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Molecular cloning and structural analysis of genes from *Zea mays* (L.) coding for members of the *ras*-related *ypt* gene family

(small guanine nucleotide binding protein/*ras* gene superfamily)

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ABSTRACT **We have isolated, cloned, and characterized two cDNAs from *Zea mays* (L.), denoted yptm1 and yptm2, encoding proteins related to the ypt protein family. Amino acid similarity scores with YPT1 from yeast and ypt from mouse are in the range of 70% for yptm1 and 74% for yptm2, respectively, whereas similarities with p21 ras and other ras-related proteins are <40%. Most amino acid residues showing identity are clustered in the GTP/GDP binding domain. In addition, two cysteine residues close to the C-terminal ends, known to be palmitoylated and necessary for membrane binding in all eukaryotic ras-related proteins that have been characterized so far, are conserved in the maize genes as well. Northern blot hybridization analysis of poly(A)$^+$ mRNA from etiolated maize coleoptiles revealed single mRNA species of approximately the same size as the isolated cDNAs. The gene for yptm1 is expressed at very low levels in maize coleoptiles and tissue culture cells. The gene for yptm2 is expressed at higher levels and is differentially represented in RNAs isolated from various organs of maize plants, with its highest level in leaves and flowers. The structural similarity of the genes identified suggests that they could be involved in the control of secretory processes.**

It has been established in animal and fungal cells that guanine nucleotide-binding regulatory (G) proteins exchange bound GDP for GTP thus initiating interaction with the corresponding effector protein(s). The intrinsic GTPase activity then hydrolyzes the bound GTP to GDP to return the complex to the resting state (1). All *ras* or *ras*-related genes code for small G proteins with molecular weights of 20,000–28,000 that share structural and biochemical homologies (2). In the past few years >20 small G proteins, including members encoded by the *ras* oncogene family, have been characterized by molecular cloning, but little is known of their specific functions (3–10). Although the products of the *ras* oncogenes are implicated in regulation of mammalian cell proliferation, other members of this family may have roles in vectorial membrane traffic (11). It was found that GTP analogs affect intra-Golgi vesicle transport and regulated secretion (12, 13). Moreover, mutations in the yeast genes encoding the ypt1 and SEC4 protein disrupt membrane traffic within the Golgi complex (14, 15) or from the Golgi complex to the plasma membrane (6, 16). The *YPT1* gene product, besides sharing GTP-binding and activation properties with other members of the *ras* gene family, plays an essential role in both mitotic and meiotic stages of the *Saccharomyces cerevisiae* life cycle (17). Expression of *YPT1* mutant alleles results in a dominant lethal phenotype with defects in microtubule organization and function (18). Recent evidence suggests that the *YPT1* gene product could be involved in Ca^{2+} metabolism (14). Analysis of cell-free protein transport has further indicated that the ypt1 protein is an essential component for the vesicular transport of proteins from the endoplasmic reticulum to the Golgi complex (19).

Due to their fundamental role in controlling basic cellular functions, it is expected that members of this ubiquitous gene family may also play an important role in plant growth control. In the present study we have searched for *ras*-related genes in plants. We describe the structural and molecular analysis of cDNA clones‡ from *Zea mays* encoding ras-related proteins that are highly homologous to the ypt proteins.

