COMMENTARY

Negative regulation of eukaryotic transcription

MARIA E. JACKSON

Beatson Institute for Cancer Research, Cancer Research Campaign Beatson Laboratories, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland

Introduction

One of the most fascinating questions currently facing biologists is how eukaryotic cells are able to control so precisely the levels of gene expression required for determination of cell fate and cell proliferation. Gene expression must be switched on and off according to developmental programs; failure to follow such programs accurately can result in gross abnormalities, as occur, for example, in the homeotic mutants of *Drosophila* (Sanchez-Herrero, 1988). The cells of the adult must also be able to respond to signals; for example, by changing their rate of proliferation or the quantity of secreted products. Much of the regulation of gene expression occurs at the level of transcription, with a hierarchy of coarse and fine controls that together determine the transcriptional activity of each gene. Protein factors binding to the DNA appear able to alter the rate of transcription by alterations in chromatin configuration or interactions with the transcription complex itself. Early work on eukaryotic transcriptional regulation was concentrated almost exclusively on transcriptional activators, but in the past three years there has been a profusion of literature describing negative regulatory elements. This review concentrates on the better-characterised systems and the most recent work; additional references may be found in earlier reviews by Levine and Manley (1989) and Renkawitz (1990). The first section deals with the role of chromatin structure in repression, followed by an analysis of the different mechanisms of negative regulation. The ensuing sections discuss the seemingly paradoxical observations that repressors can act as transactivators and vice versa, and finally how these diverse mechanisms of negative regulation fulfil the differing needs of the cell in control of gene expression during development, differentiation and responding to the environment.

The role of chromatin structure in negative regulation

Heterochromatin represents the ultimate state of gene repression. In *Drosophila*, chromosomal rearrangements that place euchromatic genes next to heterochromatin generate the phenomenon of position effect variegation (PEV), a result of variation in the degree of propagation of the heterochromatic state into the normally euchromatic adjacent sequences (reviewed by Henikoff, 1990). Thus in the case of the *white mottled* mutation, individual patches (clones) of cells in the eye exhibit different colour phenotypes depending on whether the *white* gene has been inactivated by packaging into heterochromatin. The search for suppressors and enhancers of PEV has identified several *Drosophila* loci whose products are involved in either chromatin condensation or decondensation (Tartof *et al.* 1989; Wustman *et al.* 1989). The appearance of clones of cells of a single phenotype in PEV implies an early event during development followed by epigenetic inheritance of the condensed or decondensed state. Similarly, heritable repression of *Drosophila* homeotic genes during development occurs *via* the action of the *Polycomb* group of proteins; the Polycomb protein itself has been shown to possess a 37 amino acid homology (the 'chromo domain') to the PEV modulator HP1 (reviewed by Paro, 1990). cDNAs encoding chromo domain proteins have been cloned from human and mouse libraries, and the chromo box motif is present in multiple copies in the genomes of organisms from both the plant and animal kingdoms (Singh *et al.* 1991). Thus the modulation of higher order chromatin structure, and the epigenetic inheritance of the chromatin state plays an important role in gene repression during development.

The higher order chromatin structures must be unravelled for transcriptional activators to bind and gene expression to occur, and this appears to be brought about at least in part by modification of the histone proteins (reviewed by Grunstein, 1990). Binding of transcription factors to promoter elements creates DNAase I-hypersensitive sites (reviewed by Elgin, 1988), but the presence of such hypersensitive sites does not always correlate with gene activity, and, indeed, some hypersensitive sites represent the binding of repressor proteins rather than activators (see for example, Sippel and Renkawitz, 1989). In addition to chromatin folding, methylation has been found to be incompatible with transcription; a methyl-CpG binding protein has been found to be a mediator of repression by methylation, although the mechanism is unclear (Boyes and Bird, 1991).

Addition of nucleosomes to *in vitro* expression systems inhibits initiation of transcription (Knezetic and Luse, 1986) but does not block chain elongation (Lorch *et al.* 1987). However, preincubation of the template DNA with TATA box binding factor (TFIID) alleviates nucleosome-mediated repression (Workman and Roeder, 1987). Nucleosome-mediated repression has also been demonstrated

Key words: chromatin, transcription, repression, silencers.

