Identification of a pollen-specific sucrose transporter-like protein NtSUT3 from tobacco

Remi Lemoine[a,b,*], Lukas Bürkle[b], Laurence Barker[b], Soulaiman Sakr[a], Christina Kühn[b], Matthieu Regnacq[a], Cecile Gaillard[a], Serge Delrot[a], Wolf B. Frommer[b]

[a]Laboratoire de Biochimie et Physiologie Végétales, ESA CNRS 6161, Bâtiment Botanique, 40, Avenue du Recteur Pineau, F-86022 Poitiers Cedex, France
[b]Plant Physiology, Zentrum für MolekularBiologie der Pflanzen ZMBP, Auf der Morgenstelle 1, D-72076 Tübingen, Germany

Abstract Pollen cells are symplasmically isolated during maturation and germination. Pollen therefore needs to take up nutrients via membrane carriers. Physiological measurements on pollen indicate sucrose transport in the pollen tube. A cDNA encoding a pollen-specific sucrose transporter-like protein NtSUT3 was isolated from a tobacco pollen cDNA library. NtSUT3 expression is detected only in pollen and is restricted to late pollen development, pollen germination and pollen tube growth. Altogether these data indicate that pollen is supplied not only with glucose, but also with sucrose through a specific sucrose transporter. The respective contribution of each transport pathway may change during pollen tube growth.
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Key words: Sucrose transport; Pollen; Tobacco

1. Introduction

Plants organs can be classified as either source (autotrophic) or sink (heterotrophic) organs, depending on their ability to perform sufficient photosynthesis to meet their need for energy and carbon skeletons. Sink organs rely on the supply of photosynthates (mainly sucrose) delivered by the phloem sap. Sucrose entry in the phloem cells of source organs has been well documented in the last years. In species transporting sucrose as the major photosynthate, sucrose enters phloem cells from the apoplasmic compartment. Sucrose molecules cross the plasma membrane of phloem cells through specific carriers. Cloning of the SUT1 gene encoding sucrose carrier by yeast complementation [1,2] was the basis for identifying sucrose carriers in other plant species [3–5]. Those carriers work as proton/sucrose cotransporters and have been localized to the phloem, either in companion cells or in sieve elements [6,7]

However, the exit of sucrose from phloem cells and subsequent import into sink organs is less clearly understood. One of the main reasons is that no general mechanism seems to describe all the different situations encountered among species (apoplastic vs. symplastic unloading; sucrose vs. hexoses as sugars taken up in sink cells in case of apoplastic unloading [8]).

In several cases, expression of sucrose transporters has also been reported in sinks [2,5,9–13]. However, in all cases, expression of the corresponding carriers was also detected in source leaves. Apoplasmically unloaded sucrose can also be cleaved by cell wall invertases and the resulting hexoses taken up by sink cells. Corresponding hexose carriers were cloned from a number of species [14] and localized mainly to sink organs. The third pathway is the symplasmic unloading of sucrose through plasmodesmata. Recent experiments with fluorescent dyes evidenced open symplasmic connections between the phloem and surrounding cells in specific sinks [15–17]. These different data illustrate the complexity of sugar movement in sink organs.

However, in the case of the male gametophyte (pollen) of higher plants, no symplasmic connections with the surrrounding tissues remain after microspores are released from the tetrad. This indicates that pollen grain and tube develop as symplasmically isolated organs. This isolation necessitates the presence of carriers to take up nutrients into the pollen. Surprisingly, the mechanisms of nutrient uptake is not known at the present time. Based on physiological data, the presence of a proton/sucrose mechanism in the pollen tube of the lily was suggested [18] but some recent data report on the cloning of pollen-specific hexose transporters in *Arabidopsis* (*AtSTP2* [19]) and petunia (*pmt1* [20]). Therefore the form of carbon uptake (sucrose vs. hexose) in the pollen is not clear at the present time.

In the following, the uptake of sucrose during pollen germination is investigated and we report the identification of *NtSUT3*, a cDNA encoding a sucrose transporter-like protein specifically expressed in the pollen of tobacco plants.

