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REVIEW

Epigenetics: Regulation Through Repression

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Epigenetics is the study of heritable changes in gene expression that occur without a change in DNA sequence. Epigenetic phenomena have major economic and medical relevance, and several, such as imprinting and paramutation, violate Mendelian principles. Recent discoveries link the recognition of nucleic acid sequence homology to the targeting of DNA methylation, chromosome remodeling, and RNA turnover. Although epigenetic mechanisms help to protect cells from parasitic elements, this defense can complicate the genetic manipulation of plants and animals. Essential for normal development, epigenetic controls become misdirected in cancer cells and other human disease syndromes.

Epigenetics has an impact on many seemingly disparate areas of scientific enterprise (*1*). Even a partial listing would include areas of applied science such as somatic gene therapy (*2*), cloning and transgenic technologies in plants and animals (*3*), aspects of cancer biology (*4*), the study of viral latency (*5*), the activity of mobile elements (*6*), genomic imprinting (*7*), and developmental abnormalities in plants (*8*) and animals (*9*). The excitement in this research area follows from the realization that diverse organisms have common molecular mechanisms that contribute to the epigenetic control of gene expression (*10*). A key element in many epigenetic effects concerns the recognition of nucleic acid sequence homologies at both the DNA and RNA levels. Exactly how this recognition occurs is presently unknown; however, DNA-DNA and RNA-RNA interactions can trigger gene silencing by inducing chromatin modifications and RNA degradation, respectively. These homology-dependent gene-silencing events appear to be closely connected to genomic and cytoplasmic defense systems that protect cells against infiltration by invasive DNA and by RNA pathogens. We discuss the possible origins and the molecular mechanisms underlying both pathways of epigenetic silencing. It is important to recognize that these apparently distinct regulatory processes are linked not only in the end result of inactivating foreign nucleic acids but also potentially through interconnected mechanisms.

Epigenetic Control

Epigenetic control of gene expression can be considered from the standpoint of normal development, which requires stable repression of genes not required in specific cell types. Many epigenetic effects, however, are observed in unusual circumstances, and these have recently provided new insights into mechanisms. The discovery of epigenetic processes based on nucleic acid sequence recognition followed from the development of methods to introduce genes into the genomes of fungi, plants, and animals. Introduced transgenes often integrated as multiple copies or were identical to endogenous sequences. Contrary to expectations, the increased gene dosage did not result in enhanced expression but in gene silencing. Subsequent work distinguished distinct nucleic sequence homology-based mechanisms that lead to transcriptional or posttranscriptional gene silencing (TGS and PTGS, respectively).

DNA-Based Mechanisms—The Power of Repeats

An important advance in epigenetics research has been the realization that interactions between repeated DNA sequences can trigger the formation and the transmission of inactive genetic states and DNA modifications. The source of this concept was influential work with two filamentous fungi, which provided precedents for how eukaryotes can treat redundant sequences by mechanisms involving the recognition of DNA repeats. The RIP (repeat-induced point mutation) phenomenon in *Neurospora crassa* and MIP (methylation induced premeiotically) in *Ascobolus immersus* result in the pairing-dependent modification of DNA sequence duplications during the sexual cycle of these organisms (*11*). These modifications protect the streamlined haploid genome from potentially deleterious recombination events and from the activity of endogenous transposable elements. During RIP, both linked and unlinked duplicated DNA sequences ranging in size from a few hundred to several thousand base pairs incur G-C to A-T transition mutations. For reasons that are not yet clear, RIP-modified sequences become substrates for de novo postreplicative enzymatic modification of DNA in which any remaining cytosines are converted to 5-methylcytosine. This extensive mutagenesis generates missense and nonsense codons that inactivate gene expression and also creates sequence divergence that can prevent homologous recombination. In MIP, sequence duplications become heavily methylated and silenced without mutation. Moreover, DNA methylation

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in *Ascobolus* can be transferred interchromosomally between paired alleles during meiosis by mechanisms related to homologous recombination. In this organism, DNA-DNA pairing can thus serve as a signal for de novo methylation of sequence duplications and a means to transmit methylation between homologous sequences on different DNA molecules.

