LETTER

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A plastidial sodium–dependent pyruvate transporter

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Pyruvate serves as a metabolic precursor for many plastid-localized biosynthetic pathways, such as those for fatty acids[1], terpenoids[2] and branched-chain amino acids[3]. In spite of the importance of pyruvate uptake into plastids (organelles within cells of plants and algae), the molecular mechanisms of this uptake have not yet been explored. This is mainly because pyruvate is a relatively small compound that is able to passively permeate lipid bilayers[4], which precludes accurate measurement of pyruvate transport activity in reconstituted liposomes. Using differential transcriptome analyses of C_3 and C_4 plants of the genera *Flaveria* and *Cleome*, here we have identified a novel gene that is abundant in C_4 species, named *BASS2* (*BILE ACID:SODIUM SYMPORTER FAMILY PROTEIN 2*). The BASS2 protein is localized at the chloroplast envelope membrane, and is highly abundant in C_4 plants that have the sodium-dependent pyruvate transporter. Recombinant BASS2 shows sodium-dependent pyruvate uptake activity. Sodium influx is balanced by a sodium:proton antiporter (NHD1), which was mimicked in recombinant *Escherichia coli* cells expressing both BASS2 and NHD1. *Arabidopsis thaliana bass2* mutants lack pyruvate uptake into chloroplasts, which affects plastid-localized isopentenyl diphosphate synthesis, as evidenced by increased sensitivity of such mutants to mevastatin, an inhibitor of cytosolic isopentenyl diphosphate biosynthesis. We thus provide molecular evidence for a sodium-coupled metabolite transporter in plastid envelopes. Orthologues of *BASS2* can be detected in all the genomes of land plants that have been characterized so far, thus indicating the widespread importance of sodium-coupled pyruvate import into plastids.

Pyruvate is an essential metabolite for many plastid-localized metabolic pathways of plants, including the C_4 photosynthetic CO_2-concentrating mechanism[1–3]. Despite the crucial role of pyruvate import into plastids, the molecule mediating pyruvate transport is still unknown. Because of its essential role in regenerating the CO_2 acceptor of the C_4 photosynthetic carbon-concentrating mechanism, the rate of pyruvate transport into mesophyll cell chloroplasts of C_4 plants exceeds the rates observed in C_3 plants by at least one order of magnitude[4,5] (Supplementary Information). Since the first report of pyruvate transport activity in C_4 plant mesophyll cell chloroplasts[6], the stimulation of its activity by light[7] and two distinct mechanisms of pyruvate uptake—one proton-dependent[8] and one sodium-dependent[9]—have been characterized in a wide range of C_4 plants[10] (Supplementary Information). Therefore, we proposed (1) that the gene encoding the sodium-dependent plastidial pyruvate transporter should be expressed at substantially higher levels in C_4 than in C_3 plants, (2) that its expression should be low in plants of the proton-dependent C_4 type, and (3) that it should be expressed commonly in plants of the sodium-dependent C_4 type[11]. To isolate genes potentially encoding the pyruvate transporter, we conducted comparative transcriptome analyses between a C_3 plant species, *Flaveria pringlei*, and the closely related sodium-dependent type C_4 plant species, *Flaveria trinervia* and *Flaveria bidentis*[12]; we used two independent strategies, namely, differential complementary DNA screening and next-generation messenger RNA sequencing.

By using these transcriptome analyses, we identified three novel C_4 species-abundant genes, which are predicted to encode putative chloroplast-targeted membrane proteins (Fig. 1a). In the *Arabidopsis* information resources, related proteins are annotated as 'bile acid: sodium symporter family proteins' (BASS2 (ref. 13)/BAT1 (ref. 14) for At2g26900 and BASS4 (ref. 13) for At3g56160) and 'sodium:hydrogen antiporter 1' (NHD1 (ref. 15) for At3g19490). Therefore we named

