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Review

Designed transcriptional regulators for trait development

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ABSTRACT

Development is largely controlled by proteins that regulate gene expression at the level of transcription. These regulatory proteins, the genes that control them, and the genes that they control, are organized in a hierarchical structure of complex interactions. Altering the expression of genes encoding regulatory proteins controlling critical nodes in this hierarchy has potential for dramatic phenotypic modification. Constitutive over-expression of genes encoding regulatory proteins in transgenic plants has resulted in agronomically interesting phenotypes along with developmental abnormalities. For trait development, the magnitude and timing of expression of genes encoding key regulatory proteins will need to be precisely controlled and targeted to specific cells and tissues at certain developmental timepoints. Such control is made possible by designed transcriptional regulators which are fusions of engineered DNA binding proteins and activator or repressor domains. Expression of genes encoding such designed transcriptional regulators enable the selective modulation of endogenous gene expression. Genes encoding proteins controlling regulatory networks are prime targets for up- or down-regulation via such designed transcriptional regulators.

Contents

1. Introduction

Developmental processes in biology are controlled by regulatory proteins that bind DNA in a sequence-specific manner and act via protein–protein interactions to influence gene expression at the transcriptional level (Fig. 1). Genes encoding such transcriptional regulators are central to the control of complex biological networks and are themselves integrated into hierarchical networks that control development and environmental responses [1]. Gene expression is also controlled post-transcriptionally via RNA-mediated processes [2]. Recent insights into the role of microRNAs derived from precursor transcripts [3] and the involvement of RNA interference (RNAi) in controlling epigenetic responses [4] have led to a whole new paradigm of developmental regulation.

Genome-wide association studies [5] and quantitative trait loci (QTL) mapping [6] have pointed toward the importance of regulatory genes and their expression for determining complex phenotypes. A diverse array of plant characteristics such as canopy architecture [7], plant height [8], floral morphology [9], fruit size [10], pigmentation [11], stress tolerance [12] and disease resistance

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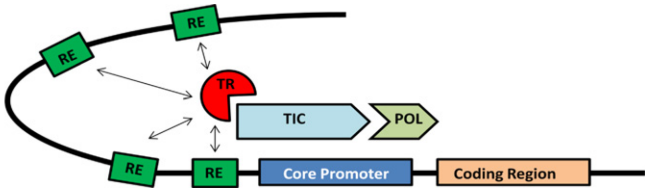

Fig. 1. Transcriptional regulation. Transcriptional regulators (TR) comprise DNA binding domains that recognize upstream regulatory elements (RE) and effector domains that interact with proteins in the transcription initiation complex (TIC) thereby controlling RNA polymerase (POL) activity.

[13], to name a few, all appear to be controlled by networks of regulatory proteins influencing gene expression cascades.

As ambitions for future crop enhancement via transgenic technology move from relatively simple traits, such as insect or herbicide resistance, to more complex traits, such as yield and environmental stress tolerance, the manipulation of developmental networks will be required. Transcriptional regulation will be one of the key levers for such control [14]. This review examines this premise and introduces the concept of modulating the expression of endogenous regulatory genes, in their 'native' context, using designed transcriptional regulators.

2. Transcriptional regulators and crop domestication

One need look no further than current domesticated crops and the mechanisms associated with their origins to appreciate the potential of transcriptional regulation for trait development. The first chapter of Darwin's *Origin of Species* [15] is entitled 'Selection Under Domestication'. Darwin starts his argument for the mutability of species and their common descent by describing the variability among current domesticated forms. He observed that many cultivated varieties of plants differ dramatically in one character but are essentially identical in others. He posits that the variation is the result of selection for incremental differences that accumulate over time. Current thinking is that Neolithic man domesticated most current crop species 10,000–15,000 years ago [16]. Although these people knew nothing about genes, they realized that whatever controlled the characteristics of plants was malleable. Under their influence, monumental genetic modifications were achieved. A comparison of the ear of modern maize with its closest wild relative, teosinte, exemplifies the scale of morphological change associated with the process of domestication [17].

