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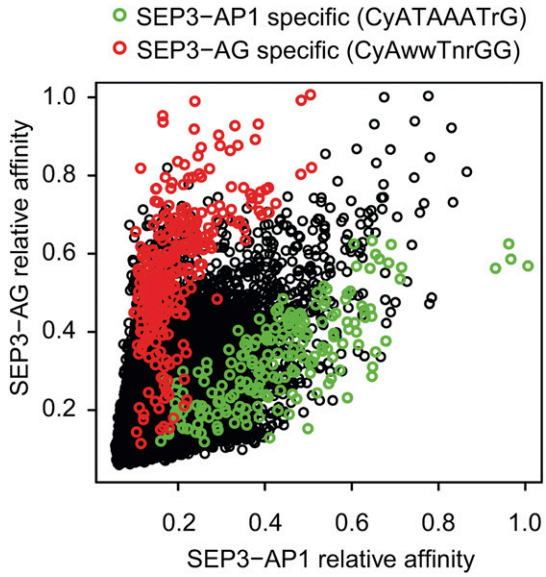
IN BRIEF

Intrafamily Protein Interactions Contribute to DNA Localization OPEN

Floral organ formation requires the expression of thousands of genes in specific spatial and temporal patterns. Several transcription factors bearing a MADS DNA binding domain specify the identity of floral organs, as summarized in the ABCE model of flower development (Goto et al., 2001), and recent genome-wide analyses have provided detailed insights into how they regulate gene expression to orchestrate floral organ formation. Despite these efforts, it remains unclear precisely how these transcription factors select their regulatory targets.

Smaczniak et al. (2017) performed a series of experiments aimed at understanding this process more thoroughly. Using the high-throughput in vitro approach SELEX-seq (systematic evolution of ligands by exponential enrichment followed by high-throughput DNA sequencing), the authors characterized the DNA binding preferences of three MADS domain-containing transcription factors that are required for floral organ formation: APETALA1 (AP1), SEPALLATA3 (SEP3), and AGAMOUS (AG). These transcription factors belong to the same family of proteins, but their functions are quite different: AP1 controls the development of nonreproductive floral organs (sepals and petals), AG controls the formation of reproductive organs (stamens and carpels), while both proteins require SEP3 as a cofactor. The authors show that when AP1 proteins interact with each other to form AP1-AP1 homodimers, they bind to different DNA sequences than AP1-SEP3 heterodimers. Similarly, AG-AG homodimers bind to different DNA sequences than AG-SEP3 heterodimers. In addition, the degree of DNA bending intrinsic to these sequences influences their interactions with heterodimers.

A recurring difficulty when interpreting these protein-DNA interactions is that they poorly relate to activity of the associated gene. That is, when a bona fide protein-DNA interaction is disrupted, often the activity of the associated gene does not change. By accounting for the heterodimerization of these transcription factors, Smaczniak et al. report a significant improvement of these correlations. From the SELEX-seq data, the authors identified sequences with high affinity to either AP1-SEP3 or AG-SEP3 complexes (see figure). They then compared these sequences with previously generated ChIP-seq data for AP1, SEP3, and AG (Ó'Maoiléidigh et al., 2013; Pajoro et al., 2014) and selected bound sequences that were more similar to SELEX-seq-derived AP1-SEP3- or AG-SEP3-specific sequences (as defined by their different consensus sequences; see figure). Next, they compared the genes associated with AP1-SEP3 or AG-SEP3 binding specificity with organ-specific gene activity data. They found that genes associated with sequences predominately selected by AP1-SEP3 complexes tend to be expressed in the sepals and petals, whereas genes associated with sequences predominately selected by AG-SEP3 are more likely to be expressed in stamens and carpels.



DNA specificity plot comparing the relative binding affinities of SELEX-seq sequences selected by SEP3-AG (*y* axis) and SEP3-AP1 (*x* axis). Each point represents a unique sequence; black points represent all sequences. SEP3-AP1-specific sequences are in green, and SEP3-AG-specific sequences are in red. Consensus sequences derived from the SELEX-seq experiments are indicated at the top of the figure. (*Reprinted from Smaczniak et al. [2017], Figure 3A.*)

The MADS domain protein family have been intensively studied for several decades, yet we continue to garner fundamental insights into biology by studying them. Over 20 years ago it was suggested that the selection of specific DNA sites by different MADS domain proteins was insufficient to explain how they specify floral organs (Riechmann and Meyerowitz, 1997), and this has been supported by more recent in vivo data (Ó'Maoiléidigh et al., 2013). Smaczniak et al. provide a nuanced interpretation of these data, which highlights the importance of heteromeric complex formation. Although the correlation between the authors' in vitro data and previously published in vivo data was significant, several hundred DNA sequences interact with these transcription factors in vivo, which, as acknowledged by the authors, were not explained by SEP3 heterodimerization. Future experiments will explore the reasons behind this, which may relate to interactions between MADS domain proteins and other classes of transcription factor.

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