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Review

Review: Artificial transcription factor-mediated regulation of gene expression

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The transcriptional regulation of endogenous genes with artificial transcription factors (TFs) can offer new tools for plant biotechnology. Three systems are available for mediating site-specific DNA recognition of artificial TFs: those based on zinc fingers, TALEs, and on the CRISPR/Cas9 technology. Artificial TFs require an effector domain that controls the frequency of transcription initiation at endogenous target genes. These effector domains can be transcriptional activators or repressors, but can also have enzymatic activities involved in chromatin remodeling or epigenetic regulation. Artificial TFs are able to regulate gene expression *in trans*, thus allowing them to evoke dominant mutant phenotypes. Large scale changes in transcriptional activity are induced when the DNA binding domain is deliberately designed to have lower binding specificity. This technique, known as genome interrogation, is a powerful tool for generating novel mutant phenotypes. Genome interrogation has clear mechanistic and practical advantages over activation tagging, which is the technique most closely resembling it. Most notably, genome interrogation can lead to the discovery of mutant phenotypes that are unlikely to be found when using more conventional single gene-based approaches.

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1. Introduction

The phenotype of any given organism results from a complex interplay between its genome and the mechanisms that led to the expression of its genes. This interplay is characterized by intricate

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feedback loops that generate the essential robustness of the phenotype. The feedback loops must also allow for flexibility when endogenous or exogenous stimuli demand for specific phenotypic adaptations. The metaphor of Waddington's epigenetic landscape [1], a model describing the different developmental paths that an embryonic cell can take toward differentiation, is still very much relevant to modern developmental genetics. The stability of gene expression patterns controlled by established epigenetic cues enables cells to withstand most of the random biotic and abiotic noise. However, when a key determinant is able to induce a crucial epigenetic change, cells and organisms might be forced into a different state or developmental program. This epigenetic view of the regulation of gene expression complements the view where genetic variation is the source of phenotypic variation; genetic variation is futile when not expressed. The phenotype of a cell can be regarded as being the product of the epigenetic landscape, genome wide transcription patterns and variation at the sequence level at any given stage of development. Fundamental research on these processes has allowed us to gather knowledge on which genes or sets of genes are involved in phenotypes of interest. In this review, we address several means of placing phenotypes under artificial control by employing artificial transcription factors (TFs) as tools for regulating the expression of endogenous genes in plants.

1.1. Regulation of gene expression

The short sequence upstream of the transcription start site that in eukaryotic genes contains the binding sites for general transcription factors and RNA polymerase II [2] is often referred to as the "minimal promoter" of a gene. More gene-specific regulatory sequences can be found in the DNA sequence upstream of this minimal promoter. It has become common practice in the field of plant molecular biology to designate a rather arbitrary DNA fragment of one to a few kilobase (kb) pairs long and located upstream of the translational start site as the "promoter" of a gene. Plant molecular biologists are usually aware of the fact that many more regulatory sequences exist at greater distances at both the 5′ and the 3′ ends of a gene as well as within its coding sequence that contribute to the precise level of gene expression. Short statements regarding "promoter activity" usually refer to the contribution of at most a few kb of upstream DNA sequence on to the regulation of transcription levels. Within the context of artificial TF-mediated regulation of gene expression, it would be better to employ the term "gene control region" rather than "promoter". This control region is usually defined as the portion of a eukaryotic gene containing the core promoter as well as any other regulatory sequences that control or influence transcription of that gene. Within the control region, the eukaryotic core promoter is defined as the region that can be bound by the general transcription factors required for RNA polymerase II-dependent transcription initiation at the transcription start site, thus equaling the "minimal promoter" mentioned above. Apart from the core promoter, the control region contains enhancer en silencer sequences [3]. These regulatory sequences are potential docking sites for more specific transcription factors that can affect the number of transcription starts at the core promoter per unit of time. The regulatory sequences can be present *in cis* of the start site, within a distance of a few kb from the core promoter, or be located at much larger genomic distances where the term "*in cis*" gradually becomes practically irrelevant. In the latter cases, these regulatory elements are absent from the relatively short PCR-generated DNA sequences taken for the "promoter" in more pragmatic approaches. When discussing the effects of artificial TFs, it is much more appropriate to acknowledge all interactions that are formed within the larger gene control region.

The conserved Mediator complex is also required for successful initiation of RNA polymerase II-dependent transcription at core promoters in eukaryotes. The Mediator complex functions as a highly complex co-activator of transcription, interacting with the protein domains of RNA polymerase II holoenzyme and general transcription factors. Mediator also interacts with the more specific transcription factors binding to sequences outside of the core promoter. Without the stimulatory contribution of the latter proteins, RNA polymerase II is unable to initiate gene transcription [4,5]. The Mediator complex can thus be thought of as a platform for integrating or relaying signals that can stimulate the initiation of transcription in the regulation of gene expression [4]. However, once the factors conducive for transcription are present and the expression of genes has been switched on in a stable manner, one could imagine that further information and activity is needed to subsequently decrease transcriptional activity or even switch off the expressed genes when this would be required, such as during developmental processes. Accumulating evidence connects the Mediator complex with epigenetic regulation, recruiting factors and enzymes that lead to the deposition of epigenetic molecular markers associated with gene silencing [6,7].

1.2. Chimeric transcription factors

Transcription factors contain a DNA binding domain and a domain that is able to affect transcriptional regulation. Such "effector" regulatory domains increase or decrease the number of transcriptional starts of a gene when bound to DNA at an appropriate position in the gene control region. The effector domain can be envisaged as directly interacting with one or more of the general transcription factors and/or RNA polymerase subunits at the transcription start site or indirectly by recruiting proteins that make these essential contacts.

The use of these effector domains has been reported in connection with natural transcription factors. Plant transcription factors equipped with signature DNA binding domains were fused to a small C-terminal peptide domain that inhibits gene expression [8,9]. This strategy is aimed at turning natural transcriptional regulators into dominant repressors of gene expression that specifically bind to the gene control region of their natural target genes. Changes in the phenotype are readily observed due to loss-of-function mutations resulting from the reduced expression of the genes that are under control of the transcription factors being experimentally manipulated. This strategy is termed Chimeric REpressor gene Silencing Technology (CRES-T) [10]. A system involving fusions with activating effector domains instead of repressing domains could also be envisaged, where an enhancing transcription factor would then affect transcription at its natural target loci in a positive manner.

In the CRES-T technology, as well as in its possible derivatives, DNA binding properties of natural TFs form the basis for the mode of action of these chimeric proteins. The artificial TFs discussed below allow for recognition of any target site of choice to affect the transcriptional activity of genes of interest at the control regions of their normal genomic position. However it is necessary to address relevant target sites within the control region to specifically regulate the expression of endogenous genes of interest. A technique that employs naturally occurring DNA binding domains is hardly an option. Even if a binding site for a known transcription factor would be present, such sites are usually of low complexity and occur at many positions within the genome. This could possibly affect the transcriptional regulation of a host of genes that are normally under control of this particular transcription factor. Custom made site-specific DNA binding domains are required to address unique sites within the genome. The molecular details of systems that allow for site-specific protein–DNA recognition have become understood

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