Recognition and inactivation of repeated DNA sequences have also been shown to occur in plants, *Drosophila*, and mammals, inspiring the acronym RIGS, for repeat-induced gene silencing (*12*). Unlike the case in filamentous fungi, repeats are not invariably silenced and methylated in plants and animals, nor do they undergo rapid point mutation. Therefore, even though sensing of sequence homology is a common element in RIGS and RIP/MIP, strictly identical processes do not appear to be involved. In plants, multiple closely linked copies of a sequence (cis inactivation) or multiple copies on different DNA molecules (trans inactivation) can target promoter methylation and TGS (Fig. 1). Trans inactivation involves an internally repetitive silencing locus that spontaneously acquires an inactive methylated state, which it imposes on a normally active, homologous target locus (*13*). Although trans inactivation resembles the meiotic transfer of methylation between alleles in *Ascobolus*, it differs in that it affects unlinked loci in diploid somatic cells. Results from a number of studies in plants have focused attention on inverted DNA repeats (IRs) as a key signal for silencing through methylation (*13*, *14*). IRs can pair in cis to become methylated autonomously, and they appear to be particularly potent trans silencers, possibly because they have special abilities to pair with a homologous partner. Direct physical evidence is still lacking for somatic pairing of dispersed repeats in plants and other higher eukaryotes, although homologous DNA sequences can be seen pairing within a chromosomal context: for example, the polytene chromosomes of *Drosophila*, in sister chromatids at mitosis and occasionally in interphase cells (*15*). Homologous regulatory DNA sequences also interact during the "transvection" phenomenon in *Drosophila*, in which certain alleles of a given locus, in a heterozygous combination, are able to complement one another provided that they are juxtaposed by somatic chromosome pairing (*16*). As discussed below, an alternative to DNA-DNA pairing as a sequence-specific, trans-acting methylation signal is an interaction between DNA and an aberrant or double-stranded (ds) RNA.

The transcription of a gene depends not only on the DNA sequence and availability of sequence-specific regulatory factors but also on the presentation of genes within the complex architecture of the chromosome (*17*). Conserved chromatin components and modifications direct the chromosomal silencing of DNA repeats in plants, animals, and fungi. Methylation induced by repeats can template chromatin modifications and TGS as indicated by the ability of the methyl-DNA–binding protein MeCP2 to recruit histone deacetylases (*18*). A direct link between DNA methylation and chromatin structure has been revealed by the identification of the *Arabidopsis ddm1* (deficient in methylation) gene as a component of a chromatin remodeling complex (*19*). The discovery of an *Arabidopsis* chromomethylase gene suggests the targeting of methylation through the chromodomain, which recognizes chromatin components (*20*). Silencing of repeated transgenes in *Drosophila*, an organism that does not methylate its DNA, is dependent on the chromodomain Polycomb group proteins, which also have a role in developmentally regulated gene silencing (*21*). *Drosophila* transgene repeat arrays can interact to induce cis and trans silencing dependent on association of the Polycomb group proteins with chromatin (*22*).

