

Short communication

A ubiquitously expressed MADS-box gene from *Nicotiana tabacum*

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Received 30 November 1993; accepted in revised form 6 March 1994

Key words: tobacco, transcription factor; MADS box; ubiquitous expression

Abstract

Experiments in *Antirrhinum majus*, *Arabidopsis thaliana* and *Petunia hybrida* have demonstrated that putative transcription factors of the so-called MADS-box family play an important role in determining floral organ identity. Such regulatory genes are transiently expressed in small numbers of cells in the floral apex. Here we describe the isolation of a cDNA from *Nicotiana tabacum* coding for a MADS-box protein which is expressed in both the floral and vegetative organs of the plant.

The MADS-box is a DNA-binding domain consisting of 55 highly conserved amino acids. In plants it is found in a growing number of putative transcription factors. Most of these proteins are exclusively or predominantly expressed during floral organogenesis. In fact, the prevailing theory at present postulates that their precisely regulated expression is a key factor in the control of floral development [1, 7]. Two MADS-box genes have been described which are also expressed during vegetative development, viz. AGL3 in *Arabidopsis* and TM3 in tomato. Partial sequences of both genes have been published [4, 6]. The tomato TM3 gene is probably a 3'-truncated clone as the sequence terminates in a poly(A) tail without a preceding stop codon [6].

In order to study the role of MADS-box pro-

teins in vegetative development we decided to isolate the tobacco homologue of the tomato TM3 gene. Two oligonucleotide primers were synthesized, one identical to nucleotides 174 through 197 and the other complementary to nucleotides 439 through 458 of the published tomato TM3 sequence. These oligonucleotides were used to PCR-amplify a 285 bp fragment from a first-strand cDNA derived from *Lycopersicon esculentum* cv. Moneymaker vegetative shoot tip RNA. The cloned fragment, identical in sequence to that already published (data not shown), has been used in *in situ* hybridization experiments to show that TM3 is uniformly expressed in the vegetative tomato shoot apical meristem [2]. We then used the tomato TM3 clone to isolate a full-length cDNA clone from a *Nicotiana tabacum* SR1

| | | | | | |
|-----------------|-------------------|--------------------|-------------------|-------------------|-------------------|
| | 1 | | | | 50 |
| TobMADS1 | MVRGKTQMR | IENATSRQVT | FSKRRNGLLK | KAFELSVLCD | AEVGLVIFSP |
| TM3 | MVRGKTQMR | IENATSRQVT | FSKRRNGLLK | KAFELSVLCD | AEVGLIIFSP |
| Agl3 | MgRGKVelkR | IENkinRQVT | FaKRRNGLLK | KAYELSVLCD | AEIaLiIFSn |
| Agamous | sgRGKieikR | IENtTnRQVT | FcKRRNGLLK | KAYELSVLCD | AEVaLivFSS |
| Apetala3 | MaRGKiQikR | IENqTnRQVT | ySKRRNGLfK | KAhELtVLCD | ArVsiimFSS |
| | 51 | | | | 100 |
| TobMADS1 | RGKLYEFASS | SMQEIIERYK | RHTKDKVQPE | NQVGEQNLQH | MQHAAASLMK |
| TM3 | RGKLYEFASS | StQEIIrgnK | RHTKDrVQPE | NQaGpQyLQy | MQHeAAAnLMK |
| Agl3 | RGKLYEFqdy | lklksrveil | qHsqrhllgE | elsemdvnel | e.Hlerqvda |
| Agamous | RGrLYEysnn | SvkgTIERYK | kaisDnsn.t | gsVaEiNaQy | yQqesAkLrq |
| Apetala3 | snKLhEyiSp | n.ttkeivdl | yqTisdVdvw | atqyErmqet | krkilettrn |
| | 101 | | | | 150 |
| TobMADS1 | KIELLEESKR | KLLGEGQLQSC | SLVELQQIEK | QLERSVSTIR | ARKIQVFKEQ |
| TM3 | KIELLEtaKR | KfLGEGLQSC | tLqEvQQIEK | QLERSVgtTIR | ARKIQVFKEQ |
| Agl3 | slrqirs | | | | |
| Agamous | qIIsiqnSnR | qLmGETigSm | SpkELrnLEg | rLERSitrRIR | skKnellfse |
| Apetala3 | lrtqikqrlg | ecLdElDiqe | lrrledemEn | tfklvrerkf | kslgnqiett |
| | 151 | | | | 200 |
| TobMADS1 | IERLKEKEKI | LASENAILRE | KFGGLQQRQG | SSGEKEGEAL | CTESSEKSDV |
| TM3 | vERLK | | | | |
| Agamous | Iqymqkrevd | LhndnqILRa | Kiaenernnp | SislmpGgsn | yeqlmpppqt |
| Apetala3 | kkknKsqqdI | qknliheLel | raedphyglv | dnGgdydsvL | gyqiegsray |
| | 201 | | 220 | | |
| TobMADS1 | ETELFIGPPE | CRIRRPLQN | | | |
| Agamous | qsqpFdsrny | fqvaalqpNn | | | |
| Apetala3 | alrfhqnhhh | yypnhgLha | | | |

