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Epigenetic control of transcription

Introduction: the genetics of epigenetics

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Abstract. *Cis*-acting binding sites for transcription activators cannot explain the patterned expression of genes during development, nor a large range of phenomena that result from gene transplacement. Heritable states of transcriptional regression or activation sequences are influenced not only by the chromosomal context of the promoter but also by modifications of histones and DNA, and long-range interactions between distant chromosomal elements. The molecular dissection of these epigenetic phenomena has become an exciting topic of reserch, revealing highly conserved mechanisms at work in chromatin-mediated gene control.

Key words. Epigenetics; chromatin; transcription; silencing; acetylation; dosage compensation; methylation; paramutation.

Epigenetic gene control is, by definition, a modulation of gene expression achieved by mechanisms superimposed upon that conferred by primary DNA sequence. Very often epigenetic events result in different patterns of expression for different copies of the same gene in a given cell nucleus. Although this field focused for many years on the description of unusual genetic phenotypes and their patterns of inheritance, the last few years have seen an explosion of information on the molecular nature of epigenetic control. Moreover, what seemed before to be discrete unlinked phenomena now appear to be related at the most fundamental levels. The packaging of nucleosomes into inactive or active domains, the patterns of modification of core histone N-termini and on DNA itself, and the influence of subnuclear organization all appear to be highly conserved and necessary mechanisms for long-range, epigenetic gene control in eukaryotes. Common mechanisms are at work in yeast, plants, flies and man, and molecular genetic tools have finally opened the field to an exceptionally fruitful cross-feeding of techniques and ideas.

It is therefore highly appropriate to present here a collection of reviews devoted to chromatin-mediated gene regulation, drawing on expertise from fields as diverse as histone modification in *Tetrahymena*, plant gene methylation, fly development and mammalian oncogenesis. In the following few pages we will summarize the areas of chromatin-mediated gene control in which major advances have been made in the last 2 years, and direct the reader to the appropriate reviews in which the subject is discussed in more detail.

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Histone acetylation patterns and the responsible enzymes

The recognition that specific acetylation patterns on the N-terminal tails of core histones characterize regions of either elevated or repressed expression [1] paved the way for the more precise analyses of histone tail function of recent years. A major advance in this field, however, has been the identification of specific enzymes that acetylate (HATs) and deacetylate (HDAs) highly conserved lysine residues within the histone N-terminal tails. The discovery that HATs are important components of transcriptional regulatory machinery lent immediate relevance to these tail modifications, both as regulators of nucleosomal packaging and as binding sites for silencing factors. Finally, the finding that HATs and HDAs might modify transcription factors, in addition to histones, provides a guarantee for future excitement in this area. Since histones are the basic building blocks of all chromatin packaging, their patterns of acetylation are relevant to control mechanisms from yeast to man. Consistently, both the enzymes and their targets show high degrees of sequence conservation. Recent findings relevant to histone acetylases are covered in Mizzen and Allis in this issue, while the importance of specific patterns of histone tail modification for dosage -compensation events in fly development are detailed in Bryan Turner's review. Understanding the inheritance of acetylation patterns through meiotic and mitotic division will be a major goal in the near future for those interested in explaining heritable patterns of gene control.

The mechanisms of silencing at mating-type and subtelomeric loci in yeast

The packaging of heterochromatin-like structures appears to be based on a cooperative 'spreading' of multiprotein complexes that interact with nucleosomes. Recent progress in the molecular analysis of the protein constituents of repressed domains in yeast have provided the field with a paradigm for the propagation of such packaging complexes. In brief, it was known that certain histone protein domains were specifically required for silencing in yeast, including residues 16–29 at the H4 N terminus and residues 4–20 at the H3 N terminus. Surprisingly, despite the similarly charged nature and extended structure of the H2A and H2B N termini, their deletion does not affect silencing (reviewed in ref. 2). Cross-linking data now argue convincingly that complexes involving Rap1 and silent information regulators Sir2, Sir3 and Sir4 propagate along the nucleosomes to repress or sequester the region from the transcriptional machinery [3–5]. Indeed, Sir3 and Sir4 interact directly with the silencing domains of H3 and H4 in vitro and in vivo as demonstrated by immunolocalization of Sir proteins in mutant strains.

