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Plant synthetic promoters and transcription factors

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Synthetic promoters and transcription factors (TFs) have become incredibly powerful and efficient components for precise regulation of targeted plant transgene expression. Synthetic promoters can be rationally designed and constructed using specific type, copy number and spacing of motifs placed upstream of synthetic or native core promoters. Similarly, synthetic TFs can be constructed using a variety of DNA binding domains (DBDs) and effector domains. Synthetic promoters and TFs can provide tremendous advantages over their natural counterparts with regards to transgene expression strength and specificity. They will probably be needed for coordinated transgene expression for metabolic engineering and synthetic circuit applications in plants for bioenergy and advanced crop engineering. In this article we review the recent advances in synthetic promoters and TFs in plants and speculate on their future.

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Introduction

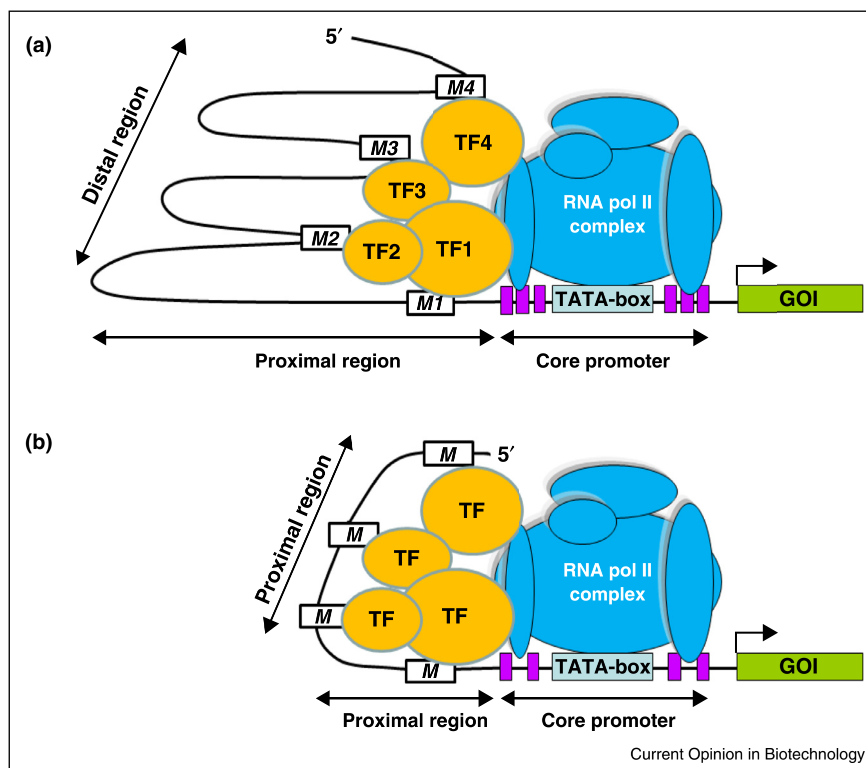
Transcriptional regulation plays an important role in gene expression, which is mainly controlled via the interactions between gene promoters and transcription factors (TFs), and between TFs and chromatin-modifying machinery. TFs bind to specific promoter sequences that comprise *cis*-regulatory elements (or motifs) to determine the temporal and spatial features of gene expression (Figure 1a). Native promoters have a dispersed arrangement of motifs that are seemingly non-conserved among genes with similar expression patterns [1]. Thus, the type, copy number and spacing of motifs within a promoter can be reorganized, which is the basis of synthetic promoter construction [2–6]. Therefore, synthetic promoters offer the prospect of streamlined constructs with specified expression patterns. Whereas promoters can essentially be synthesized *de novo*, synthetic TFs have been designed using an effector (activation or repression) domain and various existing proteins with DNA binding features, in which the DNA binding function can be altered to bind to preselected genomic loci [2,7,8]. Since the early 2000s, synthetic promoters have been used in plant genetic engineering and continue to advance in design and utility in plant synthetic biology [2–4]. Synthetic TFs are more recent arrivals in plant biotechnology, but will probably be necessary to enable metabolic engineering in plants [9,10]. In this review we will discuss the current status and trends in synthetic promoters and TFs in plants. Furthermore, we will speculate on their future utility.