Journal of Cell Science 100, 1–7 (1991)

in vivo in *Saccharomyces cerevisiae*, where either histone gene mutations or overexpression of the histone pairs H2A/H2B or H3/H4 were shown to suppress transcription-defective mutations of *HIS2* and *LYS4* (Clark-Adams *et al.* 1988). Similarly H4 depletion has been demonstrated to cause activation of *S. cerevisiae PHO5* even under high phosphate conditions when the gene is normally repressed (Han *et al.* 1988). Thus alteration of the stoichiometry of the histones and presumably therefore the disruption of chromatin structure can lead to increased gene expression.

There is evidence that ease of nucleosome displacement plays an important role in transcriptional regulation, with transcription factors competing with nucleosomes for binding sites within promoter elements. Positioning of a high-affinity nucleosome binding site close to the TATA box of *PHO5* was found to prevent induction of transcription, whereas the substitution of a DNA fragment with low nucleosome affinity at the same site led to a weak constitutive gene activity (Straka and Horz, 1991). Thus not all nucleosomes are equivalent with respect to transcription factors. Another indication that nucleosome displacement is an important step in gene activation is the observation of a DNA binding protein, GRF2, which excludes nucleosomes from the upstream activator sites (UASs) of the *S. cerevisiae GAL* gene cluster (Fedor *et al.* 1988) and several other genes (Chasman *et al.* 1990). Although GRF2 is itself only a weak activator, it potentiates activation by UAS binding transactivators. Similarly, by *in vitro* nucleosome reconstitution Pina *et al.* (1990) demonstrated that whilst the nucleosome-bound MMTV promoter is inaccessible to NF1, steroid hormone receptors are nevertheless able to recognise the histone-bound DNA and alter the nucleosome structure; this observation led the authors to propose that NF1, which is essential for MMTV transcription, can subsequently bind to the promoter where it would displace the steroid hormone receptor (Bruggemeier *et al.* 1990). Acidic activators have also been shown to function in alleviation of nucleosome-mediated repression *in vitro* (Workman *et al.* 1991); evidence that the same acidic activators recruit TFIIB to promoters in a nucleosome-free *in vitro* system (Lin and Green, 1991) reflects a second aspect of activator function, which may be an integral part of the former.

In addition to generalised repression by nucleosomes, there is one example where a precise interaction with histone H4 is essential in establishing repression. Mutation of the conserved amino terminus of H4 leads to the specific activation of the *S. cerevisiae* silent mating loci (Kayne *et al.* 1988); this activation can be suppressed by mutations of one of the mating type locus repressor proteins SIR1 (Stone *et al.* 1991) or SIR3 (Johnson *et al.* 1990), indicating that silencing requires interaction between H4 and SIR1 and SIR3.

The above lines of evidence demonstrate that both the precise positioning of nucleosomes in the vicinity of transcriptional promoters and the strength of the nucleosome–DNA interaction can have considerable influence on promoter activity. Nuclear proteins that are able to alter either of these parameters would therefore act as either activators or repressors of transcription.

Mechanisms of transcriptional repression

Several modes of action have been identified for proteins that down-regulate transcription (Fig. 1). These are: competition for transactivator binding sites; sequestering of transactivator proteins; masking of the transactivator activation domain; and lastly silencing, which operates *via* DNA elements that show position and orientation independence and appears, at least in some cases, to involve alterations in chromatin structure. Other mechanisms of repression may also occur; for example, transcriptional interference from adjacent promoters (Wu *et al.* 1990).

Competition

Repressors may compete for DNA binding with either the transcription complex itself, or with activators of transcription (Fig. 1C). Tetramers of the SV40 T antigen bind cooperatively to three adjacent sites in the SV40 early promoter, occluding both the TATA box and the early mRNA 5′ cap position, thus repressing transcription (Rio *et al.* 1980). However, it is rather more common for negative factors to compete for activator binding sites. Heat shock factor (HSF) binding to heat shock element 2 (HSE2) of the *S. cerevisiae SSA1* gene encoding HSP70 is important for both basal as well as heat-inducible expression. Park and Craig (1989) characterised an upstream repression sequence (URS) that has a 4 bp overlap with HSE2 and represses HSE2-dependent basal level transcription. Gel retardation assays identified both an HSF–DNA complex and a URS binding factor–DNA complex, but not an HSF–URS binding factor–DNA complex, consistent with a model in which HSF and URS binding factor compete for their partially overlapping cognate sites.

