Repression of Transcription in Yeast

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One of the ways prokaryotes differ from eukaryotes is in the structure of the regions that control transcription of their genes. In prokaryotes, control of transcription typically depends on a short sequence upstream of a gene. This sequence contains a binding site for RNA polymerase, and may also contain nearby or overlapping sites where regulatory proteins bind to DNA. Repressor proteins, when present, bind to sites on the DNA close enough to block the binding of RNA polymerase to the promoter. By contrast, transcriptional control regions in eukaryotes are typically much larger, and sequences important for negative regulation can lie hundreds of nucleotides from the startpoint of transcription. This difference in the structure of the regulatory regions raises the possibility that prokaryotes and eukaryotes use different methods to repress transcription of their genes.

Regulatory regions of many genes in the yeast Saccharomyces cerevisiae conform to the eukaryotic pattern. The DNA upstream of a yeast gene contains distinct elements that can be separated by hundreds of nucleotides: stretches of DNA called upstream activation sites (or UAS), and stretches of DNA closer to the startpoint of transcription that often include the sequence TATA (see figure, line 1). Activation of transcription depends on the binding of proteins to the UAS (see, for example, Giniger et al., Cell 40, 767–774, 1985). Repression of transcription, in the cases described below, depends on the action of proteins at sites other than the UAS.

A Model System

One approach to understanding how negative regulation might work in eukaryotes has been to use a prokaryotic protein to repress a yeast gene (Brent and Ptashne, Nature 312, 612–615, 1984). E. coli LexA protein synthesized in yeast can enter the nucleus and bind a lexA operator. When the operator is inserted in the GAL1 promoter between the UAS and the TATA region, LexA represses GAL1 transcription (see figure, line 2). Depending on the position of the lexA operator within the GAL1 promoter, the factor of repression varies from 5- to 10-fold. No repression is observed when the operator is placed near to, but upstream of, the UAS.

A Repression System Native to Yeast

The best-studied case of transcriptional repression in yeast depends on the product of the MATα2 gene (α2). Genes that are not transcribed in cells of the a mating type (a-specific genes) are kept repressed by a process that requires α2. Genes that are not transcribed in diploid cells (haploid-specific genes) are kept repressed by a process that requires α2 as well as the product of another gene, MATα1 (α1). α1α2 repression is more efficient when the site of action is between a UAS and a TATA region than when it is upstream of a UAS (Siliciano and Tatchell, Cell 37, 969–978, 1984; Miller et al., Nature 314, 598–603, 1985). Establishment of α1α2 repression can occur rapidly, and does not require a new round of cellular DNA synthesis (Miller and Nasmyth, Nature 312, 247–251, 1984).

This issue of Cell contains a paper by Johnson and Herskowitz that begins to show how α2-dependent repression works. The authors have purified an α2-β-galactosidase fusion protein that represses α-specific genes in vivo, and have shown that it binds to a site upstream of the α-specific gene STE6. The purified protein protects a 33 bp region upstream of STE6 from digestion by DNAase I; this region is homologous to sequences found upstream of other genes repressed by α2 (see also Miller et al., 1985, op. cit.). When the protected sequence from STE6 is inserted upstream of the CYC1 gene, α2 represses CYC1 transcription. The degree of repression depends on the location of the sequence: when it is inserted between the UAS and TATA region, α2 represses transcription by a factor of 100–200; when it is inserted upstream of the UAS, transcription is repressed by a factor of 15–20 (see figure, lines 3 and 4).

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protein binds DNA upstream of the haploid-specific \textit{HO} gene in vitro (Johnson and Herskowitz, op. cit.), but repression of \textit{HO} in vivo requires \textit{a}1.

Mutations in a gene whose product helps \textit{a}2 repress \textit{a}-specific genes have not been reported. However, such mutations may have escaped detection either because they affect an essential gene or because their effects are subtle. Like LexA binding, \textit{a}2 binding without an ancillary factor might still repress, albeit with diminished efficiency, those \textit{a}-specific genes whose \textit{a}2 binding site lies between the UAS and the TATA region.

It is not clear how DNA binding by LexA or \textit{a}2 results in repression. A repressor protein (with or without cofactors), could interfere sterically with binding of positive regulatory proteins to the UAS or TATA or could hinder the movement of an RNA polymerase molecule from the UAS to the startpoint of transcription. Alternatively, if transcription normally requires the formation of a bend in the DNA that allows proteins bound to the UAS to touch proteins bound further downstream, a repressor might work by blocking correct formation of that bend.

\textbf{A Different Repression System}

Repression of the silent copies of the mating type locus seems to proceed by another mechanism. Efficient repression depends on the products of at least 4 genes (variously called \textit{SIR} or \textit{MAR}), which require for their action a site on the DNA that has been called a "silencer" (Brand et al., Cell, 41, 41–48, 1985; see figure, line 5). \textit{SIR}-dependent repression acts over a distance of thousands of nucleotides, acts on several different promoters, and can act on at least two promoters at once. This type of repression functions whether the site of action is located upstream or downstream of a gene and the factor of repression is at least 100. When functional \textit{SIR3} protein is removed from the cell, derepression occurs within minutes and does not require passage through the cell cycle; establishment of \textit{SIR}-dependent repression, however, requires passage through S phase (Miller and Nasmyth, 1984, op. cit.). The silencer contains an ARS (autonomously replicating sequence), which may be a chromosomal origin of replication. These facts are consistent with a model in which repression results from creation of a new chromatin structure that prevents DNA near the silencer from being efficiently transcribed.