Fusarium graminearumのTRI6 zinc finger domainの核移行と安定性

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Nuclear localization and relative stability of the zinc finger domain of TRI6 trichothecene regulator

Keywords
Cys₂His₂ zinc finger protein; enhanced green fluorescence protein (EGFP); Fusarium graminearum; mycotoxin; transcription factor; trichothecene biosynthesis

We examined the localization of the full-length protein and zinc finger domain of the trichothecene regulator, TRI6. When fused to an enhanced green fluorescent protein (EGFP) gene, nuclear accumulation of the zinc finger domain, but not the full-length protein, was observed.

Fusarium graminearum produces trichothecene mycotoxins in infected cereal grains and poses serious threats to food safety. Trichothecene production is regulated by a Cys₂His₂ zinc finger transcription factor, TRI6, which recognizes a consensus sequence of YNAGGCC. TRI6 regulates not only trichothecene (Tri) genes, but also mevalonate pathway and other genes. Localization analyses with reporters such as enhanced green fluorescent protein (EGFP) are often used to unveil the regulatory mechanism of transcription factors. However, previous attempts to detect a GFP signal from the TRI6 fusion proteins were unsuccessful.

In our previous study, a strong TEF1α promoter was connected to the TRI6:EGFP fusion gene (PrEFa_Tri6:EGFP cassette) in a homologous integration vector and targeted downstream of the Tri102 locus in the genome of the TRI6 null mutant (parental strain; JCM 9873). Regardless of constitutive expression of TRI6:EGFP in the resulting transformant (strain 3#-1) cultured in 100 ml of YS_60 medium in a 300 ml Erlenmeyer flask (weakly-inducing culture condition), the fusion transcription factor did not provoke the biosynthesis of 15-acetyldeoxynivalenol (15-ADON) even in 100 ml of YS_60 culture medium (Fig. 1A; upper panel). Transformation was also conducted using another ectopic integration vector, pPrEFa_Tri6_ZF::EGFP, which contains only the zinc finger domain of TRI6 (Supplementary Fig. 1). One of the selected PrEFa_Tri6_ZF::EGFP transformants accumulated a significant amount of mRNA when cultured in both trichothecene-non-inducing YG [0.5% (w/v) Difco™ yeast extract, 2% (w/v) glucose] and trichothecene-inducing YS_60 media, as did the pPrEFa_Tri6::EGFP transformant (Supplementary Fig. 2; see Supplementary Fig. 3 for experimental detail).

Western blot analyses with an anti-EGFP antibody demonstrated that both fusion proteins were present in their intact forms, but with different degrees of fragmentation depending on the medium used for culturing (Fig. 1A; lower panel; see Supplementary Fig. 3 for experimental detail). A previous immunoprecipitation study failed to detect the full-length TRI6 C-terminally fused to GFP in the total protein extract of the fusion gene overexpressor, also suggesting instability of TRI6 within F. graminearum cells. The use of a different protein extraction reagent (TRizol® reagent [Fischer Thermo Scientific] in this study versus non-denaturing buffer in the previous study) may account for the successful detection of TRI6:EGFP in this study.

When cultured in trichothecene-non-inducing YG medium, a strong western blot signal corresponding to the size of the full-length TRI6:EGFP was detected. This was accompanied by a much more intense signal of the 31.9 kDa fragment (Fig. 1A; marked by an asterisk). TRI6:ZF::EGFP was much more resistant to proteolytic cleavage, as evidenced by the overwhelming proportion of the intact fusion protein. Interestingly, the same 31.9 kDa fragment was detected on the blot of TRI6:ZF::EGFP (Fig. 1A), suggesting the presence of a common internal cleavage site within the zinc finger domain (Fig. 1B; marked by an arrow). The signal intensity decreased significantly when the transformants were cultured in the trichothecene-inducing YS_60 medium. Indeed, the intensity of the full-length TRI6:EGFP band detected from the 100 ml YS_60 culture was much less than that with YG culture without 15-ADON production (Fig. 1A). These results suggest that the quality of TRI6 structure, but not the quantity of TRI6, within fungal cells is critical for the induction of trichothecene biosynthesis (Fig. 1A).

Despite significant improvement in the production of trichothecene by TRI6:EGFP in comparison with our
previous constitutive expresser strain 3#-14, only marginal fluorescence was observed in the nucleus under an epifluorescence microscope (Fig. 1C). In contrast, nuclear localization of the TRI6 zinc finger domain was evident as revealed by accumulation of marked green fluorescence that merged with 4,6-diamidino-2-phenylindole (DAPI) fluorescence (Fig. 1C). Thus, the putative nuclear localization signal predicted to exist within the zinc finger domain (Fig. 1B) may function to import TRI6 into the nucleus. Consistent with the results of the western blot analyses, the intensity of the TRI6_ZF::EGFP fluorescence was more prominent with the YG culture.

In conclusion, the results of the current study demonstrated that the instability of TRI6 fused to EGFP was significantly abrogated by truncating the N-terminus portion that constitutes the transcription factor activation domain. The presence of the activation domain appears to destabilize TRI6 by stimulating proteolytic cleavage within the zinc finger domain.

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Supplementary Materials

Supplementary materials may be found in the online version of this article:
Supplementary Fig. 1 Construction of ectopic integration vector pPrEFla_Tri6::EGFP and pPrEFla_Tri6_ZF::EGFP.
Supplementary Fig. 2 Northern blot analysis of the transformants.
Supplementary Fig. 3 Experimental flow chart.
Supplementary Fig. 4 Structure and sequence of pAn-Tef-hph.

References

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トリコトセン生合成制御因子TRI6の全長およびzinc finger domainの局在解析を試みた。緑色蛍光タンパク質EGFPとの融合実験でTRI6 zinc finger domainは核への蓄積が確認されたがTRI6全長は局在解析が困難であった。

キーワード：転写因子：トリコトセン生合成：マイコトキシン（かび毒）：緑色蛍光タンパク質：Cys2His2型Znフィンガータンパク質：
Fusarium graminearum