Truncated and modified versions of activator proteins are also employed as negative regulators that compete for the activator binding sites. The papillomavirus E2 reading frame encodes two products: a full-length transactivator protein (E2) and a shorter molecule (sE2) lacking the N-terminal activation domain (Giri and Yaniv, 1988). These sE2 repressor proteins have intact DNA binding domains and are able to compete with the E2 transactivator molecules for the multiple E2 binding sites present in the viral DNA (Lambert *et al.* 1987). An analogous situation exists for the nuclear factor CREB, which binds as a homodimer to cyclic AMP response elements (CREs) and activates transcription (reviewed by Karin, 1989). A second CRE binding protein, CREM, shows considerable sequence homology to CREB, but unlike CREB, CREM down-regulates CRE-mediated transcriptional activation (Foulkes *et al.* 1991). CREM is able to bind to the CRE as a homodimer or as a CREB-CREM heterodimer, and in either form would compete with transactivating CREB homodimers for CRE binding (Foulkes *et al*, 1991). Similarly, a truncated Fos family member, ΔFosB, has recently been characterised as a naturally occurring product of differential splicing of the FosB transcript (Nakabeppu and Nathans, 1991). ΔFosB lacks an activation domain but nevertheless can dimerise with c-Jun; the resultant c-Jun/ΔFosB heterodimer binds to AP1 sites and can inhibit transactivation by c-Jun/c-Fos or c-Jun/FosB heterodimers (Nakabeppu and Nathans, 1991). Thus where the activator is a dimer, as in the cases of CREB and Jun/Fos, negative regulation by competition can have two aspects: competition for dimer formation combined with competition for DNA binding sites. In addition, formation of heterodimers with varying efficiency as activators may be important in regulating transcriptional activation not only by the Fos and Jun families of proteins (Schutte *et al.* 1989) but also for all multigene families of activators that heterodimerise (reviewed by Jones, 1990).

