Silencers and locus control regions: opposite sides of the same coin

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Whether or not genes are in an active or a repressed state in a cell depends on the relative effect of gene silencers and locus control regions (LCRs). Here, we suggest that these elements act as binary switches; the state that prevails (activated or repressed) probably depends on a competition between protein complex formation and the stability of the complexes formed at either of the two elements.

THE EUKARYOTIC GENOME is organized into regions with distinct structures and functions. Active genes normally reside in loosely packaged regions, whereas few active or inactive genes are located in highly condensed domains of the nucleus.

The expression of a eukaryotic gene can also be affected profoundly by its chromosomal location, for example, when genetic rearrangements place euchromatic segments into heterochromatic domains, the expression of the translocated euchromatic gene is altered in some, but not all, of a population of cells, producing a mosaic or variegated phenotype. This phenomenon was first observed in Drosophila and is referred to as position effect variegation (reviewed in Ref. 1).

Regulatory elements, such as promoters, enhancers and silencers, are required for the transcriptional regulation of genes in vivo. There are two views of how gene activation occurs: the analog view and the digital view. Studies in bacteria, as well as studies with transiently transfected eukaryotic cells, support the analog view of transcriptional activation; that is, regulatory elements are believed to mediate transcriptional activation by increasing the rate of transcription, for example, by increasing the density of transcribing RNA polymerases. However, recent data on silencers and certain classes of enhancers (present at LCRs) support the existence of a 'binary' or digital state of gene expression. In this scenario, regulatory elements determine whether the gene is on or off in an 'all-or-nothing' phenomenon2: LCRs increase the number of cells expressing a gene and act to increase the probability that a gene will be activated in the first place. When a gene has been committed for activation, the level of transcription can vary to generate different levels of transcripts.

This review will focus on recent results on the regulation of transcription at the yeast silent loci and the human β-globin locus, to provide a model of gene regulation within densely packed regions of the genome.

Enhancers and locus control regions

Much of the detailed molecular studies on eukaryotic transcriptional regulation has been devoted to two topics – how the general transcriptional machinery assembles at the promoter3 and how activator proteins activate transcription4. Both housekeeping and inducible genes appear to be packaged into chromatin structures that can be rapidly disrupted by transcription factors in concert with ATP-dependent remodeling factors such as the SWI–SNF complex5. Activation of these genes probably involves localized chromatin remodeling around the promoter and enhancer elements, followed by functional interactions between the factors bound to these sites, facilitating transcription. However, many, though not all (for example, see Ref. 6), developmentally regulated genes are packaged into repressed chromatin structures. Transcriptional activation of these genes involves the disruption of the repressed chromatin over large regions by distant complex enhancers called locus control regions (LCRs) before gene activation6 (see Table 1).

Enhancers are regulatory sites that potentiate transcription from RNA polymerase II-transcribed promoters. Enhancers can function whether located upstream or downstream of a gene, in either orientation and in both a distance- and a gene-independent manner. Enhancers are most often assayed in transiently transfected culture cells7, but studies of enhancer function in stably transfected cell lines reveal that their function can depend on where in the chromatin they are integrated. In general, enhancers cannot activate transcription when integrated into highly condensed regions of the genome (reviewed in Ref. 8).

Locus control regions appear to be required for the activation of genes that reside in densely packed regions of the chromosome, where large domains need to be opened before gene expression. Genes that reside in constitutively open chromatin domains possess enhancers, but do not appear to have LCR activity8,9. Like enhancers, LCRs contain numerous binding sites for transcription factors, but, unlike enhancers, LCRs govern gene regulation independently of chromosomal position. It has been proposed that the different elements of the LCR act together as a functional holocomplex, and mutations that weaken the holocomplex disrupt LCR function10,11, namely position-independent gene activation.

The LCR is composed of two distinct functions: one element contains enhancer activity (as defined by transient transfection assays), and the other elements are required to open repressed chromatin domains12–14. When a chromatin domain has been opened, it has been suggested that initiation of transcription from a competent gene in that domain occurs as a consequence of interactions between the LCR holocomplex and the gene promoter.