MATERIALS AND METHODS

Construction and Oligonucleotide Screening of a Maize cDNA Library. A maize coleoptile-specific cDNA library was prepared and screened with oligonucleotides as described (20, 21). The oligonucleotide sequences were 5′-ATTTTAGATACTGCTGGTCAAGAGGAGTAT-3′ (oligonucleotide 1) and 5′-GTTCCTATGGTTGTGGTTGGTAACAAATGTGATCTT-3′ (oligonucleotide 2). Oligonucleotides were synthesized on an Applied Biosystems synthesizer, deprotected, and purified by gel electrophoresis. End-labeling was done with T4 DNA polynucleotide kinase and [γ-^{32}P]ATP. Each probe (1×10^6 cpm/ml, 1 pmol/ml) was added to two sets of double replicate filters and hybridized for 24 h at 42°C, respectively. Melting temperature [$4 \times (G + C) + 2 \times (A + T)$] was calculated (22) and was 46°C and 60°C for oligonucleotides 1 and 2, respectively. Each filter was washed in a solution containing 900 mM NaCl, 90 mM sodium citrate, and 0.5% Nonidet P-40 for three 5-min periods at room temperature, for two 10-min periods at 37°C, and for one 15-min period at each of the following temperatures: 42°C, 45°C, 48°C, and 51°C. The filters were subjected to autoradiography for 24 h. Phage from plaques yielding positive autoradiographic signals with both probes were cloned.

DNA Sequence Analysis. DNA sequence analysis was carried out by the dideoxynucleotide chain-termination method. The sequence alignment was calculated using software described by Vingron and Argos (23) and improved manually. From the distance data, a phylogenetic tree was calculated using the program KITSCH from the PHYLIP package by Felsenstein (24). The tree shown is based on the one calculated by the program but redrawn by hand to scale to the correct homologies between the sequences.

RNA Blot Hybridization. RNA was fractionated in formaldehyde-containing agarose gels, transferred to nylon (Hybond N, Amersham), hybridized for 48 h at 42°C to a nick-translated denatured probe (10^8 cpm/μg), and analyzed by standard procedures (21).

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Abbreviations: G protein, guanine nucleotide-binding regulatory protein; nt, nucleotide(s).

‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. X63277 for yptm1 and X63278 for yptm2).

RESULTS

Identification of *YPT1*-Related cDNA Clones. Direct sequence comparison of members of the *ras* gene superfamily by multiple alignments of amino acids of the GTP-binding domain is presented in Fig. 1. Comparison of amino acid sequences representing the GTP-binding domain point to three consensus elements, GxxxxGKSsxl, DTAGQE, and lxgNKxDL, where lowercase letters are variable amino acids. The first two domains are involved in binding the phosphate moiety of the GTP, whereas elements located further downstream are involved in determining guanine nucleotide specificity (25, 26). To identify related members of the *ras* gene superfamily in plants, oligonucleotides corresponding to amino acids 62–70 and 114–125 were used to screen 200,000 phages. We isolated several clones giving

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1    ---------KLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYR-KQVVIDGETCLLD  Ha-ras
1    ---------KIVVVGGGGVGKSALTIQFIQSYFVDEYDPTIEDSYR-KQVVIDDKVSILD  RAS1
1    ---------KLVVVGGGGVGKSALTIQLTQSHFVDEYDPTIEDSYR-KQVVIDDEVSILD  RAS2
1    ---------KLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENY-VADIEVDGKQVELA  rho
1    ---------KLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTIKLQ  rab1
1    ---------KYIIIGDTGVGKSCLLLQFTDKRFQPVHDLTMGVEFGARMITIDGKQIKLQ  rab2
1    ---------KILIIGNSSVGKTSFLFRYADDSFTPAFVSTVGIDFKVKTIYRNDKRIKLQ  rab3a
1    ---------KLLIIGNSSVGKTSFLLRYADDTFTPAFVSTVGIDFKVKTVYRHEKRVKLQ  rab3b
1    ---------KFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGQKIINVGGKYVKLQ  rab4
1    ---------KLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTVCLDDTTVKFE  rab5
1    ---------KLVFLGEQSVGKTSLITRFMYDSFDNTYQATIGIDFLSKTMYLEDRTVRLQ  rab6
1    ---------KILLIGDSGVGKSCLLVRFVEDKFNPSFITTIGIDFKIKTVDINGKKVKLQ  SEC4
1    ---------KLLLIGNSGVGKSCLLLRFSDDTYTNDYISTIGVDFKIKTVELDGKTVKLQ  YPT1

