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NCBI Coffee Break

8 Dec 1999

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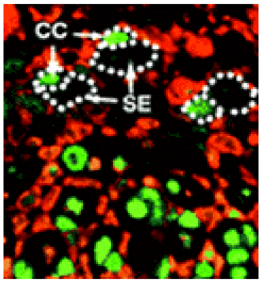

CmPP16 mRNA (green) was detected within companion cells (CC) and sieve elements (SE) of the winter squash. This suggests that the newly cloned CmPP16 acts as a plant movement protein.

Click here for more details.

Viruses provide direction on the plant information superhighway

The phloem is the long-distance transport system of plants. In addition to distributing nutrients, the phloem plays a role in transporting hormones and signaling proteins. The phloem, together with the xylem, which transports water and dissolved ions, provide the plant with its internal trafficking system.

Phloem tubes are made up of aligned cells, called sieve elements, which lack nuclei and therefore must rely on associated companion cells for physiological support and nourishment. Sieve elements and companion cells are connected by plasmodesmata - a network of tiny channels through which molecules such as sugars, hormones and amino acids travel. How these molecules are ushered via plasmodesmata into the long-distance transport systems has eluded researchers until only recently, when viruses have provided some vital clues.

Plant viral movement proteins (VMPs) have been known to exist for some time. These are proteins encoded by the virus that assist in the transportation of the viral nucleic acid around the plant. Their exact mode of action, plus the requirement (or otherwise) for coat proteins for systemic infection can vary from virus to virus. For example, cytoplasmically replicating viruses (e.g. tomato spotted wilt virus) require one or more movement proteins and a coat protein, while bipartite geminiviruses (e.g. squash leaf curl virus) require two movement proteins, but no coat protein.

The virus that has recently shed light on the plant transportation mechanism is red clover necrotic mosaic virus (RCNMV), which uses a single virus-encoded movement protein to move between cells. This VMP binds to viral RNA, and, using host-cell microfilaments, chaperones the viral RNA to the plasmodesmata. Once at the plasmodesmata, the VMP acts to somehow increase the diameter of these channels, permitting the viral nucleic acid to enter the adjoining cell. However, in order to infect a plant systemically, the red clover necrotic mosaic virus RNA must first be encapsulated by its protein coat.

Recently, a plant protein, CmPP16, isolated from *Cucurbita maxima* (winter squash), was discovered that was reported to share sequence similarity with the red clover necrotic mosaic virus VMP. If so, this suggests that CmPP16 may assist in the long-distance transport of plant RNA, using a similar mechanism to the red clover necrotic mosaic virus VMP.

Why transport RNA around the plant? One possibility is that the RNA is being used as a long-distance signaling molecule, helping to coordinate developmental processes with physiological signals. Looking at how viruses spread throughout a plant could assist in the fascinating puzzle of how different parts of

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|| Taxonomy: *Cucurbita maxima* || Taxonomy: red clover necrotic mosaic virus ||

the plant talk to each other.