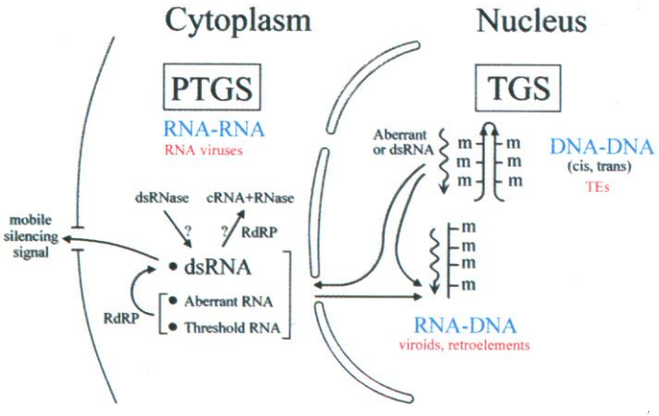

Fig. 1. Emerging view of a cellular circuit of sequence homology-based epigenetic signals (blue) and their relations to host defenses to parasitic elements (red). TGS in the nucleus is depicted as an inverted DNA repeat that pairs in cis, leading to methylation (m) of cytosine residues in promoter regions and transcriptional inactivation. The methylated inverted repeat can act in trans, possibly by DNA-DNA pairing, to induce methylation and silencing of unlinked homologous sequences. PTGS is a process of sequence-specific RNA degradation that occurs primarily in the cytoplasm. PTGS of normal mRNAs can be triggered by homologous dsRNAs, which can form from annealing of sense and antisense RNAs, by an unknown mechanism that might involve dsRNase. Antisense RNAs might be produced naturally by RdRP; possible substrates include aberrant or normal RNAs that accumulate to unacceptably high concentrations. As an alternative to the activity of dsRNases, a self-perpetuating mechanism has been proposed in which dsRNA is transcribed by RdRP to produce short antisense RNAs that would guide a single-stranded RNase to homologous target mRNAs and target them for degradation (*57*). RNAs produced in the cytoplasm can feedback on DNA to induce epigenetic modifications. These RNAs might be double stranded. In the nucleus, aberrant or dsRNAs (wavy lines) transcribed from methylated inverted DNA repeat can possibly induce methylation of unlinked homologous DNA sequences as well as PTGS in the cytoplasm when they contain sequences present in the mature RNA. Double-stranded RNAs in *C. elegans* and probably plants serve as intercellular and systemic mobile silencing signals. For more detailed models, see (*26*). cRNA, complementary RNA.

Repeat Recognition, Methylation, and Genome Defense

The involvement of methylation in many cases of TGS in plants, fungi, and vertebrates provides a link to genome defense. Cytosine methylation in bacteria is part of the system that protects the host against bacteriophage infection. The homology between prokaryotic and eukaryotic cytosine methyltransferase (MTase) enzymes has been used to argue that the latter also functions to disable foreign or invasive DNA sequences, particularly transposable elements (TEs) and endogenous retroviruses (*23*). Eukaryotic genomes are continually exposed to the activities of these elements, which can transpose through DNA or RNA intermediates. As much as 35% of the human genome is composed of the evolutionary relics of mobile elements (*6*), and plant genomes can be even more extreme (*24*). To help to contain the spread of TEs, vertebrates and plants appear to use chromosomally based silencing through DNA methylation (Fig. 1). Sequences that resemble TEs or reflect the consequences of their activity, such as repeats, novel sequence junctions, and rearrangements, are also potential targets for methylation. As discussed below, recently discovered RNA signals for methylation could also have an origin in mobile elements, specifically retroelements, that replicate through an RNA intermediate. In several ways, methylation is a singularly unsuccessful strategy for genome defense: Plant and mammalian genomes are extensively methylated and yet have 10 times as many TEs as the invertebrates that lack methylation. It is possible that TEs have evolved to actually use methylation as a strategy to hide from the host and increase their number through normal cell division events. This strategy is comparable to lysogeny in prokaryotes, and similar strategies might contribute to viral latency in mammals (*5*).

Several model genetic systems chosen for

their small genomes and short life cycle lack DNA methylation, for example, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, and *Drosophila melanogaster*. It is likely, however, that these organisms are exceptional. At least some members of all phylogenetic groups have 5-methyl cytosine in their DNA, suggesting that methylation is ancestral (*23*). These organisms have less need for methylation because a larger fraction of the genome is continually used over short generation times, and they also have much less repetitive DNA than vertebrates and most plants (*6*, *24*). The retention of proteins related to methyltransferase and methyl CpG-binding proteins in *S. pombe* and *D. melanogaster* indicates that these organisms probably lost methylation-dependent silencing pathways, rather than never acquiring them (*25*).

RNA-Based Mechanisms

Invasive DNA poses a continual threat to genomic integrity; however, cells must also respond to intrusive RNA derived either from a virus or from the overexpression of alien DNA. Recent work has revealed homology-based mechanisms for neutralizing foreign or aberrant RNA, either through a change in RNA stability in the cytoplasm or through mechanisms that use the RNA itself to induce methylation and silencing of homologous nuclear genes.

