
A tomato cDNA encoding a biotin-binding protein

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We have recently screened a tomato leaf cDNA expression library in the phage vector Charon 16 (1) with antibodies made against chloroplast membrane proteins. The screening procedure utilized secondary antibodies conjugated to biotin and alkaline phosphatase (AP) conjugated to avidin (ABC reagent, Vector Laboratories). The nucleotide sequence of one of the clones obtained and the protein sequence of its longest, but still incomplete, reading frame (Fig. 1) showed no homology to the sequences of interest. A search of protein data banks revealed homology to several biotin-containing proteins from both animals and bacteria, and especially strong homology with the biotinyl subunit of the transcarboxylase of *Propionibacterium* (2). The biotin binding site of the latter, QOTLVLEAMKME, differs by a single residue from a similar sequence in the tomato protein. Direct screening of phage plaques with avidin-AP in the absence of both primary and secondary antibodies subsequently indicated that this tomato polypeptide directly binds avidin-AP. Our results identify one source of "false" positives in screening cDNA expression libraries with an avidin-biotin based detection system. The tomato cDNA clone, which appears to be the only one identified to date encoding a plant biotin-binding protein, is available upon request.

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  G T V V A P M V G L E V K V L V K D G E K V Q
  GGTACTGTGGTTGCACCTATGGTTGGGTTAGAGGTTAAAGTATTGGTGAAGGATGGGGAGAAAGTTCAGG   70
  _____
  E G Q P V L V L E A M K M E H V V K A P A N G Y
  AGGGACAACCTGTGTTAGTATTAGAAGCAATGAAGATGGAGCATGTAGTGAAGCACCAGCTAATGGCTA   140

  V S G L E I K V G Q S V Q D G I K L F A L K D
  TGTAAGCGGCTTGAAATCAAAGTGGGCCAATCGGTCCAAGATGGTATAAACTCTTTGCTCTCAAGGAC   210

  TGAAATATATCCTGAGGCTATGACAACATCATTCTAGACAACCTGTATGTATGACCTCCTATGAGGACTGT   280

  ACATCCACTAGGCATAAGAATAACAACATTGAGATGTAAAATCTCTTGGAGTTTCATCTTCTACATTTC   350

  AATACATTTACTTGTAATGACTTTCCAGACTCAn                                     384
  
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Figure 1: Nucleotide sequence of the tomato cDNA clone and sequence of the encoded polypeptide. The putative biotin-binding site is overlined.

References:

1. DellaPenna, D., et al., Proc. Natl. Acad. Sci. USA. 83, 6420-6424, 1986.
2. Maloy, W.L., et al., J. Biol. Chem. 254, 11615-11622, 1979.