The repressive structure mediated by Sir proteins requires *cis*-acting elements like silencers or the Rap1-binding TG_{1-3} repeats at yeast telomeres to target and nucleate Sir complex binding [6]. Intriguingly, a second type of repressed chromatin appears to propagate beyond the Sir2,3,4-containing core, propagated primarily by Sir3 [5, 7]. The details of how these *cis*- and *trans*-acting components co-operate in yeast and how they are influenced by other telomeric complexes is handled in the review by Lowell and Pillus. With the help of yeast genetics the concept of propagation of a chromatin conformation has achieved a firm molecular footing. The field now faces the important question of how higher-order structures based on the nucleosomal fibre can confer a heritable repressed state on promoters. Moreover, it remains to be seen to which degree the yeast paradigm applies to the related phenomena of position-effect variegation (PEV) and Polycomb-group regulation (Pc-G) of gene expression in flies and mammals.

Stable inactivation of developmental regulators by the Pc-G protein complexes

The stable, heritable inactivation of particular sets of genes is an important regulatory aspect in the life cycle of a eukaryotic cell, and in a multicellular organism such a feature is needed to maintain determined gene expression states over developmental time. Typically, in early embryonic development, mechanisms of pattern formation generate particular combinations of regulatory factors in each cell. Subsequently, the specific expression states must be faithfully maintained over many cell generations to permit correct structures to be made during differentiation. In *Drosophila*, genetic analyses have uncovered two classes of genes which appear to be responsible for 'freezing' such developmental decisions. The genes of the Pc-G are responsible for keeping regulatory genes (i.e. homoeotic or HOX genes) permanently inactive, while the genes of the trithorax group (trx-G) are necessary for the continuous active state. There is accumulating evidence that the proteins encoded by the two groups exert their regulatory role at the level of higher-order chromatin structure.

Based upon a variety of genetic and biochemical results, it has been proposed that the Pc-G functions as multiprotein complex that folds the chromatin of target genes into a condensed and inactive form [8]. The nature of Pc-G-dependent compaction of chromatin is still not well understood, and it is important to note that whereas RNA PolII may be excluded, the small T7 RNA polymerase can still access its promoter in such chromatin [9]. Importantly, recent advances demonstrate that there is a high degree of

conservation of both the proteins and the mechanism of homeotic gene control in mammalian systems. The recent finding that mouse homologues of Pc-G and trx-G genes affect not only HOX gene expression and axial skeleton development, but also control of proliferation and the survival of haematopoietic cell lineages [10–12], extends the field of epigenetic control to that of cell cycle regulation. These exciting developments are covered in the review by van Lohuizen in this issue.

On the other side, the highly conserved SET domain that was originally identified in a trx-G and a Pc-G protein [13] and subsequently in a modifier of PEV [14] is another convincing argument for the existence of an evolutionarily conserved mechanism for epigenetic controls. The identification of SET domain proteins in yeast, flies and man, and their effects on heterochromatin propagation, as well as on homeotic gene regulation, are discussed by Jenuwein et al. in this issue. The analysis of conserved protein motifs continues to be a major theme of epigenetic research, one that should shed light on the mechanisms underlying chromatin-mediated repression.