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Figure Coffee Break

Viruses provide direction on the plant information superhighway

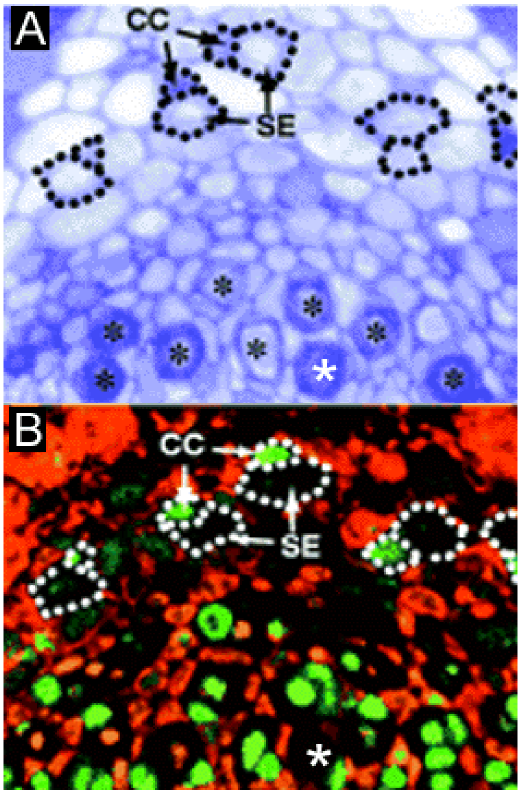
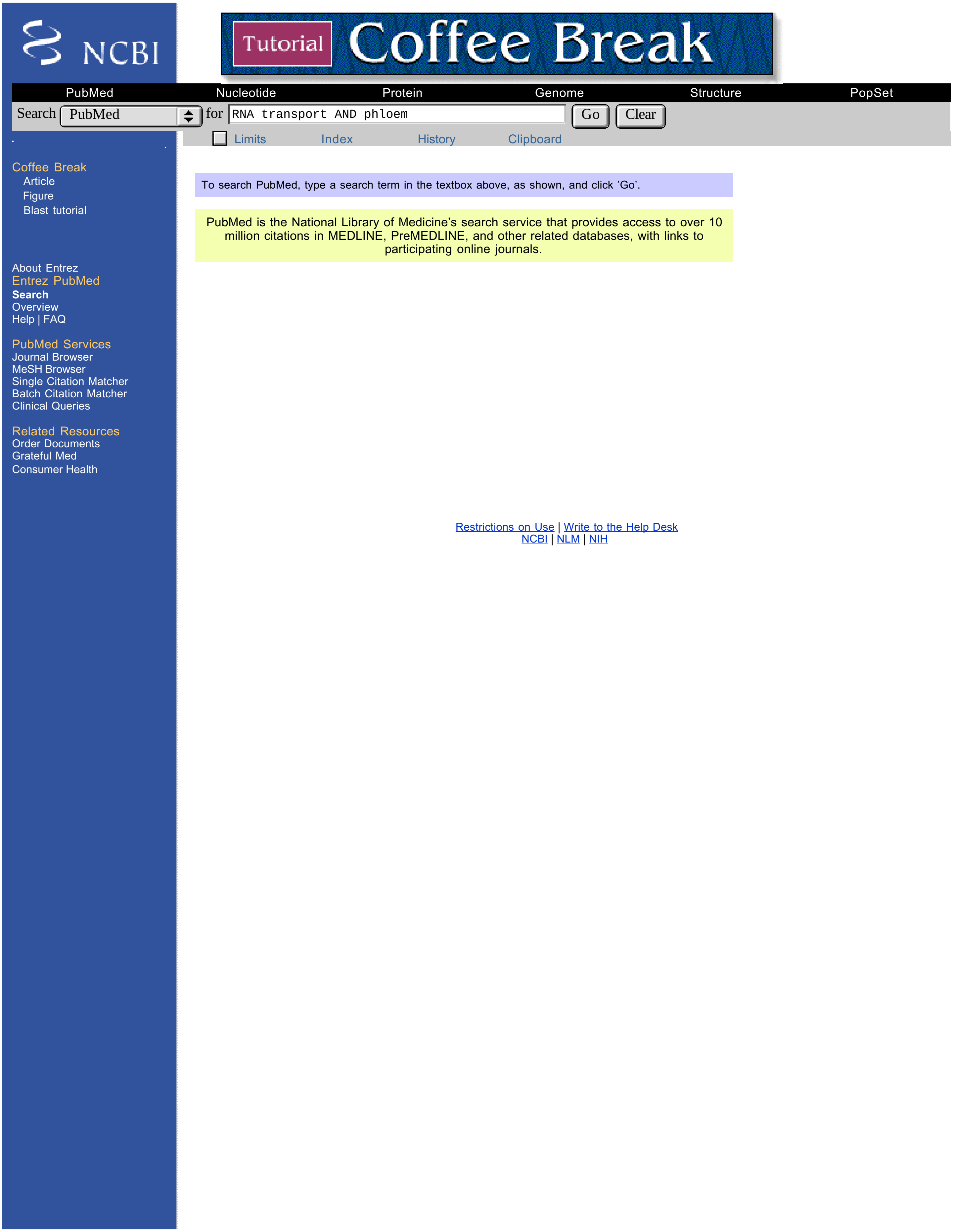


Figure 1. *CmPP16* mRNA was detected within the vascular tissue of *Cucurbita maxima* or winter squash. **(A)** This image of a transverse section of winter squash depicts the various components of the phloem. Black dots outline companion cells (CC) and sieve elements (SE) joined by fine, branched plasmodesmata. Black asterisks identify immature sieve elements; the white asterisk reflects the identical cell in images (A) and (B). **(B)** *CmPP16* mRNA (green fluorescent signal) is shown to have moved within the phloem. mRNA was found mostly in companion cells, but also in mature, functional sieve elements suggesting its movement through plasmodesmata.

(Reproduced with permission from: Xoconostle-Cazares, B., Xiang, Y., Ruiz-Medrano, R., Wang, H.-L., Monzer, J., Yoo, B.-C., McFarland, K.C., Franceschi, V.R. and Lucas, W.J. (1999) Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. Science 283, 94-98.)



Search PubMed for `RNA transport AND phloem`

To search PubMed, type a search term in the textbox above, as shown, and click 'Go'.

PubMed is the National Library of Medicine's search service that provides access to over 10 million citations in MEDLINE, PreMEDLINE, and other related databases, with links to participating online journals.

The first article in the list below discusses how mRNA molecules, transported throughout the plant by the phloem, appear to play a role in gene expression. After being ushered to distant cells, this mRNA may be translated into proteins required at the site. Use the 'Related Articles' function to expand on this theme.

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☐ **1 :** Ruiz-Medrano R, et al. Related Articles
Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants.
Development. 1999 Oct;126(20):4405-19.
PMID: 10498677; UI: 99429802

☐ **2 :** Kaltenbach M, et al. Related Articles
Flavonoid hydroxylase from Catharanthus roseus: cDNA, heterologous expression, enzyme properties and cell-type specific expression in plants.
Plant J. 1999 Jul;19(2):183-93.
PMID: 10476065; UI: 99407272

☐ **3 :** Moriau L, et al. Related Articles
Expression analysis of two gene subfamilies encoding the plasma membrane H+-ATPase in Nicotiana plumbaginifolia reveals the major transport functions of this enzyme.
Plant J. 1999 Jul;19(1):31-41.
PMID: 10417724; UI: 99348178

☐ **4 :** Lucas WJ, et al. Related Articles
Connections between virus movement, macromolecular signaling and assimilate allocation.
Curr Opin Plant Biol. 1999 Jun;2(3):192-7. Review.
PMID: 10375572; UI: 99307689

☐ **5 :** Lappartient AG, et al. Related Articles
Inter-organ signaling in plants: regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compound.
Plant J. 1999 Apr;18(1):89-95.
PMID: 10341446; UI: 99272992