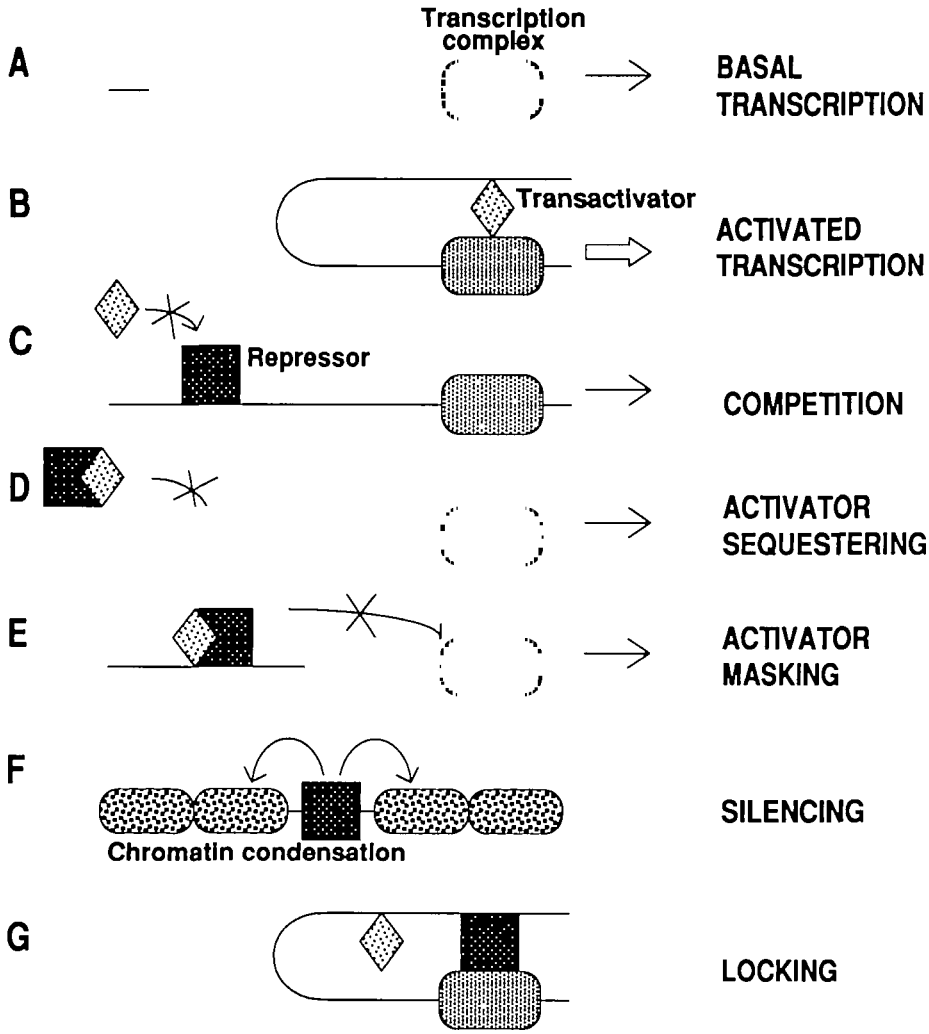

Fig. 1. Diagrammatic representation of different modes of transcriptional repression. For simplicity a single transactivator and a single repressor molecule are represented. (A and B) represent basal and activated transcription, respectively; the activator is depicted as interacting with the transcription complex and looping out the intervening DNA. (C) The activator is unable to bind to its cognate site due to the presence of the repressor molecule already bound at this site. (D) The transactivator is complexed with repressor and unable to bind DNA. (E) The transactivator is bound to its cognate site, but is complexed with DNA-bound or free repressor and thus unable to activate transcription. In C, D and E basal transcription continues, as will activated transcription from other enhancer elements in the promoter. (F) The repressor molecule promotes chromatin condensation, rendering the gene unresponsive to activation; other mechanisms of silencing may exist; for example, a repressor may also promote DNA methylation. (G) The transcription complex is locked at the promoter site by interactions with the repressor; the activator may bind DNA but has no effect.

Activator sequestering

Several transcriptional activators have been found to form inactive complexes with specific inhibitory proteins (Fig. 1D). The NF-κB transactivator is present in most cell types as a cytosolic complex with an inhibitor, I-κB; upon induction with cytokines I-κB dissociates, allowing NF-κB to enter the nucleus and activate transcription (reviewed by Lenardo and Baltimore, 1989). Zabel and Baeuerle (1990) demonstrated that I-κB is able to dissociate NF-κB from DNA *in vitro*, and proposed that after induction of NF-κB as described above newly synthesised I-κB diffuses into the nucleus where it dissociates NF-κB from its target DNA, followed by diffusion of the NF-κB/I-κB complex back into the cytosol.

An analogous situation exists for the Fos/Jun activator, whose activity is modulated by IP-1, a protein found in both the nucleus and the cytoplasm (Auwerx and Sassone-Corsi, 1991). IP-1 appears to inhibit Fos/Jun by association with the leucine zippers of Fos and Jun, thus preventing DNA binding by Fos/Jun; IP-1 is inactivated by protein kinase A (PKA)-dependent phosphorylation, whilst protein kinase C (PKC) induction activates Fos/Jun, thus indicating the possibility of cross-talk between the PKA and PKC signalling pathways (Auwerx and Sassone-Corsi, 1991).

Similarly a *Drosophila* homoeodomain protein, I-POU, has been identified that is able to dissociate the activator Cf1-a, another homeodomain protein, from DNA by the formation of non-DNA binding heterodimers (Treacy *et al.* 1991).

Activator masking

The modes of repression described above operate essentially by preventing the binding of a transactivator molecule to its cognate site. However, a class of repressor proteins also exists that does not affect transactivator binding to DNA but instead masks the activation domain (Fig. 1E), either directly or by conformational alteration, thereby neutralising activation. This mode of repression operates for the *S. cerevisiae* galactose utilisation (*GAL*) genes. In the absence of galactose the repressor GAL80, which does not itself bind DNA, forms a complex with the activator GAL4, such that GAL4 remains bound to the DNA but is unable to transactivate transcription (reviewed by Johnston, 1987). In fact, by inserting an activation domain into GAL80, Ma and Ptashne (1988) converted this repressor into an activator of promoters containing GAL4 binding sites.