1    MSNEFDYLFKLLLIGDSSVGKSCFLLRFADDSYVDSYISTIGVDFKIRTVEVEGKTVKLQ  YPTm1
1    MNPEYDYLFKLLLIGDSGVGKSCLLLRFADDSYLDSYISTIGVDFKIRTVEQDGKTIKLQ  YPTm2


51   ILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQ-YREQIKRVKDSDDVPMVL  Ha-ras
51   ILDTAGQEEYSAMREQYMRTGEGFLLVYSVTSRNSFDELLS-YYQQIQRVKDSDYIPVVV  RAS1
51   ILDTAGQEEYSAMREQYMRNGEGFLLVYSITSKSSLDELMT-YYQQILRVKDTDYVPIVV  RAS2
51   LWDTAGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVRHF--CPNVPIIL  rho
52   IWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQ-WLQEIDRYA-SENVNKLL  rab1
52   IWDTAGQESFRSITRSYYRGAAGALLVYDITRRDTFNHLTT-WLEDARQHS-NSNMVIML  rab2
52   IWDTAGQERYRTITTAYYRGAMGFILMYDITNEESFNAVQD-WSTQIKTYS-WDNAQVLL  rab3a
52   IWDTAGQERYRTITTAYYRGAMGFILMYDITNEESFNAVQD-WATQIKTYS-WDNAQVIL  rab3b
52   IWDTAGQERFRSVTTSYYRGAAGALLVYDITSRETYNALTN-WLTDARMLA-SQNIVIIL  rab4
52   IWDTAGQEGYHSLAPMYYRGAQAAIVVYDITNEESFARAKN-WVKELQRQA-SPNIVIAL  rab5
52   LWDTAGQERFRSLIPSYIRDSTVAVVVYDITNVNSFQQTTK-WIDDVRTER-GSDVIIML  rab6
52   LWDTAGQERFRTITTAYYRGAMGIILVYDVTDERTFTNIKQ-WFKTVNEHA-NDEAQLLL  SEC4
52   IWDTAGQERFRTITSSYYRGSHGIIIVYDVTDQESFNGVKM-WLQEIDRYA-TSTVLKLL  YPT1

61   IWDTAGQERFRTITSSYYRGAHGIIIVYDITDMESFNNVKQ-WLDEIDRYAN-DSVRKLL  YPTm1
61   IWDTAGQERFRTITSSYYRGAHGIIIVYDVTDQESFNNVKQ-WLNEIDRYAS-DNVNKLL  YPTm2


110  VGNKCDLAART------------VESRQAQDLARSYGI-PYIETSAKTRQGVEDAFYTL  Ha-ras
110  VGNKLDLENERQ-----------VSYEDGLRLAKQLNA-PFLETSAKQAINVDEAFYSL  RAS1
110  VGNKSDLENEKQ-----------VSYQDGLNMAKQMNA-PFLETSAKQAINVEEAFYTL  RAS2
109  VGNKKDLRNDESTKRELMKMKQEPVRPEDGRAMAEKINAYSYLECSAKTKEGVRDVFETA  rho
110  VGNKCDLTTKKV-----------VDYTTAKEFADSLGI-PFLETSAKNEKNVEQSFMTM  rab1
110  IGNKSDLESRRE-----------VKKEEGEAFAREHGL-IFMETSAKTASNVEEAFINT  rab2
110  VGNKCDMEDERV-----------VSSERGRQLADHLGF-EFFEASAKDNINVKQTFERL  rab3a
110  VGNKCDMEEERV-----------VPTEKGQLLAEQLGF-DFFEASAKENISVRQAFERL  rab3b
110  CGNKKDLDADRE-----------VTFLEASRFAQENEL-MFLETSALTGENVEEAFMQC  rab4
110  SGNKADLANKRA-----------VDFQEAQSYADDNSL-LFMETSAKTSMNVNEIFMAI  rab5
110  VGNKTDLADKRQ-----------VSIEEGERKAKELNV-MFIETSAKAGYNVKQLFRRV  rab6
110  VGNKSD-METRV-----------VTADQGEALAKELGI-PFIESSAKNDDNVNEIFFTL  SEC4
110  VGNKCDLKDKRV-----------VEYDVAKEFADANKM-PFLETSALDSTNVEDAFLTM  YPT1