☐ **6 :** Crawford KM, et al. Related Articles
Phloem transport: Are you chaperoned?
Curr Biol. 1999 Apr 22;9(8):R281-5. Review.
PMID: 10226019; UI: 99244957

☐ **7 :** Ryabov EV, et al. Related Articles
A plant virus-encoded protein facilitates long-distance movement of heterologous viral RNA.
Proc Natl Acad Sci U S A. 1999 Feb 16;96(4):1212-7.
[Record as supplied by publisher]
PMID: 9990003

☐ **8 :** Xoconostle-Cazares B, et al. Related Articles, Protein, Nucleotide
Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem.
Science. 1999 Jan 1;283(5398):94-8.
PMID: 9872750; UI: 99102700

☐ **9 :** Shakya R, et al. Related Articles, Protein, Nucleotide
Characterization of source- and sink-specific sucrose/H+ symporters from carrot.
Plant Physiol. 1998 Dec;118(4):1473-80.
PMID: 9847123; UI: 99063785

10 : Bick JA, et al. Related Articles, Protein, Nucleotide
Expression analysis of a sucrose carrier in the germinating seedling of Ricinus communis.
Plant Mol Biol. 1998 Oct;38(3):425-35.
PMID: 9747850; UI: 98418622

11 : Lu JM, et al. Related Articles
His-65 in the proton-sucrose symporter is an essential amino acid whose modification with site-directed mutagenesis increases transport activity.
Proc Natl Acad Sci U S A. 1998 Jul 21;95(15):9025-30.
PMID: 9671798; UI: 98338035

☐ **12** : Ishiwatari Y, et al. Related Articles
Rice phloem thioredoxin h has the capacity to mediate its own cell-to-cell transport through plasmodesmata.
Planta. 1998 May;205(1):12-22.
PMID: 9599802; UI: 98262536

☐ **13** : Chiou TJ, et al. Related Articles
Sucrose is a signal molecule in assimilate partitioning.
Proc Natl Acad Sci U S A. 1998 Apr 14;95(8):4784-8.
[Record as supplied by publisher]
PMID: 9539816

☐ **14** : Kuhn C, et al. Related Articles
Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements.
Science. 1997 Feb 28;275(5304):1298-300.
PMID: 9036853; UI: 97190102

☐ **15** : Ding X, et al. Related Articles
Invasion of minor veins of tobacco leaves inoculated with tobacco mosaic virus mutants defective in phloem-dependent movement.
Proc Natl Acad Sci U S A. 1996 Oct 1;93(20):11155-60.
PMID: 8855325; UI: 97008149

☐ **16** : Frias I, et al. Related Articles, Protein, Nucleotide
A major isoform of the maize plasma membrane H(+)-ATPase: characterization and induction by auxin in coleoptiles.
Plant Cell. 1996 Sep;8(9):1533-44.
PMID: 8837507; UI: 96434540

☐ **17** : Hause B, et al. Related Articles
Developmental and tissue-specific expression of JIP-23, a jasmonate-inducible protein of barley.
Plant Cell Physiol. 1996 Jul;37(5):641-9.
PMID: 8819310; UI: 96416427

☐ **18** : Gahrtz M, et al. Related Articles, Protein, Nucleotide
A phloem-specific sucrose-H+ symporter from Plantago major L. supports the model of apoplastic phloem loading.
Plant J. 1994 Nov;6(5):697-706.
PMID: 8000426; UI: 95093474

☐ **19** : Riesmeier JW, et al. Related Articles
Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning.
EMBO J. 1994 Jan 1;13(1):1-7.
PMID: 8306952; UI: 94139651

☐ **20** : Riesmeier JW, et al. Related Articles, Protein, Nucleotide
Potato sucrose transporter expression in minor veins indicates a role in phloem loading.
Plant Cell. 1993 Nov;5(11):1591-8.
PMID: 8312741; UI: 94146554

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Revised: December 3, 1999.

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This is the query page for a BLAST search. The sequence of the phloem protein, CmPP16, from *Cucurbita maxima* is entered in the textbox below. The database to be searched can be selected from the following pull-down menu, as can the format that the sequence is submitted in. Click on the "Submit Query" button below the textbox to find similar sequences to this query in the database.

Database: nr

The amino acid query sequence is filtered for low complexity regions by default.

Enter here your **amino acid sequence** as: Sequence in FASTA format

```
>gi|4164539|gb|AAD05496.1| phloem protein
MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVA
KNAGPNPLWDEKFKFLAEYPGSGGDFHILFKVMDHDAIDGDDYI
GDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAHKIHFKGEIEVGV
SFKLQGGGGCGGCYPWEN
```

Submit Query   Reset

Please read about FASTA format description

The options below are for advanced users. In this example, the number of descriptions and alignments to be displayed have been changed to 50 and 10, respectively. If no parameters are changed or selected, then BLAST is run in default mode.

Advanced options for the BLAST server:

Expect: 10   Filter: default   ☐ NCBI-gi   ☑ Graphical Overview

Descr iptions: 50   Alignments: 10

Expect value for inclusion in PSI-BLAST iteration 1: 0.001

Other advanced options:

Submit Query   Reset

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Tutorial **Coffee Break**

Query= gi|4164539 *Cucurbita maxima* phloem protein (150 letters)
Database: Non-redundant GenBank CDS translations++PDB+SwissProt+SPupdate+PIR 397,667 sequences; 121,708,859 total letters

Below are the results from a PSI-BLAST (see Altschul et al., 1997) search of the non-redundant database using *Cucurbita maxima* phloem protein as the query sequence.