Sequence-specific RNA degradation. A homology-based PTGS process that targets specific RNAs for degradation has been discovered in diverse organisms. This process is variably known as cosuppression or sense suppression in plants (*26*), quelling in *Neurospora* (*27*), and RNA-mediated genetic interference (RNAi) in *C. elegans* and other invertebrates (*28*). Although it is not yet certain that identical mechanisms operate in all cases, each involves the induction of sequence-specific RNA turnover by the introduction of several hundred bases of RNA sequence present in the mature target mRNA. These RNA sequences can be microinjected into a cell or transcribed from a transgene. The trigger for RNA turnover is best characterized in *C. elegans*, where it consists of dsRNA (*28*) (Fig. 1). Double-stranded RNA, which might be specifically degraded by ds ribonuclease (RNases) or used as a template for RNA synthesis, can be produced by annealing of sense and antisense transcripts or by transcription through an inverted DNA repeat (Fig. 2). Although dsRNA can also provoke PTGS in plants, there are cases that are not readily accounted for by dsRNA, suggesting the action of alternative inducers that have been loosely termed "aberrant" RNAs (*26*). Aberrancy can have either a quantitative or a qualitative basis. PTGS involving highly expressed single-copy transgenes has inspired threshold models, in which RNA turnover is triggered above a certain cytoplasmic level of normal RNA, perhaps because stability factors are limiting. Other examples of cosuppression in plants involve negligibly transcribed or promoterless transgenes that are almost always arranged as inverted repeats (*14*). Although low levels of dsRNA could be generated by transcription through the inverted repeat, an alternate suggestion is that the inverted repeat pairs with the homologous endogenous gene, provoking methylation or chromatin condensation (or both) of the transcribed region. RNA transcribed from this modified copy by flanking plant promoters could be terminated prematurely or extended abnormally, with the resulting aberrant RNA triggering turnover of similar RNA sequences (Fig. 1).

Insights into the mechanisms of PTGS are coming from analyzing mutants, which have been generated so far in *Neurospora* and *Arabidopsis*. The first identified mutant, *qde1* (quelling-defective) in *Neurospora*, has been found to encode a protein with homology to RNA-directed RNA polymerase (RdRP) (*27*). RdRP has played a central role in models of PTGS in plants to account for the sequence specificity of RNA degradation. RdRP could synthesize antisense transcripts from aberrant sense RNAs, leading to the production of dsRNA that triggers PTGS through a mechanism that possibly involves further RdRP activity (*26*, *29*) (Fig. 1). Intriguingly, *qde1* and RdRP homologs have been found in a number of plants, including *Arabidopsis*, as well as in *C. elegans* and fission yeast, suggesting a common PTGS mechanism involving dsRNA in all of these organisms. Further details about the mechanisms of PTGS should come from the analysis of additional *qde* mutants and *Arabidopsis sgs* (suppressor of gene silencing) mutants (*30*), as well as the recovery of PTGS mutants in other organisms.

Two remarkable features of PTGS are the amplification and cell-to-cell movement of the sequence-specific signal (*26*, *28*). Amplification is most apparent in *C. elegans*, where the concentration of dsRNA required to initiate turnover is much lower than the concentration of the target RNA. Amplification or some kind of relay system also operates in plants. The synthesis of antisense and sense copy RNAs by RdRP would be a means to amplify the signal. Cell-to-cell movement of the silencing agent has been observed in plants and *C. elegans*. In plants, long-distance transport occurs through the vascular system and is thought to involve a mobile silencing signal containing dsRNA (*26*, *29*) (Fig. 1).