Silencers and the silent domain

Heterochromatin in Drosophila and larger eukaryotes (reviewed in Ref. 15) is defined by cytological criteria that, owing to the limits of light microscopy, are not applicable in yeast. Nevertheless, there are both genetic and biochemical data indicating that the silent domains are heterochromatin-like structures in yeast16, and are the focus of this discussion.

Silencing in the yeast Saccharomyces cerevisiae is observed at the two transcriptionally repressed copies of the mating type alleles known as HMLα and HMRα. In addition, transcriptional silencing is also observed at telomeres17.
Repression at these loci involves the combined action of regulatory sites (silencers) and proteins that act at these sites. The silencers for HML and HMR contain binding sites for Rap1p and Ab1p, and an autonomous regulatory sequence (ARS) element, which binds the origin recognition complex (ORC). The silencer acts in both a distance- and an orientation-independent manner to repress transcription, suggesting that the repressed state emanates bidirectionally and is somehow propagated along the DNA. Furthermore, repression is not restricted to the mating type genes, as numerous RNA Pol II and Pol III promoters are repressed when positioned at HMR. The DNA of the silent domain encompasses about 3–4kb of DNA and is packaged into an inaccessible chromatin structure, as determined by its inaccessibility to various enzymatic probes.

Several genes and their products have a role in silencing HML and HMR including the SIR1–SIR4 (silent information regulator) gene products. Additional factors such as the histones H3 and H4 are also required for silencing, the positively charged amino-terminal tails of which have been shown to interact in vitro with Sir3p and Sir4p (Ref. 19). It is likely, however, that Sir1p interacts with the ORC bound to the silencer.

Establishment, maintenance and inheritance of silencing

Maintenance. A popular view of silencing is that the silent domain is maintained in a transcriptionally repressed state through interactions between the proteins bound to the silencer and the proteins associated with nucleosomes. However, it has been demonstrated that when a repressed domain is established, physical linkage of the silencer to the silent domain is not required for maintenance of silencing. Separation of the HMRE-silencer from the silent domain using specific enzymes in vitro has no effect on the repressed chromatin structure of the silent domain. Similar results have been obtained in vivo where the silencer was separated by recombination.

While the covalent linkage of the silencer to the silent region is not required for maintaining the repressed state, inactivation of the proteins that mediate silencing leads to an immediate disintegration of the repressed state. The use of conditional alleles of Sir3 (Refs 21, 22) and ORC2 (Ref. 23) revealed that disruption of either Sir3p or Orc2p function leads to an immediate derepression of the silent genes at various stages of the cell cycle.

Establishment of silencing is defined as the switch that leads an active HM locus to become transcriptionally silent (Fig. 1). When a silent domain has been experimentally derepressed, the repressive chromatin structure of the domain can be established only following passage through S phase. While the S-phase event that is required for the establishment of the silent state is not known, efficient establishment does require the silencer elements and Sir1p and might be a consequence of replication-associated chromatin assembly.

Mutations in the proteins that bind the silencer lead to reduced silencing. Studies on sirl mutant strains provided the first model of the mechanism of establishment of the repressed state. Unlike mutations in sir2, sir3 and sir4, mutations in sir1 cause only a partial derepression (80% derepression) of the silent mating-type loci, as measured within a population of cells. However, detailed single cell analysis indicates that in a population of sir1 mutants, 20% of the cells are totally repressed and 80% are completely derepressed at HML. Mutations in the Rap1 protein, which binds HMRE-silencer (Ref. 25), also leads to two populations of cells – one repressed and one derepressed – as do mutations within the cis-acting elements at HML-E and at the HMRE-silencer. These results can best be explained by a binary model of gene regulation. In this model, gene activation or repression is an all-or-nothing phenomenon, in which silencers act by increasing the probability that the gene is repressed in any given cell.

