?). An unliganded nuclear-hormone-receptor complex may interact with this complex which directs an interaction with mSin3 and HDAC, leading to de-acetylation of the surrounding promoter DNA, and repression of transcription. (b) Acetylation. Interaction of a liganded nuclear hormone receptor complex to the same Mad-Max complex, directs interaction with p/CAF and p300, leading to acetylation of the surrounding DNA and access of other TFs to the promoter. Acetylated nucleosomes are shown red. Deacetylated nucleosomes are grey.

recently been identified to contain silencers. Where possible, a defined binding sequence and repressor have also been listed. The Table does not represent an exhaustive list of identified silencers, but it does provide a useful reference for researchers working in this field.

Although there appears to be a difference in the mechanism of action between 5' promoter silencers and intronic silencers, and possibly in exon-located silencers, this detailed examination of silencer and repressor mechanisms reveals only two distinct functional groups: (1) the classical, position-independent silencer, and (2) the position-dependent silencer. Orientation and/or promoter independence does increase the complexity of transcriptional regulatory mechanisms, but does not add to the two groups outlined above.

Many previous reports have discussed the two basic repressor mechanisms summarized in the present review. In these reports, there has been a tendency to implicate the repressor complex in determining the mechanism of repression rather than the promoter sequence. In the present review we have attempted to highlight the importance of the structure, configuration, relative position, flanking sequences and orientation of the promoter sequence itself, with respect to repressor function.

We have shown that TFs often have the potential to act as repressors or enhancers of transcription, and that it is the promoter element which governs TF function. Therefore, we propose that the DNA sequences themselves be referred to as

Table 1 Recently identified silencers

Abbreviations: BP, binding protein; HR, hormone receptor; GR, glucocorticoid receptor.

Gene	Location	Binding sequence	<i>cis</i> -Acting repressor	Reference
Rat dopamine β -hydroxylase	−212/−388	NRSE	NRSF	[137]
Human plasminogen activator inhibitor type-2	−1977/−1675	CCTCTCTAGAGAG?	Unknown	[88]
Chicken lysozyme	−2350/−2331	TTGACCCCAGCTGAGGTCAA	NEP1/CTCF	[201]
Human sperm histone H2b-1	−103/−75	TGATTGGTAGATTATCATGTCGCGCT	CDP	[202]
Human apolipoprotein A-II	+50/+150	Not yet defined	Unknown	[123]
<i>Pisum</i> Trg-31	−1066/−559	Not yet defined	Unknown	[203]
<i>Methanococcus maripaludis</i> nitrogen fixation	+2/+18	CCGGAAN _x TTCCG	Unknown	[204]
Rice glutelin Gt3	−482/−320	Not yet defined	Unknown	[205]
Human papilloma late mRNA	Upstream of poly(A)	79 bp region?	U2AF ⁶⁵ ?	[27]
Human γ D promoter	−88/−71	CCCTGCCCCCGCGGG?	Unknown	[206]
T-cell receptor V β 2.2	−845/−884	TTAGAAT?	Unknown	[116]
Human CD4	+88/+103	CACCA?	Unknown	[125]
Human neuronal α 1-chimaerin	+40/+110	Not yet defined	Unknown	[102]
Mouse bone morphogenetic protein 4	−2095 and −865	GGGCCAAAGG(T/G)CA	COUP TFI	[207]
Mouse mammary-tumour-virus long terminal repeat	−394/−381	GAGAAAGAAAAGA	Unknown	[208]
Human insulin	−279/−258	C(3)GTCTCCTGGAGACA(3T)G(6C)AG?	GR?	[209]
Rat osteocalcin	+118/+124	TTTCTTT	Unknown	[210]
Chicken ovalbumin	−239/−220	TCTCTCCNA	Unknown	[89]
Human collagen type 4	+1429/+1450	GCTTGGACTTGCGCGCCCGAGA	SILBF	[122]
Human c-Fes	+441/+454	CTCCGTGTGAGCGA	Unknown	[124]
<i>Acanthamoeba</i> TATA BP	−21/+1	CAAATCAACAAAAAATTGGCCC	TPBF	[171]
Human thyrotropin β	−480/−128	Multiple sites	Oct 1	[82]
Rat serine-protease inhibitor 2.3	3' UTR	348 bp region	Unknown	[126]
Human platelet-derived growth factor A-chain	−1418/−1388	GGGGAGGGGG	Unknown	[90]
Rat kallikrein	−739/−472	Not yet defined	Unknown	[211]
<i>N</i> -Acetylglucosamine-1-phosphate transferase	−1057/−968	AGGAA or GAAAC?	Unknown	[211]
Rat m4 muscarinic acetylcholine receptor	−861/−840	GGAGCTGTCCGAGGTGCTGAA	NRSF	[138]
Human Pi Class glutathione S-transferase	−97/−90	GGACCTCT	Unknown	[107]
Rat angiotensin II type 1a receptor	−456/−442	TAATCTTTATTTTA	53 kDa?	[212]
Mouse thyroid HR β 1	−901/−887	GGGCGG	GCF or WTP?	[213]
Mouse thyroid HR β 1	−924/−916	CCTCTCCCA	Unknown	[213]
Human T-cell activation gene 3	−2002/−1983	CCCCATTCT	Unknown	[214]
Rat PAP1	−180/−153	Not yet defined	Unknown	[117]
Fatty acid synthase	−319/−301	CCGCGGGGGTGGGGGGTG	Unknown	[215]
Bovine growth hormone	−336/−240	AGAATGGGG?	YY1?	[216]
Rat 3 α -hydroxysteroid dehydrogenase	−755/−498	Not yet defined	Oct-1?	[217]
Human hypoxanthine phosphorylase transferase	−510/−451	GGAAGCC	Unknown	[114]
Human synapsin I	−235/−199	TTCAGCACCCNCGGACAGNGCC	NRSF	[139]
Mouse major inducible <i>Hsp70</i>	−1844/−800	Not yet defined	Unknown	[129]
Streptococcal plasmid pIP501 repR promoter pII	−1377/−1348	CGTG	copR	[218]
Rat CYP1A1	−808/−851	ATGCAAAACAT	Oct 1	[219]
Human c-myc	−336/−327	TAAACGTA	Octamer BP?	[220]
Rat cytochrome P-450 CYP1A1	−873/−851	CCTCCACTCT (NeRD)	Unknown	[221]
Avian embryonic β -type globin	+545/+880	Not yet defined	YY1	[115]
Human platelet-derived-growth-factor A-chain	+1605/+1630	CGGGGAGGGGAGTGGGGGACGCC	Unknown	[222]
Human interleukin-2	−110/−101	ACAGGTAA	Unknown	[223]
Rat m4 muscarinic acetylcholine receptor	−574/−550	GGAGCTGTCCGAGGTGCTGAA	NRSF	[142]
Human interleukin-8	−92/−85	AACGTTA	10.4kDa?	[166]
Human interferon- γ	−211/−186	GGCATAATGGGTCTGTCTCATCGTC	YY1	[200]