The E value is a statistical measure of likelyhood that the sequences listed below are truly similar to the query, rather than found by chance alone. The lower the E value, the greater the confidence that the protein found is a biologically significant match.

E-value threshold for inclusion in PSI-Blast iteration 1: 0.001
E-value threshold for inclusion in PSI-Blast iteration 2: 0.001

PSI-BLAST constructs a similarity matrix from the significant hits found in a BLAST search and uses this matrix to search the database in a second round of BLASTing. Each round of PSI-BLAST that uses the matrix to search the database is called an iteration.

Distribution of 169 Blast Hits on the Query Sequence

Mouse-over to show defline and

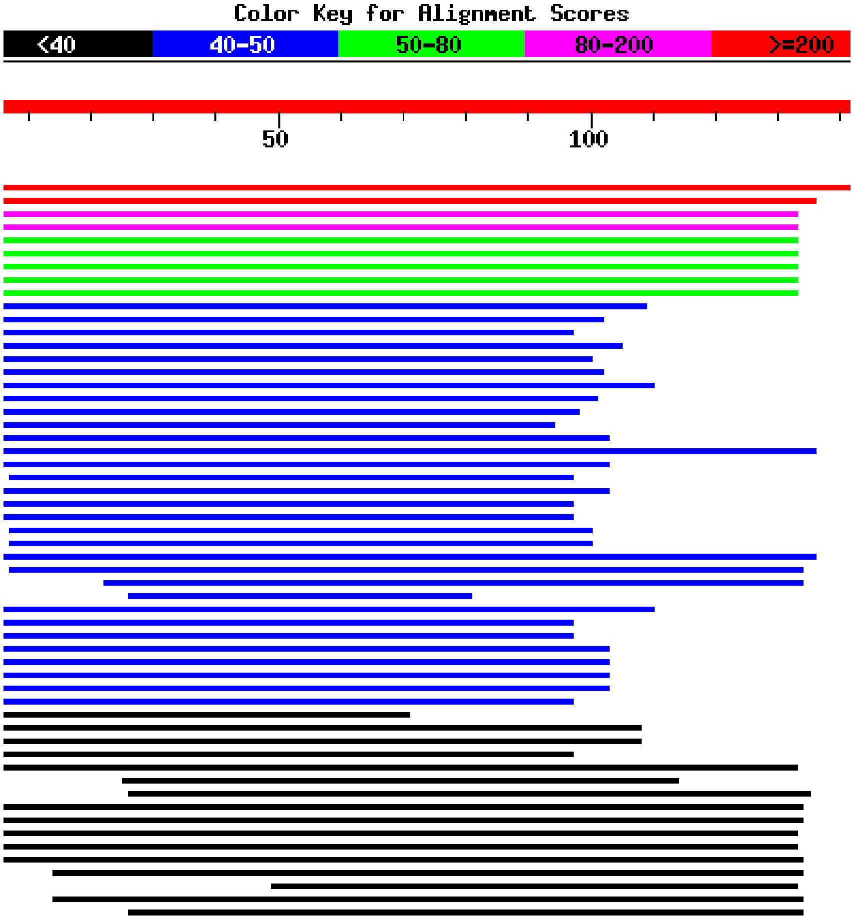

Figure legend

The above summary of the BLAST results represents proteins that match the query sequence as colored bars, with the most similar hit uppermost and appearing in red. Pink, green, blue and black bars follow, representing proteins in decreasing order of similarity. Hatched areas indicate a gap in similarity i.e., two or more distinct regions of similarity were found within the same protein hit. Moving the mouse over the bars will display the name of the matching protein found in the textbox above.

Click here to view alignments.

BLAST Alignments

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About the alignments

The match with the top score is to itself (reassuringly) - the lower the e-value or the higher the score, the higher the chance that the similarity is biologically relevant. Clicking on the scores will take you to the alignments for each of the top ten hits (as requested in our query). Clicking on the ID numbers on the left will display the sequence record at the NCBI site.

Back to BLAST result

or

Click here to see the analysis of this BLAST search.

Sequences with E-value BETTER than threshold

```
                                                                 Score    E
Sequences producing significant alignments:                      (bits) Value

 gi|4164539  (AF079170) phloem protein [Cucurbita maxima]          300  5e-81
 gi|4164541  (AF079171) phloem protein [Cucurbita maxima]          266  9e-71
 gi|2920839  (U95136) Os-FIERG2 gene product [Oryza sativa]        106  1e-22
 gi|2920837  (U95135) Os-FIERG1 gene product [Oryza sativa]        100  6e-21
 gi|3603473  (AF090698) elicitor-responsive gene-3 [Oryza sativa]   80  1e-14
 emb|CAA10133|  (AJ012692) hypothetical protein [Cicer arietinum]   69  2e-11
 gb|AAD45283.1|  (AF152601) unknown [Zea mays]                      66  1e-10
 gi|1498055  (U64437) novel protein [Zea mays]                      65  3e-10
 dbj|BAA24382|  (AB001389) CLB1 [Lycopersicon esculentum]           51  6e-06
 emb|CAA17535.1|  (AL021960) putative protein [Arabidopsis thal...  50  1e-05
 gi|4193489  (AF099138) GLUT4 vesicle protein [Rattus norvegicus]   49  2e-05
 emb|CAA71759|  (Y10787) hypothetical protein [Sporobolus stapf...  48  4e-05
 gi|3935150  (AC005106) T25N20.14 [Arabidopsis thaliana]            48  5e-05
 dbj|BAA34467.1|  (AB018290) KIAA0747 protein [Homo sapiens]        48  5e-05
 gi|3785983  (AC005560) hypothetical protein [Arabidopsis thali...  48  5e-05
 gb|AAD55497.1|AC008148_7  (AC008148) Hypothetical protein [Ara...  46  1e-04
 ref|NP_003485.1|PDYSF|  Dysferlin >gi|3600028 (AF075575) dysfe...  46  2e-04
 gb|AAD55494.1|AC008148_4  (AC008148) Unknown protein [Arabidop...  44  6e-04
 gi|3850588  (AC005278) Contains similarity to gb|AB011110 KIAA...  44  6e-04
```

