Arbuscular mycorrhizal fungi (AMF) are important biotrophic organisms, which live in symbiosis with ~80% of land plants, forming a mycorrhiza (i.e., a root colonized by a symbiotic fungus). AMF affect plant biodiversity, as well as the variability and productivity of ecosystems (1, 2). As few as ~150 fungal species are known to form arbuscular mycorrhiza (AM) in the roots of a vast number of plant species. No evidence for recombination has been found in the fungi, suggesting that they reproduce clonally and have been asexual for the entire period of their association with plants (3). Recently, AMF were placed into a new monophyletic group, the phylum *Glomeromycota*, which probably originated from the same ancestral group as the *Ascomycota* and *Basidiomycota* (4) ~1,400–1,200 million years ago and is much older than the earliest land plants, which appeared ~800 million years ago and whose primitive root systems were associated with ancestral AMF. AMF may, thus, have played a crucial role in facilitating the colonization of land by plants (5–8). It is also assumed that the ancient signaling pathways evolved in AM symbiosis were recruited subsequently for the establishment of the evolutionary younger legume-Rhizobia nodulation symbiosis (9). Today, despite the large number of plant species forming AM associations worldwide, only two major morphological types have been defined: the *Arum* and *Paris* types, respectively. In *Arum*-type mycorrhizae, fungal hyphae spread between cortical cells and form short-lived, heavily branched symbiotic structures (arbuscules) within cells. In *Paris*-type mycorrhizae, cortical cells are colonized by intracellularly growing thick coiled hyphae, which occasionally form fine arbuscule-like ramifications (10).

**Materials and Methods**


**Plant Growth Conditions.** Stock cultures of wild-type and transgenic potato plants were maintained in tissue culture by using MS medium (15) supplemented with 2% sucrose. In pot culture, inoculation of potato plants with all AMF isolates except *G. margarita* was performed as described (16). For *G. margarita*, potato plantlets were cocultivated with sunflower, which served as nurse plants. Each pot, containing the potato–sunflower combination, was inoculated with 15–20 spores of *G. margarita*. Composite plants of *L. japonicus* and *M. truncatula* (see below) were introduced to already-established mycorrhizae of *G. mosseae* on *P. lanceolata*, growing in a soil–quartz sand mixture. Plants were harvested 2–6 weeks after inoculation. Freshly collected potato roots were frozen in liquid N₂ and stored at ~80°C for subsequent RNA isolation, or they were immediately stained for β-glucuronidase (GUS) activity and for visualization of intraradical fungal mycelium (17).

**RT-PCR.** RT-PCR was performed as described (18) by using gene-specific primers, as described in Supporting Methods, which is published as supporting information on the PNAS web site. GenBank accession nos. for StPT3, Sipt1, MtPT4, MtPT1, and PHT2;1 are AJ318822, AF156695, AY116210, AF000354, and AF533081, respectively.

**Plant Hairy-Root Cultures.** Agrobacterium rhizogenes were transformed with pBin19 carrying *StPT3* promoter-GUS (16), MtPT4...
promoter-GUS, OsPT11 promoter-GUS, and StPT3 promoter-
Fluorescent Timer chimeric genes, respectively, by means of
electroporation. To initiate hairroot formation, plant tissues
were inoculated with overnight cultures of *A. rhizogenes* grown
on solid YEB medium. Potato and carrot hairy roots were
generated as described (19) by using tubers and tap roots,
respectively. Sterile potato plantlets were also used for the
formation of hairy roots. The apical part of the plantlet was
excised and the sectioned stem surface was inoculated with *A.
rhizogenes*. Inoculation of *L. japonicus* and *M. truncatula* sec-
tioned seedlings with *A. rhizogenes* as described (20)
allowed generation of vigorous composite plants consisting of
wild-type shoots and hairy roots harboring appropriate con-
structs, which developed from the inoculation site. All inocu-
lated plant material was kept in a growth chamber at low light
at 23°C until hairy roots appeared. Formed hairy roots were
transferred aseptically to nutrient medium, containing 100 mg/
liter ampicillin. We selected 10–15 independently transformed
hair root of each plant and inoculated them with AMF (see
below). Mycorrhizal hairy roots exhibiting strong reporter gene
expression were selected, amplified, and used for further
experiments.

