

AraC/AvrXa10- a New Transactivator for Plant Functional Genomics

Tran Ngoc Thach¹, Frances Shannon², and Andrzej Kilian³

¹Cuu Long Delta Rice Research Institute, Omon, Cantho, Vietnam. ²John Curtin School of Medical Research, Australian National University. ³Center for the Application of Molecular Biology to International Agriculture (CAMBIA), Australia

Transactivators are proteins which recognize and bind to a specific DNA sequence within a promoter and enhance the expression of genes nearby through a process of transactivation. Thanks to this ability, transactivators have been used as an alternative tool for studying gene function in plants along with other molecular mutagenesis methods such as T-DNA insertion, *Ac/Ds* transposon, retro-transposon, RNAi.

Transactivator-based method in rice, known as *transgenomics* (Koerniati *et al.*, 2002), is currently is based on transactivator GAL4/VP16. Although it has been widely used as a transactivator in *Drosophila* and other organisms, its application in plants however, may be obstructed by problems such as the methylation of its 17 nucleotides long DNA recognition sequence and the public acceptance of its origins (viral). Furthermore, the results of observation of performance of this protein in rice at CAMBIA indicated that its DNA recognition sequence was not specific because of being recognized and bound by endogenous proteins. Thus, it necessitates the development of new transactivators for plants.

Transactivators are normally modular in structure and consist of at least two domains; a DNA binding domain and an activation domain. In this study, the strategy for developing new transactivators relied on the replacement of these two domains among natural occurring candidates. Modular plasmids and workable systems in rice and tobacco were developed for testing of candidates for these two domains based on their ability to express a reporter gene under the control of a corresponding DNA recognition sequence. A newly developed transactivator, AraC/AvrXa10, was selected from among five candidates for the DNA binding domain (*Ac* transposase from maize; AraC, Cro (λ) and GusR from *E. coli*, and I-PpoI from slime mold) and four candidates for the activation domain (AvrXa10 from *Xanthomonas oryzae* *pv. oryzae*, Dof1 and Viviparous1 from maize, and RisBz1 from rice). AraC/AvrXa10 contains no potential site for methylation in its DNA recognition sequence and has better activity and more specificity than GAL4/VP16.

This new transactivator uses “public domain” components and can be provided to users without “Freedom-to-Operate” restriction, which currently limits the use of the patented GAL4/VP16. Thus, it was concluded, that AraC/AvrXa10 is working well and provides an alternative transactivator in transgenomics approach for gene function study and plant improvement.