PTGS in plants has been directly linked to a natural form of RNA virus resistance (*31*). Inoculation with viruses containing sequences homologous to nuclear genes is associated with PTGS of the gene and recovery from viral infection. These results provide the clearest association between a homology-based gene-silencing process and host defense (*32*). They also localize the RNA degradation step of PTGS in plants to the cytoplasm (Fig. 1), because the RNA viruses involved replicate their genome in this compartment. Migration of the silencing signal over long distances in plant phloem also recalls virus movement. Recently, viral proteins that suppress PTGS (*33*) and a plant counter-counter-defense have been described (*34*). Although dsRNA has been implicated in a general antiviral response in vertebrate cells, the plant mechanisms differ in their sequence specificity. The failure to detect RdRP homologs in humans might be a reflection of these different antiviral strategies.

RNA-directed DNA methylation. Although TGS and PTGS are most simply viewed as distinct phenomena involving DNA-DNA and RNA-RNA interactions, respectively, increasing evidence indicates that nuclear and cytoplasmic processes can be connected through aberrant, possibly dsRNA molecules (Fig. 1). One link is through RNA-directed DNA methylation, which can involve an RNA made in the nucleus or cytoplasm. A second possible link that remains to be confirmed experimentally is the transcription of aberrant RNAs from methylated DNA templates. These aberrant RNAs have been postulated to trigger RNA turnover in the cytoplasm and methylation of unlinked homologous DNA copies.

The ability of RNAs produced in the cytoplasm to feedback and induce epigenetic

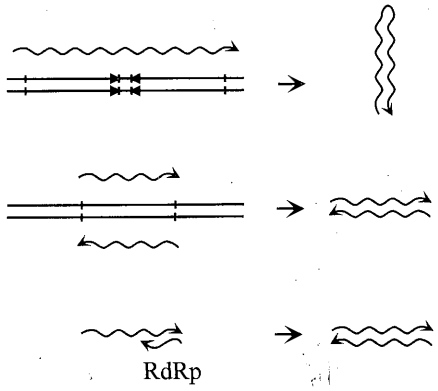

Fig. 2. Production of dsRNA. Wavy lines represent RNA, and straight lines represent DNA. (**Top**) Transcription through an inverted DNA repeat produces an RNA that can pair intramolecularly to form a dsRNA. (**Middle**) Transcription from opposite DNA strands produces overlapping sense and antisense RNAs that can anneal to form dsRNA. (**Bottom**) RdRP can synthesize antisense or sense copy RNAs from an RNA template in the cytoplasm. These RNAs can anneal to form dsRNA.

changes on DNA has been most clearly shown in plants, where nuclear transgenes became methylated only subsequent to the initiation of transgene PTGS in the cytoplasm by an RNA virus engineered with transgene sequences (*35*). That dsRNAs might be the actual inducers of DNA methylation has been suggested by the ability of viroids, a plant pathogen consisting solely of a noncoding RNA duplex, to trigger methylation of homologous nuclear DNA (*36*). Double-stranded RNA transcribed in the nucleus from an inverted repeat has also been shown to induce methylation of unlinked homologous DNA sequences in a nonpathogenic plant system (*37*). Indirect evidence that dsRNAs can heritably influence DNA in *C. elegans* is the persistence of some cases of RNAi into advanced generations, long after microinjected RNAs would be degraded or diluted out (*28*). It is not known how dsRNAs might target DNA modifications. Perhaps their double strandedness makes them appear DNA-like, such that interactions with homologous DNA mimic DNA-DNA pairing, a potential signal for de novo methylation in higher eukaryotes.

PTGS and RNA-directed DNA modifications can be considered in the context of host defenses to transposable elements (Fig. 1). As described above, vertebrates and plants use DNA methylation to transcriptionally suppress promoters in these elements (*6*). Invertebrates either lack, or rely less on, methylation to restrict mobile elements and appear to use a combination of TGS- and PTGS-like processes to limit their spread (*38*). Transposable elements could supply methylation signals through either DNA-DNA pairing or, in the case of retroelements, an RNA secondary structure that triggers methylation of homologous DNA. Viroids may target DNA methylation as a consequence of their duplex RNA structure. The capacity to direct methylation and silencing of plant sequences might be the basis of viroid pathogenicity (*36*).

