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Review

Molecular Concept of Transcription Factor in Crop Plant

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Abstract: A transcription factor regulates gene expression in crop plants at the transcription level. Typically, a transcription factor comprises domains that facilitate regulatory activities, including a transcription regulatory region, a DNA-binding region, an oligomerization site, and a nuclear localization signal region. All domains determine the functional activities of genes, including gene function, characteristics, nuclear localization, and regulation of transcription factors. Transcription factors inhibit or activate the expression of a gene through binding the functional domain of the promoter cis-acting element or their interaction with other proteins. However, Transcription factor function and structure have become crucial roles in plant molecular biology in the upcoming breeding research era.

Keywords: transcription factor; DNA binding region; cis-acting element; rice

1. Introduction

Transcription factors are trans-acting factors or proteins that interact with cis-regulatory elements at the promoter region of a gene (Khan et al. 2023). Their interaction acts as a switch-on / switch-off system for the gene at the transcription level (Cramer 2019). Many transcription factor genes have been studied or cloned in rice that control transcription activities in the genome for activation/ suppression of traits such as cold tolerance, high salt, heavy metal toxicity, bio fortification, osmotic stress, pathogenic resistance, insect pest, heat stress, and some to change the plant architecture. These transcription factors control the quantity of expression of all living and nonliving things. The structure of transcription factors shows that they have four functional areas: a DNA-binding region, a transcriptional regulatory region (which can be either an activation or inhibitory region), and a nuclear localization signal (Bushweller 2019; Cramer 2019). These functional regions control the transcriptional regulation by binding with promoter cis-elements or by interacting with the domains of other transcription factors (Khan et al. 2023). Most transcription factors only have one area where they can bind to DNA (Mitsis et al. 2020). But some transcription factors, like GT2 AP2 from Arabidopsis thaliana and rice (Oryza sativa), have two places where they can connect to DNA. Some transcription factors don't have DNA-binding regions or transcriptional control regions. By working with transcription factors that have the above functional domains, they control gene transcription (Bushweller 2019).

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2. Structure and Function of Transcription Factors

2.1. DNA Binding Region

The DNA-binding region, also called the DNA-binding domain, is the chain of amino acids that the transcription factor uses to find and attach to the DNA part that acts in cycles. (Jie et al. 2020). The amino acid sequence within DNA DNA-binding region of a specific transcription factor type remains consistent. The parts of plant transcription factors that usually bind DNA are the bZIP domain, the zinc finger domain, the MADS domain, the 1YC domain, the 4MYB domain, the homeodomain, and the AP2/EREBP domain 7. Several subgroups can be made out of some of these domains based on the count and position of conserved amino acid residues in these distinct regions. For instance, the arrangement of cysteine (C) and histidine (H) residues can be used for subclassification. Transcription factors with a zinc finger region can be broken down into three groups such as C, H,

and C. Recently, the HC, C, CHC, and CHC5 subclasses have been identified as new DNA-binding domains in plant transcription factors. These include the ARF region of Arabidopsis thaliana ARFI transcriptional factor in maize (*Zea mays*) VP1 and the B3 region of PvAlf transcription factor in beans (Phaseolus vulgaris). They are very good at finding and attaching to cis-acting elements because of their DNA-binding domain's specific amino acid patterns. (Hajheidari and Shao-Shan 2022).