The interaction of *myc*-PRF (*myc*-plasmacytoma repressor factor) with the transactivator CF1 (common factor 1) (Riggs *et al.* 1991) in the murine *myc* promoter may also represent activator masking. *myc*-PRF is expressed in terminally differentiated B-cells where it represses c-*myc* transcription. The binding sites for CF1 and *myc*-PRF are adjacent and *myc*-PRF actually stabi-

lises the interaction of the transactivator CF1 with its cognate site (Kakkis *et al.* 1989).

Silencing

DNA binding repressors may act by competition with or masking of transactivators as described in the previous sections, and in order to operate in this way the repressor binding sites must have a specific positional relationship with the activator binding sites. The fourth type of transcriptional repressor acts *via* DNA elements that are position independent, and by analogy with enhancers are termed silencers (Brand *et al.* 1985). In the same way that multimerisation of enhancer elements amplifies transcriptional activation, multimerisation of silencer elements or modules thereof has been shown to augment the negative effect. A single copy of a 40 bp silencer element from the chicken vimentin gene promoter can reduce expression from the HSV TK promoter by 30% whilst four tandem copies of the same element reduce expression by 90% (Farrell *et al.* 1990). A silencer of the chicken lysozyme gene present 2.4 kb upstream from the start site has a modular structure, with two elements F1 and F2, which can function individually in repressing transcription from a minimal TATA promoter, although repression is greater when multimers of F1 and/or F2 are present (Baniahmad *et al.* 1990). The mechanism of action of these silencers is not yet clear; however, in *S. cerevisiae* there is mounting evidence that silencing is a direct result of alterations in chromatin structure (Fig. 1F).

The *S. cerevisiae* mating type locus *HMR* 'E' silencer (Fig. 2) functions in either orientation, on either side of the promoter, can be moved to a distance of 2.6 kb from the transcription start site and will repress heterologous promoters (Brand *et al.* 1985). Three elements, A, E and B, have been identified for the *HMR* 'E' silencer; these elements show some functional redundancy but the individual elements alone cannot silence transcription (Brand *et al.* 1987). The A element possesses autonomously replicating sequence (ARS) activity to which B also contributes, E is a RAP1 (repressor-activator binding protein) binding site, and both the E and B elements are able individually to activate transcription from heterologous promoters (Brand *et al.* 1987). Two possible mechanisms for mating type locus silencing were proposed by Shore and Nasmyth (1987): first, that repression is propagated from the silencer at replication and the enhancer elements E and B ensure that replication initiates within the silencer ARS; second, that RAP1 may complex with another DNA-bound protein(s) to create a transcriptionally inactive chromatin loop and, indeed, RAP1 protein together with a nuclear scaffold extract is able to reconstitute a chromatin loop from *HMR* DNA *in vitro* (Hofmann *et al.* 1989). However, the role of the ARS sequence remains unclear: for the mating type locus *HML* 'E' silencer (Fig. 2), which possesses an ARS element (A), a RAP1 binding site (E) and an uncharacterised element (D), recent evidence suggests that the replication initiation function of the ARS element is not required *per se*, but that the ARS element may function in some other capacity in the silencer (Mahoney *et al.* 1991). Nevertheless, DNA replication is required for re-establishment of repression in a $sir3^{ts}$ strain returned to the permissive temperature (Miller and Nasmyth, 1984), and mutations of CDC7, a kinase required for initiation of DNA replication, can suppress silencer-defective *HMR* mutants (Axelrod and Rine, 1991).

In *S. cerevisiae* cells of mating type α, the α2 repressor protein expressed from the active mating type locus represses expression of **a**-specific genes by binding to the α2 operator sequence positioned about 200 bp upstream from the initiation codon of these genes (reviewed by Herskowitz, 1989). When the α2 operator is placed upstream or downstream from the *S. cerevisiae* CYC1 UAS, expression from the CYC1 promoter is repressed in **a** cells, thus the α2 operator shows position independence (Keleher *et al.* 1988). Roth *et al.* (1990) have demonstrated that the α2 repressor is able to position nucleosomes on DNA adjacent to the α2 operator; thus it appears that α2 repressor functions by altering the chromatin structure of neighbouring DNA.

