Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8309–8311, July 1999

Commentary

On the mechanism of silencing in *Escherichia coli*

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The processes that control gene expression and those that control genome segregation are usually taught in different lectures, from different chapters of the textbook, and often in different courses. However, recent studies of the segregation of certain bacterial plasmids have turned up fascinating evidence of how the two processes might be coupled. These studies have led to a model of prokaryotic gene inactivation that has most of the hallmarks of eukaryotic silencing. A paper in this issue by S.-K. Kim and J. C. Wang (1) has forced a reevaluation of the mechanism at play in *Escherichia coli* and raises important questions as to the extent that eukaryotic silencing may operate by a related mechanism.

The Distinction Between Repression and Silencing. To understand silencing in *E. coli*, we must first appreciate the distinction between silencing and other mechanisms of repressing gene expression. Repression of gene expression, as shown in studies of the now legendary *lac* and λ repressors, involves a sequence-specific DNA-binding protein whose recognition sequence, known as the operator, is physically close to a promoter sequence bound by RNA polymerase. Occupancy of the operator by the repressor has a direct effect on the ability of RNA polymerase to transcribe the adjacent gene. Although there are many details yet to be determined about the mechanism of prokaryotic repressors (2), the theme of intimate contacts between the repressor and RNA polymerase is likely to apply to all cases.

Repression in eukaryotes shares some themes with prokaryotic repression but with interesting differences. The operators of eukaryotic genes, also known as upstream regulatory sequences or upstream repression sequences, are DNA sequences occupied by sequence-specific DNA-binding proteins. Rather than contacting RNA polymerase directly, the sequence-specific binding proteins, at least in some cases, recruit a protein complex that itself mediates repression by deacetylating the histone tails of nucleosomes (3). The acetylation of lysine residues in histone tails is thought to weaken the interaction with DNA by neutralizing the charge, thus reducing the electrostatic attraction of the positively charged amino termini with DNA. Thus, deacetylation would be expected to increase repression. Different DNA-binding proteins can recruit the same species of repression complex to different genes. Thus, many examples of eukaryotic repression have a common mechanism, with specificity achieved by controlling the recruitment of the complex to particular genes.

Silencing is a mechanism of blocking gene expression that is distinctly different from classical prokaryotic repression and at least superficially distinct from eukaryotic repression. Silencing blocks gene expression through the assembly of a portion of a chromosome into a structure that precludes the interaction of sequence-specific DNA-binding proteins with their cognate sequences (4, 5). In this regard, RNA polymerase is a casualty of silencing rather than an active participant in the process. Silencing is mediated by regulatory sites, known as silencers (6), which bind specific proteins and, in ways not fully understood, lead to the bidirectional inactivation of gene expression and other sequence-specific interactions in their vicinity. Silencing and silencing proteins spread from a silencer into the surrounding sequences until, apparently, contained by a boundary element (7). In the most thoroughly studied contexts, eukaryotic silencing seems to be synonymous with formation of heterochromatin, the highly condensed structure of chromatin first described for the regions near centromeres of *Drosophila*. Indeed, when genes are translocated adjacent to centromere heterochromatin, their expression is inactivated (8).

Bacterial Partitioning Sequences Act Like Silencers. Bacterial cells lack anything obviously resembling the eukaryotic spindle apparatus, which ensures that both daughter cells at each cell division inherit a complete copy of the genome. Rather, bacterial sister chromatids are attached to specific sites near each end of the elongating bacterial cell. The process of cell elongation, combined with negative supercoiling of DNA, and the action of chromosome-condensation proteins together achieve chromatid segregation. For plasmids whose copy number is high, such as ColE1 replicated plasmids, no special mechanism is needed to explain how both daughter cells get a share of the plasmids. However, there are two well studied low-copy plasmids that use a special mechanism for their efficient partitioning in cell division. One such plasmid is the F (fertility) plasmid, whose presence or absence defines the sex of a bacterial cell. The other plasmid is actually a special quiescent intermediate of the bacteriophage P1 life cycle, in which a circularized phage chromosome is propagated as a plasmid. Both the F and P1 plasmid are present in roughly one copy per cell; thus special mechanisms have evolved to ensure efficient partitioning of the plasmids during cell division (9).