2. Materials and methods

2.1. Plant material
Tobacco plants (*Nicotiana tabacum* cv. Samsun NN) were grown in 25 cm diameter pots containing soil in a 16 h light/8 h dark cycle in a greenhouse. Plants were watered daily and supplemented three times a week with a 0.1% (w/v) solution of Peter Professional (20+20+20, Scotts Europe, The Netherlands). Pollen grains were harvested twice a day from mature dehiscent anthers: pollen was separated from anthers by vortexing in an Eppendorf tube, frozen in liquid nitrogen and stored at −80°C until use.

2.2. In vitro pollen germination and sucrose uptake experiments
Pollen grains were germinated on 20 ml of the medium described in [21]. For some experiments, sucrose in the medium was replaced with glucose or glucose+fructose. At selected times, germinated pollen were separated from ungerminated ones by filtration on a 80 μm nylon mesh (Millipore, St Quentin en Yvelines, France). Germinated pollen were rinsed with 3×50 ml of a germination medium where sorbitol replaced sucrose and polyethylene glycol (uptake medium). The collected germinated pollen were carefully resuspended in 4 ml of uptake medium. For uptake studies, 0.4 ml of resuspended pollen was incu-

*Corresponding author. Fax: (33) 5 49 45 41 86.
E-mail: remi.lemoine@campus.univ-poitiers.fr

Table 1
Effect of sugar composition on percent germination of tobacco pollen

Sugar in the medium	Amount (%, w/v)	Concentration (mM)	Germination (% of germinating pollen)
Sucrose	5	146	78 ± 11
Glucose+fructose	2.5 ± 2.5	139 ± 139	66 ± 12
Glucose+fructose	1.25 ± 1.25	69 ± 69	47 ± 10
Glucose	5	277	22 ± 10

The medium used is as described in [21] except for the sugar composition. All measurements were made after 2 h germination and repeated twice (mean ± S.D.) on independent batches of pollen.

bated at 28°C and supplemented with 8 µl of a 50 mM [U-^{14}C]sucrose solution (Amersham, Les Ulis, France) to give a 1 mM sucrose solution (final specific activity: 1.34 MBq/mmol). After 1 min, germinated pollen were separated from the incubation medium by vacuum filtration on glass fiber filters (Sartorius, Palaiseau, France) and rinsed with 3×4 ml of chilled incubation medium without sucrose. Invertase activity measurements were run according to [22].

2.3. Isolation of a genomic clone for SUT3

A genomic library from Nicotiana plumbaginifolia in λDASH II (Stratagene, La Jolla, CA, USA) was screened with the tobacco NtSUT1 as probe [5] at reduced stringency (hybridization in a buffer containing 25% formamide at 42°C, washing in 2×SSC at 56°C). A hybridizing clone (NpSUT3) was purified and analyzed further.

2.4. RNA isolation, cDNA library construction and Northern blot analysis

RNA was isolated from the different plant organs according to the procedure described in [23]. Germinated pollen were separated from ungerminated pollen grains as described above. Pollen mRNA was prepared from 1 mg total pollen RNA using Dynabeads (Dynal, New York, NY, USA) as described in [5]. An oriented cDNA library was constructed using the UNI ZAP kit (Stratagene). The library contained a high proportion of recombinants with an average insert size of 1.45 kb. The library was plated (400 000 PFU) and hybridized to genomic NpSUT3 under high stringency. Five strongly hybridizing cDNA clones were identified. All cDNAs were sequenced from the 5'-end and were shown to encode the same gene just differing in the length of the respective 5'-ends. The longest cDNA was sequenced completely on an ABI 310 (Perkin-Elmer, Weiterstadt, Germany). Since the sequence of NpSUT3 was highly similar to the newly identified cDNA, it probably represents an ortholog and thus was named NtSUT3.

Northern analysis was carried out on Hybond N membranes (Amersham, Les Ulis, France) according to the manufacturer's protocol. Unless stated otherwise, 20 µg total RNA was separated on denaturing 1% agarose gels, transferred to a nylon membrane (Hybond N) and hybridized to a ^{32}P-labelled probe (a 1.1 kb EcoRI fragment of NtSUT3). Hybridization was done overnight at 65–68°C. The final wash was in 0.5×SSC, 0.1% SDS for 20 min at 68°C. Detection was done on Hyperfilm β max (Amersham, Les Ulis, France) or by imaging (Instant Imager, Packard Instruments, Rungis, France).