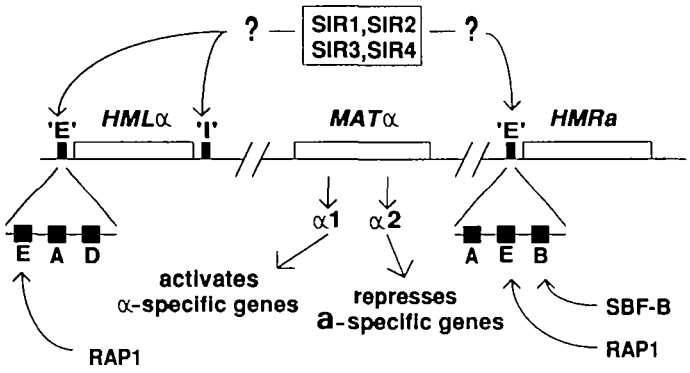

Fig. 2. The mating type loci on *S. cerevisiae* chromosome III in the α mating type configuration, where the active locus *MAT* expresses α1 and α2 products. During mating type switching the gene cassette at *MAT* is replaced by copying a cassette from one of the silent mating type loci *HML* or *HMR*. Silencer elements are shown as filled boxes. A, ARS sequence; B, SBF-B (ABF-1) binding site; D, uncharacterised element; E, RAP1 binding site; I, distal silencer for *HMLα*. Proteins SIR1–SIR4 are required for silencing, but their interaction with the silencer elements is not yet fully understood. See text for further details and references.

The overlap between transactivators and repressors

Although activation and repression of transcription are functionally opposite, it appears that some proteins can participate in either activity. It has been known for some years that bacteriophage λ repressor can activate transcription of its own gene whilst repressing other phage gene expression (see Hochschild and Ptashne, 1988) and an analogous duality of function exists for some eukaryotic transcription factors. Elements E and B of the *S. cerevisiae HML* silencer were identified as enhancer elements (Brand *et al.* 1987). Similarly, the F2 module of the chicken lysozyme silencer is equivalent to an inverted thyroid hormone response element, and in the presence of thyroid hormone F2 can activate transcription (Baniahmad *et al.* 1990). The F1 module will synergise in both the positive and negative activities of F2. DNA context, protein conformation and protein–protein or protein–cofactor interactions are all factors that may influence whether a DNA binding protein activates or represses transcription in a given situation. For example, proteins that influence nucleosome positioning (Fedor *et al.* 1988; Roth *et al.* 1990) could thereby either exclude or assist the

binding of transcription factors to adjacent DNA, depending on the placing of the nucleosome relative to activator sites. Another mechanism whereby known transactivators may act as repressors is by binding site competition. The bovine papillomavirus type 1 (BPV1) E2 product has been characterised as a potent transactivator; however, at the BPV1 P1 promoter the E2 transactivator is able to repress transcription by preventing the binding of a cellular transcription factor that is essential for promoter activity (Stenlund and Botchan, 1990). Similarly, the thyroid hormone receptor (TR) will still bind to DNA when the two half sites of its palindromic recognition sequence are separated by 3 bp; however, in this situation TR fails to activate transcription, possibly due to conformation changes brought about by binding to the expanded site, and indeed will compete with an activating oestrogen receptor binding at the same site (Glass *et al.* 1988). Thus there is no clear-cut division of transactivators and repressors; rather, there appears to be a region of overlap between the two classes of proteins.

