Exploring and explaining epigenetic effects

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Epigenetic phenomena in diverse organisms comprise some of the most intriguing and actively investigated problems in genetics. The term 'epigenetics' was introduced by Conrad Waddington to describe changes in gene expression during development. Nowadays, epigenetics in the Waddington sense refers to alterations in gene expression without a change in nucleotide sequence. However, this definition is so broad that an issue of Trends in Genetics devoted to epigenetics would read more like a modern biology textbook than a series of critical reviews. A more focused description of epigenetics refers to modifications in gene expression that are brought about by heritable, but potentially reversible, changes in chromatin structure and/or DNA methylation. This issue of Trends in Genetics explores both old and new epigenetic phenomena, such as paramutation and genomic imprinting, respectively. Although no consensus has yet emerged about the source of epigenetic effects, common threads connecting many of the phenomena suggest to us that host responses to mobile elements provide a unifying theme.

In applied research, host reactions to invasive elements might be provoked by genetic transformation, causing methylation-associated gene silencing that can frustrate attempts to engineer plants and animals genetically. Altered gene expression can also be the consequence of processes that do not involve nuclear epigenetic inheritance. Cosuppression in plants is a post-transcriptional silencing phenomenon that is due primarily to enhanced cytoplasmic turnover of transgene and homologous endogenous gene RNAs (Ref. 2). Although co-suppression has not yet been detected in animal cells, the phenomenon of 'quelling' in Neurospora provides a parallel (E.U. Selker, this issue). Priors represent a form of cytoplasmic inheritance involving a heritable change in state (in this case, protein conformation), but this behavior is clearly demarcated from cases of nuclear epigenetic inheritance (J.B. Hollick, J.E. Dorweiler and V.L. Chandler, this issue).

Epigenetic effects usually involve gene silencing. In any differentiated cell, most genes are normally active and many of the molecular components involved in generating and maintaining this state are being discovered by studying epigenetic phenomena in yeast and Drosophila. In yeasts, where powerful genetic and biochemical approaches can be combined, the study of mating type silencing, and telomeric- and centromeric-position effects has allowed a thorough analysis of individual constituents of multienzyme complexes that establish the silenced state. New epigenetic effects at several loci have indicated that these classical silencing phenomena might only represent the tip of the iceberg. The dynamics of silencing, including protein modifications and changes during the cell cycle, account for much of the recent excitement in this field (J.M. Sherman and I. Pili, this issue).

In Drosophila it is possible to investigate protein components of silencing complexes in the context of well-studied developmental pathways. There are dozens of genetically identified proteins that are involved in silencing the same target genes. These heterogeneous proteins might interact at composite binding sites and nucleate the formation of silencing complexes encompassing nearby sequences. The 'stickiness' required for this to occur might also operate between homologs and even between unrelated sites (V. Pirotta, this issue). This general model might account for the ability of heterochromatin to silence reporter genes, a phenomenon known as position effect variegation (PEV).

In many organisms, gene silencing is associated with repeated DNA sequences. Heterochromatin in higher eukaryotes comprises many types of repeats, including simple sequence arrays and inactive mobile elements. However, in filamentous fungi, repetitive sequences are almost nonexistent owing to the occurrence of two processes, RIF (repeat-induced point mutation) and MIP (methylation-induced premeiotically). Linked and unlinked sequence duplications can both be modified, genetically by RIF and epigenetically by both RIF and MIP. These processes clearly play genome defense roles because they only occur during a brief period of the sexual cycle and not during vegetative growth. Mobile elements are the likely natural targets (E.U. Selker, this issue).

Fungi also have epigenetic phenomena that resemble those seen in higher eukaryotes. For example, meiotic transversion is a normal gene regulatory process that takes place across homologs, similar to the numerous examples of transversion between mutant alleles in Drosophila. Furthermore, epigenetic states can be meiotically inherited in fission yeasts, as is observed with paramutation in plants, which involves the meiotically heritable weakening of one allele by the other after their interaction in the heterozygote. Such meiotic heritability is a hallmark of many plant epigenetic phenomena, and is not observed for post-transcriptional silencing seen in cosuppression.