**Monoxicenix Cultures.** Monoxicenix cultures of various fungal isolates
on plant hairy roots were established as described for AMF (13,
21). Medium for cultivation of potato hairy roots was supplied
with 3.6 mg/liter KH₂PO₄ and 7.5 g/liter sucrose, whereas carrot
hairy roots were grown on 4.8 mg/liter KH₂PO₄ and 10 g/liter
sucrose. Media were solidified with 0.4% (wt/vol) sucrose. We
selected 10–15 independently transformed hairy roots of each plant
and inoculated them with AMF (see below). Mycorrhizal hairy roots
exhibiting strong reporter gene expression were selected, amplified,
and used for further experiments.

**Histochemical Analysis.** For GUS assays, fresh root material was
incubated in 0.1% Magenta-β-d-glucuronide cyclohexylammoni-
um salt (Biosynth, Basel) and 0.1% Triton X-100 in 0.05 M
sodium phosphate buffer (pH 7.2) either at room temperature
for 30 min to 1 h for plant roots colonized by AMF or at 37°C
overnight for all other root–fungus associations after vacuum
infiltration. To visualize fungal hyphae, the stained material was
transferred directly to 10% KOH (wt/vol) at 90°C for 1 h to clear
the tissues, acidified with 1% HCl (vol/vol), and then counter-
stained for 1–3 h at 90°C with 0.05 or 0.3% trypsin blue,
respectively, dissolved in lactoglycerol (lactic acid/glycerol/
water, 1:1:1 vol/vol/vol). Excess staining was removed by im-
mersing the samples in 50% glycerol. Colonized cortex cells were
separated carefully from roots. Stained root material was analyzed
by light microscopy.

**Confocal Microscopy.** Fluorescence in mycorrhizal plant roots was
visualized by using two confocal laser scanning microscopes: DM
IRBE and TCS SP (Leica, Deerfield, IL) or an LSM 510 META
(Zeiss) under oil with a ×20 or ×40 objective, respectively, at
excitation and emission wavelengths of 488 and 568, 488–530,
and 575–620 nm (for DM IRBE and TCS SP) or 488 and 543,
505–530, and 560–615 nm for LSM 510 META for green and red
fluorescence, respectively. The data were processed by using the
NT (Leica) or the LSM 510 META (Zeiss) software, respectively.
Images were assembled by using PHOTOSHOP 7.0 (Adobe Systems,
Mountain View, CA).

**Phylogenetic Footprinting.** The phylogenetic relationship between
the coding sequence of the Ph1 family genes StPT3 (AJ318822),
LePT1 (tomato; *Lycopersicon esculentum*; Avraham A. Levy,
Weizmann Institute of Sciences, Rehovot, Israel, personal com-
munication), MtPT4 (GenBank accession no. AY116211),
OsPT11 (GenBank accession no. AF536971), ARAth;Phl1;6
(At5g43340), and ARAth;Phl1;8 (GenBank accession no.
At1g20860) (*Arabidopsis thaliana*; ref. 23), and the AMF-
inducible glutathione S-transferase *MgSt1* (*M. truncatula*; Gen-
Bank accession nos. AY134608 and AY134609; ref. 24) was
analyzed by using FOOTPRINTER 2.0 Web serve (available at http://bio.cs.washing-
ton.edu/software.htm) (25), a computer algorithm designed to
identify conserved motifs in noncoding regions of orthologous
genomes from many species. Motifs were identified by using a motif
size of 8–10 with a maximal parsimony score of 0–2 in a
subregion of 1,000, allowing for motif losses. The promoter
sequences were then scanned manually for variations of the six
resulting motifs.

See Supporting Methods for further description of the methods
used in this study.

**Results and Discussion**

Several hundred-million years of coevolution of plants and AMF, the worldwide distribution of AMF, and the absence
of host specificity in plant–AMF interactions suggest that the
cascade of events leading to the establishment of the functional
AM symbiosis is conserved between modern, evolutionary dis-
tant plant species. To test this possibility, promoter sequences
from mycorrhiza-specific phosphate transporter genes from the
two eudicot species potato (*S. tuberosum, StPT3*) (16) and the
model legume *M. truncatula* (*MPT4*) (26), as well as from the
monocot rice (*Oryza sativa, OsPT11*) (27) were fused to the GUS
reporter gene and the constructs were introduced into hairy
roots of five different plant species via *A. rhizogenes*-mediated
root transformation. Subsequently, the roots were challenged