3. Results

3.1. Germination of pollen and sucrose uptake

Because of the recent description of hexose carriers ex-

pressed in the pollen tube, the germination of tobacco pollen on different media was studied. Results in Table 1 were obtained by germinating pollen grains on the medium described in [24] where sucrose was replaced either by its cleavage products (glucose and fructose) or by glucose alone. As already noted [21] sucrose is the best sugar for in vivo germination of pollen. Glucose and fructose at the concentration expected if sucrose is completely cleaved by an invertase [20] were less efficient than sucrose alone (15% reduction). This was even more true when total glucose and fructose were equimolar to sucrose (1.25+1.25%): the percent of germination dropped from 78 to 47%. Glucose alone was a very poor substrate for pollen germination and those pollen that germinated often had their pollen tube burst. This differs from the results obtained in petunia [20] where glucose was as good a substrate as sucrose.

Germination experiments indicated that sucrose was the best substrate, but this did not preclude that the cleavage products were taken up by growing pollen. Therefore, sucrose and hexose uptake was investigated into pollen germinated for 2 h (pollen is autotrophic due to stored products) and 8 h (pollen is heterotrophic). Sucrose and hexoses were taken up and uptake rates increased with time (Table 2). Increased uptake was paralleled by a large increase in cell wall invertase activity. The rather high uptake of fructose after 2 h germination has not been reported yet. But again, sucrose transport could have been a result of cleavage and subsequent hexose uptake, as those sugars were readily taken up. The following experiment was thus conducted: 1 mM [^{14}C]sucrose was fed to pollen, alone or in the presence of either 10 mM sucrose or 10 mM glucose. The rationale was that, if sucrose is cleaved by an invertase before uptake, glucose would be more inhibitory than sucrose. In pollen germinated for 8 h, sucrose uptake was inhibited to 67% of control in the presence of 10 mM sucrose, and to 50% in the presence of 10 mM glucose. Reciprocally, glucose uptake was more inhibited by a 10-fold glucose excess (35%) than by a 10-fold sucrose excess (0%). It was therefore concluded that part of the sucrose present in the medium was taken up without cleavage. To get more direct evidence of sucrose uptake, the transporter involved was cloned.

Table 2
Uptake of 1 mM radiolabelled sucrose, glucose and fructose in tobacco pollen germinated for 2 and 8 h

	2 h germination	8 h germination
Sucrose uptake (nmol/mg protein/min)	5.6 ± 0.6	12.8 ± 1.4
Glucose uptake (nmol/mg protein/min)	8.0 ± 0.5	9.2 ± 1.6
Fructose uptake (nmol/mg protein/min)	8.6 ± 0.9	N.D.
Acid invertase activity (µmol sucrose cleaved/mg protein/min)	19.5 ± 1.5	47.0 ± 2.7

The corresponding values for acid invertase activity are indicated. Experiments were run twice (mean ± S.D.) as described in Section 2. N.D.: not determined.

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            AGG CAA TCC AGA AAA GAC AAG AGG GAA AAA GCA AGA GAT CAA GAA CTG GAG AAG
                10          19          28          37          46          55

AGA AAG AGC TTT TCT TTC TTC TTT CTC TCT GGC GCT CTA CAA GGG CGC CAG AGG TCT TTT
        64          73          82          91         100         109

TTC CTC TTA GAG TTA TTA ATC ATT TTG AAA AAG GAA AAA GAA AAG GAA GAG AGA ACA
        124         133         142         151         160         169
                                                                          M   T
AGA ACA TCT CCT CTC TCT GTC TAT TAC TAC GTT TTT CCT TTT TCA GTA CCT TAA ATG ACC
        184         193         202         211         220         229

  K   S   Y   I   T                           M   E   S   G   S   M   E   M
AAG TCA TAC ATA ACT TAA ACA CGG ATT GAT ATT TCA ATG GAG AGT GGT AGT ATG GAA ATG
        244         253         262         271         280         289

  K   V   Q   Q   P   Y   L   A   V   P   V   L   P   P   R   W   K   I   V   M
AAA GTT CAA CAA CCA TAT TTA GCT GTT CCA GTA CTG CCA CCA CGT TGG AAG ATA GTA ATG
        304         313         322         331         340         349

