

## **Molecular Cloning, Expression, and Characterization of an Adenylyl Cyclase-associated Protein from *Gossypium arboreum* Fuzzless Mutant**

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CAP, an adenylyl cyclase-associated protein, is predicted to be involved in cytoskeletal organization and signal transduction. Recently, we found that CAP may play an important role in fuzz-like fiber cell initiation in cotton. For the further research, we isolated two CAP homologues from wild type cotton *Gossypium arboreum* L. (DPL971) and its natural fuzzless mutant (DPL972). The gene consisted of an open reading frame of 1,416 nucleotides encoding a protein of 471 amino acid residues with a calculated molecular weight of 50.6 kDa. The amino acid sequence of the cloned CAP showed 74% homology to that of *Arabidopsis thaliana*. RT-PCR and Northern blotting results revealed that this *G. arboreum* CAP specifically expressed in ovules from -1 to 4 DPA (days post-anthesis). In addition, the transcripts of this CAP decreased gradually in the fuzzless mutant after 4 DPA. Interestingly, a conserved position was found among CAPs where polar substitution took place. At this position, the conserved uncharged unpolar Ala44 (A) of GaCAP was replaced by a Thr (T) in GaCAPm. It would be interesting to know whether this conservative substitution has structural and functional impacts on the GaCAPm. To characterize the GaCAP, the cloned CAP gene was expressed under the T7 promoter of the expression vector, pET-28a (+), in *Escherichia coli* BL21 (DE3) pLySs, and it was purified using His Bind column chromatography. The purified protein can bind with the actin in vitro indicating that the recombinant cotton CAP is functional. In summary, our results provided evidence that the expression of CAP reaches a peak during the ovule development and CAP may have crucial functions in the fiber cell initiation. And, we described a procedure to produce high yielding pure protein through one chromatographic step, which was suitable for further structure-function.