Gene Activation and Gene Silencing

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Twenty-five years ago the field of eukaryotic gene regulation was in its infancy. Paradigms driving experiments came primarily from work with prokaryotes and the phage that infected them (12). These paradigms, pioneered by Jacob and Monod, were that genes contained promoter sequences to which RNA polymerase bound and cis-acting sequences to which gene- or pathway-specific trans-acting factors bound. The cis-acting sequences typically were located very near to the transcription start site. The function of activators and repressors could be modulated by specific physiological conditions, with their binding influencing the efficiency of RNA polymerase activity in a positive or negative manner. The development of recombinant DNA enabled the cloning of eukaryotic genes, whereas the development of transformation methods allowed the introduction of modified genes, providing tests of these ideas.

In this perspective we will focus on the key concepts that have emerged from studies of gene regulation in plants, focusing on regulatory mechanisms operating on nuclear endogenous genes and transgenes. These studies have revealed that the prokaryotic paradigm is applicable, but it is not the complete story. Plant researchers have discovered mechanisms superimposed upon the DNA sequence-mediated controls. Studies of these epigenetic phenomena, transposable element cycling, paramutation, and transgene silencing, have revealed novel, previously unimagined mechanisms operating in eukaryotic cells.

PROKARYOTIC PARADIGM IS APPLICABLE TO PLANT GENES

Cis-Acting Sequences

In plant systems, the typical approach to identify key cis-acting regions of promoters has been to fuse candidate sequences (and mutated versions) to reporter genes and reintroduce these constructs into plant cells. Transient assays and stable transgenic lines have been employed. Results from a variety of plants revealed that most plant genes are organized similarly to other eukaryotic genes. Most genes have core promoter elements and enhancer sequences located most frequently in the 5′-flanking regions, which when fused to reporter genes can confer appropriate tissue-specific, developmental, or physiological expression (for review, see 10). However, rare exceptions of regulatory sequences within exons, introns, and 3′-flanking regions have been reported over the years. In mammals and *Drosophila melanogaster*, the regulatory sequences can often be quite far from the transcription start site, whereas in yeast *Saccharomyces cerevisiae* and plant genes they are more commonly within a few kilobase pairs of the transcription start site (10, 16, 18).

Trans-Acting Factors

Most of the work on the basal transcription factors has been done in animal and yeast systems. A major focus of researchers studying plants has been on identifying transcriptional activators and repressors, which function through sequence-specific binding to the DNA near the gene they control. The typical approach to identify the trans-acting factors uses both biochemical and genetic assays. Biochemical assays are routinely used to identify candidate proteins that bind to the sequences of interest. Further mutational work is required to demonstrate that these proteins are binding specifically to important regulatory sequences and that these proteins are the biologically relevant regulatory proteins. This has been demonstrated for numerous regulatory proteins in several plant species (for review, see 15). Essentially all categories of DNA-binding motifs and transcriptional activation domains identified in animal and yeast systems also occur in plants. One class of transcription factors, WRKY, has been found only in plants (3). The activity of transcription factors can be regulated by dimerization with other proteins, nuclear localization, posttranslational modifications, and the binding of small molecules (15).

EPIGENETIC REGULATION

Early genetic studies primarily in *D. melanogaster* and maize (*Zea mays*) suggested that the above paradigm from prokaryotes might not be the whole story. Early geneticists studied several examples in which regulation was variable, unstable, but herita-

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ble. These phenomena include transvection and position effect variegation in *D. melanogaster* (for review, see 22), the cycling of transposable element activity, and paramutation in maize (2, 5, 8). Numerous models were discussed, each with the common theme that global chromosomal levels of control were operating. Within the past 5 years there has been a virtual explosion of studies on chromatin level control and how this is integrated with the previously studied transcriptional activators and repressors. The bulk of the biochemical studies, carried out in yeast, *D. melanogaster*, and animal systems, suggest that eukaryotes operate with a fundamentally different logic than prokaryotes, due to the extensive compaction of their DNA into chromatin (19). Chromatin-remodeling machines are very important for eukaryotic gene regulation. Determining the mechanisms through which these remodeling machines are communicating with basal and gene-specific transcription factors to alter chromatin is an active area of investigation. The major contributions of plant researchers have been in the area of genetic dissection of epigenetic phenomena, as discussed below.

