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Changes in starch and inositol 1,4,5-trisphosphate levels and auxin transport are interrelated in graviresponding oat (*Avena sativa*) shoots

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ABSTRACT

This study was conducted to unravel a mechanism for the gravitropic curvature response in oat (*Avena sativa*) shoot pulvini. For this purpose, we examined the downward movement of starch-filled chloroplast gravisensors, differential changes in inositol 1,4,5-trisphosphate (IP_3) levels, transport of indole-3-acetic acid (IAA) and gravitropic curvature. Upon gravistimulation, the ratio for IAA levels in lower halves versus those in upper halves (L/U) increased from 1.0 at 0 h and reached a maximum value of 1.45 at 8 h. When shoots were grown in the dark for 10 d, to deplete starch in the chloroplast, the gravity-induced L/U of IAA was reduced to 1.0. *N*-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA), both auxin transport inhibitors, significantly reduced the amount of gravitropic curvature and gravity-induced lateral IAA transport, but did not reduce the gravity-induced late change in the L/U ratio of IP_3 levels. U73122, a specific phospholipase C (PLC) inhibitor, decreased gravity-induced curvature. Because U73122 reduced the ratio of L/U of IAA imposed by gravistimulation, it is clear that IAA transport is correlated with changes in IP_3 levels upon gravistimulation. These results indicate that gravistimulation-induced differential lateral IAA transport may result from the onset of graviperception in the chloroplast gravisensors coupled with gravity-induced asymmetric changes in IP_3 levels in oat shoot pulvini.

Key-words: graviperception; gravitropic response; signal transduction.

INTRODUCTION

Gravitropic responses of plants are mediated by a cascade of biophysical and biochemical events that occur during three stages, namely gravity perception, gravity signal transduction and differential cell growth (Kaufman *et al*. 1995). For gravity perception, the starch–statolith hypothesis indicates that the gravisensors in roots are starch-filled amyoplasts, and in shoots, starch-filled chloroplasts (Sack 1997; Weise *et al*. 2000). This hypothesis is supported by the fact that starchless or low-starch mutants of *Arabidopsis thaliana* and tobacco (*Nicotiana tabacum*) show no or very slow gravitropic curvature responses (Kiss *et al*. 1997; Sack 1997; Vitha *et al*. 1998). Song *et al*. (1988) showed that the size of starch grains in chloroplasts was enhanced in pulvini when sucrose was added to barley stems. This treatment resulted in an increase in the gravitropic curvature response. Quantitative studies show that the amounts of starch present in the chloroplast gravisensors of oat shoot pulvini following light versus dark pretreatments of shoots are strongly correlated with the extent of gravitropic curvature that occurs in pulvini of these shoots (Chang *et al*. 2001). These lines of evidence lend credence to the idea that starch-filled chloroplasts in shoots of green plants are indeed the gravisensors when plants are gravistimulated.

Regarding the gravity signal transduction pathway, recent studies suggest that K^+ (Philippar *et al*. 1999), H^+ (Scott & Allen 1999; Johannes *et al*. 2001), reactive oxygen species (ROS) (Joo, Bae & Lee 2001) and inositol 1,4,5-trisphosphate (IP_3) (Perera, Heilmann & Boss 1999; Perera *et al*. 2005) are possible second messengers in this pathway. Calcium/calmodulin (Belavskaya 1996; Trewavas & Malho 1997) and protein phosphorylation/dephosphorylation (Chang *et al*. 2003) are also likely components of the signal transduction pathway. In oat shoots, the IP_3 levels were higher in graviresponding pulvini than in vertical control pulvini at very early stages (1–3 min) of the gravitropic response (Perera *et al*. 2001). They showed that during the period of 10–50 min after the start of gravistimulation treatment, the levels of IP_3 were greater in lower halves than in upper halves of pulvini. This timescale for the change in oat (Perera *et al*. 2001) was much shorter than that of maize (Perera *et al*. 1999). These findings suggest that IP_3 acts as

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an important component of the gravity signal transduction pathway in both maize and oat shoot pulvini.

Polarity is the unique feature of auxin transport and its cellular and molecular mechanism is well documented (Muday, Peer & Murphy 2003). Gravity-induced differential cell growth has been explained according to this polar auxin transport mechanism. An influx carrier, AUX1, and an efflux carrier, AGR1/EIR1/PIN2/WAV6, were identified and their roles in the root gravitropic response were well established (see Muday 2001). However, only a few related findings have been reported for shoot gravitropism so far (Friml *et al*. 2002).

In monocot shoots, lateral auxin transport is responsible for the asymmetric growth of graviresponding organs, such as maize coleoptiles (Parker & Briggs 1990; Iino 1991) and oat shoot pulvini (Kaufman *et al*. 1995). In the oat shoot pulvinus, gravistimulation treatment causes asymmetric movement of indole-3-acetic acid (IAA) across the pulvinus towards the lower side (Brock *et al*. 1991). This movement occurs as early as at 30 min after the initiation of gravistimulation. At 3 h after the initiation of gravistimulation treatment, the IAA level was found to be 2.5 times greater in the lower halves than that in the upper halves of pulvini (Kaufman *et al*. 1995).

There are still many unanswered questions regarding: (1) the kinetics and/or extent of asymmetric changes that occur in starch, IP_3 and IAA levels in graviresponding cereal grass shoot pulvini; and (2) possible linkages between these changes in relation to gravity perception, signal transduction and response. The present study attempts to address these issues. Using the oat shoot pulvinus as a model system, we show that gravity-induced changes in starch and IP_3 levels are closely related and are required for IAA lateral transport to occur in graviresponding oat shoot pulvini. The significance of these observations in relation to the gravity-mediated upward bending response mechanism in pulvini of cereal grass shoots is discussed.

MATERIALS AND METHODS

Chemicals

[^{3}H]-IAA (specific activity: 925 GBq mmol^{-1}) and a phospholipase C (PLC) inhibitor, U73122 and its chemical analogue, U73343, were obtained from American Radiolabeled Chemicals Inc (St. Louis, MO, USA) and Calbiochem-Novabiochem Co. (La Jolla, CA, USA), respectively. [^{14}C] benzoic acid (specific activity: 0.49 GBq mmol^{-1}) and other fine chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise indicated.

Plant material

Seeds of oat (*Avena sativa* cv. victory) were obtained from Svalöf, International, A. B. (Swedish Seed Association), Svalöf, Sweden. Glasshouse-grown plants were illuminated with 400 W high-pressure sodium vapour lamps (PE Lighting Systems, Grimsby, Ontario, Canada) giving a light intensity of 1000 μmol m^{-2} s^{-1} at the top of the plants. The photoperiod regime was programmed for 18 h light : 6 h dark for each 24 h cycle and the glasshouse temperatures were 27/23 °C day/night (Brock *et al*. 1991). At 45 d, the shoots were harvested by cutting them off just above the roots and placing the shoots vertically in a bucket containing ca. 10 cm of water. The same day, 9 cm stem segments (located just below the *last-formed* node/pulvinus that subtends the peduncle of the panicle inflorescence) were excised from top portions of the shoots. Each stem segment consisted of the *next-to-last formed* node/pulvinus, 3 cm stem tissue located below the pulvinus and 6 cm of leaf sheath/stem tissue located above the pulvinus. As they were cut, stem segments were kept in a vertical position in a 150 mL beaker containing 20 mL distilled water. When the beaker was packed with segments, it was placed in a refrigerator at 4 °C. Under these conditions, the gravitropic responsiveness of the segments did not decrease for up to 1 week.