‘silencers’: more specifically, classical, position-independent elements as ‘silencer elements’, and position-dependent elements as ‘NREs’. Furthermore, we propose that DNA-binding TFs be referred to as ‘repressors’ (Figure 3). For example, it is inappropriate to refer to the TF YY1 itself as an active or passive repressor, or for that matter as a repressor or enhancer. However, the YY1-binding site at −200 bp in the promoter of the interferon (IFN)- γ gene [200] is a silencer element that directs YY1 to passively repress transcription of the IFN- γ gene.

Silencers have now been documented to a sufficient extent for us to know that they can function in many different ways, with passive and active mechanisms encompassing the two general groups identified to this stage. Silencer specificity is finely

controlled, often being affected by flanking sequences, *cis*-elements, and the general structure of the promoter. Furthermore, silencers have been reported to act synergistically to intensify or modify repressor function; they may be a complex region of DNA rather than a single element, or they may function as a result of a particular DNA conformation rather than a specific sequence.

It is clear that silencers form an intrinsic part of any promoter and that repression often plays a definitive role in determining spatial and temporal gene expression. However, in a large number of promoters, silencers only provide a ‘switch’ for an enhancer to act on. In other promoters silencers may play a minimal role in determining expression levels. Transcriptional repression may

Figure 3 Silencer nomenclature and mechanisms of TF multifunctionality

(a) Repression. (1) Passive repression. A hypothetical TF (TF-X), interacts with its consensus binding sequence (GTGAAA) and physically prevents Sp1 from binding to the GC box. As a consequence expression is down-regulated. (2) Active repression. TF-X binds to the same consensus sequence, but due to interaction with different flanking sequences undergoes a conformational change which allows interaction with a CoR complex. This complex then interacts with the GTFs, preventing complete GTF assembly. (b) Activation. TF-X binds to its consensus sequence; interaction with flanking sequences produces a conformation which allows binding to a co-activator complex. The co-activator complex increases the rate of GTF assembly, and subsequent expression. TAFs, TBP, mediator and RNA pol IIa have the same colourings as in Figure 1. Other components are not coloured.

not dominate eukaryote expression, as is considered to be the case in prokaryotes. However, recent results have revealed the importance of repressors in overall eukaryote expression.

Knowledge of specific and general promoter structure and repressor function provides the basis of a powerful tool by which gene expression can be manipulated and ultimately controlled *in vivo*. Further research in understanding these elements is likely to provide valuable information which can be exploited in gene-based therapies for the treatment of cancer and other human diseases, as well as allowing a better understanding of biochemical and molecular systems.

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