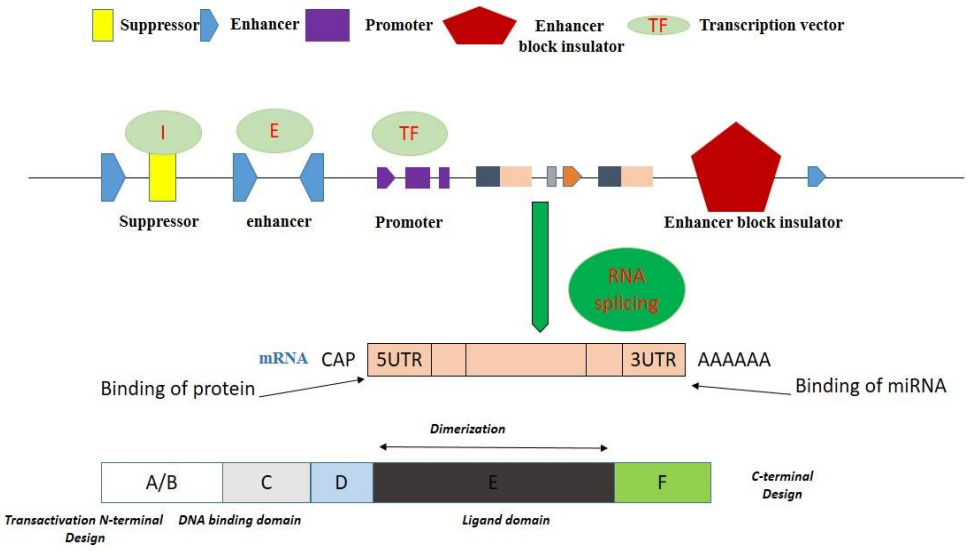

Figure 1. A schematic transcription factor structure model.

2.2 Transfer control area

2.2.1 Transcriptional Activation Region

Transcription factors differ in their transcriptional regulatory regions, which include transcriptional activation and transcription repression domains. (Cramer, 2019). These regions determine functional differences and identify various types of transcription factors from animals and Saccharomyces cerevisiae. (Bao Gia, Mark A Stamnes, and Yu Li et al. 2021). Examples include glutamine-rich domains in SPI, proline-rich domains in CTF/NF-I, and acid activation regions in GAL4 and VP16GCN4. The regions typically contain $30 \leq 100$ amino acid residues outside the DNA-binding region. Higher plant transcription factors, such as VP1 and PVALF, regulate storage protein gene expression in various plants (Niñoles et al. 2022). Their N-terminal acidic conserved amino acid sequences have transcriptional activation ability, and their homology analysis shows high homology with yeast transcription factor GCN4 and virus transcription factor VP16. Typical plant transcriptional activation regions are rich in acidic amino acids, proline or glutamine, and GBF transcription factors. However, high amino acid content does not necessarily indicate an important role (Ahmed et al. 2021).

2.2.2 Transcriptional Inhibitory Region

Bean PVALF transcription factor can trigger the expression of the cotyledon storage protein gene DLEC2. The ROM2 transcription factors of bean bZIP can combine with the regulatory element of DLEC2 to suppress the gene-activating function performed by the PVALF transcription factor. Once the N-terminal bZIP domain ROM2 of ROM2 is removed, the PVALF activation activity will be lost, and the ROM2 and PVALF activation domain junction proteins of the N-terminal bZII domain will be removed to activate the expression of DLEC2, which indicates that there is a transcriptional inhibitory region of 121 pairs of inhibitory bell barley (*Hordeum spontaneum*) in the removed ROM2N- terminal region. The study of VP1 transcription factors expressed by a-amylase gene showed that VPI also contains a domain 1131 that suppresses transcription, although many experimental results show the presence of transcriptional inhibitory regions in transcription factors (Cramer 2019). However, the structure and mechanism of the transcription factor inhibition region may be as follows: (I) Once attached to the specific promoter sites, it can hinder the binding of other transcription factors: (ii) it may inhibit transcription by repressing the action of other transcription factors, and (iii) modify advanced DNA structure (high-order structure) such that transcription is blocked.

3. Nuclear Positioning Signal Area

The nuclear localization signal region (NLS) is a region in transcription factors that coordinates their importation into the nucleus. (Lu et al. 2021). It is found in diverse transcription factors, such as GT-2 in rice, O2 in HSFA1-2 corn, tomato, and PS-IAA4 and PS-IAA6 in pea. The number of NLS varies in different transcription factors, with certain NLS, including as O2 transcription factor NLS, also present in other functional regions. (Marathe, Erich Grotewold, and Marisa S Otegui 2024). BZIP transcription factor O2 has two NLS, An and B, located between $101 \leq 135$ and 223×254 amino acid residues. The B signal region is in the same area as the DNA-binding region, indicating that the NLS plays an independent role. The B signal region in the DNA-binding region can transfer transcription factors more effectively into the nucleus. (Suter 2020).