Sequences with E-value WORSE than threshold

```
 gb|AAF13346.1|AF122022_1  (AF122022) unknown [Eufolliculina uh...  43  0.001
 gi|4099291  (U85711) phospholipase C delta-1; PI-PLC-delta-1 [...  43  0.001
 dbj|BAA86570.1|  (AB033082) KIAA1256 protein [Homo sapiens]        43  0.001
 gi|1326337  (U58746) coded for by C. elegans cDNA yk3b11.5; co...  43  0.001
 pir||PC4183  1-phosphatidylinositol phosphodiesterase (EC 3.1....  43  0.001
 pir||I61777  Munc13-3 - rat (fragment)                             43  0.002
 gi|1763306  (U75361) Munc13-3 [Rattus norvegicus]                  43  0.002
 pir||C28821  1-phosphatidylinositol-4,5-bisphosphate phosphodi...  43  0.002
 ref|NP_006216.1|PPLCD1|  phospholipase C, delta 1 >gi|1730575|...  43  0.002
 sp|P10895|PIP6_BOVIN  1-PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE ...  43  0.002
 gb|AAD20895|  (AC006234) hypothetical protein [Arabidopsis tha...  42  0.002
 gb|AAD19747|  (AF132479) Ese2L protein [Mus musculus]              42  0.002
 dbj|BAA25464|  (AB011110) KIAA0538 protein [Homo sapiens]          42  0.002
 gi|2822157  (AC004084) similar to GTPase-activating proteins; ...  42  0.002
 gb|AAD55495.1|AC008148_5  (AC008148) Unknown protein [Arabidop...  42  0.003
 gi|2425145  (AF020408) contains C2 domain similar to S. cerevi...  41  0.007
 pir||I55942  phospholipase C-delta (EC 3.1.4.-), mutant (spont...  41  0.007
 pdb|1QAS|A  Chain A, 1-Phosphatidylinositol-4,5-Bisphosphate P...  41  0.007
 pdb|1ISD|A  No definition line found >gi|1827644|pdb|1ISD|B No...  41  0.007
 sp|P10688|PIP6_RAT  1-PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE PH...  41  0.007
 ref|NP_013639.1||  Yml072cp >gi|2497080|sp|Q03640|YMH2_YEAST H...  41  0.007
 gi|1825586  (U88167) contains similarity to C2 domains [Caenor...  40  0.009
 gb|AAD20898|  (AC006234) hypothetical protein [Arabidopsis tha...  40  0.011
 emb|CAA07573.1|  (AJ007586) src2-like protein [Arabidopsis tha...  40  0.011
 gi|1922937  (AC000106) Similar to Glycine SRC2 (gb|AB000130). ...  40  0.011
 emb|CAA89822|  (Z49747) phospholipase C [Oryctolagus cuniculus]    40  0.011
  gi|2435614  (AF026214) contains similarity to C2 domains [Caen...   39  0.015
 pir||S70642  ubiquitin ligase Nedd4 - rat (fragment) >gi|12936...  39  0.015
 ref|NP_006217.1|PPLCE|  phospholipase C, epsilon >gi|2135919|p...  39  0.015
 gb|AAD29817.1|AC006264_25  (AC006264) putative C2-domain prote...  39  0.020
```

