Molecular mechanisms for the plant promoter efficiency correction

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Key message. The mutations determining functional differences between two highly identical promoters from the chickweed plant were identified. These mutations lead to the formation of hypoosmolarity-responsive cis-element within core promoter and its specific positioning relative to the other cis-elements.

Keywords: gene expression, synthetic promoters, cis-elements, genetic engineering of plants

At present, in genetic engineering there is a need for effective promoters for precise and coordinated expression of recombinant genes. This need may be satisfied with the synthetic promoters with predictable properties. Construction of such promoters, however, is restricted by shortage of knowledge on molecular functioning of plant core promoters and absence of reliable methodology of their improvement. Previously, we found that in transient expression in *Nicotiana benthamiana* plants the minimal promoter pro-SmAMP1 (having size 158 bp) from the chickweed plant (*Stellaria media* L.) was up to two times stronger than the other promoter pro-SmAMP2 (having size 161 bp) from the same plant. On the other hand, in the nutrient medium with kanamycin antibiotic pro-SmAMP2 promoter allowed to obtain up to thrice more transgenic shoots of tobacco (*Nicotiana tabacum* L.) that evidenced more constitutive expression pattern of selective gene *nptII* under its control. Meanwhile, the nucleotide sequences of these two promoters are identical by 94% and differ as little as by ten-point mutations outside the canonical cis-elements of core region, viz. TATA box, G-box, CAAT box и transcription start site (TSS). The purpose of present study was the investigation of molecular mechanisms determining the difference of properties of the two highly identical promoters. Employing site-directed mutagenesis, we created ten new synthetic variants of minimal promoter pro-SmAMP2 with substitutions or deletions of polymorphic nucleotides. New variants were assayed by transient expression of the reporter *uidA* gene in *N. benthamiana* plants and by efficiency of expression of selective *nptII* gene in transformed cells of *N. tabacum* at the nutrient medium with kanamycin. It was established that in transient expression the significant superiority of pro-SmAMP1 over pro-SmAMP2 is caused by simultaneous presence of five-point mutations between TATA box and TSS. Of these five mutations two-point substitutions result in arising of PRE cis-element (hypoosmolarity-responsive element) which is known as positive regulator from proximal region of proline dehydrogenase gene promoter in *Arabidopsis* [1]. Two-point deletions are necessary for precise positioning of PRE relative to TATA box and TSS. One more nucleotide substitution probably provides the specific nucleotide context for correct functioning of PRE. Stable (though not statistically significant) positive effect with respect to the strength of the promoters was also provided by three mutations in 5'-untranslated region of *pro-SmAMP1* gene (within *pro-SmAMP1* promoter). By the selection of transgenic tobacco cells at the kanamycin-containing nutrient medium we established that the constitutive pattern of *nptII* gene expression, though insignificantly reduced by the presence of functional PRE element in the pro-SmAMP2 sequence, still remains significantly higher than in case of using pro-SmAMP1. In addition, the pro-SmAMP2 constitutive level was also reduced by another point substitution near the G-box leading to the appearance of the CAANNNNATC motif which is able to change the promoter efficiency under the control of a circadian clock [2]. As a result of experiments the new synthetic modification of pro-SmAMP2 with PRE element in the necessary position was selected. The modification is not inferior to pro-SmAMP1 in transient expression and has the constitutive level comparable to pro-SmAMP2. Thus, the presence and the precise positioning of PRE cis-element between TATA box and TSS of core plant promoter is able to change its properties, including strength and constitutive, significantly. The found combination and mutual positioning of cis-elements may be used for the property's correction of the other plant promoters for sake of coordination of their work.

The reported study was funded by RFBR according to the research project № 19-016-00067.

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