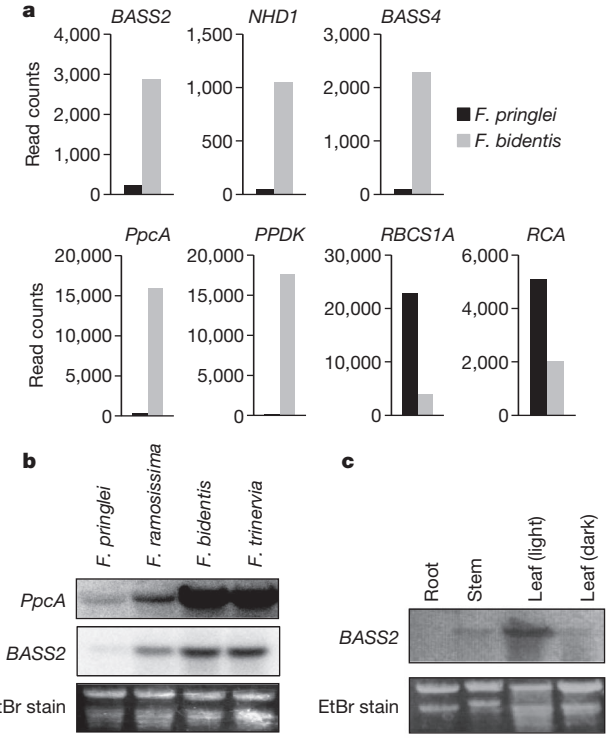

Figure 1 | The novel C_4-associated transcripts encoding plastid-targeted membrane proteins. a, From the RNA-seq data set of *Flaveria* species, *BASS2*, *BASS4* and *NHD1* expressions (top row) were compared (bottom row) with those of C_4 genes encoding PEPC (*PpcA*) and PPDK (*PPDK*) and C_3 genes encoding RubisCO small subunit (*RBCS1A*) and RubisCO activase (*RCA*). Each read count is indicated. **b**, **c**, *BASS2* and *PpcA* transcript levels in the indicated four *Flaveria* species (**b**) and in the indicated tissues of *F. trinervia* (**c**). Leaf (light), leaves taken during light exposure; leaf (dark), leaves taken during the dark period. Ten micrograms of total RNA were loaded in each lane for RNA gel-blot analyses. Radiolabelled *PpcA* and *BASS2* of *F. trinervia* were used as probes. The ethidium bromide (EtBr)-stained RNA gels are shown as loading controls in the lower panels.

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them *F. trinervia* BASS2, *F. trinervia* BASS4 and *F. bidentis* NHD1. Because *BASS2* and *NHD1*, but not *BASS4*, were commonly upregulated in another sodium-dependent type C_4 species, *Cleome gynandra* (Supplementary Information), compared to a C_3 species *Cleome spinosa*[16] (Supplementary Fig. 1 and Supplementary Information), we focused on *BASS2* and *NHD1*.

Except for the putative chloroplast-targeting peptide region, the remainder of the primary structure is highly conserved in all known plant BASS2 proteins (Supplementary Fig. 2a and Supplementary Information). The *F. trinervia BASS2* gene encodes a protein that features a predicted amino-terminal chloroplast-targeting peptide, 7–9 predicted transmembrane regions, and a cluster of charged amino acids at its carboxy-terminal end (Supplementary Fig. 2b). Although six related genes are found in the genome of *A. thaliana*, the proteins most closely related to *BASS2* form a distinct clade (Supplementary Fig. 3), suggesting that these proteins might share a common biochemical function. Transcripts encoding BASS2 are extremely abundant in the C_4 species *F. trinervia* and *F. bidentis*, highly abundant in the C_3/C_4 intermediate species *Flaveria ramosissima*, and not very abundant in the C_3 species *F. pringlei* (Fig. 1b). In *F. trinervia*, *BASS2* transcripts accumulated specifically in leaves, in particular during the light period (Fig. 1c).