119  VGNKCDLAENRA-----------VDTSVAQAYAQEVGI-PFLETSAKESINVEEAFLAM  YPTm1
119  VGNKSDLTANKV-----------VATETAKAFADEMGI-PFMETSAKNATNVQQAFMAM  YPTm2


156  VREIRQHKLRKLNPPDESG----------------------PGCMSCKCVLS--------  Ha-ras
157  IRFYSLIRLVRDDGGKYNSMNRQLDNTNE..(107aa)..RKESSGGCCIIC--------  RAS1
157  ARFYTLARLVRDEGGKYNKTLTENDNSKQ..(120aa)..SKSGSGGCCIIS--------  RAS2
169  TRFETATRAALQVKKKKK-------------------------GGCVVL---------  rho
157  AAEIKKRMGPGATAGGAEKSNVKI-----------QSTPVKQSGGGCC-----------  rab1
157  AKEIYEKIQEGVFDINNEANGIKIGPQHAATNASHGGNQGGQQAGGGCC-----------  rab2
157  VDVICEKMSESLDTADPAVTGAKQGPQLTDQQAPPHQD----------CAC---------  rab3a
157  VDAICDKMSDSLDTDPSMLGSSKNTRLSDTPPLLQQN----------CSC----------  rab3b
157  ARKILNKIESGELDPERMGSGIQYGDAALRQLRSPRRTQAPSAQE--CGC----------  rab4
157  AKKLPKNEPQNPGANSARGGGVDLTEPTQPTRNQ-------------CCSN---------  rab5
157  AAALPGMESTQDRSREDMIDIKLEKPQEQPVSE-----------GGCSC----------  rab6
156  AKLIQEKIDSNKLVGVGNGKEGNISINSGSGNSSK----------SNCC----------  SEC4
157  ARQIKQSMSQQNLNETTQKKEDKGNVNLKGQSLTNT--------GGGCC----------  YPT1

166  SAAIKKSKAGSQAALERKPSNVVQMKGRPIQQEQQK--------SSRCCST---------  YPTm1
166  AASIKDRMASQPAAANARPATVQIR-GQPVNQKTS-----------CCSS---------  YPTm2
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FIG. 1. Alignment of the amino acid sequences of the conserved GTP-binding domain of ras protein family. All amino acid sequences are from the PIR or the EMBL sequence data bases. Dashes indicate gaps introduced for optimal alignment.

strong hybridization signals. Two of the clones isolated were chosen for further analysis. Both clones, yptm1 and yptm2, were subcloned into pUC118 and the DNA sequences of both strands were determined. The DNA sequences for both clones are shown in Figs. 2 and 3. Nucleotide sequence analysis of yptm1 gave a 624-nucleotide (nt) open reading frame, with 192 nt in the 5′ flanking and 144 nt in the 3′ untranslated region followed by 33 adenosine residues (Fig. 2). Similarly, nucleotide sequence analysis of yptm2 demonstrated a 609-nt open reading frame, with 116 nt in the 5′ flanking and 257 nt in the 3′ untranslated region followed by 82 adenosine residues (Fig. 3). The 5′ untranslated regions are G + C-rich (55% and 63%, respectively). The nucleotide sequences surrounding the initiator codons, 5′-cGcaATGa and 5′-cGacATGa, respectively, are consistent with the proposed eukaryotic translation initiation consensus sequence of (A/G)NNATGg, where lowercase letters are variable amino acids (27, 28).