Inhibitor pretreatments, gravistimulation treatment protocol and measurement of gravitropic curvature

The pulvini of oat stem segments were gently abraded with an aqueous paste of silicic acid (H_2SiO_3) by rotating them three or four times between the thumb and the forefinger. This abrasion treatment did not cause any change in the ratio of IAA and IP_3 levels between the upper and lower halves of shoot pulvini compared with the ratio of non-abraded samples following gravistimulation treatment. Using a mild vacuum, abraded stem segments were infiltrated for 2 min with a solution containing 0.1 M sucrose and 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulphonic acid (Hepes)–NaOH buffer (pH 7.5) in the presence or absence of solutions of U73122, U73343, *N*-naphthylphthalamic acid (NPA), 2,3,5-triiodobenzoic acid (TIBA), okadaic acid (OA) or $LaCl_3$. The segments were then kept in a vertical position for 2 h (24 h in case of $LaCl_3$) in the solution containing one of the inhibitors in the dark at room temperature.

For gravistimulation treatment, 80 or more stem segments were placed horizontally, side-by-side, between paper towels saturated with 0.1 M sucrose and 50 mM Hepes–NaOH (pH 7.5) buffer and held in this horizontal position between two glass plates that were taped together (Fig. 1). The upper side of each segment was marked with a dot, using a blue Sharpie pen, just above each pulvinus. The pulvini were placed just outside the edges of the plates so that upward bending was unimpeded. The glass plate 'sandwich' was then placed in a Plexiglas box. The Plexiglas box was covered by placing an equal-sized Plexiglas box in an inverted position on top of the lower box. Relative humidity was maintained at 100% by the addition of 1 cm water to the bottom of the lower tray. After various periods of incubation in the dark at 25 ± 1 °C, the gravitropic curvature response of stem segment pulvini was determined with a protractor.

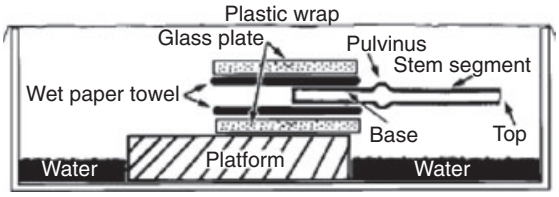

Figure 1. Illustration of a Plexiglas growth chamber in an incubator programmed at 25 °C. Oat shoot segments are placed horizontally for gravistimulation or are oriented vertically for the control.

IP_3 assays

For analysis of IP_3 content, at each time period, 8–10 p-1 pulvini were harvested and stored at –80 °C. The tissue was ground to a fine powder in liquid N_2 and added to a pre-weighed tube containing 500 µL of ice-cold 20% (v/v) perchloric acid ($ClHO_4$). After incubation on ice for 20 min, proteins were precipitated by centrifugation at 2000 *g* for 15 min at 4 °C. The supernatant was transferred to a new tube and adjusted to pH 7.5, using ice-cold 1.5 M KOH in 60 mM Hepes containing 5% (v/v) of universal pH indicator dye (Fisher Scientific, Loughborough, Leicestershire, UK). The neutralized samples were assayed for IP_3, using a [^{3}H]I(1, 4, 5) P_3 receptor binding assay kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). Assays were carried out along with controls for complete and non-specific binding according to the manufacturer's instructions by using 50 µL of sample per assay in a total assay volume of 200 µL. The IP_3 content of each sample was determined by interpolation from a standard curve generated with commercial IP_3. The presence of IP_3 in the samples was verified by pretreatment with recombinant inositol polyphosphate-5-phosphatase I according to Perera *et al.* (1999). The phosphatase treatment eliminated > 90% of the IP_3 from the oat samples.

Starch analysis

The methods employed for starch analysis are essentially those of Hubbard, Pharr & Huber (1990) and are as follows: (1) 0.2–0.4 g of fresh oat shoot pulvinus tissues was ground in a mortar in 2.8–3.5 mL of 80% ethanol; (2) this was followed by heating the ground sample in a hot (80 °C) water bath for 5 min, then centrifuging it at 1000 g for 10 min; (3) the tissue was extracted three times as above and the supernatants were discarded; (4) 0.2 M KOH was added to the remaining pellets, followed by immersion of the tubes in a boiling water bath for 30 min; (5) after the pHs were adjusted to 7.0 with 1.0 M acetic acid, each sample was treated with 3 mL of dialysed amyloglucosidase (240 U mL^{-1}) with stirring, then placed in a water bath at 55 °C for 1 h with occasional stirring; (6) the samples were next placed in a boiling water bath for 5 min, cooled and centrifuged at 18 000 g for 10 min; (7) the supernatants were decanted, filtered if necessary, made to a known volume and analysed for D-glucose content using the hexokinase-glucose-6-phosphate dehydrogenase method (Hubbard *et al.* 1990). This method is based on the conversion of the glucose-6-phosphate to 6-phosphogluconolactone in the presence of NADP and glucose-6-phosphate dehydrogenase, and hence to 6-phosphogluconate in the presence of phosphoglucoseisomerase. Absorbance was read at 340 nm against distilled water after 30 min at room temperature. All reagents used in this starch analysis were purchased from Sigma Chemical Co.

It should be noted that starch in pulvinus tissue occurs primarily in the chloroplasts. These chloroplasts are located in groups of cells lying just inside each longitudinal vascular bundle of the pulvinus and *not* in other cells that make up the pulvinus tissues (Song *et al.* 1988; Kaufman *et al.* 1995). Thus, the starch, as analysed by the mentioned protocol, is essentially that which occurs in the pulvinus chloroplasts.

Assay of lateral IAA transport

This assay was performed according to the method of Brock *et al.* (1991). [^{3}H]-IAA (specific activity: 925 GBq mmol^{-1}) was incorporated into agar (1.5% w/v) to give a final concentration of 2×10^{-7} M, pH 5.5 ($2 \times 2 \times 1$ mm) donor block. In case of the respective treatments, 0.1 mM U73122, 0.1 mM U73343, 0.1 mM NPA, 0.1 mM TIBA, 10^{-7} M OA or 0.1 mM $LaCl_3$ was included. Labelled donor blocks were placed on both sides of vertically located pulvini to pre-load the pulvinus with [^{3}H]-IAA. Oat stem pulvini were gently abraded, as described previously, for an efficient uptake of the labelled IAA. After 2 h, the donor blocks were replaced with unlabelled agar blocks, and the pulvini were either placed horizontally or left upright. At designated time points, the pulvini were cut into upper and lower halves. Radioactivity of pulvinus halves and agar blocks was measured by the use of liquid scintillation spectrophotometer (model LSC-6500; Beckman, Fullerton, CA, USA), following overnight equilibration in scintillation fluid.

Gene analysis

Three early auxin-responsive genes, *AsIAA1*, *AsIAA2* and *AsIAA3*, that are homologous to Aux/IAA gene families (Abel & Theologis 1996) were cloned from oat stem pulvini by RT–PCR with degenerate primers (Fujii *et al.* 2000). These genes were cloned from the pulvinus after being treated with 10^{-4} M IAA for 2 h. The expression level of these genes in lower and upper sides of gravistimulated pulvini was determined by RT–PCR at various time intervals during gravistimulation. The primers used for RT–PCR were as follows: 5′-AGGTCGAGGCAAAGCA-3′ and 5′-AAGT TCAGCCCCGCTTTTA-3′ (*AsIAA1*), 5′-AAGACCAA GGCGGGAGAG-3′ and 5′-GTACTCTTGATCTTGA-3′ (*AsIAA2*), 5′-AAACCGAGAAGCAGCAG-3′ and 5′-ATCCAGTCTCCGTCCTT-3′ (*AsIAA3*). A similar analysis was performed in both sides of the pulvini of vertical shoots; this expression level was defined as the control.

AsIAA1, *AsIAA2* and *AsIAA3* were deposited as AY847062, AY847063 and AY847064 to GenBank, respectively.