We assessed BASS2 protein levels using a polyclonal antiserum directed against the C-terminal charged amino acid cluster, PIPVDDKDDFKE, which is identical among the BASS2 proteins of three *Flaveria* species (Supplementary Fig. 2a). Only one protein band, with an apparent molecular mass of 28 kDa, was detected (Fig. 2a). The signal was highly abundant in the C_4 species *F. trinervia* and *F. bidentis*, whereas it was faint in the C_3 species *F. pringlei*. Among various species, the BASS2 level gradually increased in accordance with the evolutionary sequence from C_3 to C_4 in the genus *Flaveria*[12] (Supplementary Fig. 4 and Supplementary Information). BASS2 was clearly detectable in the mesophyll cell chloroplast fraction (Supplementary Fig. 5), which was confirmed by immunohistochemical analyses (Fig. 2b). In addition to the signal from the mesophyll cell chloroplasts, a significant signal was also observed in bundle sheath cell chloroplasts, indicating a function of the BASS2 protein in both cells. The localization of BASS2 in plastids was also evidenced by the green fluorescent protein (GFP) fluorescent image of a BASS2–GFP fusion protein, which was stably introduced into transgenic tobacco plants (Supplemental Fig. 6). A cross-reacting protein band was clearly detectable in both monocotyledonous and dicotyledonous C_4 plant species for which a sodium-dependent mode of pyruvate transport was previously established[10] (Fig. 2c). There was no immuno-detectable protein in proton-dependent type C_4 plants, including maize, which is consistent with previous analyses of the maize chloroplast envelope proteome, in which BASS2 and NHD1 were not detected[5,17] (Supplementary Information).

We sought to confirm the biochemical function of BASS2 in a heterologous expression system. Recombinant BASS2 protein was expressed in *E. coli*, and was found to be predominantly localized to

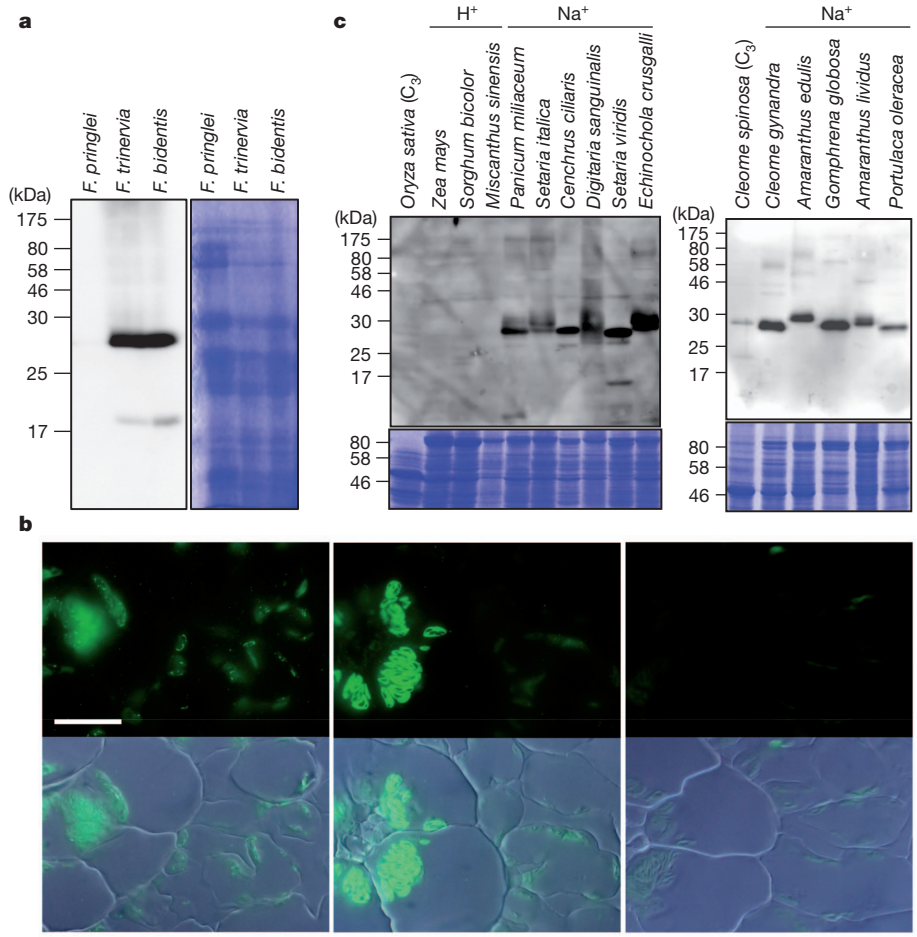

Figure 2 | BASS2 protein levels. **a**, BASS2 protein levels in three *Flaveria* species. Left, immunoblot analysis; right, Coomassie brilliant blue (CBB)-stained gel used as a loading control. **b**, Top, fluorescent immunohistochemistry on transverse sections of the leaves of *F. trinervia* using anti-BASS2 antiserum (left), anti-RubisCO antiserum (centre) or without the anti-serum (right). Bottom, merged figures with fluorescence view and differential interference view. Scale bar, 50 μm. **c**, Generality of BASS2 protein in arbitrarily selected monocotyledonous (left panel) and dicotyledonous (right panel) C_3 and C_4 plants. The biochemical classifications of pyruvate uptake are indicated at the top (H^+, Na^+). Top, western blotting data; bottom, CBB staining used as a loading control.