Translation terminates at a TGA (position 817, Fig. 2) or TAA (position 727, Fig. 3) stop codon. No canonical polyadenylylation signals typical for other eukaryotic genes are present in either cDNA (29, 30). The motifs AAAAT, located 39 and 133 nt upstream of the poly(A) tail, are possible polyadenylylation signals. Several sequence motifs have been shown to play a role in the 3′ processing of mRNA (31, 32). The consensus sequence YGTGTTYY (where Y is a pyrimidine) is located ≈30 base pairs 3′ of the polyadenylylation signal in most mammalian genes analyzed (33). Good homology to this consensus sequence is found for yptm1 87 base pairs (TGTGTTTC, position 876) and for yptm2 23 base pairs (TGTGAACA, position 961) downstream from the polyadenylylation signal.

Structural Analysis of the yptm-Encoded Proteins. The amino acid sequences deduced from the DNA sequences for yptm1 and yptm2 are shown in Figs. 2 and 3. Assignment of the initiator codons is based on DNA sequence analysis. The predicted proteins have 208 and 203 amino acids and calculated molecular weights of 23,283 and 22,475, respectively. We compared the derived amino acid sequences with se-

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1 GCTCAGCTGTGTTTCGACCTGCGTGCGTGCTTGAGAGAGAGGCGACTTCCCGGAGTTTAG 60

  CATCTTCTTCTCTCTCAGTCTCACCCTGTCCCTCGCGAAAGATCTCCATACTTCCCTCCT 120

  CTCTTCCCGCACTGTTCTCGGAATCTCTCTTCTACTCCGCGCTGTTCTTGGGATCGAAAG 180

  GTGGGAAGCGCAATGAGCAACGAGTTCGATTACCTGTTCAAGCTTCTCCTGATCGGCGAC 240
                M  S  N  E  F  D  Y  L  F  K  L  L  L  I  G  D
                1

  TCCTCGGTGGGCAAGTCCTGCTTCCTCCTCCGCTTCGCTGACGACTCCTACGTGGACAGC 300
   S  S  V  G  K  S  C  F  L  L  R  F  A  D  D  S  Y  V  D  S
                           25

  TACATCAGCACGATCGGCGTCGACTTTAAAATCCGCACGGTCGAGGTGGAGGGCAAGACC 360
   Y  I  S  T  I  G  V  D  F  K  I  R  T  V  E  V  E  G  K  T
                                          50

  GTAAAGCTGCAGATTTGGGACACAGCAGGGCAGGAGCGGTTCAGGACCATCACGAGCAGC 420
   V  K  L  Q  I  W  D  T  A  G  Q  E  R  F  R  T  I  T  S  S
                                                      75

  TACTACAGAGGAGCTCACGGGATAATTATTGTTTATGACATCACGGACATGGAGAGCTTC 480
   Y  Y  R  G  A  H  G  I  I  I  V  Y  D  I  T  D  M  E  S  F

  AACAACGTGAAGCAGTGGCTTGACGAGATCGACCGATACGCCAACGACAGCGTGCGCAAG 540
   N  N  V  K  Q  W  L  D  E  I  D  R  Y  A  N  D  S  V  R  K
            100

  CTTCTTGTTGGTAACAAATGTGATCTGGCCGAGAACAGGGCTGTCGATACTTCAGTAGCA 600
   L  L  V  G  N  K  C  D  L  A  E  N  R  A  V  D  T  S  V  A
                           125

  CAGGCTTACGCTCAAGAGGTAGGCATCCCGTTCCTCGAAACGAGCGCTAAGGAGTCGATC 660
   Q  A  Y  A  Q  E  V  G  I  P  F  L  E  T  S  A  K  E  S  I
                                          150

  AACGTCGAGGAGGCGTTCTTGGCAATGTCTGCTGCAATTAAGAAAAGTAAAGCAGGGAGT 720
   N  V  E  E  A  F  L  A  M  S  A  A  I  K  K  S  K  A  G  S
                                                      175