Replication of experiments and statistical analysis of data

All experiments of the oat shoots were performed at least three times. In every experiment, at least 20 shoots were used. To test for significance of the data, mean values were calculated with Student's *t*-test according to http://graphpad.com/quickcalcs/. A P value of < 0.05 was considered to be significant unless otherwise indicated.

RESULTS

Insights on the gravisensing process: dark treatment decreases levels of starch in oat shoot pulvinus gravisensors and shoot gravitropic curvature response

A previous study with barley (*Hordeum vulgare*) shoot pulvinus (Song *et al*. 1988) showed that starch statoliths (starch-containing chloroplasts) in gravistimulated shoot pulvini did not sediment if starch levels in these statoliths are decreased by placing pulvinus-containing segments in the dark. As a consequence, graviperception can be blocked and little or no negative gravitropic bending occurs. These observations on barley shoot pulvini are corroborated here with similar studies on oat shoot pulvini. When oat shoots are gravistimulated for 18 h after being subjected to dark pretreatment for different periods of time, their gravitropic curvature is significantly reduced as compared with shoots that underwent a corresponding light pretreatment (Fig. 2a). Internodal extension growth in these experiments was unaffected by vertical versus horizontal orientation of the stem segments. In each case, internodal extension in the light or dark varied between 8 and 10 mm (data not shown). The reduction in the gravitropic curvature of both dark- and light-pretreated shoots is positively correlated with the time of pre-incubation (Fig. 2a).

Parallel with the analysis of the gravitropic responses of light and dark pretreated oat shoot pulvini, we performed quantitative analyses of starch in the pulvini using similar kinetics. The results show that dark-pretreated pulvini had significant lower levels of starch than light-pretreated ones (Fig. 2b) over the entire time of pretreatment (0–7 d). The greatest rate of decrease in starch level occurred between 0 and 2 d of dark pretreatment, as did also, the rate of gravitropic curvature.

Insights on the linkage between gravisensing process and the signal transduction process: movement of starch statoliths is needed for changes in IP_3 levels to occur upon gravistimulation treatment

Based on the results presented in Fig. 2, we next determined the effects of dark and light pretreatments on gravity-induced IP_3 changes. Oat shoot segments were pre-incubated in the dark or light for 0, 2, 5 or 7 d and then gravistimulated for 1 or 40 min. These represent the times for gravity-induced early or late changes of IP_3 level, respectively (see below or Perera *et al*. 2001). The results presented in Fig. 3 show changes in the lower halves versus upper halves (L/U) ratio of IP_3 levels imposed by gravistimulation for 40 min with increasing time of dark/light pretreatment. Furthermore, the L/U ratio was lower in

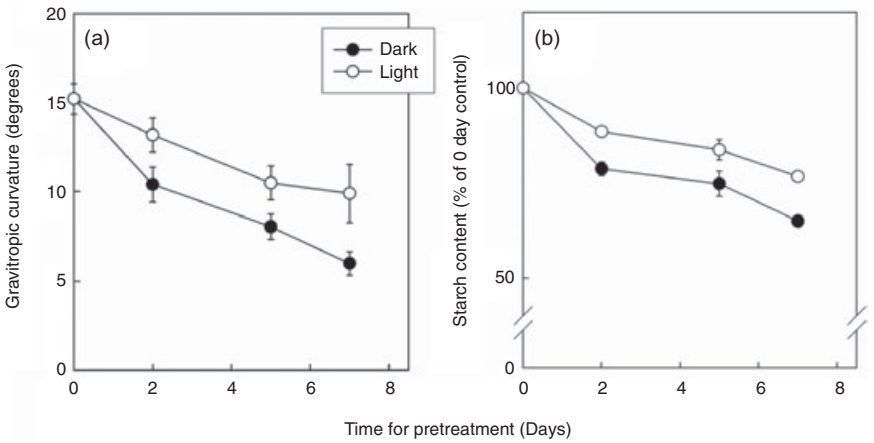

Figure 2. Effect of dark pretreatment on gravitropic curvature (a) and on starch levels (b). In (a), oat shoot segments were incubated in the dark for 0, 2, 5 and 7 d, then were gravistimulated for 18 h. In (b), the shoot segments were incubated in the dark for 0, 2, 5 and 7 d. The pulvini were then collected at these times, frozen on dry ice and stored at –80 °C for subsequent starch analysis. Vertical bars represent SE of means from four experiments. In (a), results for dark-treated segments are significantly different ($P < 0.01$ except at 2 h where $P < 0.001$) from those for light-treated segments at every time point except for the 0 h point. In (b), results for dark-treated segments are significantly different ($P < 0.001$ except at 7 h where $P < 0.01$) from those for light-treated segments at every time point except for the 0 h time point.

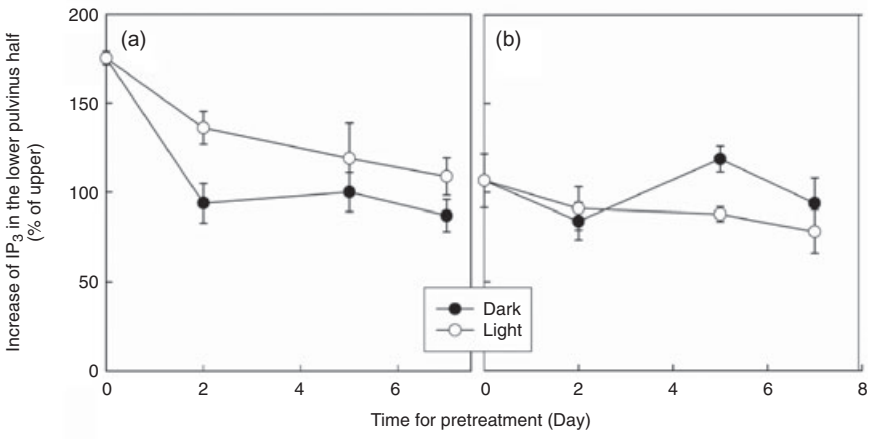

Figure 3. Effect of dark pretreatment on changes in inositol 1,4,5-trisphosphate (IP_3) level. Oat shoot segments were incubated in the dark for 0, 2, 5 and 7 d, then were gravistimulated for 40 min (a) or 1 min (b). After the gravistimulation, oat shoot pulvini were cut into top and bottom halves, frozen on dry ice, then stored at –80 °C for subsequent IP_3 analysis. Vertical bars represent SE of means from three experiments. The ratio of left versus right halves of control pulvini is around 1.0. IP_3 levels in vertical controls were 220 ± 40 and 260 ± 40 pmol g^{-1} fresh weight (FW) for light and dark for 0 d, respectively. In (a), results for dark-treated segments are significantly different ($P < 0.05$ except 2 d where $P < 0.01$) from those for light-treated segments at every time point except the 0 h point. In (b), results for dark-treated segments are significantly different ($P < 0.01$) from those for light-treated segments only at the 7 h time point.

dark-pretreated pulvini than in light-pretreated ones – reaching a value of ca. 1.0 (100%) following 5 or 7 d of pre-incubation under both conditions (Fig. 3a). However, such an effect was not observed in oat shoots gravistimulated for 1 min (Fig. 3b). To confirm the dark effect, we tested the effects of OA, a specific inhibitor of protein phosphatase 1 and 2a, and of $LaCl_3$, a calcium channel blocker, on gravity-imposed L/U ratio of IP_3 because these drugs elicited a decrease in starch content in graviresponding oat shoot pulvini (Chang *et al*. 2001). When oat shoots were treated with these two inhibitors, both inhibitors elicited a significant reduction in gravitropic curvatures (by 73%) compared with the control (Fig. 4a). However, shoot elongation was reduced by 34 and 29% only in the presence of OA or $LaCl_3$, respectively, (data not shown) suggesting that the inhibitors affect the gravitropic response specifically. Furthermore, the two inhibitors decreased gravity-imposed L/U ratio of IP_3 to ca. 1.0 (Fig. 4b). These results indicate that there is a close relationship between gravity-induced changes in starch content, elicited by light or dark pretreatment, and IP_3 level manifested after 40 min of gravistimulation treatment in oat shoot pulvini.