Paramutation is a fascinating phenomenon because it demonstrates that some alleles and homologous unlinked loci can interact in trans, resulting in persistent changes in expression after the interacting genes are inherited separately in progeny. Paramutation thus transgresses Mendel's first law, which disallows residual influences on alleles following their segregation into different gametes. Initially believed to affect only a few plant genes, paramutation might be more common than previously imagined. Recent examples include...
transgenes, which when locally repeated can even silence a nonallelic partner. The mechanisms(s) of paramutation are not yet known, but different cases of paramutation might be due to distinct mechanisms (J.B. Hollick, J.E. Dorweiler and V.L. Chandler, this issue). Several (but not all) examples of paramutation involve methylation, which might be revealing the action of a genome defense system directed against invasive sequences, such as transgenes or mobile elements.9,10 One possible mechanism, interallelic methylation transfer, received strong support from a recent study showing that the imposition of methylation from one allele to another in a filamentous fungus is mechanistically related to gene conversion.10

The function of DNA methylation has been a controversial subject since it was first proposed to play a role in the regulation of gene expression during development.11,12 Although the original experiments suggesting that changes in methylation are important in development were performed in mice, some of the most direct evidence to support this proposal comes from studies in Arabidopsis where it has been possible to reduce levels of DNA methylation in a nonlethal manner. Among the most striking effects are floral homeotic transformations that are associated with ectopic expression of floral homeotic genes (E.J. Richards, this issue). Although these observations have been interpreted as proving that methylation is necessary for normal development13, an alternative interpretation is that demethylation activates promoters of transposons that are normally silenced by methylation, thereby driving ectopic expression of neighboring genes.14 Remnants of long-terminal-repeat retrotransposons in promoters of plant genes can act as negative regulatory elements15, and these might be activated upon genome-wide partial demethylation.

The ubiquitous role of DNA methylation in development is being further questioned by recent work in mice indicating that methylation is crucial only in somatic cells, because null mutations in the only known DNA methyltransferase gene still permit considerable early development.16 Gametic imprinting, that is, the expression of a gene from only one parental chromosome, depends on methylation. Imprinting might have no intrinsic role in mammalian development, but has evolved in placental mammals as a consequence of their special reproductive features (R. Jaenisch, this issue). Reciprocal imprinting of genes for growth factors and their receptors has suggested a parental conflict hypothesis, which has received some recent support (R. Jaenisch, this issue). This hypothesis is also consistent with an epistasis mechanism to explain the loss of imprinting of the IGF2 gene in the Beckwith–Wiedemann syndrome (W. Reik and E.R. Maher, this issue). However, other data (discussed in Ref. 18) do not corroborate the parental conflict hypothesis. The matter should be resolved by determining whether the orthologs of imprinted mouse and human genes are also imprinted in marsupials, which have underdeveloped extraembryonic membranes.19 The finding that at least one naturally imprinted gene might have retrotransposed suggests that imprinting might have originated as a genome defense response.21 (J.A. Yoder, C.P. Walsh and T.H. Bestor, this issue).

The most radical challenge to the developmental paradigm for methylation comes from the astounding realization that the bulk of cytosine methylation in mammalian DNA is found in transposons (J.A. Yoder, C.P. Walsh and T.H. Bestor, this issue). Methylation can be especially effective against retroelements because they are unable to transpose when silenced. Based on the distribution of methylated cytosines, the primary function of methylation in mammals appears to be suppression of parasitic sequence elements (J.A. Yoder, C.P. Walsh and T.H. Bestor, this issue). Unlike CpG-depleted exonic DNA, transposons are rich in this dinucleotide, which is the substrate for DNA methyltransferase.

What was thought to be a global methylation pattern arising from the broad distribution of CpGs in vertebrate genomes appears, instead, to reflect the dispersion of transposable elements within introns and between genes. Therefore, cytosine DNA methylation serves to contain transposable elements in filamentous fungi, plants and mammals. This defensive strategy seems to be effective because an organism that lacks DNA methylation, Drosophila, is extremely susceptible to transposon-induced genome damage.

Given that cytosine methylation can protect genomes from mobile elements and that a substantial number of epigenetic effects involve methylation, it is worth considering whether many epigenetic phenomena are not actually a consequence of this genomic immune system. Genes associated with transposable elements could be mistakenly identified as genome invaders and become methylated and silenced. Epigenetic phenomena that cannot involve methylation,
for example, PEV in Drosophila, might nevertheless involve a genome defense system against transposons, which are major constituents of heterochromatin. Moreover, retrotransposons specifically accumulate on a chromosome that is in the process of becoming heterochromatic. Formation of heterochromatin at transposon arrays in Drosophila (Fig. 1) and other artificial repeat arrays in vertebrates would be a manifestation of this genome defense response, because condensation should inhibit transposition and local repeat expansion. Even in yeast, which has relatively few mobile elements, some newly described epigenetic phenomena involve transposon-derived sequences that might activate as yet uncharacterized genome defense systems.

The proposed widespread role of transposons in provoking epigenetic effects extends the legacy of Barbara McClintock, who not only discovered transposable elements and detailed their epigenetic behavior in plants, but also first suggested their possible involvement in paramutation and PEV (Ref. 30). Natural epigenetic phenomena thus appear to reflect another profound way that transposable elements have shaped and influenced genome evolution.

References

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