Inheritance. The transcriptional state of the silent domain is also stably inherited through multiple mitoses. The stable transcription state of a gene is presumably disrupted in every cell cycle during replication and, consequently, to inherit the repressed state, silencing would have to be efficiently re-established in every cell cycle. Mutations in sirl as well as in the components of the silencer slightly affect the mitotic inheritance of the transcriptional state. In sirl mutant cells, greater than 90% of the repressed mother cells gave rise to repressed daughter cells. As the transcription state of a gene is disrupted during S phase, the mechanism by which the silent state is stably inherited in these mutant cells is not well understood.

However, deletion of the silencer from a silenced chromatin domain in cells progressing through the cell cycle leads to derepression of the silent domain within a single generation, indicating that the intact silencer is required for the efficient inheritance of the repressed chromatin structures following replication. The silencer appears to provide the genomic memory that promotes the reformation of the silent chromatin in the progeny. In the absence of a silencer, the probability of inheriting the silent state in the daughter cells is nonexistent, whereas cells in which sir1 and rap1 are mutated can still generate the repressed state, which can then be faithfully propagated to the progeny, but the inheritance of the silent state in every cell cycle is more prone to failure, presumably because the stability of the protein complex at the silencer is weakened and the probability of inheriting the repressed state is thus reduced. Therefore, silencers can be considered as elements that efficiently ensure gene repression.

Heterochromatin and transcription activation

Transcriptional activation of a gene is determined in part by the formation of an LCR-mediated holocomplex, which most likely increases the probability, frequency and duration of expression of a gene at a specific locus. This hypothesis is borne out by results from recent studies of mutations that weaken the β-globin LCR holocomplex. The studies suggest that mutations decrease the

| Table I. Characteristic features of enhancers, focus control elements (LCRs) and silencers |
|---------------------------------|-----------------|-----------------|
| Function                        | Enhancers       | LCR             | Silencers       |
| Orientation                     | Activate        | Activate        | Repress         |
| Location                        | Both            | –               | Both            |
| Distance of action              | 5' and 3'       | Large           | Moderate        |
| Gene specificity                | Some Pol II     | Pol II          | Pol II and Pol III |
| Position independence           | No              | Yes             | Yes             |
| Chromatin remodeling            | Localized       | Large domains   | Moderate domains |

*With respect to gene. **Pol II and Pol III refer to RNA polymerases II and III.*
A schematic representation of the maintenance, establishment and inheritance of silencing during the cell cycle. Nucleosomes are represented as circles, the SIR protein complexes are depicted as horizontal ellipsoids, while the silencer-associated proteins (Rap1p, Abf1p and the six-subunit origin recognition complex) are shown as vertical ellipsoids.

stability of interactions between the LCR holocomplex and the gene promoter, probably by decreasing the frequency or duration of such associations and thereby reducing transcription. This would therefore manifest itself as fewer cells in the population possessing a potentially active gene, rather than all the cells expressing the gene at intermediate levels. Thus, the LCR determines gene expression by determining the frequency and duration of the active state rather than controlling the rate of transcription directly. These results are analogous to the results obtained in yeast, where mutations in the silencer lead to two populations of cells, one with the domain repressed and one with the domain derepressed). Both sets of results support a binary model of gene regulation.

This model is also supported by studies that distinguish expressing cells from non-expressing cells. In stable transfection experiments, reporter genes (neo) linked to an enhancer (from the β-globin LCR) generate many more G418-resistant colonies compared to the reporter gene alone. However, when the amount of correctly initiated mRNA was measured from individual clones, it was revealed that the enhancer did not further enhance mRNA levels over that seen with the promoter alone. Similar results were obtained with transgenic mice: in mice carrying a transgene with the LCR, all the cells expressed the gene. However, mice that had lost the LCR exhibited a mosaic expression pattern, with some cells expressing the transgene and others not. In those cells within the mosaic that expressed the transgene, the level of expression was comparable to that in cells containing the LCR.