Alignments

```
 gi|4164539 (AF079170) phloem protein [Cucurbita maxima]
```

```
           Length = 150

 Score =  300 bits (759), Expect = 5e-81
 Identities = 142/150 (94%), Positives = 142/150 (94%)

Query: 1   MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFL 60
           MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFL
Sbjct: 1   MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFL 60

Query: 61  AEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAH 120
           AEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAH
Sbjct: 61  AEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAH 120

Query: 121 KIHFKGEIEVGVSFKLQXXXXXXXXXYPWEN 150
           KIHFKGEIEVGVSFKLQ         YPWEN
Sbjct: 121 KIHFKGEIEVGVSFKLQGGGGCGGCYPWEN 150

 gi|4164541 (AF079171) phloem protein [Cucurbita maxima]
           Length = 138

 Score =  266 bits (672), Expect = 9e-71
 Identities = 125/137 (91%), Positives = 132/137 (96%)

Query: 1   MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFL 60
           MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGP+P+W+EKFKFL
Sbjct: 1   MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPDPIWNEKFKFL 60

Query: 61  AEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAH 120
            EYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDV+NLLAEGVRKG SE+PPRMY VLAH
Sbjct: 61  VEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVQNLLAEGVRKGWSELPPRMYQVLAH 120

Query: 121 KIHFKGEIEVGVSFKLQ 137
           KI+FKGEIEVGV F+ Q
Sbjct: 121 KIYFKGEIEVGVFFQRQ 137

 gi|2920839 (U95136) Os-FIERG2 gene product [Oryza sativa]
           Length = 156

 Score =  106 bits (261), Expect = 1e-22
 Identities = 51/133 (38%), Positives = 78/133 (58%), Gaps = 1/133 (0%)

Query: 2   GMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFLA 61
           G G++EVHL+  KGL  +D L K IDPY  + ++ QER S VA++ G NP W+E FKF
Sbjct: 3   GSGVLEVHLVDAKGLTGNDFLGK-IDPYVVVQYRSQERKSSVARDQGKNPSWNEVFKFQI 61

Query: 62  EYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAHK 121
               + G   +  ++MDHD    DD++G+  I+V +L++ G+  G  EM    + V+
Sbjct: 62  NSTAATGQHKLFLRLMDHDTFSRDDFLGEATINVTDLISLGMEHGTWEMSESKHRVVLAD 121

Query: 122 IHFKGEIEVGVSF 134
             + GEI V ++F
Sbjct: 122 KTYHGEIRVSLTF 134

 gi|2920837 (U95135) Os-FIERG1 gene product [Oryza sativa]
           Length = 159

 Score =  100 bits (247), Expect = 6e-21
 Identities = 49/135 (36%), Positives = 77/135 (56%), Gaps = 2/135 (1%)

Query: 2   GMGMMEVHLISGKGLQAHDPLNK--PIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKF 59
           G G++EVHL+  KGL  +D L +   I PY  + ++ QER S VA++ G NP W+E FKF
Sbjct: 3   GSGVLEVHLVDAKGLTGNDFLGEIGNIHPYVVVQYRSQERKSSVARDQGKNPSWNEVFKF 62

Query: 60  LAEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLA 119
                 + G   +  ++MDHD    DD++G+  I+V +L++ G+  G  EM    + V+
Sbjct: 63  QINSTAATGQHKLFLRLMDHDTFSRDDFLGEATINVTDLISLGMEHGTWEMSESKHRVVL 122

Query: 120 HKIHFKGEIEVGVSF 134
               + GEI V ++F
Sbjct: 123 ADKTYHGEIRVSLTF 137

 gi|3603473 (AF090698) elicitor-responsive gene-3 [Oryza sativa]
           Length = 144

 Score = 79.6 bits (193), Expect = 1e-14
 Identities = 45/134 (33%), Positives = 76/134 (56%), Gaps = 12/134 (8%)

Query: 1   MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFL 60
           M  G +EV L+  KGL+  D L   +DPYA +  + QE+ S VA   G +P W+E F F
Sbjct: 1   MVQGTLEVLLVGAKGLENTDYLCN-MDPYAVLKCRSQEQKSSVASGKGSDPEWNETFMFS 59

Query: 61  AEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAH 120
             +  +     ++ K+MD D+   DD++G+  I ++ +  EG       +PP +Y+V+
Sbjct: 60  VTHNAT----ELIIKLMDSDSGTDDDFVGEATISLEAIYTEG------SIPPTVYNVVKE 109

Query: 121 KIHFKGEIEVGVSF 134
           +  ++GEI+VG++F
Sbjct: 110 E-EYRGEIKVGLTF 122
```