  CAGGCAGCCCTGGAGAGGAAGCCCTCCAATGTAGTTCAGATGAAAGGGCGGCCGATCCAG 780
   Q  A  A  L  E  R  K  P  S  N  V  V  Q  M  K  G  R  P  I  Q

  CAAGAGCAGCAGAAGAGTAGTAGATGCTGTTCAACATGAGGCACAGCAATGTCTGTCTTT 840
   Q  E  Q  Q  K  S  S  R  C  C  S  T  *
            200                    208

  GGAGAATCAGTAAAATGATTATCTGCTAGACTAGATGTGTTTCTCTGTCTTCGTTTGTTT 900

  TGAGACACATATATATAGTTGCGAAAATGCGGTGGTGAGAAACTCGAAATGAATCTGAGC 960

  CAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 996
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FIG. 2. Nucleotide and deduced amino acid sequence of a cDNA encoding yptm1 protein. Numbering of the nucleotides progresses positively in the 5′ → 3′ direction beginning with nt 1 of the initiator codon.

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GCTCTCGCTCTCTCGTCCCCCTCAGATCCACCGACTCGCTCTGAGTCTCTGACCGCCCCT 60

TCCGTCGTCCCCGTTCGAAGGCGCGGCAAGCAGCGATTCAGCCAGATTCCATTCGACATG 120
                                                          M
                                                          1

AATCCCGAGTACGACTACCTTTTCAAACTTCTGCTTATTGGTGATTCTGGTGTTGGGAAA 180
 N  P  E  Y  D  Y  L  F  K  L  L  L  I  G  D  S  G  V  G  K

TCATGCTTGCTTCTCAGATTTGCGGATGATTCATATTTGGACAGCTACATCAGCACAATT 240
 S  C  L  L  L  R  F  A  D  D  S  Y  L  D  S  Y  I  S  T  I
          25

GGGGTTGATTTTAAAATTCGGACAGTAGAGCAAGATGGGAAGACCATAAAACTTCAAATT 300
 G  V  D  F  K  I  R  T  V  E  Q  D  G  K  T  I  K  L  Q  I
                   50

TGGGATACTGCTGGGCAAGAGCGCTTCAGGACCATCACTAGCAGCTACTACCGTGGAGCT 360
 W  D  T  A  G  Q  E  R  F  R  T  I  T  S  S  Y  Y  R  G  A
                                  75

CATGGAATCATTATTGTATATGACGTGACAGACCAAGAAAGCTTCAATAATGTGAAGCAA 420
 H  G  I  I  I  V  Y  D  V  T  D  Q  E  S  F  N  N  V  K  Q
                                                   100

TGGTTAAATGAAATTGATCGTTATGCAAGTGACAATGTTAACAAGCTCCTTGTTGGGAAC 480
 W  L  N  E  I  D  R  Y  A  S  D  N  V  N  K  L  L  V  G  N

AAGAGCGACCTAACTGCCAACAAAGTTGTGGCAACTGAGACAGCAAAGGCATTTGCTGAT 540
 K  S  D  L  T  A  N  K  V  V  A  T  E  T  A  K  A  F  A  D
          125

GAGATGGGCATCCCGTTCATGGAGACGAGTGCCAAAAACGCCACCAACGTCCAGCAGGCC 600
 E  M  G  I  P  F  M  E  T  S  A  K  N  A  T  N  V  Q  Q  A
                   150

TTCATGGCTATGGCTGCATCCATCAAGGACAGGATGGCCAGCCAACCAGCCGCGGCCAAC 660
 F  M  A  M  A  A  S  I  K  D  R  M  A  S  Q  P  A  A  A  N
                                  175

GCAAGGCCAGCGACGGTGCAGATCCGCGGGCAACCCGTCAACCAGAAGACGTCTTGCTGC 720
 A  R  P  A  T  V  Q  I  R  G  Q  P  V  N  Q  K  T  S  C  C
                                                   200