Both F and P1 have a DNA sequence element with a function akin to the centromere of eukaryotic chromosomes. In the case of F, the centromere-like partitioning site is called *sopC* (9), and in the case of P1, the site is called *parS* (10). Both sites contain multiple recognition sequences for a protein that acts at that site to partition the plasmids. *sopC* contains 12 tandem binding sites for SopB protein and *parS* contains multiple binding sites for ParB.

In previous work, both the Wang group (10–12) and the Yarmolinsky group (13) found that the presence of a partition site on a plasmid or chromosome led to the silencing of genes within up to 10 kbp of the partitioning site. Silencing in each case depends on expression of sufficiently high levels of SopB or ParB, respectively. This silencing of gene expression in the case of SopB also blocked association of DNA gyrase and DNA adenine methylase with the silenced sequences (13). Reversible crosslinking experiments established that ParB binds throughout the silenced region, far beyond the ParB-binding sites in the *parS* site (10). The block to gene expression mediated by the two partitioning sites fulfills most of the criteria used to define eukaryotic silencing.

Models for Prokaryotic Silencing. Collectively, the two studies offer three possible models for how the silencing induced by the partition sequences may operate. (*i*) The partitioning site nucleates the binding of SopB or ParB, which then forms a continuous protein fiber extending bidirectionally

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along DNA, precluding sequence-specific interactions between other proteins and their recognition sequences. (*ii*) Localized microcondensation of the region around the partitioning site. (*iii*). Sequestration of the partitioning site to a subcellular compartment that excludes accessibility of other proteins to their cognate sequences.

Although both groups initially favored a protein filament model, recent studies have led the Wang group to favor the sequestration model, at least for the case of *sopC*–SopB silencing. Three clues have led to this view. First, electron microscopic studies of SopB bound to *sopC* show SopB bound to its recognition sequences, but the DNA with saturating amounts of bound SopB lacks any properties reminiscent of a protein filament, even in the presence of high concentrations of SopB protein (11). Second, high-resolution studies of the subcellular localization of a SopB–GFP fusion protein shows that the protein is not evenly distributed throughout the cell. Rather, the protein occupies positions approximately one-quarter of a cell length proximal to each end of elongated cells (12). Thus, SopB has the potential capacity to localize DNA sequences bound to subcellular compartments.

The study in this issue (1) provides the third clue and extends the analysis of SopB-mediated silencing in two important ways. First, fusion of SopB to either of two unrelated DNA-binding domains causes silencing of genes near binding sites for the fusion partner. Thus, there is nothing special about the manner in which SopB binds DNA that is essential to the silencing mechanism. Second, fusions containing only the first 82 aa of the 323-aa SopB protein joined to either DNA-binding domain are capable of mediating silencing. These 82 aa are also sufficient to localize SopB or a GFP fusion partner to subdomains within *E. coli* cells. One would not expect these 82 aa, which lack the SopB DNA-binding domain, to be adequate to form protein filaments, particularly when joined to either of two different unrelated fusion partners. This fact further challenges the protein filament model.

Instead, Kim and Wang (1) suggest that the amino-terminal 82 aa of SopB protein serves as a homing factor that sequesters DNA bound by SopB to localized membrane components near the cell poles. As a result, localized patches of SopB in wild-type cells or patches of fusion proteins in their study are formed at specific positions. Once SopB recruits *sopC* into the patches, additional SopB protein or other DNA-binding proteins in the same patch could bind to adjacent DNA sequences through nonspecific interactions. The authors point out that this model is adequate to explain the data for silencing at *parS* as well.