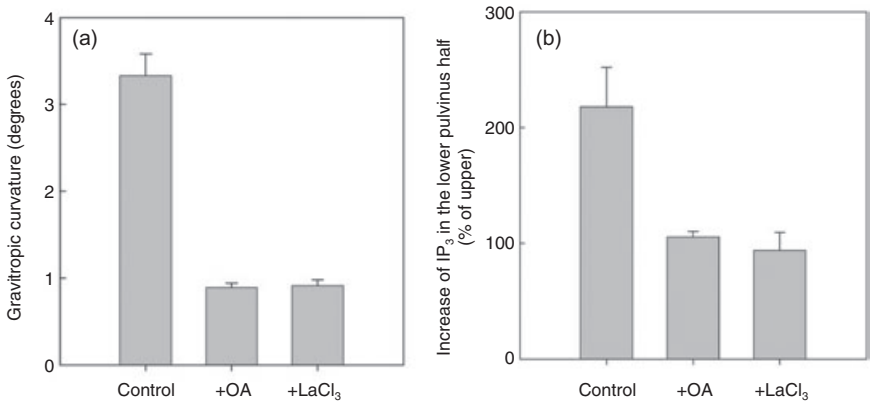

Figure 4. Effect of okadaic acid (OA) and $LaCl_3$ on (a) the gravitropic response and (b) changes in inositol 1,4,5-trisphosphate (IP_3) level upon the gravistimulation treatment. Oat shoot segments were abraded with silicic acid briefly and vacuum infiltrated in a solution containing 5 mM KH_2PO_4 (pH 5.5) with or without 1 μM OA or 10 mM $LaCl_3$ for 2 min. They were then incubated in the same solution for 2 h in the dark at room temperature. After the treatment, oat shoot segments were positioned horizontally for 30 min. After the gravistimulation, the upper and lower halves of pulvini were cut directly onto ice, then frozen at –80 °C for subsequent IP_3 analysis. The experiment was repeated four times using 10 segments for each time point. Values denote mean ± SE. The ratio of left versus right halves of control pulvini is around 1.0. IP_3 levels in vertical controls were 540 ± 90, 510 ± 110, 390 ± 50 pmol g^{-1} fresh weight (FW) for 0.1% dimethyl sulphoxide (DMSO) control, OA and $LaCl_3$, respectively.

Table 1. Effects of auxin transport inhibitors on the gravity-induced lateral auxin transport

Time for gravistimulation	L/U ratio		
	Control	+NPA	+TIBA
40 min	1.22 ± 0.04	1.02 ± 0.01[a]	0.99 ± 0.01[a]
2 h	1.30 ± 0.03	1.09 ± 0.02[a]	1.08 ± 0.03[a]

Oat shoot segments were pre-incubated in a solution containing 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulphonic acid (Hepes)–NaOH (pH 7.5) and 0.1 M sucrose, in the presence or absence of 0.1 mM *N*-naphthylphthalamic acid (NPA) or 2,3,5-triiodobenzoic acid (TIBA). After the pre-incubation treatment, the stem segments were placed horizontally for 40 min or 2 h. Values denote means ± SE of four independent experiments.
[a]Significantly different compared with the control ($P < 0.001$) by Student's *t*-test.
L/U, lower halves versus upper halves.

Further insights on the linkage between gravisensing process and the signal transduction process: graviperception is required for lateral IAA transport to occur in graviresponding oat shoots

Here, we set out to determine whether or not graviperception, downward movement of starch statoliths and changes in IP_3 levels are required for lateral auxin (IAA) transport to occur after the onset of gravistimulation treatment. To do this, we needed to verify whether or not lateral transport of auxin is required for the gravitropic response to occur (Brock *et al*. 1991). Auxin transport inhibitors, including NPA and TIBA, reduced gravitropic curvature (38.4% by 0.1 mM NPA; 78.7% by 0.1 mM TIBA) at 24 h after the initiation of the gravistimulation treatment (data not shown). To monitor lateral auxin transport, we measured the radioactivity of [^{3}H]-IAA in upper and that in lower halves of the pulvini after the onset of gravistimulation treatment. When we compared gravity-imposed L/U ratio of the control with that in the presence of NPA or TIBA, the ratio decreased from 1.22 to nearly 1.0 at 40 min after the initiation of the gravistimulation treatment (Table 1). This inhibition persisted, even after 2 h of gravistimulation. As a negative control, we measured lateral transport of [^{14}C]-benzoic acid following gravistimulation treatment. While [^{3}H]-IAA was distributed in favor of the lower half with an L/U ratio of 1.2–1.3, [^{14}C]-benzoic acid appeared to move without any polarity in the pulvini upon gravistimulation; it showed an L/U ratio of 1.0 (Table 2). Table 2 also shows that clinorotation of the shoots negated the increase in the L/U ratio of [^{3}H]-IAA distribution. Based on these results, we conclude that the observed downward movement of [^{3}H]-IAA is specifically imposed by gravity in gravistimulated oat shoot pulvini. These results also indicate that our assay method for characterization of lateral auxin transport is valid and that our results support the observations of Brock *et al*. (1991).

In previous reports, Song *et al*. (1988) and Chang *et al*. (2001) suggested that gravistimulated oat shoot pulvini perceive the gravity signal via sedimentation of starch-filled chloroplasts. To determine if graviperception, mediated by starch gravisensors, is coupled to lateral auxin transport, we decreased the starch levels by incubating oat shoots in the dark for 6 d prior to gravistimulation treatment (see Fig. 2b). Table 3 shows that dark pretreatment of vertically held oat shoots reduced the L/U ratio of IAA of oat pulvini to ca. 1.0 following gravistimulation treatment. This result clearly shows that gravisensing exerted by starch-filled chloroplasts is required for IAA lateral transport to occur in graviresponding oat shoot pulvini. How this gravisensing process leads to the establishment of IAA asymmetry is still an unsolved question.

Insights into the signal transduction process: gravity-elicited increase in the L/U ratio of IP_3 levels is required for lateral IAA transport to occur

Perera *et al*. (2001) showed that gravity-imposed L/U ratio of IP_3 levels was reduced in the presence of U73122, an action inhibitor of PLC, during 10–50 min (late phase) after the start of the gravistimulation treatment in oat shoot pulvini. Such a reduction was found neither during the initial 3 min (early phase) of gravistimulation, nor in the

Table 2. Negative controls for gravity-induced lateral auxin transport

Time for gravistimulation	L/U ratio		
	Gravistimulation [^{3}H]-IAA	Gravistimulation [^{14}C]-benzoic acid	Clinorotation [^{3}H]-IAA
40 min	1.20 ± 0.02	0.94 ± 0.03[a]	0.99 ± 0.01[a]
2 h	1.33 ± 0.03	1.02 ± 0.04[a]	1.03 ± 0.04[a]

Oat shoot segments were pre-incubated in a solution containing 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulphonic acid (Hepes)–NaOH (pH 7.5) and 0.1 M sucrose, in the presence or absence of [^{14}C]-benzoic acid or [^{3}H]-indole-3-acetic acid (IAA). After the pre-incubation treatment, the stem segments were subjected to clinorotation at 1 g or were placed horizontally for gravistimulation for 40 min or 2 h. Values denote means ± SE of four independent experiments.
[a]Significantly different compared with the control ($P < 0.001$) by Student's *t*-test.
L/U, lower halves versus upper halves.