**Table 1. Induction of mycorrhiza-specific phosphate transporters in*
plant species of different taxonomic positions**

<table>
<thead>
<tr>
<th>Plants studied</th>
<th>Promoter region fused to GUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>StPT3</td>
</tr>
<tr>
<td><strong>Eudicotae</strong></td>
<td></td>
</tr>
<tr>
<td>Asteridae</td>
<td></td>
</tr>
<tr>
<td>Solanales</td>
<td></td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>+, G.m., P</td>
</tr>
<tr>
<td>Petunia hybrida</td>
<td>+, G.m., I</td>
</tr>
<tr>
<td>Apials</td>
<td></td>
</tr>
<tr>
<td>Daucus carota</td>
<td>+, G.L., P</td>
</tr>
<tr>
<td>Rosidae</td>
<td></td>
</tr>
<tr>
<td>Fabales</td>
<td></td>
</tr>
<tr>
<td>Lotus japonicus</td>
<td>+, G.mos., I</td>
</tr>
<tr>
<td>Medicago truncatula</td>
<td>+, G.mos., I</td>
</tr>
</tbody>
</table>

Potato (*S. tuberosum*) and carrot (*D. carota*) transgenic hairy roots. *L.
japonicus*, and *M. truncatula* composite plants with transgenic hairy roots
and transgenic petunia (*Petunia × hybrida*) were generated by means of agробac-
terial transformation with constructs carrying promoter regions of mycor-
rhiza-specific phosphate transporters from potato (*StPT3*; 1.72 Kb), *M. trunca-
tula* (*MPT4*; 0.87 Kb), and rice (*O. sativa*) (*OsPT11*; 0.8, 1.54, and 3.16 Kb)
fused to the GUS reporter gene. Roots were inoculated with one of three AMF
species: *G. m. gigaspora*; *G. i. Glomus intraradices*; and *G. m.*
*Glomus mosseae*. Mycorrhizal roots were stained for GUS activity. +, Positive
GUS staining in root zones colonized by AMF; −, absence of GUS staining.
P and I indicate formation of either Paris- or intermediate-type AM, re-
spectively.

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with three different species of AMF, and GUS expression was monitored (Table 1). In summary, promoters of eudicot species directed GUS expression in a mycorrhiza-specific manner in the eudicot host plants, whereas the promoter from the monocot rice was not regulated correspondingly in the eudicot plants (Table 1). Microscopic inspection revealed that GUS expression invariably colocalized to intraradical AMF mycelium (data not shown). These data suggest that signal perception and the intracellular signal transduction pathway mediating the mycorrhiza-specific regulation of phosphate transport are conserved in at least three orders of the plant kingdom, i.e., the Solanales, Apiales, and Fabales, which are distributed between the two subclasses Rosidae and Asteridae within the eudicots. The evolutionary distance between rice and potato apparently did not allow conservation of the respective regulatory mechanisms, although both species exhibit mycorrhiza-specific phosphate transport (16, 27). Promoter-dissection studies indicated the presence of a regulatory domain within a 129-bp region of the StPT3 promoter. Truncated versions of the StPT3 promoter lacking this region failed to direct GUS reporter gene expression on AM fungal colonization of potato roots with G. mosseae, whereas the presence of this region, irrespective of the length of the upstream sequence, was necessary to activate GUS expression (data not shown). Phylogenetic footprinting was performed to identify conserved candidate regulatory elements in nonconserved promoter sequences of different phosphate transporter

Table 2. Induction of StPT3 expression in potato inoculated with fungi from different taxonomic positions