  V   A   S   I   A   A   G   V   Q   F   G   W   A   L   Q   L   S   L   L   T
GTG GCC TCC ATA GCT GCT GGA GTT CAA TTC GGA TGG GCA CTT CAG CTC TCT TTG TTA ACT
        364         373         382         391         400         409

  P   Y   L   Q   L   L   G   I   P   H   R   Y   A   S   I   I   W   L   C   G
CCT TAT TTA CAG CTA CTT GGA ATT CCA CAC AGA TAT GCT TCA ATT ATA TGG CTT TGT GGA
        424         433         442         451         460         469

  P   I   S   G   M   I   V   Q   P   A   V   G   Y   L   S   D   N   L   S   S
CCT ATT TCT GGA ATG ATT GTT CAG CCT GCG GTT GGT TAT TTA AGT GAC AAC CTC TCC TCC
        484         493         502         511         520         529

  T   F   G   R   R   R   P   F   I   A   A   G   S   S   L   V   A   V   A   V
ACC TTT GGC CGC CGT CGG CCT TTC ATT GCC GCC GGC TCC TCC CTT GTC GCC GTC GCC GTC
        544         553         562         571         580         589

  I   F   I   G   F   A   A   D   I   G   H   A   F   G   D   P   L   D   T   K
ATC TTC ATT GGC TTT GCT GCT GAT ATT GGA CAT GCT TTT GGT GAT CCT CTT GAT ACA AAA
        604         613         622         631         640         649

  T   K   P   L   G   I   I   T   F   I   V   G   F   W   Y   L   D   V   A   N
ACT AAG CCG CTT GGC ATT ATT ACT TTT ATC GTT GGG TTT TGG TAC CTT GAT GTC GCT AAC
        664         673         682         691         700         709

  N   M   L   Q   G   P   C   R   A   F   L   A   D   L   S   G   G   K   A   C
AAC ATG TTA CAG GGT CCG TGC AGA GCA TTC TTG GCT GAT CTC TCC GGC GGG AAA GCC TGT
        724         733         742         751         760         769

  R   I   R   T   G   Q   S   C   Y   A   F   F   M   A   V   G   S   I   L   G
CGG ATA AGG ACG GGA TCT TGC TAC GCA TTC TTC ATG GCG GTG GGA AGC ATT CTG GGG
        784         793         802         811         820         829

  N   A   A   G   S   Y   S   H   L   Y   T   I   F   P   F   T   K   T   E   A
AAC GCC GCC GGT TCA TAT TCT CAC CTC TAC ACC ATC TTC CCC TTC ACA AAG ACA GAA GCC
        844         853         862         871         880         889

  C   G   V   Q   C   A   N   L   K   S   C   F   L   I   S   V   V   L   L   L
TGT GGT GTT CAA TGT GCA AAC CTA GCA AGT TGT TTT CTG ATC TCA GTA GTT TTA TTG CTC
        904         913         922         931         940         949

  T   L   T   T   L   A   L   T   A   V   D   E   K   V   L   P   Q   K   D   H
ACT TTA ACA ACC TTA GCC TTA ACA GCT GTG GAT GAA AAA GTG CTG CCA CAG AAA GAT CAT
        964         973         982         991        1000        1009

  F   I   N   S   E   Y   L   G   S   S   G   K   K   G   G   L   L   F   F   G
TTC ATC AAC AGT GAA TAT TTG GGT TCA TCA GGA AAA AAA GGA GGA TTG TTA TTT TTT GGA
       1024        1033        1042        1051        1060        1069

  E   M   F   E   A   L   K   H   L   P   R   S   V   W   I   L   L   M   V   T
GAG ATG TTT GAA GCT CTA AAG CAT TTG CCA AGA TCA GTG TGG ATT CTT CTA ATG GTC ACA
       1084        1093        1102        1111        1120        1129

  A   V   N   W   I   A   W   F   P   F   T   L   Y   G   T   D   W   M   G   K
GCT GTA AAC TGG ATT GCT TGG TTT CCT TTT ACC TTG TAT GGC ACT GAC TGG ATG GGC AAA
       1144        1153        1162        1171        1180        1189