the plasma membrane (Supplementary Fig. 7a, b). In comparison to the empty-vector control, *E. coli* cells expressing BASS2 showed significantly higher rates of pyruvate uptake (Fig. 3a, b). Even without the addition of sodium chloride, some pyruvate uptake was observed after extended incubation, which is possibly due to the presence of trace amounts of sodium ions introduced by the *E. coli* culture. Notably, another monovalent cation, lithium, could not substitute for sodium as reported previously[9] (Fig. 3b).

Next, we simultaneously expressed *BASS2* and *NHD1* in *E. coli* (Supplementary Fig. 7a, b) and performed uptake experiments (Fig. 3c). Although significant sodium-dependent uptake was always observed, pyruvate uptake in the absence of externally added sodium chloride was observed in some experiments. As to this experimental variation, we noticed that the NHD1 protein levels were quite variable among independent experiments, whereas the BASS2 protein levels were highly reproducible (Fig. 3d). In the presence of sodium chloride, the uptake activity was always correlated with the BASS2 protein level. On the other hand, in the absence of applied sodium chloride a correlation with NHD1 protein levels was observed. These results indicate that NHD1 could establish a sodium gradient across the *E. coli* membrane, thereby promoting BASS2-dependent pyruvate uptake.

Whereas the physiological function of sodium-dependent pyruvate uptake is well established for C_4 plants (Supplementary Information), the role of plastidial pyruvate uptake in C_3 plants is not well characterized. Publicly available microarray data indicate that the *A. thaliana BASS2* orthologue, *At2g26900*, is highly expressed in developing leaves, whereas expression in mature leaves is low[13,18] (Supplementary Fig. 8), which was also confirmed by promoter::reporter analyses and by immunoblot analysis (Supplementary Fig. 9). In two independent mutant lines, named *bass2-1* and *bass2-2* (Supplementary Fig. 10a), no *BASS2* expression was detected (Supplementary Fig. 10b, c). These mutant plants did not show an apparent phenotype under normal growth conditions (Fig. 4c). Isolated chloroplasts from 5-day-old wild-type plants displayed sodium-dependent pyruvate uptake activity in the dark, whereas pyruvate was taken up without simultaneous addition of sodium in the light, as reported in sodium-dependent C_4 plants[9,10] (Fig. 4a). Its specific activity was calculated at 4.53 μmol per mg chlorophyll per hour, which is approximately one-fifth of the average activity (23.6) observed in sodium-dependent C_4 plants[10] (Fig. 4b). On the other hand, chloroplasts isolated from the *bass2* mutants entirely lacked sodium-dependent pyruvate uptake activity (Fig. 4a, b).

Pyruvate is one of the two crucial substrates of the methyl erythritol phosphate (MEP) pathway[2]. If BASS2 is required for fuelling the MEP pathway, *bass2* mutants should be more sensitive to mevastatin, an inhibitor of hydroxymethylglutaryl-CoA reductase (the rate-limiting enzyme of the cytosolic mevalonic acid pathway), than the wild type (Supplementary Fig. 11). Indeed, in the presence of 50 nM mevastatin, a slight growth retardation was observed in the wild type, as reported previously[19]. This growth retardation was more severe and a yellow-leaf phenotype was observed in both *bass2* mutants (Fig. 4c). This mevastatin-sensitive phenotype of *bass2* mutants strongly indicates that BASS2 functions to supply pyruvate to the MEP pathway.