When an open chromatin domain has been established, LCR enhancers, like silencers, are also required for the stable mitotic inheritance of this domain. Analysis of clones bearing independent integration events of the reporter gene revealed that when an active state is established, the presence of an LCR element reduced the rate of transcriptional inactivation of the linked reporter. However, subsequent removal of the β-globin LCR enhancer from the linked reporter gene by recombination increased the rate of transcriptional repression at these loci. This shows that deletion of the LCR enhancer does not result in immediate repression of transcription, but merely increases the rate of repression. Thus, LCRs are required for the efficient establishment of an active state in a repressed chromatin environment and are also required for the efficient inheritance of the active state in daughter cells. These functions of an LCR can therefore be viewed as being antagonistic to those of silencers.

**Competition between activators and repressors**

The relative concentrations and affinities of transcription factors and repressors has been proposed to determine the transcriptional state of a gene (Fig. 2). In vitro studies support this model and a similar picture emerges from in vivo studies of gene regulation at yeast telomeres. Like the silencers at HML and HMR, telomeres are sites of silenced chromatin. Recent data suggest that
the upstream activating sequence (UAS) of a gene is the major determinant for effective transcriptional regulation at the telomere.

Pprlp is a transcriptional activator that binds the UAS of the URA3 gene to modulate its transcription. When URA3 is translocated to the telomere, its basal level of transcription is strongly repressed. The silent chromatin also prevents transcriptional activation of the telomeric URA3 gene by Pprlp, specifically during the G1- and early S phases of the cell cycle. However, following replication of the DNA, Pprlp can activate transcription of the telomeric URA3 gene. These data support a model in which the fate of a gene is determined by a competition between the formation of a stable active transcriptional complex and the formation of the normal silenced chromatin structure either during or soon after replication.

This hypothesis is reinforced by the analysis of extragenic suppressors that restore silencing in strains containing a defective HMR-E silencer. These studies identified mutations in cell-cycle regulatory genes (such as CDC7, as well as various cyclins) and genes encoding transcription factors (for example, SWI6 and SIR3). One model suggests that these mutants act by slowing the progression of the cell cycle, thus changing the relative concentrations of activators and repressors, and providing a greater opportunity for the repressed state to reform following its disruption during S phase.

The competition and sequestration of silencing factors to specific subnuclear compartments might further affect the establishment of silencing. The observation that a physical proximity between telomeres and HMR increases silencing at HMR, coupled with the observation that increased telomeric length leads to derepression of HMR, support a model of dynamic equilibrium between silencing at both the telomeric and the HM loci. Furthermore, the demonstration that telomeres are clustered at the nuclear periphery, raises the possibility that subnuclear compartmentalization also influences silencing.

The observation that the silent loci are late-replicating and that silencing depends critically on the proper dosage of Sir4p (changes of twofold are deleterious) along with the demonstration that Sir3p is a limiting component in the cell, raises the possibility that the level, activity and compartmentalization of proteins during the cell cycle might play an important role in the switch between repressed and derepressed states.

A synthesis
Silencers appear to have properties and functions similar, but antagonistic, to LCRs. Any model of silencer and LCR function must take into consideration the numerous general properties of these two elements — namely that they function bi-directionally in a position- and distance-independent manner on both homologous and heterologous promoters. Because silencers and LCRs act as binary switches in a particular cell, leading to a repressed or activated state, it is suggested that these two cis-acting modules might have antagonistic functions that are executed by similar molecular mechanisms. In the case of transcriptional repression, the formation of a stable complex at the silencer probably leads to the recruitment of proteins involved in the formation of the silent chromatin domain. In the case of transcriptional activation, the formation of a complex at the LCR leads to the opening of the repressed chromatin domain allowing for the promoter to function. The molecular mechanisms by which entire domains are either repressed or activated remain obscure, but the state that prevails might depend on a competition between the formation of the two complexes (at silencers and LCRs) (Fig. 2). The most likely time for the occurrence of this competition would be during S phase and the period immediately following it, before chromatin maturation when the transcription state of a gene is disrupted and has to reform. The timing of replication of a particular gene coupled with the relative concentrations of activators and repressors in specific compartments of the nucleus could affect the outcome of this competition.

