LETTER TO THE EDITOR

REPLY: The BIF Domain is structurally and functionally distinct from other types of ACT-like domains

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We welcome the discussion on the BIF/ACT-like domain in plant bHLH proteins and hope this will help to clarify their functions and structures distinct from those of other types of ACT-like proteins. The bHLH proteins are found in major eukaryotic groups and play important roles in many plant developmental processes and environmental responses (Feller et al., 2006; Pires and Dolan, 2010). The model plant \textit{Arabidopsis thaliana} has 159 bHLH members; in particular, DYSFUNCTIONAL TAPETUM1 (DYT1) is an early regulator of normal tapetum and pollen development (Zhang et al., 2006; Feng et al., 2012; Zhu et al., 2015).

\textit{DYT1} expression and function are precisely regulated by a set of interactions: on one hand, protein products of DYT1 downstream genes \textit{bHLH010/089/091} interact and form a feed-forward loop with DYT1 to control the proper expression of shared downstream anther genes (Zhu et al., 2015); on the other hand, bHLH010/bHLH089/bHLH091 facilitates the stage-dependent nuclear-specific localization of DYT1 in a positive feed-back regulatory loop.
enhancing DYT1 function (Cui et al., 2016).

In addition to the bHLH domain, DYT1 and its homologs share a second domain close to its C-terminus with a predicted secondary structure of $\beta\alpha\beta\alpha\beta\alpha$ ($\alpha$ for an $\alpha$ helix and $\beta$ for a $\beta$ strand) (Figure 1). This C-terminal domain of DYT1 is required for the interaction with and the nuclear localization by bHLH010/bHLH089/bHLH091, and transcriptional activation activity and in vivo function of DYT1 (Cui et al., 2016); this domain in DYT1 and homologs was named the BIF domain for bHLH protein interaction and function and also found in over one third of Arabidopsis bHLH proteins (Cui et al., 2016).

Previously, a domain with similar predicted $\beta\beta\alpha\beta\beta\alpha$ structure was found in the maize bHLH transcription factor R and some additional maize and Arabidopsis proteins and was referred to as an ACT-like domain (Feller et al., 2006) or ACT-domain (Kong et al., 2012). However, there are substantial functional and structural differences (see below) between the C-terminal BIF/ACT-like domain of plant bHLH proteins (Feller et al., 2006; Kong et al., 2012; Cui et al., 2016) and the original ACT domain and previously reported ACT-like domain in other proteins (Chipman and Shaanan, 2001; Siltberg-Liberles and Martinez, 2009; Lang et al., 2014).

The ACT domain and previously reported ACT-like domains are found in functionally diverse proteins. The ACT domain, which was named after the three founding members Aspartate kinase, Chorismate mutase and TyrA (prephenate dehydrogenase), is found in a variety of enzymes involved in amino acid and purine metabolism and is known to bind amino acids in negative feedback-regulation of such enzymes (Schuller et al., 1995; Aravind and Koonin, 1999; Grant et al., 1999; Chipman and Shaanan, 2001; Grant et al., 2001). The ACT domain was
thought to have evolved independently from the catalytic domain of amino acid metabolic enzymes (Hsieh and Goodman, 2002). The ACT domain is found in Arabidopsis amino acid metabolic enzymes such as Aspartate kinase, 3-phosphoglycerate dehydrogenase, and acetohydroxyacid synthase regulatory subunit, with suggested regulatory functions (Chipman and Shaanan, 2001; Lee and Duggleby, 2001; Hsieh and Goodman, 2002; Mas-Droux et al., 2006). In addition, the ACT domain is present in ACT domain repeat (ACR) proteins in plants, with multiple members in both Arabidopsis and Oryza sativa including the OsACR7 and OsACR9, which likely act as the glutamine signal sensors (Hsieh and Goodman, 2002; Hayakawa et al., 2006; Liu, 2006; Kudo et al., 2008; Sung et al., 2011; Takabayashi et al., 2016)). The ACT and ACT-like domains in the regulatory domains of E. coli threonine deaminase have similar βαββαβ secondary structures (Schuller et al., 1995; Aravind and Koonin, 1999; Chipman and Shaanan, 2001; Lang et al., 2014). Another domain was considered to be an ACT-like domain because of a predicted βαββαβ secondary structure similar to that of ACT-proteins, but it was named RAM, for regulation of amino acid metabolism (Ettema et al., 2002).

By contrast, the BIF/ACT-like domains in plant bHLH proteins are predicted to share a ββαββα structure, which differs from the βαββαβ structure in the position of 4 out of 6 motifs (Feller et al., 2006; Kong et al., 2012; Cui et al., 2016). At the sequence level, there are very low levels of amino acid sequence identities, with only 4.6% between the ACT domain of 3-phosphoglycerate dehydrogenase and the ACT-like domain of threonine deaminase, and only 6.6% between the ACT domain and the ACT-like RAM domain; these percentages are not
statistically different from that between two random sequences. The levels of amino acid sequence identities between the ACT-like domains in plant bHLH proteins and the ACT domains are also very low, such that the DYT1 and R proteins are not found in the pfam database as having an ACT-like domain.

In short, the “ACT-like domain” is a general term for various domains that show similar structural similarities to the ACT domain, whereas the “BIF domain” is a more specific term referring to those in DYT1 and other plant bHLH proteins with distinct function in dimerization and nuclear localization of interacting proteins and the ββαββα topology. We consider the domain in plant bHLH proteins sufficiently different from others to warrant a distinct name.

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Figure 1. The Location and Position (with $p$-value) of DYT1 Orthologs-Conserved Motifs in DYT1 sequence.

A schematic diagram representing the $\alpha$-helices (magenta box) and $\beta$-sheets (green arrows) in DYT1. (B) A block diagram showing the 8 best non-overlapping tiling of motifs from the analysis of the DYT1 amino acid sequences and those of 54 DYT1 orthologs; the conserved motifs (M1~M8) are indicated as boxes with different colors. The length of each motif is relative to the length of DYT1 sequence, and the height of each box provides an indication of the significance of the match. (C) The location and amino acid sequences of each motif. Position $p$-value (the probability of a single random subsequence of the length of the motif scoring at least as well as the observed match) is indicated above each motif. The secondary structure of DYT1 was predicted in website: https://www.predictprotein.org; and the motif analysis was performed using MEME software (Bailey and Elkan, 1994).


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