4. Oligomerization Sites

An oligomerization site is a functional place where different transcription factors can work together (Lai et al. 2019). They have fairly stable chains of amino acids, and most of them take on a certain shape in space and are connected to the DNA-binding area. In the case of MADS transcription factors, the oligomerization area has two a-helixes and two B-folds. The oligomerization area of bZIP transcription factors has a zipper structure, and the oligomerization area of bHHL transcription factors has a spiral-ring-helix structure. The yeast two-hybrid system (Two-hybrid system) showed that the B-Peru transcription factor in maize might interact with the C1 transcription factor's DNA-binding area, making it easier for C1 and DNA to bind (Bai et al. 2020). Genetic research and temporary expression studies (transient expression assay) also reveal the C1 transcription factors have an impact only, in activating transcription when they bind to B-Peru. Its spiral-ring-helix domain is very important for B-Peru to connect to C1 and help start transcription. The oligomerization region of transcription factors does affect their activity, according to the previously described research, even though the precise role of the helix-ring-helix domain among B-Peru and C1 is still unclear (Lai et al. 2019).

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5 The Activity of Transcription Factors

The regulation of transcription factor genes is regulated by cell development, the external environment, and other factors, and the activity of transcription factors is also regulated by many factors (Weidemüller et al. 2021).

5.1 Post-translation Modification

The activity of transcriptional factors is regulated greatly by post-translational modification. In addition to regulating the nuclear import of transcription factors, the phosphorylation modification of translated proteins can alter the activity of transcription factors or their capacity to bind to DNA (Zhang et al. 2019). For instance, purified casein kinase II (CKII)-catalyzed phosphorylation and the nuclear extract of Arabidopsis thaliana can enhance the DNA binding capacity of GBF1 transcription factors in Arabidopsis thaliana (Dorone 2020). Phosphorylation following translation also controlled the activity of the O _ 2 (OPAQUE-2) transcription factor, which controls the expression of the gliadin gene in maize seeds. It was found that eight amino acid sites in O2 could be phosphorylated by CKII. Six of these loci were located in the transcriptional activation region. SDS-PAGE electrophoresis also showed that there were many phosphorylation homologues (phosphorylated isoform) in O2 (Channaveerappa et al. 2019). Among them, only non-phosphorylation homologues (non-phosphorylated isoform) and $1 \leq 2$ amino acid residues phosphorylation of low phosphorylation homologues (hypophosphorylated isoform), and DNA binding to its high phosphorylation homologues (hyperphosphorylated isoform) cannot bind to DNA, but must be treated with phosphatase before they can bind to DNA (Gil and Paola 2019). The proportion of different phosphorylation forms still changed day and night. During the day, the O2 content of low phosphorylation homologues was high, the storage protein gene expression was active, the nocturnal high phosphorylation homomorphism content increased, the activation ability to gene transcription decreased, and the storage protein synthesis decreased (Richardson and Ryan 2023; Wani and Vinay 2020). Organisms can ensure the regular and quantitative synthesis of proteins through the diurnal variation of this O2 phosphorylation homologue (Tan et al. 2020).

5.2 Nuclear Localization

The transcription factors play a role in the nucleus; it is very important to regulate the process of their entry into the nucleus (Janota et al. 2020). The process of transcription factor entering nucleus is regulated by external stimulation, cell cycle, development stage and so on (Theilgaard-Mönch et al. 2022; Wang 2021). Although nuclear pores can allow 40-60 ku proteins to spread through, this study have shown that the process of proteins passing through nuclear pores is mostly active.

The process of transcription factors entering the nucleus is carried out through one to several nuclear localization signal regions (Liu et al. 2022). NLS first binds to a nucleophilic protein located at the nuclear pore by interacting with the receptor protein NBP (NLS-binding protein), and then moves through the nuclear passage with the help of the transfer function of the nucleophilic protein (Jardine 2020). The transcription factors without NLS region enter the nucleus by interacting with the transcription factors with NLS region (Lu et al. 2021). The process of transcription factor entry is also affected by post-translation phosphorylation modification and intermolecular interaction (Chen et al. 2020). The phosphorylation and dephosphorylation of NLS and its side sequences is an important way to regulate the movement of transcriptional regulator across the nuclear envelope (Muñoz-Díaz and Julio 2022).

5.3 Dimerization Effect

Transcription factors are crucial for plant development and growth by modulating the expression of diverse functional genes (Khan et al. 2018). They interact with other proteins through oligomerization regions, affecting their ability to bind to DNA, binding mode, and location in cells. Plant transcriptional regulators, including O2, PvALF, VP1, and EMBP1, contain bZIP domains and can be regulated by forming heterodimers with other proteins (Niñoles et al. 2022). Three bZIP proteins (OsZIPs) were isolated from rice, which may enhance the association of EMBP1 to the EM promoter, while Histone H 1 has shown to increase the EMBP-1 interaction with its specific promoter binding site (Niñoles et al. 2022).