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References
2 Walters, M. C. et al. (1996) Genes Dev. 10, 185-195
9 Robertson, G. et al. (1996) Nucleic Acids Res. 24, 1465-1471
11 Milot, E. et al. (1996) Cell 87, 105-114
13 Ellis, J. et al. (1996) EMBO J. 15, 562-568
14 Burgert, J. et al. (1995) Genes Dev. 9, 3083-3096
19 Hecht, A. et al. (1995) Cell 80, 583-592

Figure 2
A diagrammatic representation of the binary model of gene expression. In this model, a competition between activators and repressors is proposed leading to the all-or-nothing change in chromatin states and transcription activation. The blue dashed line reflects the concentration of activator protein, the stability of binding as well as the duration of binding of the activator to a particular gene sequence, while the red dashed line reflects concentration, affinity and duration of binding of the repressor to the gene. The green line indicates the probability of a gene becoming potentiated for transcription activation during development and differentiation.
Histone acetylation: chromatin in action

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Histone acetylation acts as a landmark and determinant for chromatin function. Active roles in the transcription and assembly of chromatin have been discovered for histone acetyltransferases and deacetylases. This review highlights these roles and discusses their significance for the maintenance of cell differentiation.

Chromatin is not a static entity where DNA is packaged up and forgotten by the molecular machinery controlling transcription, replication, recombination and repair. Instead, chromatin structure is dynamic, accommodating the need for DNA to partake in the various functions that require it as a template.

In the past, changes in chromatin conformation were usually viewed as a consequence of DNA being used for some purpose, but recently, a remarkable series of observations challenge this view. Here, we discuss the exciting progress in the identification and characterization of chromatin-modifying proteins, particularly acetyltransferases and deacetylases. The precise roles of these histone-modifying enzymes in transcription are not yet known and this review includes speculative ideas about their possible functions. For example, components of the basal transcriptional machinery, transcriptional coactivators and co-repressors have been found to directly modify histones. Histone acetyltransferases and deacetylases might exert regulatory functions at the level of promoter recognition, activation by upstream activators or in the process of initiation or elongation by RNA polymerase. This review focuses on only some of these possibilities, in particular, the establishment and the maintenance of a transcriptionally competent chromatin environment. These biochemical observations complement existing structural and genetic data that establish chromatin as an integral regulatory component of the transcription process.

Chromosomal replication and repair has been found to require a molecular chaperone that shares a common subunit with particular histone acetyltransferases and deacetylases, and that also interacts with a tumor suppressor. Other links between histone acetylation and tumorigenesis exist: for example, the adenovirus oncoprotein E1A has been shown to modulate the interactions between acetyltransferases and deacetylases, and fusions between the genes that encode two such putative histone acetyltransferases are associated with certain cancers.

Recent studies also support an essential role for histone acetylation in the establishment and maintenance of a differentiated phenotype. This surge of new information implicates histone acetylation and the associated chromatin conformational transitions as having a central role in cell and developmental biology.

This review attempts to integrate the phenomenon of histone acetylation with the molecular events that it appears to regulate. We will describe: (1) some of the potential mechanisms by which histone acetyltransferases regulate transcription; (2) the potential roles of components of histone acetyltransferases and deacetylases used in chromatin assembly in monitoring chromosomal integrity during the cell cycle; and (3) we will speculate on the significance of histone acetylation for the maintenance of the differentiated state and of aberrant acetylation for cell transformation.

What does histone acetylation do to chromatin?

Before discussing the various proteins that acetylate and deacetylate the core histones, it is useful to know what structural changes might occur within chromatin as a consequence of acetylation. Each core histone has two domains: a histone fold domain, which is involved in histone–histone interactions and in wrapping DNA in nucleosomes, and an amino-terminal tail domain that lies on the outside of the nucleosome, where it can interact with other regulatory proteins and with DNA. The amino-terminal tail domains are lysine-rich and are targets for acetylation. Acetylation greatly reduces the affinity of the histone H4 tail for DNA. The physical consequences for nucleosomal integrity of acetylating all of the histone tails in the absence of any other proteins are relatively minor. However, there is a modest reduction in the wrapping of DNA around the histone octamer and nucleosomes pack together less efficiently in arrays (for reviews, see Refs 12, 13).