

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

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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 glycine-rich 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 formation.

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 anther-specific genes in attempts to understand the molecular

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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 characterization 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 pBlue-script SK(- and +) vectors (Stratagene). This cloned frag-

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 N-terminal 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.

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References

- Aguirre PJ, Smith AG (1993) Molecular characterization of a gene encoding a cysteine-rich protein preferentially expressed in anthers of *Lycopersicon esculentum*. *Plant Mol Biol* 23: 477–487
- Altschul SF, Gish W, Miller W, Myers W, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Altschul SF, Madden TL, Schäffer A, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Bucciaglia PA, Smith AG (1994) Cloning and characterization of *Tag 1*, a tobacco anther β -1, 3-glucanase expressed during tetrad dissolution. *Plant Mol Biol* 24:903–914
- Chen R, Aguirre PJ, Smith AG (1994) Characterization of an anther- and tapetum-specific gene encoding a glycine-rich protein from tomato. *J Plant Physiol* 143:651–658
- Denecke J, Rycke R, Botterman J (1992) Mammalian- and plant-sorting signals for protein retention in the endoplasmic reticulum form a closely related epitope. *EMBO J* 11:2345–2355
- Foster GD, Robinson SW, Blundell RP, Roberts MR, Hodge R, Draper J, Scott RJA (1992) *Brassica napus* mRNA encoding a protein homologous to phospholipid transfer proteins is expressed specifically in the tapetum and developing microspores. *Plant Sci* 84:187–192
- Heijne G von (1985) Signal sequences: the limits of variation. *J Mol Biol* 184:99–105
- Hesse T, Feldswisch J, Balshüsemann D, Bauw G, Puype M, Vanderckloov J, Löbler M, Klämbt D, Schell J, Palme K (1989) Molecular cloning and structural analysis of a gene from *Zea mays* L coding for a putative receptor for the plant hormone auxin. *EMBO J* 11:2453–2461
- Hird DL, Worrall D, Hodge R, Smartt S, Wyatt P, Scott R (1993) The anther-specific protein encoded by the *Brassica napus* and *Arabidopsis thaliana* *a6* gene displays similarity to β -13-glucanases. *Plant J* 4:1023–1033
- Koltunow AM, Truettner J, Cox KH, Wallroth M, Goldberg RB (1990) Different temporal and spatial gene expression patterns occur during anther development. *Plant Cell* 2:1201–1224
- Nacken WKF, Huijser P, Beltran H-P, Saedler H, Sommer H (1991a) Molecular characterization of two stamen-specific genes *tap1* and *fill* that are expressed in the wild type but not the *deficiens* mutant of *Antirrhinum majus*. *Mol Gen Genet* 229:129–136
- Nacken WKF, Huijser P, Saedler H, Sommer H (1991b) Molecular analysis of *tap2* an anther-specific gene from *Antirrhinum majus*. *FEBS Lett* 280:155–158
- Rochester DE, Winter JA, Shah DM (1986) The structure and expression of maize genes encoding the major heat shock protein hsp70. *EMBO J* 5:451–458
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463–5467
- Schrauwen JAM, Mettenmeyer T, Croes AF, Wullems GJ (1996) Tapetum-specific genes: what role do they play in male gametophyte development? *Acta Bot Neerl* 45:1–15
- Seurinck J, Truettner J, and Goldberg RB (1990) The nucleotide sequence of an anther-specific gene *Nucleic Acids Res* 18: 3403
- Wright SY, Suner M-M, Bell PJ, Vaudin M, Greenland AJ (1993) Isolation and characterization of male flower cDNAs from maize. *Plant J* 3:41–49