<table>
<thead>
<tr>
<th>Taxonomic position (kingdom/phylum/order/family)†</th>
<th>Fungal species</th>
<th>Fungal status</th>
<th>GUS activity*</th>
<th>Mycorrhiza type (only for AMF)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oomycota</td>
<td>Phytophthora infestans</td>
<td>Pathogen</td>
<td>−, ms</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>Rhizopus stolonifer</td>
<td>Pathogen</td>
<td>−, ms</td>
<td></td>
</tr>
<tr>
<td>Zygomycota</td>
<td>Paraglomus brasilianum</td>
<td>−</td>
<td>+, s</td>
<td>P; ah, ch</td>
</tr>
<tr>
<td>Archaeosporales</td>
<td>Archaeospora nicolsonii</td>
<td>−</td>
<td>+, s</td>
<td>P; ah</td>
</tr>
<tr>
<td>Diversisporales</td>
<td>Acaulospora delicata</td>
<td>−</td>
<td>+, s</td>
<td>P; ah</td>
</tr>
<tr>
<td>Diversisporales</td>
<td>Glomus sporum</td>
<td>−</td>
<td>+, s</td>
<td>P; ah</td>
</tr>
<tr>
<td>Gigasporaceae</td>
<td>Gigaspora margarita</td>
<td>−</td>
<td>+, ms</td>
<td>P; ch</td>
</tr>
<tr>
<td>Glomeraceae (Glomus-group A)</td>
<td>Glomus caledonium</td>
<td>Mutualistic symbiont</td>
<td>+, ms</td>
<td>l; ah, ch, ih</td>
</tr>
<tr>
<td></td>
<td>Glomus geosporum</td>
<td>−</td>
<td>+, s</td>
<td>l; ah, ch, ih</td>
</tr>
<tr>
<td></td>
<td>Glomus intraradices</td>
<td>−</td>
<td>+, ms</td>
<td>l; a, ah, ch, ih</td>
</tr>
<tr>
<td></td>
<td>Glomus mosseae</td>
<td>−</td>
<td>+, ms</td>
<td>l; a, ah, ch, ih</td>
</tr>
<tr>
<td>Glomeraceae (Glomus-group B)</td>
<td>Glomus etunicatum</td>
<td>−</td>
<td>+, ms</td>
<td>P; ch</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>Piriformospora indica</td>
<td>Plant growth-promoting endophyte</td>
<td>−, m</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Rhizoctonia solani</td>
<td>Pathogen</td>
<td>−, ms</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Binucleate Rhizoctonia sp.</td>
<td>Neutral endophyte</td>
<td>−, s</td>
<td>—</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Nectria venticosa</td>
<td>Pathogen</td>
<td>−, ms</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Phialocephala fortinii</td>
<td>Mutualistic symbiont to weak pathogen</td>
<td>−, m</td>
<td>—</td>
</tr>
</tbody>
</table>

*Potato plants and/or hairy roots harboring the StPT3 promoter–GUS reporter gene construct were inoculated with 17 strains of root-colonizing organisms. GUS activity in colonized tissues corresponds to the induction of StPT3 expression. +, Positive GUS staining in root zones colonized by AMF; −, absence of GUS staining.
†Systematics of AMF is given according to ref. 4.
‡Order and family names are given only for AMF.
§GUS activity assays were performed in monoxenic (m), soil (s), or in both cultures (ms).
| Details of AM morphology: P, Paris-type AM; I, intermediate-type AM; a, arbuscules; ah, arbusculate hyphae; ch, coiled hyphae; ih, intercellular hyphae. |
genes and one AMF-inducible gene of unrelated function. From six motifs found within the 129-bp regulatory region of StPT3, the CTTC motif was abundant in the promoters of mycorrhiza-specific phosphate transporters from eudicot tomato (LePT4; R. N. and M. B., unpublished data), and M. truncatula (MtPT4), and an AMF-up-regulated glutathione S-transferase (MtGst1) from M. truncatula, whereas it was absent in the OsPT11 promoter from monocot rice, the promoters of the flower-specific ARAth;Ph1:6 gene, and the promoters of the root-specific and phosphate starvation-inducible ARAth;Ph1:8 gene, respectively, from A. thaliana (23) (Fig. 1 and see Table 3, which is published as supporting information on the PNAS web site). Of all promoters analyzed, the TAAT motif was present exclusively in the AMF-up-regulated Ph1 family genes from eudicot potato, M. truncatula, and tomato, respectively. In the MtGst1 promoter, only one of six motifs was conserved, whereas four elements were conserved in the ARAth;Ph1:8 promoter. None of the motifs was present in the flower-specific ARAth;Ph1:6 gene from A. thaliana. Ultimately, it must be elucidated whether these motifs alone or in combination specify root and/or mycorrhiza specificity to StPT3 expression and have functions in other mycorrhizal plant species that are similar to functions in potato.