For the efficient import of pyruvate, a mechanism to export sodium cations from the chloroplasts would be needed. On the basis of our data, we propose that sodium-dependent pyruvate transport into mesophyll cells of certain C_4 plant species is facilitated by a two-translocator system, consisting of a sodium:pyruvate cotransporter (BASS2) and a sodium:proton antiporter (NHD1) (Supplementary Fig. 12a). In addition, the molecular mechanism of the light-driven uptake of pyruvate into isolated chloroplasts in C_4 plants[9,10] and in the C_3 plant *A. thaliana* without exogenous sodium ions (Fig. 4a) can be explained by this coupling reaction, as follows. Under light conditions, proton uptake

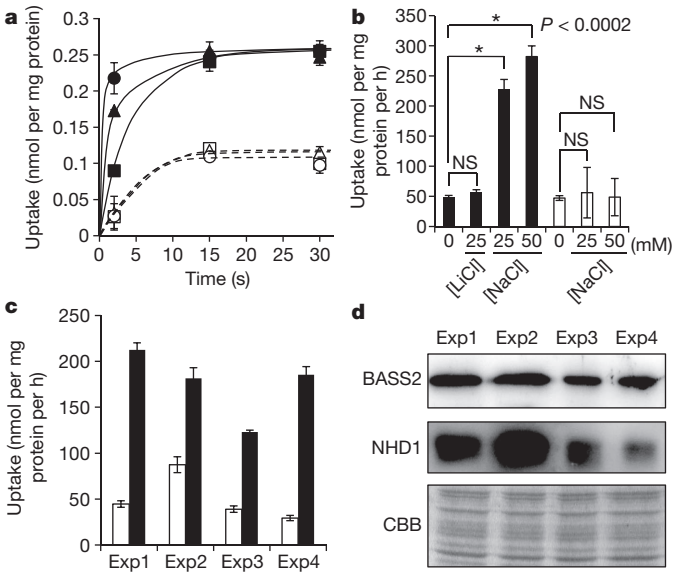

Figure 3 | Pyruvate uptake activity. **a**, Whole *E. coli* cells with (filled symbols) or without (open symbols) BASS2 recombinant protein were incubated with 0 mM (squares), 25 mM (triangles) or 50 mM (circles) NaCl. Data are mean ± s.d., $n = 4$. **b**, Uptake activities of BASS2-expressing *E. coli* (black columns) and empty vector control (white columns) at the initial time point under indicated concentrations of LiCl or NaCl. Data are mean ± s.d., from four independent experiments. *P* values were produced by Student's *t*-test. NS, not significant. **c**, Uptake activities of the dual-expressing *E. coli* in the absence (white columns) and the presence (black columns) of NaCl. Mean values of four experimental repeats of four independent experiments are indicated, with s.d. **d**, The recombinant proteins detected by BASS2-peptide-antibody (upper panel) or by the fused artificial tag (middle panel). The lower panel indicates CBB staining used as a loading control.