Table 3. Dark pretreatment-reduced indole-3-acetic acid (IAA) lateral transport

	Control	Dark pretreatment
L/U ratio		
40 min	1.22 ± 0.02	0.99 ± 0.03[a]
2 h	1.31 ± 0.04	1.02 ± 0.05[a]

Oat shoot segments were incubated in the dark at room temperature (25 °C) for 6 d. After this pretreatment, the shoots were gravistimulated for 40 min or 2 h. Values denote means ± SE of four dependent experiments.
[a]Significantly different compared with the control ($P < 0.001$) by Student's *t*-test.
L/U, lower halves versus upper halves.

presence of U73343, the chemical analogue of U73122. This indicates that the gravity-imposed late change in L/U ratio of IP_3 levels is U73122 specific.

Based on these results, we postulated that gravity-elicited lateral transport of IAA could be decreased in the presence of U73122 if the gravity-elicited increase in the L/U ratio of IP_3 levels is required for lateral IAA transport. Our results clearly show this to be the case. U73122 significantly inhibits gravity-induced IAA lateral transport (Fig. 5a); at the same time, it also significantly reduces the amount of gravitropic curvature that occurs after 8 h of gravistimulation treatment (Fig. 5b). However, stem elongation was not affected by the presence of U73122 (data not shown). On the other hand, the inactive analogue, U73343, which only slightly inhibited IAA lateral transport (Fig. 5a), had little effect on the extent of gravitropic curvature (Fig. 5b). These results support the view that the L/U ratio of IP_3 levels elicited by gravistimulation treatment is required for lateral IAA transport to occur.

Because lateral IAA transport is elicited in the pulvini by gravistimulation treatment, it was predicted that there would be differential expression of one or several auxin-responsive gene(s) between the upper and lower halves of the pulvini. Among cloned auxin-responsive genes, including *AsIAA1* (*A. sativa* Aux/IAA), *AsIAA2* and *AsIAA3* from oat shoot pulvinus, only *AsIAA2* responded to exogenously applied IAA (data not shown). Upon gravistimulation treatment, a greater level of expression of *AsIAA2* was found in lower than in upper halves of oat pulvini at 40 min and at 2 h after the start of the gravistimulation treatment (Fig. 6a). However, such differential expression between the lower and the upper halves of the pulvini was not found at 1, 3 and 20 min following the initiation of gravistimulation treatment. This differential gene expression pattern of *AsIAA2* disappeared following treatment with the PLC inhibitor, U73122, but was clearly expressed at 2 h after the start of the gravistimulation treatment in the presence of the analogue, U73343 (Fig. 6b). Once again, these results confirm the idea that gravity-induced changes in the IP_3 level are required for downward movement of IAA in graviresponding oat shoot pulvini.

The reverse possibility that gravity-induced lateral auxin transport affects changes in IP_3 levels was also tested. For

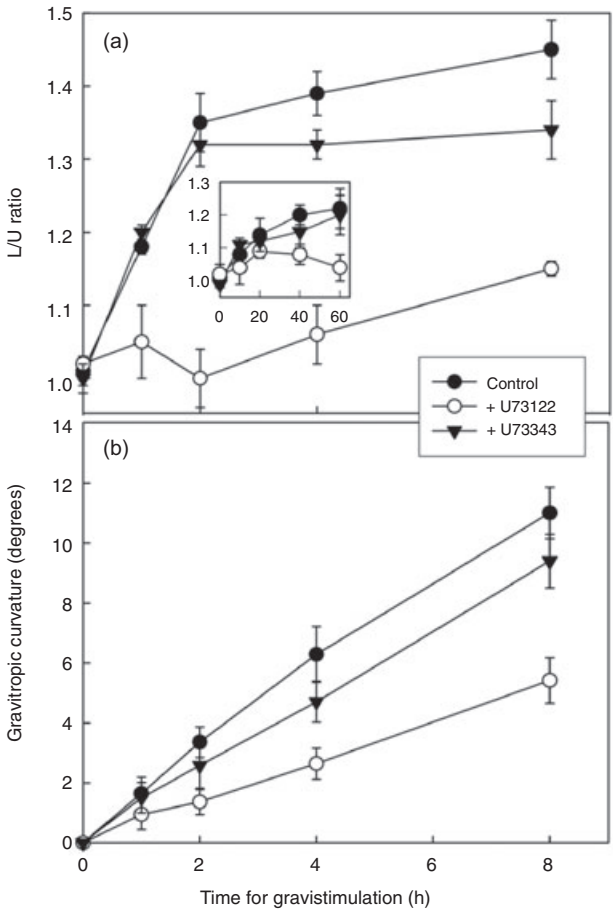

Figure 5. Time course for the effects of phospholipase C (PLC) inhibitors (U73122 and U73343) on (a) gravity-induced lateral transport of indole-3-acetic acid (IAA) and (b) the gravitropic response. In (a), the oat shoot pulvini were abraded and vacuum infiltrated with 2×10^{-7} M [^{3}H]-IAA ± 0.1 mM U73122 or U73343 for 2 min; then, the pulvinus tissues were attached to 1.5% (w/v) agar blocks containing 5 mM potassium/phosphate buffer (pH 5.5), 2×10^{-7} M [^{3}H]-IAA ± 0.1 mM U73122 or U73343, and were incubated in the dark at room temperature for 2 h. The oat segments were gravistimulated for 0, 10, 20, 40 and 60 min (inset) or 0, 1, 2, 4 and 8 h after the pre-incubation. After the gravistimulation, the pulvini were cut into halves and subjected to liquid scintillation spectrometry. In (b), oat shoots were pretreated and gravistimulated as in (a) except that the pulvini were pre-incubated in a solution containing the same chemicals cited for (a). Bars on the graphs (a and b) denote SE of three independent experiments. In (a), results for U73122-treated segments are significantly different ($P < 0.001$ except at 1 h where $P < 0.05$) from those of the control at every time point except at 0 h point. In the inset, P values are < 0.05 and 0.01 at 40 min and 1 h, respectively, when U73122 treatments are compared with the control. Differences at other time points in the comparison between U73122 treatments and the control and all time points in the comparison between U73343 treatments and the control are not significantly different. In (b), results of U73122-treated segments are significantly different ($P < 0.001$) from those of the control at every time point except at the 0 and 1 h points. Differences of all time points for the comparison between U73343 treatments and the control are not significantly different.

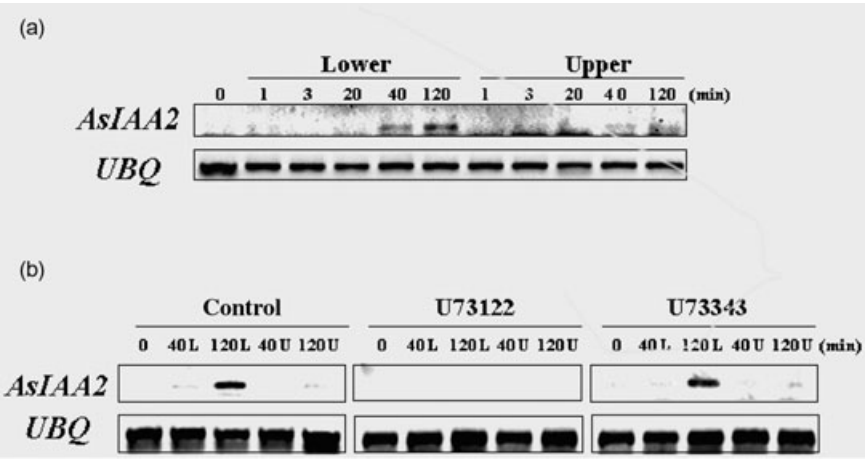

Figure 6. Time course for the effects of phospholipase C (PLC) inhibitors (U73122 and U73343) on the differential expression pattern of *IAA2* gene. Oat shoot pulvini were incubated and treated as described in Fig. 5. In (a), oat shoot segments were gravistimulated for 0, 1, 3, 20, 40 and 120 min. Then, the pulvini were cut into halves by halves, immediately placed on dry ice, then stored at –80 °C. In (b), the segments were pretreated with PLC inhibitor or analogue for 2 h and then gravistimulated for 0, 40 and 120 min. After these respective times, the pulvini were cut into halves (designated as upper and lower halves), immediately placed on dry ice, then stored at –80 °C. Total RNAs were extracted from the collected halves and were subjected to Northern blot hybridization. The photographs in (a) and (b) represent typical results from three independent experimental trials in which all show identical patterns. Expression of the *UBQ* gene was presented as a control.