TCGTCTTAAGCTTGCCTGATTTCTCTGGTTCCTTGTGACTATTACTACCGTTTGTGCGAG 780
 S  S  *
   203

CATGCAAATGTATTTGTTGCATTATTGCTGATAGCATCTGTTCGTTGGCCAGATGAGTAG 840

AGAGCTGAAAAAATGTTAAGAACACCATAGAGAGAAGTCTATCCGTGCTCTCTCAACTTA 900

AGGAGCAAACAGCAAGACTTCATTTTTTTCTACTTTACTGTTTATTAGTCGCGTTACATT 960

TGTGAACAGTTATTGTAATGTCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1020

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1065
```

FIG. 3. Nucleotide and deduced amino acid sequence of a cDNA encoding yptm2 protein. Numbering of the nucleotides progresses positively in the 5′ → 3′ direction beginning with nt 1 of the initiator codon.

quences of other ras-related protein sequences deposited in data banks. A comparative analysis showed an unexpectedly strong similarity of the maize ypt proteins to the *ras* gene superfamily. The yptm2 protein shows a higher level of similarity to the mouse ypt protein or rab1 protein than to the yeast protein. Further inspection of the homologous region at the protein sequence level confirmed that the homology is confined mainly to the GTP-binding domain. At the C terminus, the predicted ypt proteins contain two cysteine residues followed by hydroxylated amino acids. A similar sequence, containing two cysteine residues, one of which is needed for posttranslational lipid binding and subsequent membrane anchoring, is found at the C terminus of all members of the *ras* gene superfamily (34, 35). To obtain an evolutionary relationship of ras-related proteins, a multiple sequence alignment was prepared based on distance measurements (23). The tree in Fig. 4 shows that rab1 from rat, ypt from yeast, and ypt from maize form a group of proteins within the ras family distinct from other rab or ras proteins. As can be seen from the alignment in Fig. 1, the regions that are likely to be involved in GDP/GTP binding are highly conserved whereas in other positions conservation only within particular subgroupings can be seen. Thus alignment within this tree is according to function and not to species grouping.

Northern Blot Analysis. By using yptm1 cDNA as a probe, a weak 1-kilobase signal was detected in poly(A)$^+$ mRNA from maize coleoptiles (data not shown). A predominant transcript of ≈1 kilobase was detected in Northern blot analysis of poly(A)$^+$ mRNA from coleoptiles using yptm2

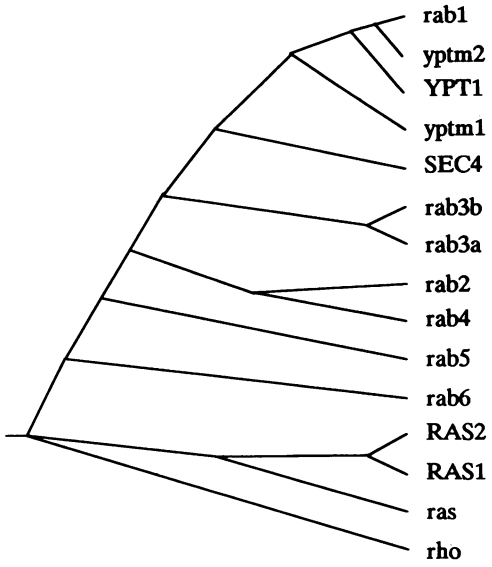

FIG. 4. Unrooted phylogenetic tree of ras-related amino acid sequences.

cDNA as a probe (Fig. 5). To analyze organ-specific expression of the gene for yptm2, total RNAs from various maize organs were separated on an agarose gel, blotted onto nitrocellulose, and hybridized to a ^{32}P-labeled yptm2 probe. Levels of yptm2 transcripts were quantified by laser scanning densitometry and normalized to the relative level of hybridization to 18S rRNA. The level of expression is low in stems. Intermediate levels of expression are observed in roots, leaves, callus suspension culture cells, and coleoptiles, from 3.7- to 6.2-fold in comparison to stems. We find highest expression of the gene for yptm2 in flower tissues (10-fold in comparison to stems). We also analyzed the expression of *ypt*-related genes in other plant species. However, as shown in Fig. 5 (lanes f, g, and h), we were not able to detect hybridizing signals with yptm2 cDNA as a probe in total RNA from various other plants.

DISCUSSION

Here we have used an oligonucleotide strategy to search for members of the *ras* superfamily in plants. We were able to