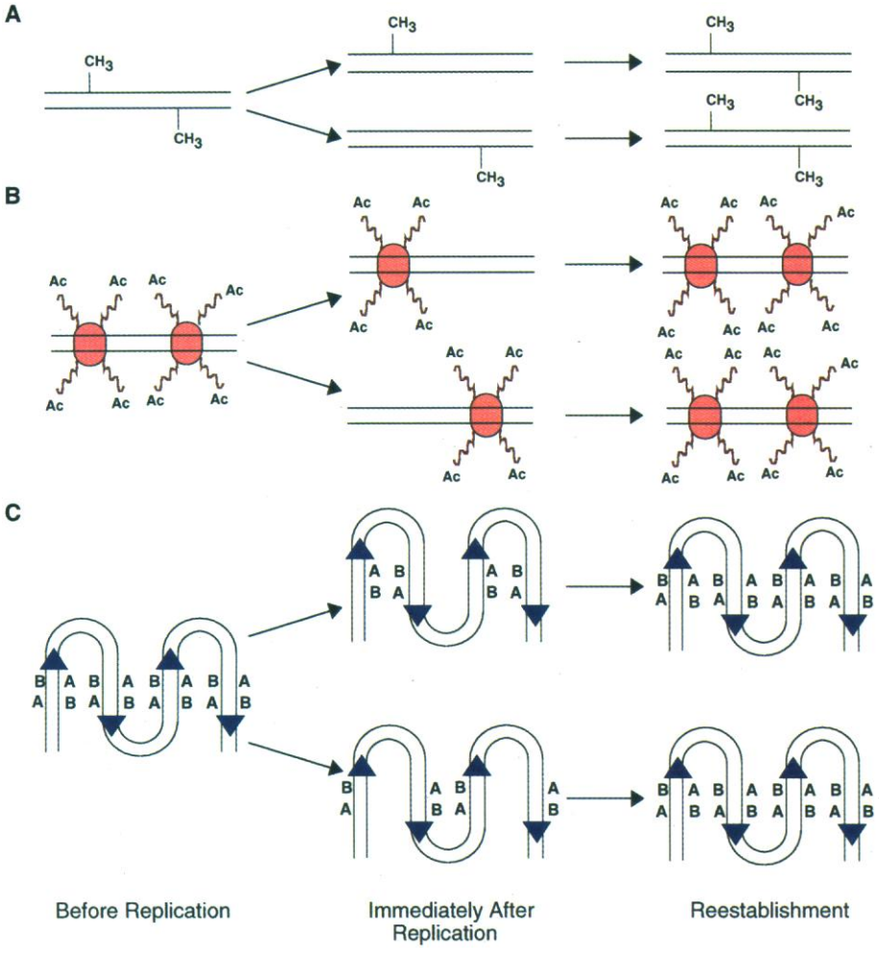

Fig. 3. Mechanisms mediating the inheritance of epigenetic effects at the DNA level. (**A**) A symmetrically methylated CpG dinucleotide will segregate one methyl CH_3 to both daughter chromatids after replication. If this hemimethylated DNA is preferentially recognized by DNA methyltransferase sequestered at the replication fork, then symmetric methylation of CpG dinucleotides will be rapidly established. DNA methylation will then target chromatin modification. (**B**) Modified histones and associated acetyltransferases (Ac) on the parental chromosome will be segregated to both daughter chromatids randomly. The enzymes that are retained in contact with the histones may locally modify chromatin to retain effects on transcription on both chromatids. (**C**) High local concentrations of regulatory trans-acting factors (AB) localized at DNA repeats (arrows) may facilitate the sequestration of free factors from the nucleoplasm on both daughter chromatids after replication.