PEV and other roles for heterochromatin

As discussed in the review by Jenuwein et al., it is impossible to discuss Pc-G and trx-G regulatory mechanisms without breaching the subject of position-effect variegation. Indeed, genetic and immunological evidence suggests that the repression mechanism supported by Pc-G members shows many of the hallmarks of heterochromatin-induced repression, which is best exemplified by PEV in flies. PEV involves a local change in chromatin structure that represses the transcription of genes brought into the vicinity of heterochromatin, usually by a chromosomal rearrangement. Heterochromatin appears to 'spread' in *cis* across a euchromatin/heterochromatin boundary, causing repression of a normally active gene in a variable (cell-specific or clone-specific) manner. Almost every eukaryotic cell has heterochromatin, which was classically defined as the deeply staining, highly condensed part of the interphase chromosomes, and these domains typically show late replication during S phase, an absence of meiotic recombination and an almost complete absence of transcriptional activity. In *Drosophila*, genetic screens identified several components of heterochromatin (known as Su(var) and E(var), or collectively as modifiers of PEV). Recent progress in understanding the interplay of these factors and the exciting idea that the availability of factors that either suppress or promote heterochromatin may be developmentally regulated [15] is reviewed in by Lu and Eissenberg in this issue.

Perhaps the most dramatic progress in understanding the cellular role of heterochromatin has come from quite different studies, those probing nuclear organization and meiotic chromosome segregation [16–20]. In *Drosophila*, heterochromatin comprises primarily the simple repeats at centromeres, the Y chromosome and various repetitive domains on chromosome 4. These are dispersed throughout the embryonic nucleus, with no particular subnuclear localization [17], although the centromeric heterochromatin is clustered together in the polytene chromosomes of larval salivary glands [21]. Although it is well established that the insertion of a euchromatic gene in proximity to heterochromatin results in its variegated expression, an unusual example of PEV induced by a dominant allele of the *brown* gene has shed light on the importance of nuclear organization in repression mechanisms. $brown^D$ (bw^D) results from the insertion of a large block of heterochromatin into the coding sequence of brown. In bw^+/bw^D heterozygous flies, this insertion acts in *trans* to provoke the variegated expression of the homologous wild-type copy of the gene. The *brown* locus (bw^+) itself is located at the tip of the right arm of chromosome 2, far from the centromeric heterochromatin. However, using fluorescence in situ hybridization, two groups have shown that in heterozygotic cells showing the variegated phenotype, both copies of brown are closely associated with the centromeric heterochromatin of chromosome 2 [16, 17]. This association requires known modifiers of PEV, like the heterochromatin-binding protein HP1. Apparently, the heterochromatic insertion at $brown^D$ interacts with centromeric heterochromatin, and the somatic pairing of homologous chromosomes forces a centromere-proximal localization of the wild-type allele. This suggests that *trans*-repression is a consequence of sequestering the wild-type gene into a specific heterochromatic 'compartment'.

Further evidence that heterochromatin plays a key role in enabling chromosomes to 'talk' to each other are studies of achiasmatic meiotic segregation by Hawley, and centromere studies by Hennikoff (reviewed in ref. 22). These laboratories have elegantly demonstrated that the meiotic pairing of nonhomologous chromosomes is mediated by the patterns of centromeric heterochromatin maintained on the chromosomes, enabling proper meiotic segregation in the absence of recombination. This identifies an important function for heterochromatin, and it will be interesting to see what effect the modulators of PEV have on this process.

Cis-acting sequences: nucleation sites and boundaries

Inherent in the model of linear transmission of an inactive chromatin state is the idea of *cis*-acting sequence elements that either target the silencing complexes to a particular domain, act to enhance the propagation of the repressed state along the chromatin fibre or act to protect an adjacent (i.e. active) domain

from the action of another such element. These functions are not identical, and can be referred to as 'nucleation' sites, 'propagation' elements and 'boundaries', respectively. Recently, through genetic studies and the analysis of reporter gene constructs in transgenic flies, both the *cis*-regulatory sequences that maintain transcriptionally inactive states of homeotic genes, the Pc-G response elements (PREs) (reviewed in ref. 23), and elements that faithfully reproduce the boundaries of homeotic gene expression domains (e.g. the Fab7 element) have been characterized. In some cases, elements are clustered and functions may even overlap. Intriguingly, a third class of *cis*-acting sequences that do not function per se as Pc-G recruitment sites, but which play a role in the stable and heritable silencing of genes in fly development, have been characterized and dubbed "maintenance elements". When placed near a targeted Pc protein, these aid in the assembly and propagation of the repressed state [24, 25]. The complex boundary elements of the homeotic bithorax complex in flies are reviewed in this issue by Mihaly et al.