  E   V   Y   G   G   R   V   R   D   G   N   L   Y   V   L   C   M   S   L   G
GAG GTA TAT GGG GGC AGA CTT AGG GAC GGG AAT CTA TAC AAT AAA GGA GTA CAT GCA GGT
       1204        1213        1222        1231        1240        1249

  V   F   G   L   L   S   S   V   V   L   C   L   H   S   L   G   V   E   C
GTA TTT GGG CTT CTG TTG AGC TCA GTG GTG CTA TGT TTA ATG TCA CTG GGA GTG GAG TGT
       1264        1273        1282        1291        1300        1309

  V   G   K   W   L   G   G   A   K   R   L   W   G   I   V   N   F   I   L   A
GTT GGA AAG TGG CTA GGT GGT GCA AAA AGG CTA TGG GGG ATT GTC AAT TTC ATC TTG GCT
       1324        1333        1342        1351        1360        1369

  I   C   L   A   M   T   V   F   V   T   K   H   A   D   K   S   R   R   Y   D
ATT TGC TTG GCC ATG ACT GTT TTT GTT ACA AAA ATG GCA GAC AAA TCA CGG CGA TAC GAC
       1384        1393        1402        1411        1420        1429

  G   D   G   E   L   L   P   P   D   Q   G   V   K   I   S   A   L   L   L   N
GGC GAC GGC GAA CTT CTG CCG CCG GAT CAA GGT GTC AAG ATC AGT GCC TTG CTT CTT AAT
       1444        1453        1462        1471        1480        1489

  A   V   T   G   I   P   L   A   V   L   Y   S   I   P   F   A   H   A   S   I
GCT GTT ACG GGA ATT CCT CTA GCA GTC CTT TAT AGC ATC CCA TTT GCT ATG GCA TCT ATA
       1504        1513        1522        1531        1540        1549

  Y   S   S   N   V   G   A   G   Q   G   L   S   Q   G   V   I   N   L   A   I
TAT TCC AGT AAT GTT GGG GCA GGG CAA GGT CTA TCA CAA GGA GTC ATA AAT CTT GCA ATA
       1564        1573        1582        1591        1600        1609

  V   V   P   Q   T   L   V   S   I   S   G   R   P   D   A   L   F   G   G
GTT GTC CCA CAG ACG TTA GTG TCA ATT AGT GGG AGG CCA TTT GAT GCA TTG TTC GGA GGA
       1624        1633        1642        1651        1660        1669

  G   N   L   P   A   F   V   A   G   A   V   A   A   A   V   S   G   I   L   A
GGG AAC TTG CCG GCG TTT GTG GCG GGA GCA GTT GCA GCT GCA GTC AGT GGT ATT TTG GCA
       1684        1693        1702        1711        1720        1729

  L   T   L   L   P   S   P   T   E   P   C   Q   T   Y   P   H   F   R   R   C
CTC ACA TTG TTG CCT TCT CCT ACT GAG CCA TGC CAA ACA TAT CCC CAT TTT CGC CGT TGC
       1744        1753        1762        1771        1780        1789

  F   P   M   T   K   Y   W   T   S   Q   N   K   F
TTC CCA ATG ACA AAA TAC TGG ACA TCA CAA AAC AAG TTT TGA TCA CTA ATT TTT TTT CTT
       1804        1813        1822        1831        1840        1849

TCT TTT TGT GCA TAA TGC AGA ATT AGA GTA ACA CAG TTT TGA AGG TTT TCC TGC CTT CTG
       1864        1873        1882        1891        1900        1909

TTA ATG GTT GTA CAA AAT AAT GAT GTT AGA TCG TAC TAT TAG TAT ATG TTA TTT AAA AGG
       1924        1933        1942        1951        1960        1969

CTG ACA GAG TCC AAT TCC TCT CAA AAA AAA AAA AAA AAA AAA AAA A
       1984        1993        2002        2011        2020
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3.2. Identification of NtSUT3

A genomic library from *N. plumbaginifolia* in λDASH II (Stratagene, La Jolla, CA, USA) was screened with the tobacco *NtSUT1* cDNA as probe [5]. A positive clone (named *NpSUT3*) was further analyzed. A 1.4 kb *Eco*RI fragment hybridized with a *NtSUT1* probe in Southern blots [5]. Sequencing showed that this fragment was homologous, but not identical in the coding region to *NtSUT1*. A 300 bp fragment (*Eco*RI/*Bgl*II digest from *NpSUT3*) corresponding to a coding region showing homologies to the 5′-end of NtSUT1 was used to screen a cDNA library from mature pollen grains of *N. tabacum* in λZAPII.