Transposable Element Cycling

The cycling of transposable elements between active and inactive states was first reported by Barbara McClintock over 40 years ago. She reported that *Spm* elements could undergo heritable but reversible shifts in activity. Often these shifts in activity would occur in predictable patterns during development, and be reversible or cyclical. However, they could also be essentially irreversible, resulting in cryptic, silent elements. In the 1980s several laboratories, investigating several different classes of transposable elements in maize, demonstrated that the most consistent molecular correlate with silencing was cytosine methylation (for review, see 5). These cyclical events are thought to represent the elements escaping from cellular control mechanisms that function to keep repetitive elements silenced. A consequence of packaging transposable elements into inactive chromatin could be a reduction in both mutation rates and ectopic recombination events between repeated sequences.

Paramutation

Paramutation is a violation of Mendel's law of segregation, which states that two alleles segregate from each other unchanged. All examples of paramutation involve an interaction between alleles that leads to a meiotically heritable reduction in the expression of one of the alleles. Paramutation was first described for two maize genes in the 1950s by Alexander Brink and Edward H. Coe, Jr. In the 1990s, paramutation was shown to occur at another maize gene, and several transgenes (see below). Where it has been tested, paramutation is associated with reduced transcription and altered chromatin structure (for review, see 2). There are numerous differences between the phenomenology among the different genes and whether paramutation correlates with DNA methylation and the presence of repeated sequences within the genes (for review, see 2). Despite these differences, the recent isolation of a mutation that affects paramutation at multiple loci and transposon silencing suggests a common underlying mechanism (for review, see 2). The mechanism is unknown, but the current favored model is altered chromatin structural changes (2, 8).

Transgene Silencing

Throughout the past 11 years, a wide range of gene silencing phenomena in plants have been revealed by extensive studies on transgene expression (for review, see 4). Silencing can be transcriptional (transcriptional gene silencing [TGS]) or posttranscriptional (posttranscriptional gene silencing [PTGS]). It can affect single transgene copies or unlinked partners (including transgenes, endogenous genes, or viruses) through homology-dependent processes that can be reciprocal (cosuppression) or unidirectional (a transgene is silenced by another expressed transgene or by a replicating virus).

TGS

Transcriptional silencing typically is associated with DNA methylation within the promoter region and, when tested, alterations in DNaseI hypersensitivity, indicative of altered chromatin (for review, see 4). Although many transcriptionally silenced transgenes have complex structures with multiple copies integrated into a single genomic site, simple single-copy insertions can also be transcriptionally silenced. Two nuclear proteins required for TGS have been identified. DDM1 is a chromatin-remodeling protein belonging to the SNF2/SWI2 superfamily. Its impairment releases both TGS and methylation of transgene arrays (9) and silent retrotransposons (7). The impairment of MOM1, a novel nuclear protein, releases TGS but not methylation of transgene arrays, suggesting that TGS could operate through methylation-dependent or -independent pathways (1). Several examples of transgenes that are transcriptionally silenced undergo paramutation-like behavior in that they can silence homologous sequences located in either allelic or nonallelic positions (for review, see 4, 8). As proposed for paramutation, this silencing could result from the transfer of altered chromatin structural changes (Fig. 1A). TGS can also result from expression of dsRNA derived from promoter sequences (11; Fig. 1B), suggesting that like PTGS, TGS can be mediated by dsRNA (see below).