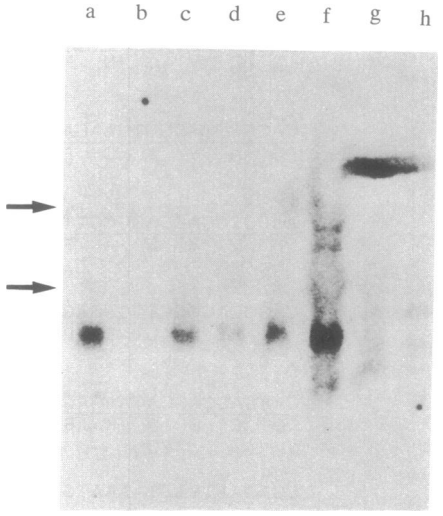

FIG. 5. Organ-specific expression of yptm2 mRNA. Total RNA was fractionated in a formaldehyde gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled yptm2 cDNA. Lanes: a, tassel; b, stem; c, root; d, coleoptile; e, callus suspension culture; f, *Arabidopsis thaliana*; g, *Nicotiana tabacum*; h, *Datura innoxia*.

isolate several cDNAs from a maize coleoptile-specific cDNA library coding for members of the ras-related ypt protein family. Similarity to the Ha-ras protein is 35% and restricted to amino acids in the GTP-binding domain. The homologies are localized in four main blocks corresponding to amino acid residues 18–21, 62–68, 121–124, and 151–153 (using the numbering of the yptm proteins as a reference), which have all been implicated in the binding of GDP/GTP (26). The spacing of regions of highest identity and hydrophobicity demonstrate strong functional similarity for GTP binding and hydrolysis within this family. Amino acids 37–48, which are probably located within the flexible effector loop, are thought to mediate interactions with corresponding effector proteins (26). It is therefore not surprising that precisely these amino acids are very different in yptm proteins from those of ras proteins. The sequence from amino acid 166 to the C terminus diverges considerably within the whole ras family. The only amino acids strictly conserved within this domain are Cys-206/201 and Cys-200/201. In all ras and ras-related proteins, these cysteines are needed for palmitic acid binding and subsequent membrane anchoring. The conservation of these amino acids in the cDNAs isolated here argues that the corresponding plant proteins are localized in the membrane. In contrast to the ras proteins, no basic amino acids are found immediately on the N-terminal side of the cysteine. Moreover, downstream we find, in contrast to other members of the ras family, mostly hydrophilic amino acids. These differences may indicate variations in the specificity of plant acylases catalyzing the addition of the membrane anchor.

The identification of a series of *ypt*-related genes from plants raises the question of their function. In eukaryotes ypt proteins seem to serve apparently basic cellular functions and it is now becoming clear that, at specific steps in the secretory pathway, G proteins regulate the traffic of proteins in membrane vesicles. Genes encoding GTP-binding proteins involved in regulation of transport between early compartments of the secretory pathway have been cloned from yeast and from mammalian cells (36, 37). Members of this rapidly growing gene family of small GTP-binding proteins may be key elements in the regulation of vesicular transport and delivery of proteins to the cell surface.

The identification of ras-related proteins in maize, which apparently have homologues in other plants as well, is of particular interest. Most plant tissues respond to growth stimuli, such as auxins, by cell elongation. Active expansion of membranes requires regulated secretion and correct targeting of secretory vesicles. Thus, in plant cells, GTP hydrolysis could trigger the vectorial flow of membrane material through a metabolic cascade to the surface. This may explain why we find members of the *ypt* gene family expressed in elongating maize coleoptile cells.

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