To learn more about the specificity of StPT3 expression in various plant–fungus associations, wild-type and transgenic potato plants, as well as hairy roots harboring a StPT3 promoter-GUS chimeric gene, were inoculated with 17 species from the fungal phyla Glomeromycota (10 species), Zygomycota (1 species), Basidiomycota (3 species), Ascomycota (2 species), and the nonfungal phylum Oomycota (1 species), all of which are known to enter either mutualistic symbiotic, pathogenic, plant-growth promoting, or neutral associations, respectively, with plants (Table 2). Histochemical staining for GUS activity revealed that StPT3 promoter activity colocalized with root zones colonized by AMF (Fig. 2 C and D), as shown (16). No GUS activity was observed in noninoculated control roots, root zones of mycorrhizal plants that were not colonized by AMF, or in roots that were colonized by fungal pathogens or root endophytes other than AMF (Table 2). Interestingly, low levels of StPT3 expression were detected by RT-PCR using StPT3 gene-specific primers and RNA from noninoculated roots and leaves of potato (Fig. 3A). These low transcript levels had not been detected (16) by RNA gel blot analysis. StPT3 transcript abundance was increased exclusively in potato roots inoculated with AMF, whereas it remained low in roots of noninoculated control plants and plants inoculated with species from phyla other than the Glomeromycota (Fig. 3A). In comparison, mRNA abundance of the phosphate transporter gene StPT1 (16) was comparable in all associations (Fig. 3A). Gene-specific RT-PCR to detect MtPT4 gene transcripts in M. truncatula confirmed mycorrhiza-specific expression of MtPT4 in roots colonized by AMF (Fig. 3B) (26). In contrast to basal StPT3 expression in nonmycorrhized potato, no MtPT4 transcripts were detectable in roots of noncolonized M. truncatula and shoots, thus indicating differential regulation of StPT3 and MtPT4 expression in the absence of AMF in the respective plant species. Overall, the expression of an AM-associ-
ampli

truncatula
colonized by the AMF fungus

used.

tively, over 35 cycles.

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suggests possible phosphate transfer at the fungus–root interface of
the Paris-type mycorrhiza.

It is, however, generally known that the product of the GUS
reaction may diffuse and, thus, obscure the precise localization
of GUS expression. To confine reporter gene expression
precisely to the site of promoter activity and simultaneously obtain
information on the temporal profile of StPT3 expression, a
reporter system based on the Fluorescent Timer, a mutant form
of the DsRed fluorescent protein from the coral Discosoma, was
used. The Fluorescent Timer protein shifts fluorescence color
from green to red over time because of slow fluorophore
maturation (33). The rate of color conversion has been reported
to be independent of protein concentration and, therefore, can
be used to trace time-dependent expression. To trace time-
dependent expression, potato hairy roots harboring a StPT3
promoter–Fluorescent Timer chimeric gene colonized with G.
margarita and G. caledonium, respectively, were inspected by
laser scanning confocal microscopy. Within a single colonization
unit, differentially fluorescing cells were present. Generally,
background green autofluorescence in cortical cells originated
mainly from fungal hyphae (Fig. 4D), whereas strong red fluo-
rescence was detectable exclusively in cells colonized by AMF
hyphae (Fig. 4B and E). Neither noncolonized cortical cells nor
fungal hyphae emitted strong red fluorescence. Colonized cells
usually exhibited homogeneously distributed green and red
fluorescence independent of the type of colonization, i.e., coiled
hyphae or arbusculate hyphae (Fig. 4A, B, E, and F). Interestingly,
cells emitting strong red fluorescence were bordered by
colonized cells without red fluorescence (Fig. 4E). An overlay
of both red and green fluorescence of the same section allowed us
to see not only where in a colonization unit the StPT3 promoter
is active but also when it becomes inactive (Fig. 4F). Green
fluorescence originating from the Fluorescent Timer was ac-
panied by red fluorescence, giving rise to yellow-to-orange
cells signifying continuous promoter activity. Red fluorescent
cells denoted cells in which promoter activity had ceased after an
extended “on” period. Nonsynchronous pattern of promoter
activity was apparent among individual cells within a single
mycorrhizal unit, i.e., in morphologically similar potato cortex
cells colonized by thick-coiled hyphae from G. margarita, and
cells emitted green and red, red only, or no fluorescence (Fig.
4F), whereas in potato most cortical cells containing arbusculate

Fig. 3. Expression of mycorrhiza-inducible phosphate transporters in potato
and M. truncatula. (A) Semiquantitative RT-PCR performed with 1 µg of total
RNA extracted from potato roots (or leaves, where indicated) colonized by
fungi of different status (see Table 2). cDNAs were amplified with StPT3 and
StPT1 sequence-specific primers, respectively. (B) MtPT4 expression in M.
truncatula colonized by the AMF fungus G. mosseae. RT-PCR was performed
with 1 µg of total RNA extracted from M. truncatula tissues. cDNAs were
amplified with MtPT1-, MtPT4-, and PHT2;1-sequence-specific primers, respec-
tively, over 35 cycles. MtPT1 and PHT2;1 served as marker genes for expression
in roots and shoots, respectively. As size marker, PstI-restricted λ DNA was
used.