this purpose, IP_3 quantification was performed in graviresponding oat pulvini in the presence or absence of NPA and TIBA. We reasoned that this could shed light on the sequential relationship between late changes in IP_3 levels and IAA lateral transport in graviresponding oat shoot pulvinus. To observe gravity-induced 'early' or 'late' changes in L/U ratio of IP_3, oat shoot segments were gravistimulated for 1 or 30 min, respectively. As shown in Fig. 7, NPA and TIBA did not reduce gravity-induced late changes in L/U ratio of IP_3. Instead, these inhibitors increased the L/U. Furthermore, these two inhibitors showed little effect on the early changes, including the increase in IP_3 level (control = 630 ± 90; NPA = 890 ± 120; TIBA = 800 ± 80) and L/U ratio of IP_3 (Fig. 7) elicited by gravistimulation treatment. These results indicate that lateral auxin transport is not a prerequisite for the late changes that are observed in IP_3 levels in graviresponding oat shoot pulvini.

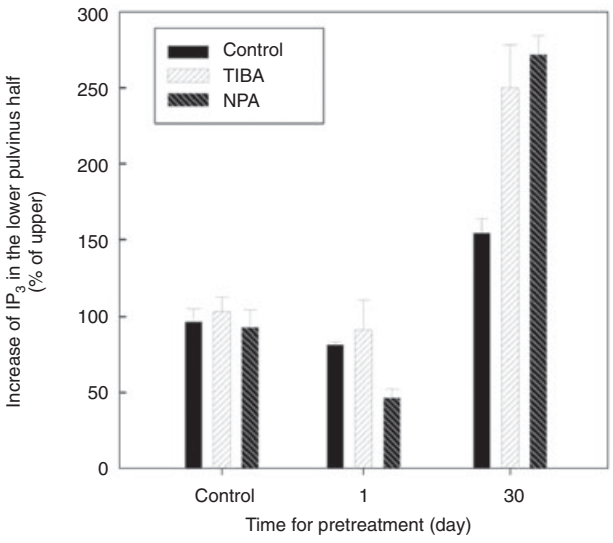

Figure 7. Effect of *N*-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) on changes in inositol 1,4,5-trisphosphate (IP_3) level upon gravistimulation treatment. Oat shoot segments were abraded with silicic acid briefly and vacuum infiltrated in a solution containing 5 mM KH_2PO_4 (pH 5.5) with or without 0.1 mM NPA or TIBA for 2 min. They were then incubated in the same solution for 2 h in the dark at room temperature. After the treatment, the oat shoot segments were positioned horizontally for 0, 1 and 30 min. After the gravistimulation, the upper and lower halves of pulvini were cut directly onto ice, then frozen at –80 °C for subsequent IP_3 analysis. The experiment was repeated four times using 10 segments for each time point. Values denote mean ± SE. The ratio for the control refers to the left versus the right halves of pulvini. IP_3 levels in vertical controls were 280 ± 70, 410 ± 100 and 430 ± 50 pmol g^{-1} fresh weight (FW) for 0.1% ethanol control, NPA and TIBA, respectively.

DISCUSSION

Gravisensing by starch statoliths is required for changes in IP_3 levels and lateral IAA transport to occur in graviresponding oat shoots

Previous work shows that the gravisensors (statoliths) in cereal grass shoots are made up of starch-filled chloroplasts that occur in aggregates of cells (statocytes) lying inside each of the vascular bundles located around the circumference of graviresponsive pulvini (Song *et al*. 1988; Chang *et al*. 2001). The signal transduction process in oat shoots involves gravity-induced changes in the level of IP_3, a possible second messenger (Perera *et al*. 2001), and gravity-induced lateral auxin transport (Brock *et al*. 1991). Because these events may be linked, we undertook the present

studies so as to determine their sequence and the nature of the interrelationships between changes in levels and structure of starch in the chloroplasts/statolith gravisensors, changes in IP_3 levels and lateral auxin transport in the oat shoot pulvini following gravistimulation treatment.

To gain an insight into the nature of these interrelationships, the first question we asked was as follows: if starch levels in the chloroplasts are reduced by placing pulvinus-containing stem segments in the dark for periods up to 7 d, could gravity-induced lateral auxin transport also be reduced? Results in Table 3 show that gravity-induced lateral auxin transport was indeed abolished when starch levels in the pulvini were decreased by physiologically imposed dark pretreatment. At 10 d, both light and dark pretreatments showed that the gravitropic curvatures were reduced to similar levels, 5.8 ± 0.6 and 6.6 ± 0.4, respectively (data not shown). This observation, coupled with the results shown in Fig. 2a, raises the possibility that the reduction of the curvatures was at least, in part, because of aging during light or dark pretreatment.

One can argue that the gravitropic curvature of dark-pretreated oat shoots is still significant (Fig. 2a) although the L/U ratio of IAA is about 1 (Table 3) after the dark pretreatment for 6 d. In Table 3, the L/U ratio of IAA was measured at 40 min and 2 h after the gravistimulation, while the gravitropic curvature (around 6 degrees) was measured at 18 h after initiation of gravistimulation treatment. Furthermore, upon 18 h gravistimulation after dark pretreatment for 6 d, the L/U ratio of IAA was around 1.35 (data not shown). Thus, this observed 16 h delay in the achievement of an L/U ratio for IAA > 1 after 6 d of dark pretreatment is closely correlated with the concomitant decrease that occurs in the amount of starch in the chloroplast gravisensors that results in a reduced gravitropic response.

The second question is this: is the starch-mediated gravisensing process responsible for the observed gravity-induced changes in IP_3 levels in graviresponding oat shoot pulvini? Figure 3 shows that the L/U ratio of IP_3 levels is decreased if the starch levels in oat shoot pulvini are reduced by dark pretreatment. In addition, OA and $LaCl_3$, which decreased the levels of starch in graviresponding oat shoot pulvini, reduced this IP_3 ratio upon gravistimulation treatment (Fig. 4). Based on these observations, we conclude that the gravisensing process, mediated by the chloroplast/statolith gravisensors, is required for both gravity-induced changes in IP_3 levels and basipetal auxin transport elicited by the gravistimulation treatment.

In graviresponding oat shoot pulvini, the graviperception phase includes an early increase and an initiation of late asymmetric changes in IP_3 levels, and initiation of lateral IAA transport

Three time parameters are important components of the graviperception phase of gravitropism. The first, *basipetal statolith descent*, refers to the time required for the starch statoliths/chloroplasts to reach the bottoms of the statocyte cells within a pulvinus once a gravistimulation treatment is imposed. The second is the *presentation time*, which refers to the amount of time a gravistimulus is needed to elicit an upward bending response. In oat shoots, the presentation time is ca. 5.2 min, as determined by clinorotation of horizontally positioned stem segments for various periods of time (Chang *et al*. 2003). The third is the *lag period* that ends with the first perceptible upward bending response in the pulvinus, as determined by the use of angular recording position transducers. In oat shoots, this lag period is of the order of 10–15 min (Chang *et al*. 2003).