Discussion

The work described above demonstrates the wide range of mechanisms of negative control of transcription, reflecting the differing needs of the cell: to prevent undesirable gene expression, to respond rapidly to stimulation, to switch off a response rapidly and to fine-tune the expression of active genes. In *S. cerevisiae* coexpression of both **a** and α mating type products results in sterility (reviewed by Herskowitz, 1989), and during development and differentiation in higher eukaryotes inappropriate gene expression is likely to disrupt cell programming. Most eukaryotic promoters contain binding sites for ubiquitous transactivators and the mere absence of tissue-specific activators may not be sufficient to prevent a basal level of transcription. Thus silencers, able to repress transcription independently of their position with respect to enhancers, might be expected to be present for many developmentally regulated genes. Chromatin loops bounded by nuclear scaffold attachment regions (SARs) appear to constitute the regulatory units of eukaryotic transcription (Phi-Van and Stratling, 1988; Sippel and Renkawitz, 1989) and exogenous genes integrated into the genome can be insulated from both activating and silencing chromosome position effects by placing SARs at either end of the gene to be introduced (Kellum and Schedl, 1991). Thus it might be envisaged that during replication when the chromatin is unfolded, and the DNA presumably becomes accessible to transcription factors, silencers would ensure that inappropriate regulatory units from SAR to SAR were returned to an inactive state. Silencing may be in part an epigenetic effect, depending on the transcriptional status of a gene in the previous cell generation: in *HML* 'E' mutants where both silenced and derepressed cells exist in the same population, the silent state is inherited (Mahoney *et al.* 1991). In this instance silencing may be analogous to the repression seen as a result of the action of the *Drosophila* position effect varigation modulators (Henikoff, 1990) and *Polycomb* group proteins (Paro, 1990) described earlier: in both cases the repressed state, once established, can be inherited epigenetically.

However, negative regulation by formation of higher order chromatin structure would be inapt in cases where a gene must respond rapidly to signalling pathways. More appropriate mechanisms would be the sequestering of activators (Lenardo and Baltimore, 1989; Treacy *et al.* 1991; Auwerx and Sassone-Corsi, 1991) or masking of activator domains (Johnston, 1987; Keleher *et al.* 1988). Following the induction phase, gene expression may be rapidly switched off by repressor even though transactivators may still be present. An additional advantage of the masking mechanism of repression is that upon induction and repressor dissociation the transactivator is already bound to the DNA and able to respond immediately. Similarly, in a 'locking' mechanism (Fig. 1G; and Keleher *et al.* 1988) a repressor molecule locks the transcription machinery at the promoter prior to activation. A well-characterised example of such a mechanism is the *lac* repressor of *Escherichia coli*, which actually increases the binding of RNA polymerase to the *lac* promoter whilst preventing initiation of transcription (Straney and Crothers, 1987). Thus upon induction and consequent removal of *lac* repressor the first round of transcription is augmented; therefore the *lac* repressor can be said to act as a transient activator (Straney and Crothers, 1987). DNA-binding transcriptional repressors may also function in holding the DNA in an open configuration to which transactivators may bind easily upon induction. Fine tuning of transcription from active genes appears to be achieved in many cases by competition between activators and repressors (Lambert *et al.* 1987; Foulkes *et al.* 1991; Nakabeppu and Nathan, 1991) or even between activators of different efficacy (Schutte *et al.* 1989). Both transactivators and repressors are also subject to activating/inactivating post-translational modifications such as phosphorylation (Barber and Verma, 1987; Auwerx and Sassone-Corsi, 1991; Boyle *et al.* 1991) and perhaps redox status changes (Abate *et al.* 1990). Several of these mechanisms may operate together: for instance, the activity of the Jun/Fos transactivator is subject to negative regulation by competition for both binding sites and heterodimer formation (Schutte *et al.* 1989; Nakabeppu and Nathan, 1991), sequestering by IP-1 (Auwerx and Sassone-Corsi, 1991) and phosphorylation state changes (Barber and Verma, 1987; Boyle *et al.* 1991). The importance of negative regulation of transactivation is highlighted by the identification of many transactivators including Fos and Jun as proto-oncogene products (reviewed by Lewin, 1991), and recent evidence suggests that the tumour suppressor gene product $p105^{Rb}$ is a negative regulator of the transactivator DRTF1 (Bandara and La Thangue, 1991).

It is increasingly apparent that eukaryotic transcription is subject to a highly complex interplay of both positive and negative interactions. Much of the current work on transcriptional regulation has focussed on the operation of individual control elements; the ultimate aim must be to understand how multiple positive and negative control circuits operate together to determine the level of gene expression during the different phases of development, differentiation and responses to the environment.

I thank Drs M. Saveria Campo and John Wyke for their valuable comments on the manuscript.

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