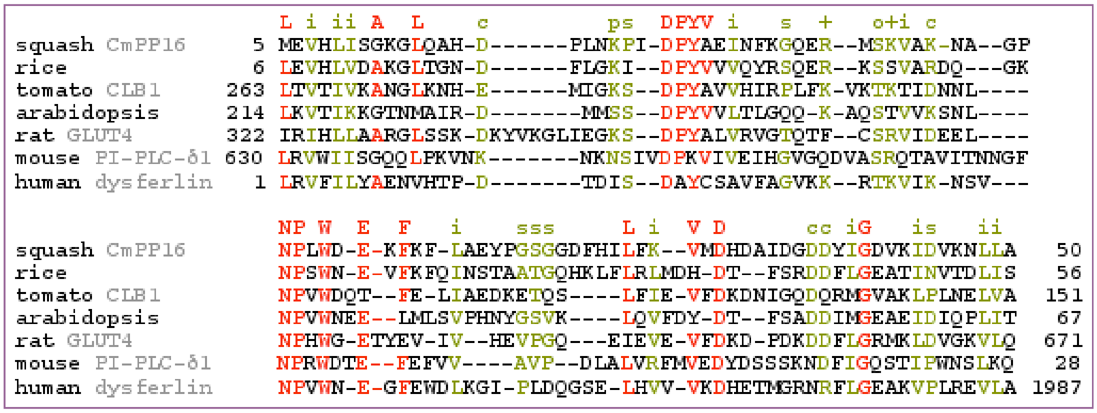
```
 emb|CAA10133| (AJ012692) hypothetical protein [Cicer arietinum]
           Length = 143

 Score = 68.7 bits (165), Expect = 2e-11
 Identities = 43/134 (32%), Positives = 71/134 (52%), Gaps = 12/134 (8%)

Query: 1   MGMGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFL 60
           M  G +EV LIS KGL+ +D L+  IDPY  ++++ QE  S V + AG NP W+E F F
Sbjct: 1   MPRGTLEVVLISAKGLEDNDFLSS-IDPYVILSYRAQEHKSTVQEGAGSNPQWNETFLFT 59

Query: 61  AEYPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAH 120
                S     +  ++M+ D  + DD +G+  I ++ +  EG       +    Y ++
Sbjct: 60  VSDSAS----ELNLRIMEKDNFNNDDNLGEAIIPLEAVFEEG------SLAENAYKLVKE 109

Query: 121 KIHFKGEIEVGVSF 134
           +  + GEI+V ++F
Sbjct: 110 Q-EYCGEIKVALTF 122

 gb|AAD45283.1| (AF152601) unknown [Zea mays]
           Length = 128

 Score = 66.3 bits (159), Expect = 1e-10
 Identities = 42/131 (32%), Positives = 71/131 (54%), Gaps = 13/131 (9%)

Query: 4   GMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFLAEY 63
           G +EV L+S KGL+  D LN  +DP+  +  + QE+ S VA  AG  P W+E F F
Sbjct: 4   GKLEVLLVSAKGLEDTDFLNN-MDPFVILTCRTQEQKSSVANGAGSEPEWNETFVFTV-- 60

Query: 64  PGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAHKIH 123
             S     +  K+MD D +  DD++G+  I ++ +  EG       +PP ++ V+  +
Sbjct: 61  --SDDTPQLHLKIMDSD-LTNDDFVGEATIPLEAVFQEG------SLPPAVHPVVKEE-K 110

Query: 124 FKGEIEVGVSF 134
           + GE+++ ++F
Sbjct: 111 YCGEVKLALTF 121

 gi|1498055 (U64437) novel protein [Zea mays]
           Length = 143

 Score = 65.2 bits (156), Expect = 3e-10
 Identities = 42/131 (32%), Positives = 71/131 (54%), Gaps = 13/131 (9%)

Query: 4   GMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFLAEY 63
           G +EV L+S KGL+  D LN  +DP+  +  + QE+ S VA  AG  P W+E F F
Sbjct: 4   GKLEVLLVSAKGLEDTDFLNN-MDPFVILTCRTQEQKSSVANGAGSEPEWNETFVFTV-- 60

Query: 64  PGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAHKIH 123
             S     +  K+MD D +  DD++G+  I ++ +  EG       +PP ++ V+  +
Sbjct: 61  --SDDTPQLHLKIMDSD-LTNDDFVGERTIPLEAVFQEG------SLPPAVHPVVKEE-K 110

Query: 124 FKGEIEVGVSF 134
           + GE+++ ++F
Sbjct: 111 YCGEVKLALTF 121

 dbj|BAA24382| (AB001389) CLB1 [Lycopersicon esculentum]
           Length = 505

 Score = 50.8 bits (119), Expect = 6e-06
 Identities = 30/131 (22%), Positives = 64/131 (47%), Gaps = 4/131 (3%)

Query: 4   GMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFLAEY 63
           G + V ++   GL+ H+ + K  DPYA ++ +   ++     +   NP+WD+ F+ +AE
Sbjct: 262 GKLTVTIVKANGLKNHEMIGKS-DPYAVVHIRPLFKVKTKTIDNNLNPVWDQTFELIAED 320

Query: 64  PGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEMPPRMYHVLAHKIH 123
             +     +  +V D D I  D  +G  K+ +  L+A+  ++ +  + P++  +
Sbjct: 321 KETQS---LFIEVFDKDNIGQDQRMGVAKLPLNELVADAAKEIELRLLPKLDMLKVKDKK 377

Query: 124 FKGEIEVGVSF 134
            +G I + V +
Sbjct: 378 DRGTITIKVLY 388

 emb|CAA17535.1| (AL021960) putative protein [Arabidopsis thaliana]
           Length = 369

 Score = 49.6 bits (116), Expect = 1e-05
 Identities = 29/108 (26%), Positives = 55/108 (50%), Gaps = 7/108 (6%)

Query: 3   MGMMEVHLISGKGLQAHDPLNKPIDPYAEINFKGQERMSKVAKNAGPNPLWDEKFKFLAE 62
           +G+++V +  G  +   D ++   DPY  +    Q+  S V K+   NP+W+E+   +
Sbjct: 212 IGLLKVTIKKGTNMAIRDMMSS--DPYVVLTLGQQKAQSTVVKS-NLNPVWNEEL--MLS 266

Query: 63  YPGSGGDFHILFKVMDHDAIDGDDYIGDVKIDVKNLLAEGVRKGKSEM 110
            P + G   +  +V D+D    DD +G+ +ID++ L+   +  G  EM
Sbjct: 267 VPHNYGS--VKLQVFDYDTFSADDIMGEAEIDIQPLITSAMAFGDPEM 312

CPU time:     0.20 user secs.       0.06 sys. secs          0.26 total secs.

  Database: Non-redundant GenBank CDS
```

```
  translations+PDB+SwissProt+SPupdate+PIR
    Posted date:  Dec 3, 1999  3:22 PM
  Number of letters in database: 132,989,671
  Number of sequences in database:  432,960

Lambda     K      H
   0.319    0.141    0.434

Gapped
Lambda     K      H
   0.270   0.0470    0.230


Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 42146255
Number of Sequences: 432960
Number of extensions: 1763079
Number of successful extensions: 2980
Number of sequences better than 10.0: 157
Number of HSP's better than 10.0 without gapping: 15
Number of HSP's successfully gapped in prelim test: 142
Number of HSP's that attempted gapping in prelim test: 2875
Number of HSP's gapped (non-prelim): 198
length of query: 150
length of database: 132,989,671
effective HSP length: 48
effective length of query: 102
effective length of database: 112,207,591
effective search space: 11445174282
effective search space used: 11445174282
T: 11
A: 40
X1: 16 ( 7.4 bits)
X2: 38 (14.8 bits)
X3: 64 (24.9 bits)
S1: 41 (21.7 bits)
S2: 66 (30.1 bits)
```