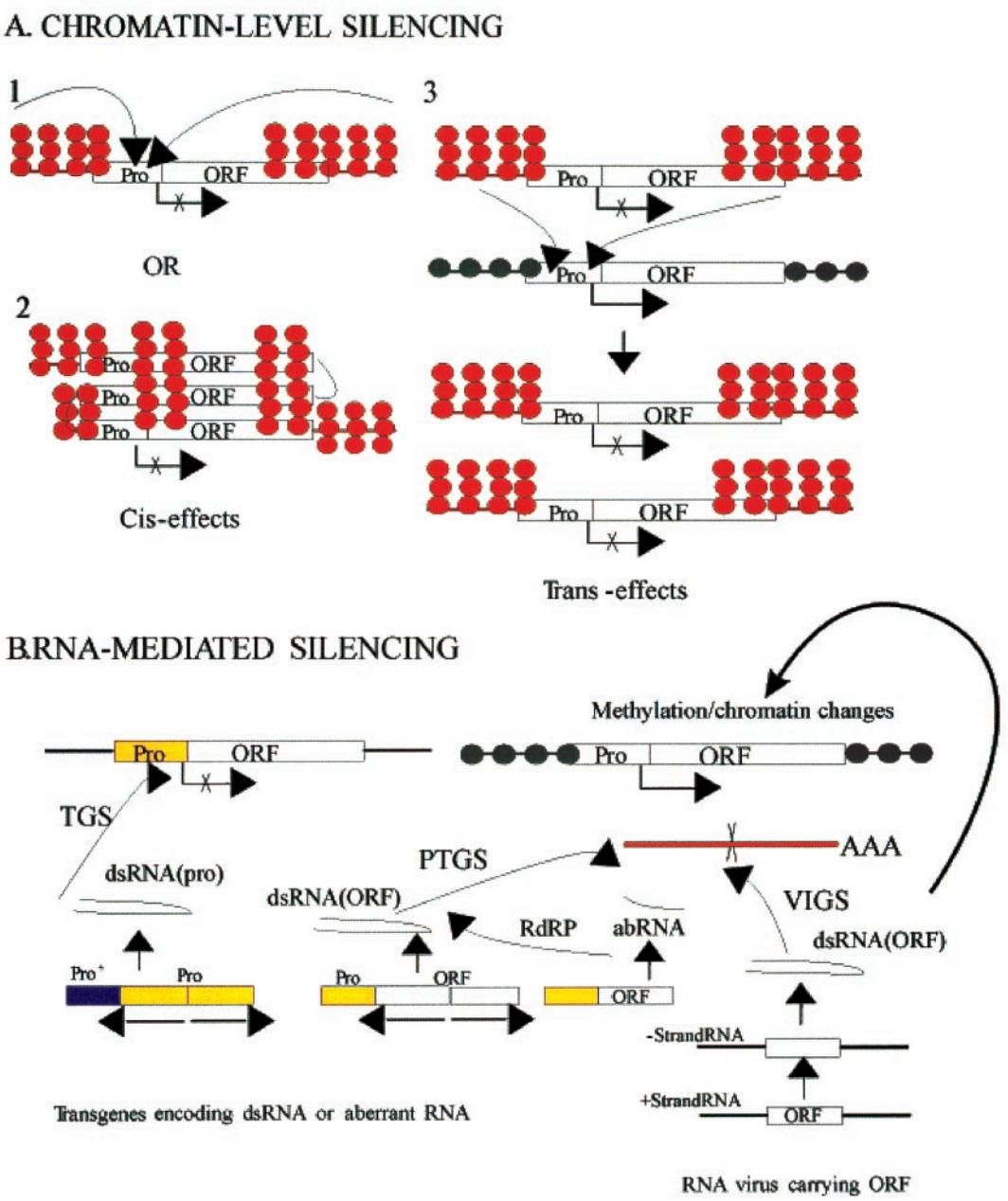

Figure 1. A, Models for cis and trans effects of chromatin structure on transcription of endogenous genes or transgenes. The red balls symbolize repressive chromatin structure, whereas the green balls symbolize a more open, transcriptionally active state. A, 1: Repressive chromatin spreading from adjacent sequences, 2: repeated sequences form repressive chromatin, and 3: trans interactions between sequences with repressive chromatin cause altered chromatin structures. B, Models for RNA-mediated silencing. The common theme is that silencing is triggered by a double-stranded RNA molecule (dsRNA). If the RNA is homologous to the promoter, TGS can occur. If the RNA is homologous to the transcribed mRNA (symbolized by open reading frame), transcription is not affected, but the RNA is degraded. Two of a variety of transgene structures are depicted that could give rise to dsRNA, either directly or via the activity of an RNA-dependent RNA polymerase (RdRP) on an aberrant RNA (abRNA). Viruses can also readily produce dsRNA through the RNA-dependent RNA polymerases they encode, resulting in virus-induced gene silencing of an endogenous gene or transgene open reading frame homologous to the engineered virus.

PTGS

Posttranscriptional silencing is a sequence-specific RNA degradation process that propagates systemically throughout the plant (for review, see 4), and correlates with the accumulation of 25-nucleotide-long RNA species (6). It was originally described as cosuppression of transgenes and homologous endogenous genes (14, 20) and it shows similarities with quelling in fungi and genetic interference by dsRNA (RNAi) in animals (for review, see 17, 21). Homologous viruses can act as targets or triggers of PTGS (and are therefore referred to as virus-induced gene

silencing) whereas nonhomologous viruses can inhibit PTGS (for review, see 4). Arabidopsis PTGS-deficient mutants are hypersensitive to infection by viruses that partially counteract PTGS but not by viruses that totally inhibit PTGS, suggesting that the issue of virus infection depends on the fight between plant antiviral defenses (PTGS) and viral anti-PTGS attacks (13). Three proteins required for PTGS have been identified. AGO1 is a protein similar to rabbit eIF2C protein (M. Fagard and H. Vaucheret, unpublished data), SGS2 is similar to tomato RNA-dependent RNA polymerase, and SGS3 is a novel protein of unknown function (13). AGO1 and SGS2 are similar to proteins required for quelling in fungi and RNAi in animals, confirming the mechanistic link between these three phenomena, and the essential role of dsRNA in targeting RNA for degradation. A summary of current models is provided in Figure 1B.