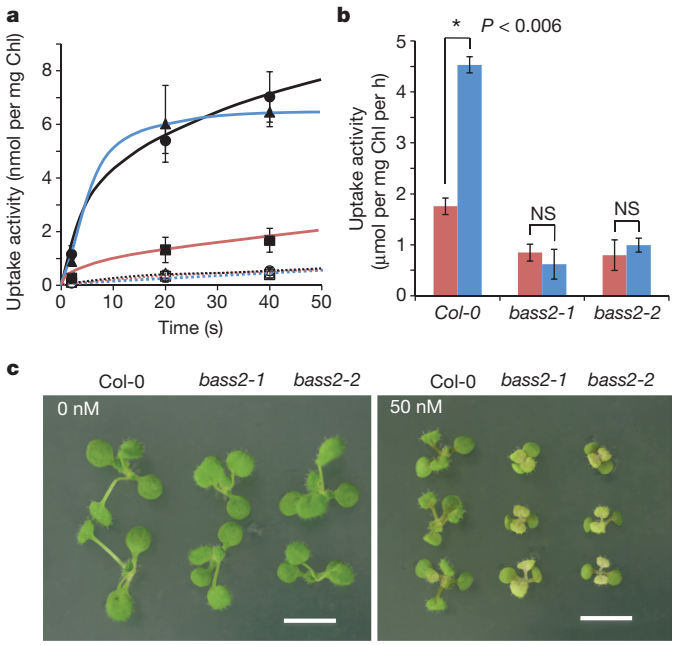

Figure 4 | BASS2 function in *A. thaliana*. **a**, Time-dependent pyruvate uptake activities of wild-type Col-0 (solid lines) and *bass2-1* (dotted lines). Isolated plastids were incubated with (triangles with blue lines) or without (squares with orange lines) 12.5 mM NaCl under dark conditions, or without NaCl under light conditions (circles with black lines). Data show mean ± s.d., $n = 3$–4. **b**, Uptake activities of wild-type Col-0 and *bass2* mutants at the initial time point (2 s). These activities were measured under near-saturating conditions with (blue bar) or without (red bar) NaCl, and mean values with s.d. were determined by three experiments performed on separate chloroplast preparations. *P* values were produced by Student's *t*-test. NS, not significant. **c**, Mevastatin-sensitive phenotype of *bass2* mutants. Plants were grown without (left) or with (50 nM) mevastatin for 10 days. Scale bar, 5 mm.

into thylakoid alkalizes the stroma[20], NHD1 exchanges sodium with protons, thereby establishing a sodium gradient across the envelope, and then BASS2 takes up pyruvate. We propose that in C_4 plants this proton gradient is maintained by stoichiometrically coupling pyruvate import to the export of phosphoenolpyruvate (PEP) (Supplementary Information and Supplementary Fig. 12a).

Extensive efforts have been made to identify organelle-localized pyruvate carriers, both in mitochondria and in plastids[21]; however, the mitochondrial pyruvate importer has remained elusive (Supplementary Information). As shown in Supplementary Fig. 12b, we demonstrate, using evidence from both gain-of-function and loss-of-function experiments, that BASS2 is a plastid-localized sodium-dependent pyruvate transporter functioning in C_4 photosynthesis and in the MEP pathway in C_3 plants.

METHODS SUMMARY

Differential screening and RNA-seq of the genus *Flaveria*. Both mRNA populations were prepared from well-expanded leaves of *F. trinervia* and *F. pringlei* sampled in the middle of the light period. Poly-A+ mRNA was isolated and used to prepare single-strand radiolabelled cDNA as described[22]. Each radiolabelled probe was hybridized to duplicate nylon membranes containing approximately 9,000 phage clones of a *F. trinervia* leaf cDNA library[23]. Except for the major C_4 marker genes encoding phosphoenolpyruvate carboxylase (PEPC), pyruvate, orthophosphate dikinase (PPDK) and NADP-ME, we isolated 56 clones as novel C_4-abundant signals, which were classified into 19 genes by determining their cDNA sequences. By comparing the cDNA fragments to the known genome of *A. thaliana*, we found the *BASS2* and *BASS4* partial cDNA sequences. The cDNA library was rescreened using these partial cDNA fragments as probes, and the full-length cDNAs were isolated. The sequences of these cDNAs were deposited in DDBJ (accession numbers: *F. trinervia BASS2*, AB522102; *F. trinervia BASS4*, AB522103).

The transcriptomes of *F. bidentis*, *F. pringlei*, *C. gynandra* and *C. spinosa* leaves were analysed by RNA sequencing (RNA-seq) as described previously[16,24]. The *F. bidentis BASS2*, and *F. pringlei BASS2* full-length cDNA sequences were obtained from the RNA-seq contig data.

Pyruvate uptake measurement. Isolated plastids from wild-type Col-0 or *bass2* mutants were incubated with 0.2 mM or 0.5 mM pyruvate (0.5 μCi [2-^{14}C]pyruvate, American Radiolabelled Chemicals, Inc.) and with indicated concentrations of NaCl in accordance with the silicone-oil bilayer method[20].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions T.Y. performed the differential screening and isolated the *BASS2* gene. M.N. and Y.O.-I. analysed C_4-abundant genes. Y.O.-I. prepared the *bass2* mutants and revealed *BASS2* expression. M.S. performed the confocal laser micro-scanning and the analyses of immunohistochemistry. J.O. and T.F. performed the pyruvate-uptake measurements on isolated chloroplasts, and A.B., A.P.M.W. and T.F. performed the *E. coli* whole-cell uptake measurements. U.G., P.W., A.B. and A.P.M.W. performed *Flaveria* and *Cleome* transcriptome analyses and the phylogenic analysis. S.H. was involved in designing the study, T.F. and K.I. designed the study, and T.F. and A.P.M.W. wrote the paper.

