SEQUENCE UPDATE

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Molecular characterization of an anther-specific gene from tobacco shows sequence similarity to a tapetum-specific gene from tomato

Received: 14 September 1999 / Accepted: 24 September 1999

Abstract We have cloned and determined the DNA sequence of the cDNA of ntGRP15. The cDNA ntGRP15 represents an anther-specific, developmentally regulated gene from Nicotiana tabacum that encodes a glycinerich protein. Northern analysis shows that the gene is specifically expressed in anthers and is stringently regulated during anther development. It appears only in anthers at the meiosis to free microspore stages of development. The encoded protein is small (12.2 kDa), has a 31% glycine content and contains a putative signal sequence. By both nucleotide and amino acid sequence alignment, the gene shows high sequence similarity to a gene previously isolated from Lycopersicon esculentum, namely, TomA92b9. High glycine content, presence of a signal sequence and similarity to the tomato *TomA92b9* gene suggests the protein functions as a structural cell wall protein, possibly involved in pollen exine forma-

Key words Glycine-rich · Tobacco · Tomato Pollen development · Signal sequence

Pollen formation is an elaborate process dependent on the accurate interplay of the developmental programs of sporophytic and gametophytic tissues in the anther. Several researchers have isolated and characterized antherspecific genes in attempts to understand the molecular

tural Experiment Station. The tobacco and tomato sequences are listed in GenBank under the following accession numbers: tobacco ntGRP15 U74325, tomato TomA92b9 Z21719.

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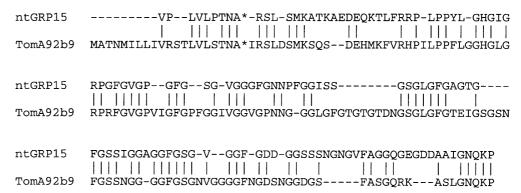
This is publication number 981210040 of the Minnesota Agricul-

mechanisms underlying pollen development (reviewed in Schrauwen et al. 1996). These genes can be grouped by function into those whose encoded proteins: (1) are thought to be lipid transfer proteins such as TomA5b (Aguirre and Smith 1993) and e2 (Foster et al. 1992); (2) are components of the exine wall including TA29 (Seurinck et al. 1990; Koltunow et al. 1990) and TomA92b9 (Chen et al. 1994); (3) are callases such as Tag1 (Bucciaglia and Smith 1994) and a6 (Hird et al. 1993) or (4) are proteins of unknown function such as tap1 and tap2 (Nacken et al. 1991a,b) and MSF14 (Wright et al. 1993). Our knowledge is as yet incomplete and the isolation and characterization of additional anther- and tapetum-specific genes will aid in the delineation of the molecular programs necessary for pollen development.

Here we report the isolation and characterizaton of an anther-specific gene from tobacco, ntGRP15. It is expressed only in anthers from meiosis to the early free microspore stages of development. It encodes a small, glycine-rich protein indicating a structural rather than enzymatic role in the anther (Schrauwen et al. 1996; Koltunow et al. 1990). The encoded protein contains an N-terminal signal peptide necessary for entrance into the secretory pathway (von Heijne 1985). There is high nucleotide sequence similarity to TomA92b9, a tomato gene specifically localized to the tapetum (Chen et al. 1994) and thought to be involved in pollen exine formation.

Nicotiana tabacum L. cv. Samsun plants were grown and the flowers developmentally staged as in Bucciaglia and Smith (1994). RNA was extracted from buds, pistils, anthers, and leaves according to Rochester et al. 1986. Poly(A)-enriched RNA was extracted and used to synthesize the first strand cDNA. PCR reactions were done using sense primer: 5'-CGGGATCCGGNGTNTGYTA-YGG-3' and antisense primer: 5'-CGGAATTCGGCC-ANCCNSWYTC-3' (where N=A,C,G or T; R=A or G; Y=C or T; S=C or G and W=A or T) as previously described (Bucciaglia and Smith 1994). The PCR reaction yielded a 245-bp fragment that was cloned into pBluescript SK(- and +) vectors (Stratagene). This cloned frag-

Fig. 1 Alignment of the amino acid sequences of ntGRP15 and TomA92b9. Vertical lines indicate amino acid identity between the two proteins. Dashes indicate gaps relative to the TomA92b9 protein sequence. The asterisk marks the putative signal sequence cleavage sites in both ntGRP15 and TomA92b9



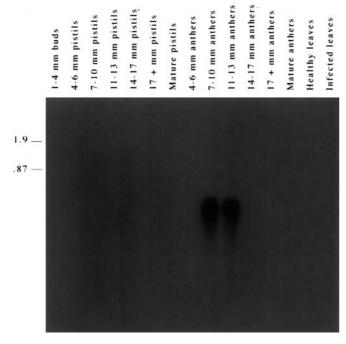


Fig. 2 Northern blot analysis of *ntGRP15* mRNA in tobacco. Each lane contains 20 μg of total RNA from: buds 1–4 mm in length; pistils or anthers from flowers 4–6, 7–10, 11–13, 14–17, and greater than 17 mm in length; mature pistils and mature anthers from flowers whose anthers were dehiscent; healthy leaves and leaves infected with TMV. The RNA was hybridized with the ³²P-labeled *ntGRP15* 245-bp PCR fragment and exposed to film for 7 days. RNA size standards in kb are shown to the *left*

ment was used as a probe in northern analysis and to isolate the 621-bp *ntGRP15* from a tobacco cDNA library.