Plants respond to various environmental, tissue, development signals during growth and development, requiring precise regulation of gene expression (Scheres and Wim 2017). The expression of the seed storage protein gene is specifically regulated by the tissue and development stage, and transcriptional regulation is essential for agricultural production. Plants also respond to environmental factors like drought, high salt, hormones, diseases, and cell development in vivo, activating RNA polymerase II transcriptional complex through trans-acting factors (Forni, and Bernard 2017). These factors activate the transcription and expression of genes, affecting physiological and biochemical aspects of the appropriate regulatory response.

Plants contain a diverse range of transcription factors, with 15% of genes encoded or may encode transcription factors. In past studies, over 30 bZIP transcription factors and over 40 AP2 / EREBP transcription factors have been isolated in Arabidopsis (Ma et al. 2024). Some transcripts are capable of regulating multiple genes, affecting the progress of diverse physiological and biochemical mechanisms (Li et al. 2018).

5.4 BZIP Transcription Factors

BZIP transcription factors is a type of transcriptional regulators which is found in animals, plants and microorganisms (Aslam et al. 2019). Plants include corn O2, Arabidopsis PosF21, wheat and rice HBP-1 and so on. The common characteristics of bZIP transcription factors are as follows: (I) the basic domain that binds to the specific DNA sequence; (ii) the Leucine zipper region involved in polymerization is closely related to the alkaline region; (iii) the N-terminal of the transcription factor contains acid activation region: (iv) binds to DNA in the form of dimer, and the alkaline region of peptide chain N-terminal binds directly to DNA. The core sequence of bZIP transcription factor recognition is ACGT, such as CACGTG (G box), GACGTC (C box), TACGTA (A box) and so on (Han et al. 2023). Few genes are triggered by light or abscisic acid (ABA) contain these components. G box elements are typically found in genes activated by ABA, auxin, jasmonic acid and salicylic acid, and also the most frequent cis acting elements in photoinduced genes. Arabidopsis HY5, GBF2 and other bZIP transcripts can precisely attach to G box elements and trigger the transcription of photoinduced genes (Stafen et al. 2022).

O2 transcription factor is not only a specific transcription factor in plant endosperm, but also an important regulator of seed storage protein synthesis (Cao et al. 2022). Its molecular mass is 47 ku, and comprises of all the three characteristics region of bZIP transcription factors, and the transcriptional activation region is located among amino acid residues 41-91 at the N-terminal. O2 not only control the developmental and tissue specific expression of crucial rice storage proteins such as a-gliadin, but also regulated the expression of cyPPDKI gene in corn b $\leq$ 32, which encodes a ribosomal inactivated egg (ribosome-inactivating protein) homologue, while cyPPDK1 encodes pyruvate orthophosphate dikinase (cytoplasmic pyruvate orthophosphate dikinase) in cytoplasm. Recently, transcription factors similar to corn O2 have been found in Sorghum vulgare, wheat, Coix (Co*ix lacryma-jobi* L.) and other plants (Wang et al. 2023). Moreover, some experiments have shown that there are transcription factors similar to O2 in rice, so it is speculated that O2 transcription factors may widely exist in cereal crops to monitor the expression of storage protein genes (J. Wang, Chen, Zhang, Meng, and Wei 2020). While, O2 can facilitates the expression of various genes at the same time, its attaching sites are different in different gene promoter regions. For example, in 22 ku gliadin gene promoter, it binds to TCCACGTAGA site, the O2 binding site in rice GluB gene promoter is GCN4 element (TGAGTCA), while in corn bo32 gene promoter region, multiple O2 binding sites are found (Kaur 2020). The results show that O2 can not only act on its cis-acting elements in the form of homodimer, but also bind to other proteins in rice (such as PBF-1, OHP1, etc.) in the form of heterodimer. O2 can be phosphorylated by nucleic acid extract of rice endosperm or recombinant of Arabidopsis thaliana CK II, and there are many phosphorylation sites in the main activation region of O2 (Rahman, 2019; Yang et al. 2022). Therefore, it is suggested that phosphorylation role is important in the regulation of O2 activity.