Author Information cDNA sequences for *F. trinervia* BASS2, *F. trinervia* BASS4 and *F. bidentis* NHD1 have been deposited in the DNA Data Bank of Japan, with respective accession numbers AB522102, AB522103 and AB642169. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to T.F. (tfurumoto@hiroshima-u.ac.jp).

METHODS

Plant growth and crude protein extraction. All plants except for *A. thaliana* and transgenic tobacco plants were grown under natural light in a greenhouse from September to October 2008 in Hiroshima, Japan. *A. thaliana* and tobacco were grown in a growth chamber under constant light at 21 °C and at 28 °C, respectively. In promoter::reporter analyses, *A. thaliana* was grown on soil for 19 days, and in the pyruvate uptake measurements plants were grown on half-strength MS agar plates for 5 days. Leaf samples were frozen in liquid nitrogen and ground with a mortar and pestle into fine powder. Crude protein was extracted with 3 volumes (w/v) of extraction buffer (50 mM Tris-HCl (pH 8.0), 0.5% Triton X-100, 0.1% Nonidet P-40, protein inhibitor cocktail (Complete-mini, Sigma), 1 mM phenylmethylsulphonyl fluoride, 1 mM dithiothreitol). The homogenates were centrifuged at 12,000*g* for 15 min, and the supernatants were used for immunoblot analysis. Immunoblot analyses were performed as previously described[25], except that the anti-BASS2-peptide polyclonal antiserum, diluted 1:5,000, was used as the first antibody.

Immunohistochemical analysis. *F. trinervia* leaves were dissected into small pieces, and transferred into vials containing the fixative solution (4% formaldehyde in 0.05 M phosphate buffer (pH 7.0)); the vials were evacuated by a water aspirator until the specimens sank to the bottom of the vials. After fixation for 8 h at 4°C, the samples were rinsed in 0.05 M phosphate buffer. Then, the samples were dehydrated in a graded acetone series and embedded in plastic resin (Technovit 8100, Kulzer) using a graded series of acetone and resin. Semi-thin sections for light microscopy were obtained with an ultramicrotome using glass knives. The tissue sections were transferred onto glass slides and dried for 20 min at 37 °C. After rinsing in PBS, the sections were incubated with 1% bovine serum albumin in PBS for 5 min. Then, sections were incubated for 1 h with the anti-BASS2-peptide antiserum diluted 1:1,000, or anti-RubisCO antiserum diluted 1:5,000, in PBS at 37 °C in a moist chamber. For the control sections, this incubation step was omitted. After three washes in PBS, the sections were incubated for 1 h with the secondary antibody conjugated with Alexa 488 fluorochrome (Molecular Probes) diluted 1:200 in PBS at 37 °C in a moist chamber. Signals were visualized by epifluorescence microscopy (E600, Nikon) and images were captured with a CCD camera (DXM 1200, Nikon).