The genes controlling some of the most important morphological changes associated with domestication have been identified and have shed light on some of the forces underlying phenotypic selection [18]. It has become evident that the differences between domesticated crops and their wild progenitors are largely the result of differences in gene expression affecting plant growth and development. A few well-studied examples highlight these differences and the importance of transcriptional control. Maize QTL analysis identified 5 genetic loci that control major morphological differences between teosinte and maize [17]. Candiate genes for three of these are transcriptional regulators; *TEOSINTE BRANCHED 1* (*TB1*) controls apical dominance [19], *TEOSINTE GLUME ARCHITECTURE* (*TGA1*) controls placement of the kernel on the ears [20] and *FLORICULA/LEAFY* (*ZFL2*) is associated with infloresence phyllotaxy [21,22]. The product of *TB1* is believed to be a transcriptional repressor that controls several key cell cycle genes [18]. Domesticated maize consists of a single shoot terminated by a tassel and short axillary branches terminated by ears and has higher levels of *TB1* expression in axillary meristems when compared to teosinte, which is highly branched [23]. Apical dominance in domesticated maize appears to have been the result of selection for higher *TB1* expression in axillary meristems resulting in less branching via suppression of lateral shoot expansion. *TGA1* encodes a transcription factor that controls the structure of the basal cupule that holds the kernel on the ear [20]. Maize has a small cupule enabling the kernel to be exposed on the surface of the ear while teosinte has a large cupule and glume that is hardened by silica [20]. The dissimilarity is thought to be the result of a single amino acid difference between the maize and teosinte alleles [20]. *ZFL2* is involved in the regulation of floral identity genes and is associated with more rows of kernels in maize than teosinte [22].

SHATTERING 4 (*SH4*) is a gene underlying a major QTL controlling seed dispersal in rice [24]. Domesticated rice, which does not shed its seed at maturity (i.e., it is non-shattering), has a mutation in the *SH4* gene. The gene product is a protein containing a *MYB3* DNA binding domain that interferes with normal abscission layer formation [25]. This loss-of-function mutation in a transcriptional regulator, *SH4*, controls the expression of key abscission layer formation genes in the flower-pedicel junction.

Fruit size in tomato is yet another important domesticated trait that appears to have been the result of selection for modified gene expression via alterations in genes encoding transcriptional regulators [26]. A major QTL controlling tomato fruit size involves a gene, *FACIATED* (*FAC*), encoding a transcription factor that controls carpel number during flower and fruit development [27]. The expression of this gene largely accounts for the observed difference in fruit morphology, i.e., down-regulation of the gene during flower development results in higher carpel number [27].

In addition to major morphological modifications, phenotypes associated with metabolic changes have also accompanied crop domestication [28]. The lack of color of domesticated rice pericarp tissue appears to be the result of a frame shift mutation in the *RED PERICARP* (*Rc*) gene which encodes a regulator of anthocyanin production [29]. Similarly, changes in flavanoid biosynthesis in tomato, eggplant and pepper fruit during domestication were the result of mutations in regulatory genes controlling this metabolic pathway [30].

One of the lessons that can be gleaned from studies of the molecular genetics of crop domestication is that mutants of genes encoding transcriptional regulators can manifest themselves as developmental variants capable of serving as sources of valuable traits. It appears that subtle changes in the timing and/or level of expression of key regulatory genes can create unique morphological variations. This insight should prove useful for future crop enhancement and points toward the potential of using transcriptional regulation as a means of generating valuable genetic variability on which to base future selection. This knowledge should be particularly powerful when combined with modern biotechnological tools enabling enhanced genomic analysis and modification.

3. Transgenic over-expression of native transcriptional regulators

Yield is an important agronomic trait and numerous transgenic strategies have been pursued with the goal of improving some of the key components [31]. These include attempts to improve tolerance to biotic [13] and abiotic [32] stresses, increase photosynthesis [33], enhance nitrogen use efficiency [34] and modify plant architecture or shade avoidance characteristics that allow increased planting density [35]. Since transcriptional regulators have profound effects on the developmental physiology of plants, it would appear as if the genes that encode them should hold great promise as candidates for transgenic expression for improving some of these traits [14]. To date, most studies designed to modify plant phenotypes with transcriptional regulators have involved ectopically