Fig. 1. Amino acid sequence comparison between TobMADS1 and selected other MADS-box proteins. The deduced amino acid sequence of TobMADS1 was aligned with tomato TM3 [6], and *Arabidopsis thaliana* Agl3 [4], Agamous [8] and Apetala3 [3] using the University of Wisconsin GCG sequence analysis software package as described [5]. Identities with TobMADS1 are in capital letters. Dots indicate gaps. TM3 and Agl3 are partial sequences.

Lambda ZapII library (Stratagene catalogue No. 936002). One positive plaque was identified amongst 3×10^5 plaques screened. The sequence of this clone, designated TobMADS1, has been deposited in the EMBL database. The cDNA contains a 5'-untranslated region of 202 nt and a 3' non-coding region of 216 nt including a poly(A) tail of 45 nt. Stop codons in the 5' end in all three reading frames clearly identify the indicated ATG as the start codon. The protein contains 219 amino acids including the initiator methionine, and it has a predicted molecular weight of 25 100 and a pI of 10.2. The 55 amino acid MADS-box domain is identical to the tomato TM3 domain except for one conservative V-to-I variation. In the remaining 100 amino acids over which TobMADS1 can be compared to the partial TM3 sequence the identity is 80%. Sequence comparison with other MADS-box containing plant genes shows high amino acid homology in the DNA-binding domain, and limited but probably signif-

icant homology outside the MADS-box (Fig. 1). It is worth noting that TobMADS1 is not par-

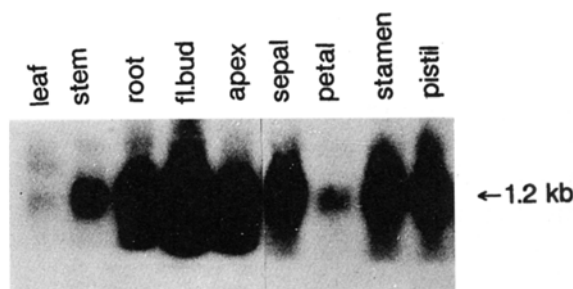


Fig. 2. Northern blot analysis of TobMADS1. Total RNA (10 μ g per lane) was isolated from tobacco organs, separated on glyoxal gels and analysed by northern blotting using an antisense riboprobe prepared from nucleotides 357 to 682 of TobMADS1, that is excluding the conserved MADS-box. 'Apex' is the vegetative shoot apex including leaf primordia up to ca. 3 mm in length. 'Floral buds' were at the 2-5 mm stage. Sepal, petal, stamen, pistil were at the stage when the flowers were 1 to 2.5 cm in length which represents developmental stages approximately to the time point of microspore mitosis.

ticularly closely related to the *Arabidopsis* vegetatively expressed AGL3 gene.

The high sequence homology with tomato TM3 makes it reasonable to suppose that TobMADS1 is its tobacco equivalent. We performed a northern blot analysis to test whether TobMADS1 is expressed in a pattern comparable to that reported for the tomato TM3 gene. As a probe a deletion derivative of TobMADS1 was used encompassing nt 357 through 682 (amino acids 52 through 160), that is, not including the conserved MADS-box domain. A database search revealed that at the DNA level this probe had no significant homology to any plant MADS-box gene other than the tomato TM3 gene. In tomato, a true diploid species, TM3 is a single-copy gene [6]; thus we anticipate two TobMADS1 genes in the closely related allotetraploid tobacco. As can be seen in Fig. 2, TobMADS1 is indeed expressed in all organs tested, both vegetative and floral. Expression in mature leaves and petals is comparatively low. However, a similar low expression was seen using a probe for translation initiation factor eIF-4A, which we routinely use as a constitutive control [5; data not shown].

We have isolated a full-length cDNA from tobacco coding for a ubiquitously expressed MADS-box-containing protein. In the future, we will attempt to alter its expression and thus study its function.

Acknowledgements

This work was supported in part by a grant from the Swiss National Science Foundation. We

thank Dr Andrew Fleming for critically reading the manuscript.

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