

The floral homeotic protein SEPALLATA3 recognizes target DNA sequences by shape readout involving a conserved arginine residue in the MADS-domain.

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Abstract

SEPALLATA3 of *Arabidopsis thaliana* is a MADS-domain transcription factor (TF) and a key regulator of flower development. MADS-domain proteins bind to sequences termed 'CARG-boxes' [consensus 5'-CC(A/T)6 GG-3']. Because only a fraction of the CARG-boxes in the *Arabidopsis* genome are bound by SEPALLATA3, more elaborate principles have to be discovered to better understand which features turn CARG-boxes into genuine recognition sites. Here, we investigate to what extent the shape of the DNA is involved in a 'shape readout' that contributes to the binding of SEPALLATA3. We determined in vitro binding affinities of SEPALLATA3 to DNA probes that all contain the CARG-box motif, but differ in their predicted DNA shape. We found that binding affinity correlates well with a narrow minor groove of the DNA. Substitution of canonical bases with non-standard bases supports the hypothesis of minor groove shape readout by SEPALLATA3. Analysis of mutant SEPALLATA3 proteins further revealed that a highly conserved arginine residue, which is expected to contact the DNA minor groove, contributes significantly to the shape readout. Our studies show that the specific recognition of cis-regulatory elements by a plant MADS-domain TF, and by inference probably also of other TFs of this type, heavily depends on shape readout mechanisms.

Identifier

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