The clone called *NtSUT3* (accession number AF149981) has an open reading frame of 1560 bp (Fig. 1). The 5′-UTR contains a small open reading frame encoding a peptide of seven amino acids. NtSUT3 shares 64.9% identity (amino acid level) with the sieve element sucrose transporter NtSUT1 [5]. The first 130 amino acids of NtSUT3 share 87% identity with NpSUT3 confirming that they are orthologous proteins.

3.3. Heterologous expression of NtSUT3 in yeast

To test for the function of NtSUT3, the cDNA was cloned as an *Xba*I/*Xho*I fragment into pMK195 [25] and the sucrose uptake-deficient yeast strain SUSY7 ura3 was transformed (Bürkle, unpublished data). However, no difference in growth on sucrose could be detected when compared to control. To exclude that remaining flanking regions of the cDNA had a negative effect on expression in yeast, a PCR fragment lacking the small upstream translated region was cloned into pMK195 and into pDR195 [25]. The plasmids differ in the promoter used to drive the expression of the cloned cDNA (*ADH1* and *PMA1* promoters, respectively). However, no growth of the transformed yeast on sucrose could be seen. Thus, despite the very high sequence similarity with other members of the SUT family, no function in yeast could be demonstrated so far. NtSUT3 thus has to be considered a putative sucrose transporter.

3.4. Organ-specific expression of NtSUT3

Preliminary results indicated a highly specific expression profile for *NtSUT3*: expression was only detected in flowers; no signal was observed in source leaves, sink leaves, stem or roots. To determine the localization of *NtSUT3* expression more precisely, the different organs of mature flowers were dissected and used for RNA extraction. The results presented in Fig. 2A show that expression of *NtSUT3* was detected in stamen and (to a much lower level) in total flower and corolla. Concerning the signal in corolla, it was certainly due to pollen contamination (see below). However, no hybridizing RNA was detected in source leaves as noted before, indicating that no cross-hybridization with *NtSUT1* occurs.

To get a precise localization of expression, the stamina were dissected into filaments, anthers, anthers depleted of pollen and isolated pollen grains (Fig. 2B). The highest amount of *NtSUT3* mRNA was detected in isolated pollen. Since removal of pollen from the anther is incomplete, weak expression was also found in anthers after thorough shaking. No expression could be detected in filaments.
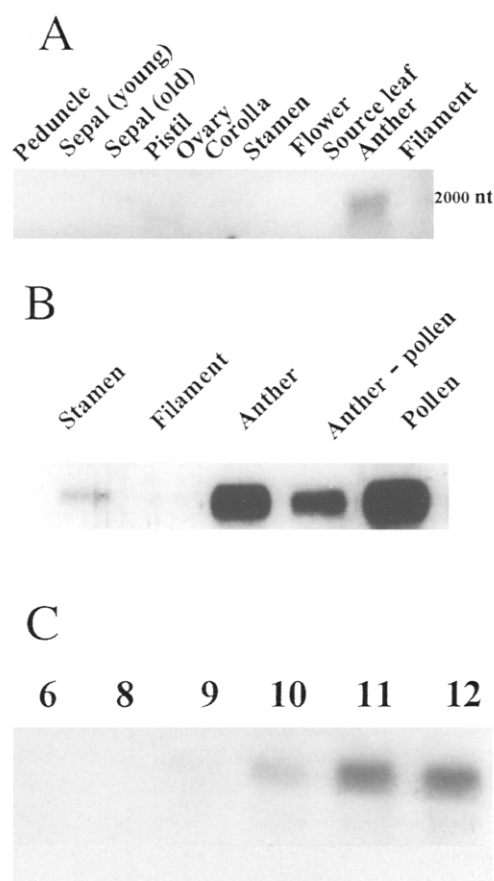
Fig. 1. DNA and deduced protein sequence of *NtSUT3*. The small upstream open reading frame is underlined.
←

A

2000 nt

B

C

6   8   9   10   11   12

Fig. 2. Expression of *NtSUT3*. For all Northern analyses, 20 µg of total RNA was loaded per lane. A: Expression of *NtSUT3* in the different parts of the flower. Source leaf RNA was included as a negative control. B: Expression of *NtSUT3* in different parts of the stamen. C: Expression of *NtSUT3* in anthers at different stages of development. Anthers were collected at stages as defined in [26].

