Gene structures in the sf21-gene family in sunflower (Helianthus annuus)

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The growth of the pollen tubes towards the ovary is influenced by compounds present in the pollen and pistil, and by products synthesized through interactions between the pistil and the growing pollen tubes (Trewavas und Gilroy 1991, Trewavas und Malho 1997). The pistil- and pollen-expressed gene sf21 encoding a 352 amino acid polypeptide was isolated by differential screening of a floral cDNA library from sunflower and characterized. The deduced polypeptide
is structurally related to the animal proteins RTP / NdrI and the vertebrate inositol 1,4,5 trisphosphate (IP3) receptor (Mignery et al. 1990, Mignery und Südhof 1990). Recent studies have shown that the sf21 gene is also related to a gene on chromosome II of Arabidopsis thaliana (Lin et al., 1999). In sunflower pistils, the Sf21 protein is localized in the nucleus of the stigma cells, and in the cytoplasm and nucleus of the stylar transmitting tissue cells (Kräuter-Canham et al., 2001). In an effort to elucidate the possible role of the pollen- and pistil-specific expressed sf21 gene in sexual reproduction of sunflower we initiated the identification of the sf21 homologous genes and their promoter regions. The sf21 probe was generated by PCR using the sf21 cDNA clone as template. The sunflower BAC library constructed from the restorer line RHA325 using pBeloBAC11 as vector and HindIII, consists of 104,736 clones. Screening of the BAC library with radiolabelled sunflower sf21 probe identified eight positive clones. To determine whether these clones represent one or more loci, DNA isolated from each clone was subjected to restriction enzyme fingerprinting with HindIII. The patterns of enzyme digestion and hybridisation with sf21 as probe differentiated all eight BAC clones. The BAC insert sizes of these clones ranged from 18.5 kb to 62.0 kb. Positive BAC clones were digested with HindIII und subcloned into pUC18. Of these eight clones, seven have already been subcloned, obtaining positive subclones with insert sizes in the range of 1.7 kb to 14.0 kb. We identified the partial structure of sf21 gene in four positive subclones by sequencing. Promoter deletion constructs using GUS as reporter gene will be made to identify promoter elements responsible for the tissue-specific expression of the gene. Transformations of tobacco and sunflower with these constructs are planned. GUS tests will evaluate the tissue specificity of each construct. Analysis of the function of the sf21 gene will be done by using Arabidopsis mutants obtained by transposon mutagenesis.