5.5 AP2/EREBP Transcription Factors

The research aims to study and explain the structure and functional activities of plant transcription factors, namely, the AP2/EREBP family, in Arabidopsis thaliana and tobacco (*Nicotina tabacum*) (Xie et al. 2019). Regulation of plant cell cycles, growth, development and reaction to the environmental stress are some of the critical processes that are orchestrated by these transcription factors. They are divided into two separate subfamilies: AP2 (APETALA2) and EREBP (ethylene-responsive element binding protein). AP2 transcription factors contain two AP2/EREBP domains, while EREBP transcription factors have one AP2/EREBP domain, which regulates plant molecular response to hormones, pathogens, low temperature, dry early, and high salt (Feng et al. 2020; Ku, Sintaha, et al. 2018; W. Xie et al. 2022).

The N-terminal region of AP2/EREBP domain is alkaline hydrophilic and Consists of three β-folds, which play a key role in identifying cis-acting elements (Ma et al. 2024; Zhou et al. 2024). The C-terminal domain also has an Amphiphilic α helix, which is expected to mediate protein-protein interaction with other proteins as transcription factors, as well as direct DNA binding.

The members of the EREBP transcription factors, tobacco EREBP1-4, tomato Pti4-6, Arabidopsis RAV1-2, AtEBP, AtERF1, DREB1A-C, and DREB2A-B have been found to be related to cell development, hormone, disease resistance, low temperature and drought, and high salt (Zuo et al. 2023). Arabidopsis thaliana factor, the AtERF is specifically bound to the GCC-box binding domain (GCC-box binding domain, GBD), which is combined with the large trench of its target sequence GCC-box by forming three reverse β-lamellae (C.-Y. Chen, Lin, Chen, and Cheng 2020).

Arabidopsis transcriptional factors such as the DREB1A and DREB2A modulate the transcription of low temperature tolerance, drought tolerance and high salinity genes (Meena et al. 2022; Zhang and Xia 2023). Their promoter regions contain dehydration-responsive element (DRE), making DREB1A overexpressed in transgenic Arabidopsis thaliana. Amino acid sequence analysis showed that both DREB transcription factors contained a C-terminal acidic transcriptional activation region and an N-terminal alkaline nuclear localization signal region (Yadav et al. 2023; Yu et al. 2024).

5.6 Activation and Inhibition of VP1 (Viviparous.1) Transcription Factors

The study reveals that a transcription factor, such as the corn transcription factor, can both activate and inhibit transcription (Kimotho, Baillo, and Zhang 2019). VP1 is a typical transcription factor that can activate wheat EM gene and corn C1 gene, as well as suppress the activity of the α-amylase gene in barley (Matilla 2024). The activation mechanism of EM gene and C1 gene by VP1 varies. C1 is a transcription factor that regulates several enzyme genes involved in anthocyanin production, and its expression can be modulated independently by VP1, by the hormone ABA, or by exposure to the light (Kim et al. 2023). VP1 can activate EM gene transcription through Sph-like factor or RY factor, mainly by acting on another protein EMBP1 to activate EM gene transcription. EMBPI, a bZIP transcription factor, that specifically interact with to two G-box- like motifs in the promoter of the EM gene. Though the B2 domain of VP1 has a weak non-specific DNA interaction ability, but it can greatly modulate the attachment of EMBP to Emla and Emlb in the

target G-box motif. While the protein GF14 present in rice indicates the possibility of its involvement in the EM gene expression activation (Huang et al. 2022). VP1 also suppresses the transcription of the α-amylase gene when seed germination accours, indicating that the inhibition and activation mechanism of VP1 on transcription are different and carried out independently (Zheng et al. 2019).

6. Conclusions

Transcription factors in a plant act as an activator or repressor of inducible gene expression by interacting with DNA and other associated proteins. The specificity of a particular cis-acting sequence to a transcription factor is determined by its DNA-binding domain, whereas transcription is either promoted or suppressed by the transcriptional regulatory domain. In addition, its activity is also regulated by nuclear localization and oligomerization. Over the past decade, molecular biology has increasingly started to focus on cis-regulatory elements in the promoters, transcription factors, and the resulting processes rather than functional genes. The identification and characterization of the structure and function of transcription factors is one of the major contents to elucidate the regulation mechanism of gene expression under varied conditions. Revealing the precise mechanism of the interaction between transcription factors and their connection with DNA, we may artificially control the expression of specific genes, so that plant gene transformation can gain favorable outcomes.

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