Taking these time parameters into consideration, the current and our previous studies show: (1) that increases in the level of IP_3 are observed in oat shoot pulvini during the first 3 min of gravistimulation, but significant upper/lower asymmetry (with significant higher levels occurring in bottom halves) in IP_3 levels are clearly evident only between 10 and 50 min after the start of gravistimulation treatment (Fig. 1 in Perera *et al*. 2001); and (2) that the L/U ratio of auxin transport is ca. 1.08 ± 0.04 at 10 min after the initiation of gravistimulation treatment (Fig. 5a inset in the present study). Parallel results were obtained in maize (*Zea mays*) shoot pulvini (Perera *et al*. 1999), except that all time parameters for the mentioned changes are much longer in maize than in oat shoot pulvini (Perera *et al*. 2001). The results obtained with oat shoot pulvini clearly indicate that the early increase in IP_3 levels, the initiation of late asymmetric changes in IP_3 levels and the initiation of lateral auxin transport all occur during the lag period of the gravitropic response, before the tissue is committed to bending. Our results also demonstrate that the IP_3 gradient precedes the lateral IAA transport in this phase.

Perera *et al*. (2005) reported that roots of *Arabidopsis* transgenic plants, which constitutively expressed the IP_3 hydrolysis enzyme, showed reduced auxin transport. This result supports our conclusion that a change in the L/U ratio of IP_3 may affect auxin transport upon gravistimulation in oat shoot pulvini.

Gravity-induced asymmetric growth of oat shoots may result from differential distribution of IAA and/or differential sensitivity to the IAA between the upper and the lower halves of pulvini

As in other plants, the gravistimulation treatment induces basipetal movement of IAA across organs where gravitropic curvature occurs. In the present study, we show that the L/U ratio of IAA is about 1.4 at 3 h after the initiation of the gravistimulation treatment (Fig. 5a). At a comparable time, Brock *et al*. (1991) obtained a value of 1.5. This confirms the fact that our results are consistent. This L/U ratio reaches 1.7–1.8 after 24 h of gravistimulation treatment (data not shown). However, this ratio is significantly lower than the L/U ratio of 2.5 for free IAA level, measured by the use of gas chromatography–mass spectrometry (GC–MS) (Kaufman *et al*. 1995). One possible explanation

for this discrepancy is that the differential release of free IAA from conjugated IAA between the upper and the lower halves may occur in the pulvini as a result of gravistimulation treatment. Therefore, it is likely that gravity-induced asymmetric growth may result from asymmetric auxin distribution, that is, high L/U ratio of IAA level imposed by both lateral IAA transport *and* differential release of the free IAA from conjugated moieties. However, it should not be ruled out that the asymmetric growth may also be because of differential sensitivity between the upper and the lower halves of pulvini to auxin as proposed previously by Long *et al*. (2002) for maize shoot pulvinus.

The well-known specific auxin transport inhibitors, NPA and TIBA, not only block auxin transport, but they also increase gravity-imposed L/U ratio of IP_3 level as shown in Fig. 7. This result is interesting, but not expected. Recently, several reports suggest that vesicle transport is involved in the shoot gravitropism of *Arabidopsis* (Kato *et al*. 2002; Yano *et al*. 2003). Furthermore, NPA-binding proteins associated with IAA transport proteins seem to circulate and become targeted to plasma membranes via vesicle transport (Muday *et al*. 2003). In addition, the gravitropic curvature in oat shoot pulvini is decreased when 0.1 mM monensin, an inhibitor of glycoprotein transport, is applied (data not shown). This suggests that NPA may affect gravity-induced vesicle transport, thereby resulting in change in the activity of membrane proteins such as PLC that generates IP_3.

It is well known that an IAA influx carrier, AUX1, and the IAA efflux carrier, AGR1/EIR1/PIN2/WAV6, are involved in root gravitropism (Dolan 1998; Muday 2001). Recently, Friml *et al*. (2002) and Noh *et al*. (2003) have suggested the possibility that PIN3 and PIN1 are also key players in mediating shoot gravitropism in *Arabidopsis*. These reports support the view that many kinds of IAA transport proteins may also be involved in gravity-induced lateral IAA transport in oat shoot pulvini. Such a possibility requires further investigation.

Components which are likely to contribute to the gravity signal transduction process in oat shoot pulvini

Calcium, as a second messenger, has been long proposed to be an essential component of signal transduction process in graviresponding shoots and roots of plants (Poovaiah & Reddy 1993; Sinclair & Trewavas 1997; Friedman *et al*. 1998; Plieth & Trewavas 2002). In oat shoots treated with calcium channel blockers, including $LaCl_3$, verapamil and ruthenium red, the gravitropic curvature is significantly reduced (Chang *et al*. 2001). These results present compelling evidence for the participation of calcium in the signal transduction pathway in graviresponding shoots of oats. In addition, these results support the notion that IP_3 molecules play essential roles in the gravitropic response because it is well known that changes in the cytoplasmic level of calcium are caused by IP_3 ions in many physiological events of plants (Drobak 1992). However, the possibility that calcium is involved in gravity-induced auxin transport in oat shoots needs to be elucidated although the possibility is shown in maize roots (Lee & Evans 1985). Rashotte, DeLong & Muday (2001) showed that a protein phosphatase is involved in the regulation of IAA transport in graviresponding *Arabidopsis* roots. In oat shoots, gravity-induced *in vivo* and *in vitro* differential phosphorylations of two soluble proteins (50 and 53 kD) were detected only when stem segments were gravistimulated (Chang *et al*. 2003). In light of the mentioned evidence, one must conclude that calcium and protein phosphorylations, in addition to IP_3, are essential components of the gravity signal transduction process in oat shoots.

Based on our studies and on other evidence, the following scenario (see model in Fig. 8) is possible in oat shoots: upon gravistimulation, gravity-induced changes in IP_3 levels (possibly via increases in cytosolic calcium levels) result in an increase in the L/U ratio of IAA molecules. This is then followed by differential phosphorylation of the gravispecific soluble 50 kD protein (Chang *et al*. 2003). However, how and when they act during signal transduction are missing links that must be addressed in future studies. Furthermore, how gravity perception leads to signal transduction in graviresponding roots and shoots is a central question that remains unsolved.

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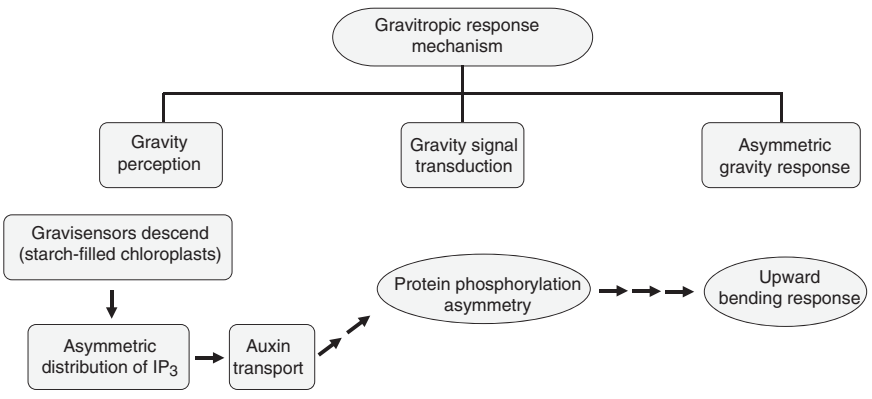

Figure 8. Model that depicts the interrelationship between changes in starch and inositol 1,4,5-trisphosphate (IP_3) levels and auxin transport in graviresponding oat shoots.

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REFERENCES

Abel S. & Theologis A. (1996) Early genes and auxin action. *Plant Physiology* **111,** 9–17.