Fig. 4. Spatial and temporal pattern of StPT3 promoter activity in mycor-
rhizal potato hairy roots. Laser scanning confocal microscopy of potato hairy
roots harboring the StPT3 promoter–Fluorescent Timer chimeric gene. Roots
are colonized by G. caledonium (A–C) or G. margarita (D–F). (A) Green
fluorescence of a transgenic root originating mainly from the Fluorescent
Timer in cells colonized by arbusculate hyphae (ah) or fungal coiled hyphae
(ch) and not from noncolonized cells (n). (B) Red fluorescence from the
Fluorescent Timer. (C) Yellow fluorescence originating from merged images in
A and B. (D) Merged images showing transmitted light, and red and green
fluorescence, respectively, of a control root harboring the StPT3 promoter-
GUS gene. Green autofluorescence originates from root hairs (rh), epidermal
cells (ec), and fungal coiled hyphae (ch). (E) Red fluorescence from the Flu-
orescent Timer. StPT3 promoter activity is localized to cortical cells colonized
by fungal coiled hyphae (ch). Colonized cells exhibit high (h), residual (r), or no (n)
Fluorescent Timer fluorescence. (F) Merged images showing both red and
green fluorescence. Colonized cells exhibit different levels of StPT3 promoter
activity; c, continuous StPT3 promoter activity leading to yellow color because
of overlay of green and red fluorescence as in C; r, Fluorescent Timer expres-
sion has ceased, and residual Fluorescent Timer protein fluoresces red; n,
colonized cell with no Fluorescent Timer expression. Images in A–C and D–F
were captured with laser scanning confocal microscopes obtained from Zeiss
and Leica, respectively. (Bars, 50 µm.)
hyphae from *G. caledonium* emitted green and red fluorescence, indicating that the *StPT3* promoter was simultaneously active in most colonized cells (Fig. 4 A–C). Somewhat weaker and green fluorescence in two cells of the same colonization unit was not related to fungal biomass (data not shown) and, therefore, suggested that in these cells *StPT3* expression has just been turned on (Fig. 4C). This dynamic event within single colonization units was previously thought to be impossible to visualize in mycorrhizal roots (16, 26), and it could reflect a different functional status of individual colonized cells within mycorrhizal units. We observed that in old monoxenic cultures of *G. margarita* and potato hairy roots, *StPT3* promoter activity ceased at the time of fungal spore formation (data not shown). Overall, these data indicate that induction of *StPT3* expression is a temporally controlled cell-autonomous process with no signaling occurring to neighboring noncolonized cells. Thus, *StPT3* expression is most likely induced during cell-to-cell contact between the two symbionts and depends on the functional state of the fungus–plant association within a single cell. A combination of different approaches, including the Fluorescent Timer technique, could shed more light on structure–function relationships within symbiotic units of a mycorrhiza.

The functional AM fungus–root interface extends far beyond the periarbuscular space and includes *Paris*-type hyphae and hyphal coils colonizing root cell walls. Our results support the hypothesis that phosphate transport in mutualistic beneficial symbioses between eudicot host plants and evolutionary ancient monophyletic AM fungi exploits conserved signal perception and transduction mechanisms. Secondly, the corresponding signal(s) is released and/or perceived at root cortex cells in a temporally defined manner in the presence of intracellular AM fungal hyphae. In our model, either an AMF-specific signal molecule or a fungal component modified by a fungal or a root cortex cell-specific secretory enzyme elicits the response to AM fungal colonization and eventually the induction of *StPT3* (and probably other symbiosis-related plant genes) (see Fig. 5, which is published as supporting information on the PNAS web site).

We thank many colleagues for supplying us with the microorganisms used in this study; Sigrid Unseld (Institute of Biology, University of Stuttgart, Stuttgart, Germany) for her Fluorescent Timer plasmid; Anna Mezzacasa and Dr. Ari Helenius (Institute of Biochemistry, ETH Zurich) for support with confocal laser scanning microscopy; and Dr. Christine Rausch (Institute of Plant Sciences, ETH Zurich) for RNA samples. The work was supported by Roche Research Foundation Grant 2001-146 (to M.B. and N.A.), Research Commission of ETH Zurich Grants TH-1/00-3 and TH-5/02-3 (to M.B. and N.A.), and Swiss National Science Foundation Grant 3100-066745 (to M.B.). Some of the monoxenic cultures had been initiated at the K. A. Timiryazev Institute of Plant Physioloogy of the Russian Academy of Sciences (Moscow) by V.K. and were supported by Russian Fund for Basic Research Grant 01-04-48320 to Dr. Inna Kuzovkina. All other work was performed at ETH Zurich.