By far the most dramatic case of a *cis*-acting sequence that establishes a repressed state is that of the Xic (X-chromosome inactivation center) locus on the inactive X chromosome in mammalian cells. There is now strong evidence that X-chromosome inactivation in mammals is likely to use a chromatin-based mechanism for repression, and that the Xist locus, which encodes a large apparently nontranslated RNA, is both necessary and sufficient to confer inactivation on adjacent sequences [26–28]. Moreover, the Xist locus and 9 kb of upstream controlling region is apparently all the cell needs to be able to 'count' the number of X chromosomes in the cell and properly inactivate all but one [29]. How this fascinating RNA is stabilized, and how it functions to initiate and propagate transcriptional repression, is the subject of the review by Brockdorff and Duthie in this issue. Indeed, studies from the laboratories of Jaenisch and of Brockdroff now indicate that there are both 'nucleation sites' such as the site of transcription of Xist, and 'propagation elements', that regulate the extent and efficiency with which this repressed state propagates from the Xic locus. What these sequences and their ligands are, and what 'boundary' elements might restrict the extent of repression, are all burning questions awaiting answers.

Silencing in plants and DNA methylation as a defence mechanism

The Xist locus is also an excellent example of a parentally imprinted gene that remains silent in oocytes but which is expressed from the paternal X chromosome in extraembryonic cell types derived from the zygote (see Brockdorff and Duthie, this volume). Indeed, methylation generally correlates with zygotic imprints that convey epigenetic regulation on specific genes, depending on the parental source of the allele. Cytosine methylation is also one of the major features conserved between plants and animals that correlates with the stable repression of genes. In plants, transgenes are nearly always silenced, although in many cases silencing has been shown to be promoted by a homology-recognizing mechanism, or to require the insertion of multiple copies of the transgene. This repeat-induced silencing appears to send or provide a signal for de novo methylation, which results in reduced gene expression. A second, seemingly unrelated, mechanism for epigenetic downregulation of transcription involves RNA turnover in the cytoplasm. Both of these mechanisms, which represent two major lines of research in epigenetic regulation in plants, are reviewed in the contribution from Matzke and Matzke in this issue. As the mechanisms of higher-order organization become clearer, no doubt there will be heightened interest in the mechanisms by which the plant cell recognizes repeated inserts and inactivates these as potentially harmful 'foreign DNA'.

Concluding remarks

We hope that the work reviewed in this issue will serve as a launching pad for many new lines of study, since the field of epigenetics now requires a massive input of biochemical and molecular explanations for the fascinating phenomena described over the last 5 or 6 decades. What are the factors that bind the *cis*-acting sequences to target repressive mechanisms to the adjacent domains and to limit the propagation of repressed chromatin? What sequences and factors serve as propagators or 'boosters' allowing the spreading of the repressed state? At what level does methylation and/or acetylation intervene in the mechanism? Do they precede establishment of repression, maintain the repressed state or are they a post facto result of the repression? Which common elements related by sequence reflect conserved function throughout the plant and animal kingdoms? How is silenced chromatin organized such that it can exclude the transcriptional apparatus? Most important, how is a chromatin state heritably maintained through meiotic and mitotic events? We hope this collection of reviews excites as much interest as the recent EMBO Workshop on Chromatin and Epigenetic Regulation, which laid the basis for this collection. The next few years promise significant progress in our understanding of chromosomes, chromatin and gene regulation, which may, in the end, lead 'epigenetics' to 'genetics' after all.

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