Belavskaya N.A. (1996) Calcium and graviperception in plants: inhibitor analysis. *International Review of Cytology* **168,** 123–185.

Brock T.G., Kapen E.H., Ghosheh N.S. & Kaufman P.B. (1991) Dynamics of auxin movement in the gravistimulated leaf-sheath pulvinus of oat (*Avena sativa*). *Journal of Plant Physiology* **138,** 57–62.

Chang S.C., Cho M.H., Kang B.G. & Kaufman P.B. (2001) Changes in starch content in oat (*Avena sativa*) shoot pulvini during the gravitropic response. *Journal of Experimental Botany* **52,** 1029–1040.

Chang S.C., Cho M.H., Kim S.-K., Lee J.S., Kirakosyan A. & Kaufman P.B. (2003) Changes in phosphorylation of 50 and 53 kDa soluble proteins in graviresponding oat (*Avena sativa*) shoots. *Journal of Experimental Botany* **54,** 1013–1022.

Dolan L. (1998) Pointing roots in the right direction: the role of auxin transport in response to gravity. *Genes & Development* **12,** 2091–2095.

Drobak B.K. (1992) The plant phosphoinositide system. *Biochemical Journal* **288,** 697–712.

Friedman H., Meir S., Rosenberger I., Halevy A.H., Kaufman P.B. & Philosoph-Hadas S. (1998) Inhibition of the gravitropic response of snapdragon spikes by the calcium-channel blocker lanthanum chloride. *Plant Physiology* **118,** 483–492.

Friml J., Wisniewska J., Benková E., Mendgen K. & Palme K. (2002) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. *Nature* **415,** 806–809.

Fujii N., Kamada M., Yamasaki S. & Takahashi H. (2000) Differential accumulation of *Aux/IAA* mRNA during seedling development and gravity response in cucumber (*Cucumis sativus* L.). *Plant Molecular Biology* **42,** 731–740.

Hubbard N.L., Pharr D.M. & Huber S.C. (1990) Role of sucrose phosphate synthase in sucrose biosynthesis in ripening bananas and its relationship to the respiratory climactic. *Plant Physiology* **94,** 201–208.

Iino M. (1991) Mediation of tropism by lateral translocation of endogenous indole-3-acetic acid in maize coleoptiles. *Plant, Cell & Environment* **14,** 279–286.

Johannes E., Collings D.A., Rink J.C. & Allen N.S. (2001) Cytoplasmic pH dynamics in maize pulvinal cells induced by gravity vector changes. *Plant Physiology* **127,** 119–130.

Joo J.H., Bae Y.S. & Lee J.S. (2001) Role of auxin-induced reactive oxygen species in root gravitropism. *Plant Physiology* **126,** 1055–1060.

Kato T., Morita M.T., Fukaki H., Yamauchi Y., Uehara M., Niihama M. & Tasaka M. (2002) SGR2, a phospholipase-like protein, and ZIG/SGR4, a SNARE are involved in the shoot gravitropism of *Arabidopsis*. *Plant Cell* **14,** 33–46.

Kaufman P.B., Wu L.L., Brock T.G. & Kim D. (1995) Hormones and the orientation of growth. In *Plant Hormones and Their Role in Plant Growth and Development* (ed. P.J. Davis), pp. 547–571. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Kiss J.Z., Guisinger M.M., Miller A.J. & Stackhouse K.S. (1997) Reduced gravitropism in hypocotyls of starch deficient mutants of *Arabidopsis*. *Plant & Cell Physiology* **38,** 518–525.

Lee J.S. & Evans M.L. (1985) Polar transport of auxin across gravistimulated roots of maize and its enhancement by calcium. *Plant Physiology* **77,** 824–827.

Long J.C., Zhao W., Rashotte A.M., Muday G.K. & Huber S.C. (2002) Gravity-stimulated changes in auxin and invertase gene expression in maize pulvinal cells. *Plant Physiology* **128,** 591–602.

Muday G.K. (2001) Auxins and tropisms. *Journal of Plant Growth Regulation* **20,** 226–243.

Muday G.K., Peer W.A. & Murphy A.S. (2003) Vesicular cycling mechanisms that control auxin transport polarity. *Trends in Plant Science* **8,** 301–304.

Noh B., Bandyopadhyay A., Peer W.A., Spalding E.P. & Murphy A.S. (2003) Enhanced gravi- and phototropism in plant *mdr* mutants mislocalizing the auxin efflux protein PIN1. *Nature* **423,** 999–1002.

Parker K.E. & Briggs W.R. (1990) Transport of indole-3-acetic acid during gravitropism in intact maize coleoptiles. *Plant Physiology* **94,** 1763–1769.

Perera I.Y., Heilmann I. & Boss W.F. (1999) Transient and sustained increases in inositol 1,4,5-triphosphate precede the differential growth response in gravistimulated maize pulvini. *Proceedings of the National Academy of Sciences of the United States of America* **96,** 5838–5843.

Perera I.Y., Heilmann I., Chang S.C., Boss W.F. & Kaufman P.B. (2001) A role for inositol 1,4,5-trisphosphate in gravitropic signaling and the retention of cold-perceived gravistimulation of oat shoot pulvini. *Plant Physiology* **125,** 1499–1507.

Perera I.Y., Hung C.Y., Brady S., Muday G.K. & Boss W.F. (2005) A universal role for inositol 1,4,5-trisphosphate-mediated signaling in plant gravitropism. *Plant Physiology* **140,** 746–760.

Philippar K., Fuchs I., Lüthen H., *et al.* (1999) Auxin-induced K^+ channel expression represents an essential step in coleoptile growth and gravitropism. *Proceedings of the National Academy of Sciences of the United States of America* **96,** 12186–12191.

Plieth C. & Trewavas A.J. (2002) Reorientation of seedlings to the earth's gravitational field induces cytosolic calcium transients. *Plant Physiology* **129,** 786–796.

Poovaiah B.W. & Reddy A.S.N. (1993) Calcium and signal transduction in plants. *Critical Review of Plant Science* **12,** 185–211.

Rashotte A.M., DeLong A. & Muday G.K. (2001) Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response and lateral root growth. *Plant Cell* **13,** 1683–1697.

Sack F.D. (1997) Plastids and gravitropic sensing. *Planta* **203,** S63–68.

Scott A.C. & Allen N.S. (1999) Changes in cytosolic pH within *Arabidopsis* root columella cells play a key role in the early signaling pathway for root gravitropism. *Plant Physiology* **121,** 1291–1298.

Sinclair W. & Trewavas A.J. (1997) Calcium in gravitropism. A re-examination. *Planta* **203,** S85–90.

Song I., Lu C.R., Brock T.G. & Kaufman P.B. (1988) Do starch statoliths act as the gravisensors in cereal grass pulvini? *Plant Physiology* **86,** 1155–1162.

Trewavas A.J. & Malho R. (1997) Signal perception and transduction: the origin of the phenotype. *Plant Cell* **9,** 1181–1195.

Vitha S., Yang M., Kiss J.Z. & Sack F.D. (1998) Light promotion of hypocotyl gravitropism of a starch-deficient tobacco mutant correlates with plastid enlargement and sedimentation. *Plant Physiology* **116,** 495–502.

Weise S.E., Kuznetsov O.A., Hasenstein K.H. & Kiss J.Z. (2000) Curvature in *Arabidopsis* inflorescence stems is limited to the regions of amyloplasts displacement. *Plant & Cell Physiology* **41,** 702–709.

Yano D., Sato M., Saito C., Sato M.H., Morita M.T. & Tasaka M. (2003) A SNARE complex containing SGR3/AtVAM3 and ZIG/VTI11 in gravity-sensing cells is important for *Arabidopsis* shoot gravitropism. *Proceedings of the National Academy of Sciences of the United States of America* **100,** 8589–8594.

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