IMPLICATIONS AND FUTURE DIRECTIONS

An eventual understanding of the homology-dependent silencing discussed above should reveal how homologous sequences interact in the nucleus and the cytoplasm to influence the regulation of each other, how heritable expression states are established, and how they are maintained through numerous cell divisions and transmitted to the next generation. These studies have important implications for understanding how gene regulation can be heritably modified, influencing development and potentially the evolution of new developmental programs.

An increasing number of proteins required for transposon cycling, paramutation, TGS, and PTGS are being identified. Their characterization will allow an understanding of these processes at the molecular level. New links among these processes and between them and related phenomena in other eukaryotes are anticipated in the coming years. Transposon cycling, paramutation, and TGS were supposed to be closely related (occurring through chromatin changes in the nucleus) and distinct from PTGS (resulting from RNA degradation in the cytoplasm). However, this paradigm is becoming suspect. Recent reports revealed a role of dsRNA and correlations with DNA methylation in both TGS and PTGS in plants (11, 21). In addition, several proteins, including a RNaseD-like protein (MUT-7) are involved in both RNAi and transposon silencing in *Caenorabditis elegans* (17).

The next few years promise to be very exciting as many important questions are answered: What is the role of DNA methylation in TGS and PTGS? What is the systemic signal for PTGS? How do homologous sequences find each other in the nucleus? How do communications between homologous sequences establish altered expression states? Once established, how are these distinct transcription states maintained through generations? Are these homology gene silencing phenomena revealing cellular mechanisms for protection against invasive DNA? Do they also play a role in fundamental developmental processes?

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