Epigenetic Control of Development

The epigenetic control of gene expression is a fundamental feature of mammalian and plant development, as indicated by developmental arrest or abnormalities in methylation-deficient mutants (*8*, *9*). Examples of sequence-identical alleles being stably maintained in different functional states in humans are X-chromosome inactivation, which serves to normalize the expression of X-linked genes in females (XX) and males (XY) (*39*), and genomic imprinting, an unusual non-Mendelian phenomenon in which alleles are expressed differently depending on their parental origin (*7*). Comparable molecular events, including methylation, histone hypoacetylation, and late replication, are involved in both X inactivation and genomic imprinting. Both DNA repeats and dsRNAs recur as possible triggers of DNA modifications. Many imprinted genes are associated with repeats, and a repeat-induced process involving L1 retroelements has been hypothesized for X-chromosome inactivation (*40*). Several examples of genomic imprinting involve overlapping sense and antisense RNAs (*41*), which could produce dsRNA that provokes methylation and allele-specific repression. The inactive X-chromosome also produces a noncoding RNA, Xist, which is required for inactivation, and its antisense counterpart, Tsix (*42*), suggesting the possible involvement of a dsRNA in X inactivation and methylation. Cis inactivation might be assured by the synthesis from opposite DNA strands of separate sense and antisense transcripts, which must be at locally high concentrations to be able to find and anneal with each other to form dsRNA. A diffusible, trans-acting signal consisting of dsRNAs could be produced by intramolecular base pairing of an RNA transcribed through an inverted DNA repeat (Fig. 2).

Heritability of Epigenetic States

To maintain the stable repression of genes required for the unfolding of developmental programs, epigenetic states must be inherited during cell division. DNA methylation provides the most direct epigenetic mechanism for the maintenance of the repressed state (Fig. 3A). Symmetrically methylated CpG dinucleotides will have one methyl cytosine segregated to both daughter DNA duplexes after replication. The resulting hemimethylated CpG dinucleotide is rapidly remethyl-

ated within one minute of synthesis. The mammalian Dnmt1 methyltransferase is sequestered at the replication fork to maintain the methylation status of DNA (*43*). Immature chromatin assembled at the replication fork is enriched in acetylated histone and provides a window of opportunity to reprogram genes after replication (*44*). If methyl CpG-binding proteins are able to bind close to the replication fork and thereby recruit histone deacetylase, the window of opportunity to reprogram genes after replication would be rapidly closed. Preexisting histones as well as other chromatin proteins present on the parental chromosome, such as Polycomb, are also likely to be retained on daughter chromatids. A random distribution of each component would be sufficient to repackage 50% of each daughter chromatid, offering considerable potential for the propagation of a gene activity state dependent on chromosomal structure. Histones having defined states of acetylation are known to be segregated in this way during chromosomal duplication and to be maintained in this state through mitosis (Fig. 3B) (*45*). Alterations in acetylation status with the use of inhibitors of histone deacetylase interfere with epigenetic controls on transcription. An additional common feature of repetitive sequences is that they contribute to high local concentrations of protein-binding sites (*46*). The assembly or aggregation of many similar nucleoprotein complexes may establish a compartment or domain within the nucleus that will facilitate reestablishment of chromatin configurations after chromosomal duplication (Fig. 3C) (*22*).

Although developmentally associated epigenetic modifications must be reset during sexual reproduction, there is a growing awareness that some acquired epigenetic states can be inherited meiotically. The classic case is paramutation, a type of trans silencing in which one allele or chromosomal locus alters the activity of a sensitive allele or locus in a way that lingers after the two segregate in progeny (*47*). First identified in plants, paramutation transgresses Mendel's first law, which states that alleles should segregate unchanged in progeny. Paramutation is not well understood but is thought to involve physical interactions that transmit one state of gene activity or silencing between alleles or loci. Although some cases involve methylation, others do not, implicating meiotically heritable changes in chromatin structure. Alleles that participate in paramutation have been suggested to be foreign in some way, and indeed rearranged TE sequences are involved in one case in maize (*24*). Meiotic inheritance of epigenetic states has been observed in mammals and in organisms that do not methylate their DNA, including fission yeast and *Drosophila* (*48*). The general extent to which acquired or induced epigenetic changes persist during meiosis is an open area of research that could have considerable implications for human genetics and disease. For example, a paramutation-like effect has been associated recently with susceptibility to human diabetes involving alleles of the insulin gene that contain variable numbers of tandem repeats (*49*).