Which model is correct? At this point, the absence of a demonstrable protein filament is a clear problem for the acceptance of that model. The sequestration model is still in need of further testing. For example, if it is possible to separate the role of the amino terminus of SopB in localization to subcellular domains from its role in silencing, the sequestration model would be seriously challenged. Localization domains in proteins are often discrete continuous sequences that are seldom, if ever, longer than 20 aa. Thus, it is conceivable that derivatives of the SopB amino terminus can be found that silence without causing subcellular localization, or vice versa. Similarly, there are, as yet, no data on whether the SopB fusion protein can actually sequester the DNA to which it is bound to these subcellular domains. Surely, identifying the proteins with which the SopB amino terminus interacts will be important in advancing our knowledge of the mechanism of silencing. If SopB is only a homing device, what other proteins are present in these patches, and how do they exclude RNA polymerase?

The Relationship Between Prokaryotic Silencing and Eukaryotic Silencing. The *Saccharomyces* mating-type genes provide one of the most thoroughly studied examples of eukaryotic silencing. The mating-type genes at the *MAT* locus are expressed, whereas those same genes at the *HM* loci are silenced through the action of silencers that flank the loci. This silencing mechanism involves the assembly of a specialized chromatin structure, made up of a protein complex, consisting of Sir2p, Sir3p, and Sir4p (14), that binds the tails of histone H3 and H4 as they protrude from the nucleosome (15). These same proteins are required for the silencing of genes placed adjacent to artificial telomeres (16); only Sir2p is also required for silencing in the rDNA (17).

In addition to their effects on gene expression, the silencing examples described above have another similarity to silencing in *Saccharomyces*. Like *sopC* and *parS*, silencers of *Saccharomyces* also have the capacity to endow plasmids with a segregation mechanism that is independent of the mitotic spindle (18). Similarly, silenced chromatin at telomeres has a spindle-independent segregation mechanism (19). Finally, although the centromeres of *Saccharomyces* have no detectably silenced chromatin, the centromeres of *Schizosaccharomyces* are silenced (20). Thus, there is a common association between sites that are silenced and sites that have a role in segregation in both prokaryotes and eukaryotes. At this point, it is unclear whether these disparate examples represent instances of independent solutions to a common problem that have taken on superficial similarity or represent a common solution to the same problem.

Could sequestration play a role in silencing in *Saccharomyces*? Localization studies have shown that the majority of Sir3 and Sir4 protein in a cell is localized to discrete foci in the nucleus that contain telomeres and the Rap1 protein, which binds telomeres (21). Similarly, Sir2 protein is located both at these foci and in the nucleolus, where Sir2p plays a role in nucleolar silencing (22). Thus the Sir proteins are positioned in the nucleus in the same locations as at least some of the sequences they silence. At this time, the significance of the foci containing Sir proteins is unclear. However, mutations in the yeast genes encoding the nonhomologous end-joining proteins Ku70 and Ku80 result in loss of telomere clustering and loss of telomere silencing (23).

If there is a fundamental relationship between the mechanism of silencing in bacteria and in *Saccharomyces*, one would expect orthologs of silencing proteins identified in one kingdom to play a role in silencing in the other. Among the Sir proteins defined in *Saccharomyces*, the Sir2 protein offers the best chance for comparative genetics. Sir2p is a member of a family of five related proteins in *Saccharomyces*. Remarkably, Sir2 orthologs have been found widely in eukaryotes, eubacteria, and archaebacteria (24). In *Saccharomyces*, Sir2p is part of a nucleosome-binding protein complex; however, as determined from the eubacterial and archaebacterial orthologs, the Sir2 family is more ancient than the nucleosome itself. Nevertheless, bacteria have at least one small positively charged protein that associates with DNA that could conceivably partner with bacterial Sir2 orthologs. With all of these parallels, it will be very interesting to determine whether the Sir2 protein of *E. coli* plays a role in the silencing phenomena highlighted here and whether it colocalizes with SopB or ParB protein. Someone should make a bacterial *sir2* mutation and evaluate its effect on silencing.

I thank Rebecca Bacon, Gloria Rudenko, and Nicholas Cozzarelli for valuable discussions and many insightful comments on the manuscript.

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