BASS2/NHD1 expression in *E. coli* and uptake measurements. The recombinant BASS2 was expressed in *E. coli* (BL21 DE3) using the pETduet-1 expression vector (Novagen), similarly to a previously reported method[22]. For preparation of the expression vector, an internal NcoI site was mutated without amino acid substitution and an artificial EcoRI site was introduced at the end of the putative chloroplast-targeting site. To introduce these mutations, two primer sets were used for PCR: 5′-AAGAATTCTGCCAAGCAGCAACAAATGTAC-3′, 5′-GTTTAAGGAATCCGTGGACTGTAGGTGTAG-3′ and 5′-GTTTAAGGAATCCGTGGACTGTAGGTGTAG-3′, 5′-CAAGTCGACTTACTCCTTGAAATCATC-3′. The amplified DNA fragment was subcloned into the EcoRI and SalI sites of pBluescript KS(-); after the sequence was confirmed to be correct, this fragment was amplified with two primers, 5′-AACCATGGGCTGCCAAGCAGCAACAAATGTA-3′ and 5′-AGGTCGACTTACTCCTTGAAATCATCTT-3′. The former includes an artificial NcoI site, and the latter includes a SalI site. After confirming its sequence, this fragment was introduced into the NcoI and SalI sites of the pETduet-1 vector. The N-terminal putative plastid-targeting peptide was removed, and the resulting mature protein was expressed without tags. The *NHD1* cDNA fragment was amplified with two primers (5′-TGCATATGGCAGAAGATAAGGCACGTGG-3′, 5′-TGCTCGAGCGAGCCCGAGATGAATGGAA-3′) from reverse-transcribed cDNA sources. The former and the latter contained an artificial NdeI site and an artificial XhoI site, respectively. After PCR, amplified cDNA fragments were subcloned into pGEM-T vector before the correct sequence was confirmed. The *NHD1* fragment was introduced into the NdeI and XhoI site of pETduet-1, resulting in a translational S-tag fusion at the C-terminus. The transgenic *E. coli* was grown in LB medium (pH 7.0), where NaCl was replaced by KCl (87 mM)[26]. When the D_{600} was approximately 0.5, 0.1 mM IPTG was added to the *E. coli* culture which was further incubated for 4 h at 25 °C (ref. 27). An artificial pH gradient was formed across the *E. coli* membrane as described previously[28]. *E. coli* was washed with inside buffer (50 mM $KHPO_4$ (pH 8.2), 0.1 mM $MgSO_4$, 0.1 mM $CaCl_2$) once, and then incubated in the same buffer containing 50 μM of carbonyl cyanide *m*-chlorophenylhydrazone and 1 mM As_2O_3 for 15 min at room temperature. After centrifugation, the *E. coli* was resuspended with reaction buffer (50 mM $KHPO_4$ (pH 7.8), 0.1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.1 mM [2-^{14}C]pyruvate) with the indicated concentration of NaCl. Two pyruvate concentrations, 0.5 mM or 0.1 mM, were adopted for the uptake reactions. The former was in accordance with the previous assay conditions using isolated plastids[8,9], and the latter was adjusted for *E. coli* whole-cell assay to avoid the rapid saturation of uptake. After incubation for the indicated amount of time, the *E. coli* suspension was used in the silicone-oil filtration-centrifugation method to stop the uptake reaction[20]. Mean values of the uptake activities and standard deviations were determined from three to four independent *E. coli* preparations.

***BASS2* expression in *A. thaliana* and *bass2* mutants.** Seeds of two independent *bass2* mutants (*bass2-1* and *bass2-2*) were obtained from the ABRC seed stock centre (SALK_101808 and SALK_098962, respectively). Each homozygote was isolated as described previously[29]. Ten-day-old, 7-day-old and 5-day-old plants were used for an RNA gel-blot (northern blot) analysis, a protein gel-blot (western blot) analysis, and for pyruvate uptake measurements, respectively. For the measurement of short periods of uptake (predicted 2 s), the silicone-oil double layer method was used, and for longer periods of uptake, the silicone-oil single layer method was used in accordance with a previous report[20]. To measure pyruvate uptake activity, two conditions were adopted: (1) as a near non-saturating condition, isolated plastids were incubated for the indicated number of seconds in reaction mixture (50 mM HEPES (KOH) (pH 7.8), 5 mM EDTA, 0.35 M sorbitol), which includes 0.2 mM pyruvate (0.5 μCi [2-^{14}C]pyruvate) with or without 12.5 mM NaCl; and (2) as a near-saturating condition, isolated plastids were incubated in the same buffer, including 0.5 mM pyruvate (0.5 μCi [2-^{14}C]pyruvate) with or without 50 mM NaCl. Sodium-dependence was measured under dark conditions. Under light conditions, approximately 50 μmol m^{-2} s^{-1} of white light was applied for 10 min before measurement on ice and during centrifugation steps. The cytoplasmic concentration of sodium in 5-day-old *A. thaliana* is not known. Thus, 12.5 mM sodium was adopted, based on the previous biochemical characterization of the pyruvate uptake activity on the plastids isolated from C_4 plants, which was about twice the K_m value for sodium (4.8 mM)[9].

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CORRECTIONS & AMENDMENTS

CORRIGENDUM

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A plastidial sodium–dependent pyruvate transporter

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In this Letter, the DNA Data Bank of Japan accession code for *Flaveria trinervia* BASS4 should be AB522103. This has been corrected online.