3.5. Developmental regulation of NtSUT3 expression

All preceding RNA blot analyses had been conducted on organs harvested at flower maturity, which in tobacco corresponds to anthesis. To follow *NtSUT3* expression during anther development, anthers were collected at the stages described in [26]. Fig. 2C presents the results obtained on RNA extracted from anthers at stages 6–12, stage 6 corresponding to pollen mitosis I and stage 12 to anther dehiscence. *NtSUT3* expression increased gradually during the very late stages of pollen maturation with a maximum at stages 11–12. Overexposure of the film (data not shown) indicated that no expression was detected before stage 9. RNA purified directly from pollen isolated from anthers at stages 6–12 gave similar results (data not shown) and no expression of *NtSUT3* was found at any stage in other floral organs (data not shown). The control of expression indicates that *NtSUT3* represents a so-called late gene [27] that is activated after pollen mitosis I (stage 6).

In pollen, a large number of genes are actively transcribed late during development and the corresponding transcripts are stored and translated during pollen germination [27]. The expression of *NtSUT3* was investigated during in vitro pollen germination (Fig. 3). The amount of *NtSUT3* mRNA detected in the pollen tube was stable between 1 and 6 h of germination and was only slightly reduced (15–20%) as compared to

that in pollen grains. Thus NtSUT3 also seems to serve nutrition during pollen tube growth.

4. Discussion

In spite of numerous data available on sugar transport in plants and the evident suitability of pollen for such studies (symplasmic isolation from surrounding tissues), the mechanism of sugar uptake is not clearly known in pollen. A proton/sucrose cotransport mechanism has been proposed in lily pollen tubes [18], whereas hexose carriers have recently been cloned and found to be specifically expressed in the pollen of petunia [20] and *Arabidopsis* [19]. Expression of *AtSTP2* is limited to early stages of gametophyte development at the beginning of callose degradation and microspore release [19] and AtSTP2 would transport callose-derived monosaccharides. *Pmt1* is expressed specifically in the male gametophyte, and high levels of mRNA accumulate in mature pollen and in pollen germinated for 13 h [20]. Our results on pollen germination confirm that sucrose is the best substrate with almost 80% germination after 2 h. Products of sucrose cleavage are partially able to sustain germination whereas glucose is not a good substrate. This finding disagrees with the data obtained for petunia pollen [20] where glucose efficiently supported germination. Whether this discrepancy is related to specific differences remains to be determined. Sugar uptake experiments indicate that sucrose in pollen tubes is, at least in part, taken up without cleavage by invertase, as already suggested in [18]. To analyze the molecular basis of sucrose transport into pollen, a SUT cDNA was cloned by screening a tobacco pollen-specific library with *NtSUT1*, a cDNA encoding the sieve element sucrose transporter from *N. tabacum* [5,7]. NtSUT3 shares 64.9% identity at the amino acid level with NtSUT1, and has all characteristics of plant sucrose transporters. Several attempts to demonstrate a sucrose transporter function in yeast failed and this could be related to features specific for *NtSUT3*. Short upstream open reading frames are supposed to regulate translation. This phenomenon has been studied extensively for the *pma3* gene of the H^+/ATPase of *N. plumbaginifolia* where disruption of a five amino acid upstream
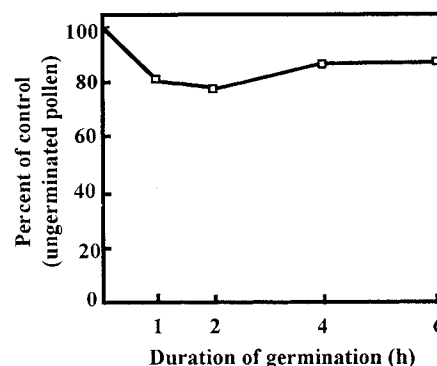