Epigenetics and Human Disease

Alterations in epigenetic controls have other profound roles in human disease. A common form of inherited mental retardation related to the Fragile X Mental Retardation gene 1 (*FMR1*) gene involves expansion and methylation of a CGG trinucleotide repeat in the 5′ regulatory region, leading to transcriptional inactivation of the *FMR1* gene. The CGG repeat is also the site of preferential chromosomal breakage. This breakage may reflect alterations in chromosomal organization that are a consequence of repeat-induced silencing (*50*). Alterations in the expression of individual imprinted loci lead to developmental abnormalities. In humans, such abnormalities include Beckwith-Wiedemann, Angelman, and Prader-Willi syndromes (*7*).

Although cancer cells often have reduced levels of 5-methylcytosine in the genome relative to normal tissues, many tumor-suppressor genes are silenced in tumor cells by de novo methylation of their promoter regions (*4*). This aberrant methylation is suggested to have a causal role at the preneoplastic stage of cancer progression. Deregulation of genomic imprinting can also play a role in cancer development, as exemplified by loss of imprinting of the *IGF2* gene in Wilms' tumor (*51*). The epigenetic silencing of the *FMR-1* and tumor-suppressor genes by DNA methylation offers the exciting clinical prospect of interfering with both the molecular pathways that target methylation per se and those that mediate transcriptional silencing dependent on the recognition of DNA methylation.

Epigenetics and Genome Evolution

Homology-based cellular defense responses, which appear to be ancient given their wide phylogenetic distribution, have possibly been recruited to regulate host gene expression. During evolution, eukaryotic genomes have expanded in size through increases in gene number and the accumulation of repetitive sequences, many of which consist of mobile elements and their degenerate remains (*6*, *24*). The spread and elaboration of epigenetic control mechanisms based on repeats and the recognition of "foreign" sequences would be compatible with this pattern of genome evolution. Homology-based silencing of duplicated genetic loci could have played a role in the genetic diploidization of newly formed polyploid genomes, contributing to their successful establishment during plant and vertebrate evolution. Transposable elements integrated into promoters of genes could alter expression patterns and attract methylation or chromatin modifications to regulate the modified promoter (*52*). RNA signaling molecules that move systemically throughout plants could modulate physiological and developmental processes by inducing PTGS at distant sites (*53*). Although much additional work remains, it is increasingly appreciated that homology-based epigenetic mechanisms have vast potential to contribute to natural gene regulation.

Outlook

Epigenetics represents a new frontier in genetics research. With the completion of genome-sequencing projects, a major challenge will be to understand gene function and regulation. Achieving this goal will require determining how epigenetic controls are imposed on genes. The various homology-based gene-silencing mechanisms and their intertwined actions are beginning to reveal a cellular circuit of controls that can be used to modify gene expression at the level of chromosomes and RNA turnover.

Epigenetic silencing mechanisms that target repeated or foreign nucleic acids can be both troublesome and useful in attempts to genetically engineer plants and animals. Unwanted transgene silencing, which can involve either methylation of DNA or PTGS of highly expressed transgenes, presents a persistent problem. Various attempts to circumvent undesired silencing include using matrix attachment or insulator regions, limiting the amount of bacterial vector DNA and repetitive elements, and restricting transgene transcription to moderate levels. On the other hand, some applications of transgenic technology in agriculture require silencing of specific genes. Hardier tomatoes, for example, have been engineered to repress a fruit-ripening gene through homology-based silencing (*54*). Cosuppression in plants and RNAi in *C. elegans* are important tools in "knockout" experiments to test gene function and are being increasingly applied to other systems. A second technology that might be affected adversely by epigenetic controls is cloning of mammals. The high abnormality and fatality rates of mammals cloned from somatic nuclei are being attributed in part to problems with genomic imprinting. This complication sounds familiar to plant biologists, who have long been aware that "clonal uniformity is the exception rather than the rule" (*55*, p. 277; *56*). A way around this obstacle to reliable cloning has not yet been devised and will require a more complete understanding of the establishment, maintenance, and resetting of epigenetic states during development and sexual reproduction.

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