Analysis Coffee Break

Viruses provide direction on the plant information superhighway

The newly discovered plant movement protein, CmPP16, contains a C2 calcium-binding motif



```
                      L i ii A  L    c         ps  DPYV i   s  +   o+i c
squash CmPP16      5 MEVHLISGKGLQAH-D------PLNKPI-DPYAEINFKGQER--MSKVAK-NA--GP
rice               6 LEVHLVDAKGLTGN-D------FLGKI--DPYVVVQYRSQER--KSSVARDQ---GK
tomato CLB1      263 LTVTIVKANGLKNH-E------MIGKS--DPYAVVHIRPLFK-VKTKTIDNNL----
arabidopsis      214 LKVTIKKGTNMAIR-D-------MMSS--DPYVVLTLGQQ-K-AQSTVVKSNL----
rat GLUT4        322 IRIHLLAARGLSSK-DKYVKGLIEGKS--DPYALVRVGTQTF--CSRVIDEEL----
mouse PI-PLC-δ1  630 LRVWIISGQQLPKVNK-------NKNSIVDPKVIVEIHGVGQDVASRQTAVITNNGF
human dysferlin    1 LRVFILYAENVHTP-D-------TDIS--DAYCSAVFAGVKK--RTKVIK-NSV---

                      NP W  E  F   i    sss     L i  V D      cc iG   is   ii
squash CmPP16         NPLWD-E-KFKF-LAEYPGSGGDFHILFK--VMDHDAIDGDDYIGDVKIDVKNLLA    50
rice                  NPSWN-E-VFKFQINSTAATGQHKLFLRLMDH-DT--FSRDDFLGEATINVTDLIS    56
tomato CLB1           NPVWDQT--FE-LIAEDKETQS----LFIE-VFDKDNIGQDQRMGVAKLPLNELVA   151
arabidopsis           NPVWNEE--LMLSVPHNYGSVK----LQVFDY-DT--FSADDIMGEAEIDIQPLIT    67
rat GLUT4             NPHWG-ETYEV-IV--HEVPGQ---EIEVE-VFDKD-PDKDDFLGRMKLDVGKVLQ   671
mouse PI-PLC-δ1       NPRWDTE--FEFVV----AVP--DLALVRFMVEDYDSSSKNDFIGQSTIPWNSLKQ    28
human dysferlin       NPVWN-E-GFEWDLKGI-PLDQGSE-LHVV-VKDHETMGRNRFLGEAKVPLREVLA  1987
```

The figure shows CmPP16 from the squash *Cucurbita maxima* aligned with a selection of similar proteins found using PSI-BLAST. In spite of extensive searches using both CmPP16 and the viral movement protein as query sequences, the sequence relationship between CmPP16 and the viral movement protein could not be found.

This part of the sequence represents a C2 domain - a well-characterized calcium-binding motif found in a variety of proteins, including phospholipases and protein kinases C. C2 domains are thought to bind phospholipids, inositol polyphosphates, and intracellular proteins, and are implicated in signal transduction and membrane trafficking.

Residues in red indicate amino acids that are conserved in 50% of known C2 domains [3], while those in dark yellow indicate amino acids with similar properties: c, charged; +, positively charged; p, polar; s, small; h, hydorphobic; i, aliphatic. The consensus sequence for this set of C2 domains is shown above the alignment. The numbers at each end of the alignment represent the number of amino acids not shown. Clicking on the protein/organism name will display the GenBank record for that sequence.

Some of the proteins included in this alignment are from other plants: an immediate-early gene elicited by a fungus from rice, a gene that is preferentially expressed in fruit tissues from tomato, and a putative protein from the model organism *Arabidopsis,* generated by the large-scale genome sequencing effort. Also included are: the GLUT4 glucose transporter from the rat, phospholipase C delta-1, an enzyme involved in signaling, from the mouse, and human dysferlin, a skeletal muscle gene that is mutated in two types of muscular dystrophy. The presence of a C2 domain in *Cucurbita maxima* CmPP16 suggests that binding phospholipids or intracellular proteins may be important for its RNA-chaperoning function. However, it remains to be experimantally shown that this is the case.

Back to BLAST result

CmPP16 was used to search the non-redundant protein sequence database using the PSI-BLAST program [4] with standard parameters. Sequences included in the multiple alignment of the C2 domain were selected from output from the first iteration. The multiple sequence alignment was constructed using ClustalW [5].

[1] Xoconostle-Cazares, B. et al. (1999) Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science* 283, 94-98
[2] Lazarowitz, S. G. (1999) Probing plant cell structure and function with viral movement proteins. *Curr. Opin. Plant Biol.* 2, 332-338
[3]Schultz, J., Milpetz, F., Bork, P. and Ponting, C.P. (1998) SMART, a simple modular architecture research tool: Identification of signalling domains. *Proc. Natl. Acad. Sci. USA* 95, 5857-5864
[4] Altschul, S. F. et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389-402
[5] Higgins, D. G., Thompson, J. D. and Gibson, T. J. (1996) Using CLUSTAL for multiple sequence alignments. *Methods Enzymol.* 266, 383-402