Flowers greater than 4 mm in length were chosen whose corresponding anthers were developmentally premeiotic to dehiscent (4–6 mm: premeiotic, 7–10 mm: meiotic, 11–13 mm: early free microspore, 14–17: late free microspore, >17 mm: microspore mitosis, mature: dehiscent). Pistils were isolated from these staged flowers. Buds 1–4 mm in length, healthy leaves, and leaves infected with TMV (tobacco mosaic virus) were also sampled. Northern blot analysis was done as described in Bucciaglia and Smith (1994).

The nucleotide sequence of the *ntGRP15* cDNA isolated from the tobacco anther library was determined in

both directions (Sanger et al. 1977) using a Sequenase 2.0 sequencing kit (U.S. Biochemical). Computer analysis of the nucleotide and inferred amino acid sequences were performed using the GCG software package. These sequences were used in BLAST searches (NCBI BLAST – Altschul et al. 1990; Altschul et al. 1997) of nucleotide and protein databases.

A Blastn (nucleotide) search identified TomA92b9 cDNA as the nucleotide sequence with the highest sequence similarity to ntGRP15. An alignment of the two nucleotide sequences exhibits 58% nucleotide sequence identity (data not shown). The protein encoded by ntGRP15 is 129 amino acids in length and contains 31% glycine residues (Fig. 1). There is a putative signal cleavage site between residues 9 and 10 (von Heijne 1985). The mature protein has a mass of 11.4 kDa. Analysis of the protein sequence indicates no ER retention signals exist in the C-terminus of the putative peptide (Hesse et al. 1989; Denecke et al. 1992). A Blastp search of protein databases retrieved several very large glycinerich proteins whose sequences were aligned to ntGRP15 solely by high glycine content. Manual alignment of the 20 highest scoring proteins in the Blastp search found that TomA92b9 had the highest overall amino acid sequence identity, 49%.

It should be noted that the *ntGRP15* probe hybridizes to a fragment of approximately 700 nt. The length of the sequenced *ntGRP15* clone (621 bp), the alignment of *ntGRP15* with *TomA92b9* and the typical length of eukaryotic signal sequences (von Heijne 1985) indicate that the cloned *ntGRP15* is approximately a full length cDNA.

Northern analysis shows that *ntGRP15* RNA is expressed only in 7–10 mm anthers and 11–13 mm flowers (Fig. 2). Anthers in 7–10 mm tobacco flowers have a fully differentiated tapetum and a pollen sac which contains microspore mother cells undergoing meiosis. Callose walls form between the microspore mother cells in anthers of 6–7 mm flowers and microspores are in tetrads by the 10–11 mm stage. At 12–13 mm, the callose wall has been degraded and the microspores released into the locule. At stages 14 mm and beyond, the tapetum begins to shrink and then degenerates (Bucciaglia and Smith 1994; Koltunow et al. 1990). At the stages of anther development in which *ntGRP15* is expressed, the tapetum is actively producing materials that, when secreted, form the outer pollen wall.

The *ntGRP15* gene encodes a protein with features that allude to its function. The mature protein is small, 11.4 kDa, based on the cDNA sequence. Such a small protein is unlikely to have an enzymatic function (Schrauwen et al. 1996). Its high glycine content suggests that the protein may serve in a structural capacity. The presence of an Nterminal signal peptide indicates the protein is secreted. The lack of any ER retention signals (Hesse et al. 1989; Denecke et al. 1992) indicates that the protein is not retained in the ER. There is substantial nucleotide and amino acid similarity to TomA92b9, a gene whose expression is tapetal-specific. Both genes are expressed during meiosis through free microspore stages of anther development (Chen et al. 1994). The proteins are of similar small size (<15 kDa) and both contain a putative signal peptide with conserved residues surrounding the cleavage site (Fig. 1). The glycine content is similar between the two mature proteins (TomA92b9 27.9% and ntGRP15 31%). Based on the features of the *ntGRP15* protein and its similarity to TomA92b9, it seems possible that ntGRP15 encodes a pollen wall protein that is synthesized in the tapetum and secreted to the developing microspores.

Acknowledgements Thanks to Jeffrey Boore, Kevin Helfenbein and Alan Wolf for critical review of this manuscript.

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