Possible involvement of *CsTypA1* in reproductive organ development of cucumber (*Cucumis sativus*)

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**Abstract**  
Cucumber (*Cucumis sativus*) is a monoecious plant that is extensively studied with respect to floral sex determination. While the genetic background, hormonal and environmental factors are well characterized, the molecular mechanisms regulating unisexual flower development are not well understood. Initially a cucumber floral bud is bisexual and has the potential to develop into a male or female flower. Using differential cDNA-AFLP analysis between plant apices of monoecious (predominantly male) and gynoecious (female) cucumber plants, we isolated a cDNA that encodes a putative GTP binding protein tyrosine phosphorylated protein A (*CsTypA1*). TypA genes are widely distributed in plants and prokaryotes. Bacterial TypA proteins are involved in protein translation and have been proposed to function as global regulators of various regulatory pathways. The role for TypA proteins in plants is unknown. In cucumber, *CsTypA1* is expressed differentially during male and female flower development and is highly expressed in the ovary particularly in the ovules. Our data imply that *CsTypA1* is involved in reproductive organ development.

**INTRODUCTION**  
Cucumber (*Cucumis sativus*) is a monoecious plant that serves as a plant model for floral sex determination and reproductive organ development. The genetic background, hormonal regulation and environmental factors involved in sex determination are well characterized in cucumber (Rudich 1990; Perl-Treves 1999). For isolation of floral sex specific genes we performed a differential cDNA-Amplified Fragment Length Polymorphism (cDNA-AFLP) analysis using plant apices at the two leaf stage taken from two near isogenic cucumber lines. Monoecious plant apices consist of bisexual flower buds and male flower buds while gynoecious plant apices consist of bisexual flower buds and female flower buds (Bai et al. 2004). We isolated a partial cDNA sequence that is highly identical to GTP binding tyrosine phosphorylated protein A (TypA) proteins (*CsTypA1*). TypA proteins form a sub-family within the ribosome binding translation factor family of GTPases (Owens et al. 2001). TypAs are found in a wide range of bacteria and plants but not in the Animalia kingdom. Bacterial TypA proteins participate in regulation of stress responses (Farris et al. 1998; Freestone et al. 1998; Kiss et al. 2004) and bacterial pathogenicity related functions in pathogenic bacteria (Grant et al. 2003) emphasizing the broad scale of their regulatory functions. TypAs are found in numerous plants and have been

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identified in green algae, moss, liverwort, gymnosperms and many angiosperm species. However, the role of TypA proteins in plants has not been studied yet. In this work we investigated the possible role of *CsTypA1* gene in cucumber female flower development.

**MATERIAL AND METHODS**

**Plant material**

Seeds of monoecious (‘Beit-Alpha’; *MMff*) and gynoecious (‘Beit-Alpha’; *MMFF*) near-isogenic cucumber plants were received from Hazera Genetics, Ltd. (Berurim M.P. Shikmim, Israel). Seeds were germinated and grown in trays or 1.5 L plastic pots containing soil mixture of peat and vermiculite (1:1, v/v). Plants were grown in a growth chamber under a 16 hrs photoperiod, at 27 ± 1°C. Under these conditions the gynoecious plants develop female flowers only while the monoecious develop mostly male flowers with occasional female flowers at later stages of development (from the 13th node onwards).

**Isolation of *CsTypA1***

A partial cDNA clone of the putative TypA was isolated using the differential cDNA-AFLP method performed as described previously (Barak and Trebitsh 2007). The full-length cDNA of *CsTypA1* was isolated by 5’ and 3’ Rapid amplification of cDNA ends (FirstChoice RLM-RACE Kit, Ambion, Huntingdon, Cambridgeshir).

**In situ hybridization**

Plant materials were fixed in 4 % paraformaldehyde, hybridization and immunological detection was described previously (Barak and Trebitsh 2007). *In vitro* transcribed antisense and sense *CsTypA1* RNA probes were labeled with digoxigenin using T7 RNA polymerase (Roche Diagnostics, Penzberg, Germany).

**RESULTS AND DISCUSSION**

**Isolation of full-length *CsTypA1* cDNA sequence**

To isolate genes that are differentially expressed during unisexual flower development we used differential cDNA-AFLP analysis (Barak and Trebitsh 2007). The full length cDNA of *CsTypA1* (2.5 kb) encodes for 598 amino acid polypeptide. There are three conserved domains of TypAs, the GTP binding site, a domain homologous to elongation factors EF-G and EF-Tu and the domain that contains the putative ribosome binding site. The conserved domains of bacterial TypAs are also conserved in plant TypA proteins. *CsTypA1* is highly identical to TypAs isolated from other plant species, ranging from 88 % identity with *Arabidopsis thaliana* TypA to 61 % identity with the green alga *Chlamydomonas reinhardtii* TypA (Fig. 1). All plant species are clustered in a single clade that includes moss, algae and flowering plants. The cucumber TypA protein is most closely related to TypA of eudicot plant species (Fig. 1). *TypA* transcripts are found in most plant databases (http://plantta.tigr.org/), yet little is known about their function in plants. The *CsTypA* polypeptide is also homologous to proteins from prokaryotes such as the cyanobacteria *Synechococcus elongates* with 51 % identity (Fig. 1). The similarity between plants and bacteria TypAs indicate their preservation along evolution.
Figure 1. Phylogenetic tree of the full-length amino acid sequences of TypA proteins. Phylogenetic analysis was performed by MEGA4. Bootstrap test results (1000 replicates) is shown next to the branches, bar indicates 0.05 substitutions per residue. Cucumis sativus (EF426245), Arabidopsis thaliana (BAB08691), Trifolium pratense (AAR17698), Oryza sativa (BAD21496), Physcomitrella patens (EDQ64428), Chlamydomonas reinhardii (XP_001700103), Synechococcus elongates (NP_387196).

Gene expression analysis

To study the possible involvement of CsTypA1 in flower development we determined its expression level during this process. CsTypA1 is differentially expressed in different stages of cucumber flower development. Notably, in situ hybridization indicated that at the sex determination stage CsTypA1 is specifically expressed in sepal and stamen primordia. Thus, in a bisexual flower bud, at the sex determination stage, CsTypA1 transcript level is high in floral buds that develop to male flowers compared to those developing to female flowers (Barak and Trebitsh 2007). In flower buds at later stages of development expression of CsTypA1 is higher in female flowers than in male flowers. In female flowers CsTypA1 transcripts are detected in the transmitting track, papilla, nectary and ovary (Barak and Trebitsh 2007). Before anthesis, CsTypA1 is strongly expressed in the ovules, specifically in the micropyle region (Fig. 2). This finding strengthens the hypothesis that CsTypA1 is involved in female flower development, in particular in the development of ovary/ovules.

CONCLUSIONS

In bacteria TypAs are considered as global regulators of translation (Grant et al. 2003; Owens et al. 2004) involved in diverse aspects of bacterial life. However, the role of TypA in plants is yet unknown. Our studies of TypA in cucumber (Fig. 2, Barak and Trebitsh 2007) indicate that the cucumber TypA is developmentally regulated and is involved in reproductive organ development. Further research is being conducted to study the function of TypA during plant developmental processes.
Figure 2. Expression of CsTypA1 in longitudinal sections of a cucumber ovary prior to anthesis. Tissue was hybridized with CsTypA1 RNA probes from the antisense strand (A) or the sense strand (B) as negative control.

Literature cited