Fig. 3. Expression of *NtSUT3* during pollen tube germination. Pollen tubes were separated from ungerminated pollen at selected times as described in Section 2. 20 µg of total RNA was loaded per lane. Expression was quantified on an Instant Imager. The results were calibrated by using actin as control (actin cDNA was isolated from the pollen-specific cDNA library, Bürkle, Stadelhofer and Frommer, personal communication). Results are expressed as percent of ungerminated pollen.

open reading frame led to a 55% increase in translation in tobacco protoplasts [28]. Furthermore, *NtSUT3* contains three in frame ATGs which might be detrimental to heterologous expression in yeast [29]. These different elements are certainly involved in translational regulation of expression and could explain the absence of functional expression in yeast. The high sequence identity with NtSUT1 and other plant sucrose carriers cDNAs argues in favor of NtSUT3 being a sucrose carrier. However, one cannot rule out the possibility that NtSUT3 does transport different substrates.

As expected from the cloning of *NtSUT3* in a pollen cDNA library, *NtSUT3* expression is highly specific. Northern analysis only detects *NtSUT3* mRNA in the pollen. *NtSUT3* expression is restricted to the very late stages of flower development (Fig. 2C) classifying *NtSUT3* as a 'late pollen gene' [27] similar to *pmt1*. *NtSUT3* is expressed when pollen grains mature in the thecal fluid of the loculus and take up sugar from this fluid [30]. No data on the exact nature of sugars taken up during pollen maturation are available except for the cloning of hexose transporters in petunia and *Arabidopsis* [19,20]. NtSUT3 could thus mediate the uptake of sucrose in the pollen grain during maturation, particularly in tobacco pollen where sucrose represents by far the major soluble sugar (sucrose content is approx. 76 ± 7 mg/g pollen, mean $\pm$ S.D. of two measurements; Lemoine, unpublished results) whereas no starch is detected by iodide staining. But *NtSUT3* expression is also detected in mature pollen grains and during pollen tube growth (Fig. 3) as for many pollen-specific genes which transcripts are translated at the time of germination [24]. *NtSUT3* expression during pollen tube growth is in accordance with uptake and germination studies. Although these results were obtained in vitro, the medium used allows a very efficient growth for up to 48 h with all morphological characteristics of in vivo pollen [21]. Altogether, this indicates that NtSUT3 could also play a role in the nutrition of the pollen tube, both in vitro and in vivo.

According to the different data available, germination and growth of pollen tubes may not be homogeneous with time. In binucleate pollen like *Nicotiana*, division of the generative cell into sperm cells occurs after several hours of germination and corresponds to the switch from autotrophic to heterotrophic growth [24]. Ylstra et al. [20] measured that sucrose was converted to hexoses in the germination medium after 13 h, but gave no indication that sucrose was cleaved directly from the beginning of germination. In tobacco pollen tube, invertase activity increases with time. This could indicate that sucrose is the preferred form of uptake during the first hours of germination, in accordance with the description of a proton/sucrose cotransport in lily pollen after 1.5 h germination [18]. However, also in lily, sucrose uptake was partially inhibited by glucose. Taken together, these data indicate that sugar transport could well be a mixture of sucrose and hexose uptake. Due to the very high growth rate of the pollen tube, it is conceivable that different uptake pathways are used for efficient nutrition.

The identification of NtSUT3 as a putative pollen-specific sucrose carrier is in accordance with the description of numerous pollen-specific genes that encode transport proteins having homologs in the sporophyte [19,20,31,32]. Nevertheless, this is the first report of a sucrose transporter-like protein specifically expressed in one single sink organ. AtSUC1 has been recently localized to the pollen grain and tube in *Arabi-*

dopsis, but also in other parts of the anther and flower (N. Sauer, personal communication) [33]. Our results based on mRNA expression are in agreement with physiological data. More experiments will be needed to determine if both transporters (NtSUT3 and AtSUC1) have the same localization. Work is under way to determine the exact role of NtSUT3 during pollen maturation and/or pollen tube development. In tobacco, reverse genetics and the accessibility to pollen for physiological analysis should give important information.

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