Synthetic promoters

A synthetic promoter consists of a core promoter and synthetic motifs for spatial and temporal control of transgene expression. Typically, a motif sequence is derived from extant sequences, but multiplied or otherwise recombined. The core promoter sequence is set ±50 bp with respect to the transcription start site (TSS), and contains a TATA-box, GA elements or a coreless region together with some core promoter elements (such as Y patch, CCAAT element, Inr element, CA element, etc. [11,12]). The distribution of core promoter elements exhibits relatively positional conservation with respect to the TSSs in plants [13]. The core promoter directs accurate transcription initiation when bound by some basal TFs, and provides little or no basal expression level [14•,15]. The most well-known core promoter is the minimal CaMV 35S promoter, which is about 54 bp in length and provides a very low basal expression and an efficient transcription initiation in both dicots and monocots. Recently, a minimal *ZmUbi1* promoter of 126 bp in length was characterized from maize *Ubiquitin-1* with a low basal expression for use in monocots [16]. Even though there is a need to identify additional core promoters from native plant genes and viruses, synthetic core promoters can obviously be produced using different TATA-box regions and core promoter elements, as demonstrated in yeast *Pichia pastoris* [17••].

The selection, copy number and spacing of *cis*-regulatory elements determine the strength, temporal and spatial expression patterns of synthetic promoters. Selection of motifs with known functions can be conducted with the help of three main databases, that is, PLACE [18], PlantCARE [19] and TRANSFAC [20]. Novel motifs can be characterized using synthetic motif library screening [21], bioinformatics-based *de novo* motif discovery

Figure 1



Schematic representations of synthetic promoters versus natural promoters. **(a)** A natural promoter contains a core promoter region, a proximal region and a distal region that typically range from 500 bp to over 2000 bp [90]. The core promoter contains core promoter elements (pink) and the proximal and distal regions are composed of various *cis*-regulatory elements. Binding of multiple transcription factors to these motifs determines the complex expression profiles of these promoters. **(b)** Synthetic promoters could be built by fusing multiple copies of a single motif (or several motifs) together with a core promoter [i.e., a TATA-box region and several core promoter elements (pink)], or purely synthetically with the help of modelling. Only user-preferred transcription factors could bind to these motifs and activate gene expression. Compared to natural promoters, synthetic promoters provide higher (or lower) expression level with high specificity, lower basal expression level, shorter length, and less sequence homology to any host genomic sequences. The transcription initiation sites of genes-of-interest (GOI) are indicated by horizontal arrows.

[22], and/or experimental approaches (i.e., 5′ and 3′ deletion, and addition of motifs individually or in combination [3]). As the deconstructive analysis of plant natural promoters for functional motif discovery has slowly increased during the past three years [23–34], the effectiveness of using computational tools for *de novo* motif discovery in plants has been experimentally demonstrated in *Arabidopsis thaliana* [35] and soybean [36].

Once motifs of interest have been selected for synthetic promoter construction, motif copy number and spacing have to be optimized. Motif copy number often correlates with an increase in synthetic promoter strength as demonstrated from work in various plant species, such as *Arabidopsis* [28], tobacco [37] and rice [38]. The motif dosage effect in synthetic promoters is not surprising, since congruent findings have been observed in native promoters in *Arabidopsis* [24••] and apple [39••], even though no dosage effects have also been observed [4]. When multiple motifs are engineered into a single synthetic promoter, proper spacing among motifs is required for the hierarchical arrangement of their corresponding TFs in order to obtain full synergistic interactions with the RNA polymerase II complex (Figure 1; [37]). Mehrotra and Mehrotra [40] demonstrated that two copies of the ACGT motif in synthetic promoters resulted in salicylic acid-inducibility in tobacco when separated by five nucleotides, but were abscisic acid-inducible when separated by 25 nucleotides.

Recent advances in plant synthetic promoter engineering are making strides to generate more constitutive, bidirectional or inducible synthetic promoters for a better transcriptional regulation of transgene expression in plants (Table 1; see [2] for more plant synthetic promoters). Most synthetic promoters tested to date were either hybrids of multiple promoter parts or fusions of specific *cis*-regulatory elements with a core promoter (Figure 1b). Curran *et al.* [41••] demonstrated that functional, purely synthetic yeast promoters could be created from various

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