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NUCLEOTIDE SUGARS AND STARCH SYNTHESIS IN SPADIX OF *ARUM MACULATUM* AND SUSPENSION CULTURES OF *GLYCINE MAX*

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Key Word Index—*Arum maculatum*, Araceae, cuckoo-pint, *Glycine max*, Leguminosae, soybean, starch synthesis, ADPglucose, UDPglucose

Abstract—The aim of this work was to determine the relative contributions of ADPglucose and UDPglucose to starch synthesis in two non-photosynthetic tissues, the developing club of the spadix of *Arum maculatum*, and suspension cultures of *Glycine max* Rates of starch accumulation during growth are compared with estimates of the maximum catalytic activities *in vitro* of ADPglucose starch synthase, ADPglucose pyrophosphorylase, UDPglucose pyrophosphorylase and UDPglucose starch synthase. The latter could only be measured at high concentrations (10–30 mM) of UDPglucose Clubs of *Arum* and cells of *Glycine* contained 292 and 6 8 nmol UDPglucose per gram fresh weight, respectively The corresponding figures for ADPglucose were 29 and 0 4 From the above data it is argued that in both *Arum* club and *Glycine* cells the activity of UDPglucose starch synthase is too low to make any quantitatively significant contribution to starch synthesis The activities of ADPglucose starch synthase and pyrophosphorylase were high enough to mediate the observed rates of starch accumulation It is suggested that starch synthesis in these tissues is via ADPglucose

INTRODUCTION

The relative contributions of ADPglucose and UDPglucose to starch synthesis in the non-photosynthetic cells of higher plants are not established [1] The evidence for a dominant role for ADPglucose, summarized by Preiss [1, 2], suffers from two weaknesses First, evidence from measurements *in vitro* of starch synthase (EC 2 4 1 21), ADPglucose pyrophosphorylase (EC 2 7 7 27) and UDPglucose pyrophosphorylase (EC 2 7 7.9) is difficult to assess because many of the measurements were made before the properties of the enzymes were established, and before the difficulties of measuring enzymes in plant extracts were fully appreciated There is little evidence of the assays being optimized or of consideration of differential losses of enzyme activity during extraction Thus, as the estimates have not been shown to reflect the maximum catalytic activities of the tissues, we may not reliably compare estimates for different enzymes, estimates for the same enzyme in tissues at different stages of development, or estimates of enzyme activity *in vitro* with rates of starch synthesis *in vivo* [3] For example, recent measurements, held to show increased activity of ADPglucose starch synthase during the formation of soybean seeds, reveal that the reported activities are capable of supporting no more than 10–20% of the starch accumulation known to occur *in vivo* [4] Further, although estimates of ADPglucose starch synthase may greatly exceed those of UDPglucose starch synthase [1], this is not always so [5] The second weakness in the argument for the dominance of ADPglucose is that there are not enough authenticated measurements to permit any generalization about the amounts of ADPglucose and UDPglucose in non-photosynthetic cells of plants The data that are available are disparate [6, 7]

In this paper we report an attempt to remedy the above weaknesses As experimental material, we chose a tissue in which starch synthesis dominates metabolism, the developing club of the spadix of *Arum maculatum*, and a tissue in which there is no such dominance, suspension cultures of soybean We estimated the rates of starch synthesis *in vivo* from measurements of starch content, compared these estimates with those of the maximum catalytic activities of ADPglucose starch synthase and pyrophosphorylase, and UDPglucose starch synthase and pyrophosphorylase, and determined the amounts of ADPglucose and UDPglucose in the cells

RESULTS

Starch accumulation

Lance [8] measured the starch content of developing clubs of *Arum maculatum*, his estimates for mature clubs have been confirmed [9] The developmental stages of the club have been arbitrarily designated α, β, γ, pre-thermogenesis and thermogenesis, and each has been characterized [10] From our measurements of fresh weight and Lance's measurements of the amounts of starch per g fresh weight, we calculated that at α stage and pre-thermogenesis, the starch contents are 24 and 284 mg per club, respectively Observation of the natural development of 11 separate plants, that were part of the native

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population used for the measurements of enzymes and substrates, showed that it took, on average, 12 days for the clubs to develop from α-stage to pre-thermogenesis Thus the minimum rate of net accumulation of starch in these clubs was 93 nmol anhydroglucose/min per club Local meteorological records showed that the average temperature in the district during the above 12 days was 12 1°

Table 1 gives the changes in fresh weight and starch content during the growth of the suspension cultures of soybean Fresh weight increased most rapidly between days 4 and 7 Starch content of the culture increased almost linearly between days 4 and 11, and the rate of accumulation, calculated over this period, was 0 47 nmol anhydroglucose/ml culture per min

Enzyme activities

The assays for ADPglucose starch synthase and pyrophosphorylase, and UDPglucose pyrophosphorylase were optimized for plant material that was in the middle of its period of maximum accumulation of starch, β to γ stage clubs, and 3 to 7-day-old cultures The following checks were made for each enzyme from *Arum* clubs and soybean cells We optimized the pH, and the concentration of each component, of the assay mixtures We showed that activity was linear with respect to time and to the amount of extract The latter was not readily achieved and the amounts and concentrations of extract specified for the assays are critical Loss of activity during extraction of the clubs and cells was investigated in the following type of recombination experiment [3] For *Arum* we prepared three samples, one of α stage clubs, one of β stage clubs, and one a mixture of equal weights of α stage and β stage clubs Activity in the extract of the mixed sample was compared with that predicted from the measurements made on the separate components of the mixture We did similar experiments with β and γ stage clubs, and with soybean cultures of different ages (4 to 6-day-old cultures were mixed with 11 to 13-day-old cultures) The values obtained for the mixed samples were always within 18% of those predicted, and generally within 10% For ADPglucose starch synthase we also optimized the pH, and the concentration of the components, of the extraction medium For this enzyme in *Arum* we made the following additional checks Activity was not enhanced by including dithiothreitol (5–20 mM) in either the assay or the extraction medium, nor by centrifugation of the extracts (30 000 *g* for 15 min) and separate assay of the pellet and supernatant Evidence that the assay for ADPglucose starch synthase measured incorporation into starch was obtained by incubating the methanol-insoluble water-soluble product of the assay with amyloglucosidase (EC 3 2 1 13) and α-amylase (EC 3 2 1 1) This treatment released all of the label in the products from *Arum* and from soybean as [^{14}C]glucose

Table 1 Growth and starch accumulation by suspension cultures of soybean

Age of culture (days)	Fresh weight (mg/ml culture)	Starch (μg/ml culture)
Initial	24 9 ± 4 9	94 ± 6
4	62 9 ± 5 5	237 ± 24
7	142 8 ± 13 3	591 ± 94
11	170 1 ± 16 8	1011 ± 219
14	187 5 ± 15 8	1165 ± 165

Four separate cultures were harvested at each time, weight and starch content of all four were determined and values are means ± s e m

Our estimates of enzyme activities are in Tables 2 and 3 The rates are expressed per club, and per ml of culture, to allow comparison with the rates of starch accumulation In addition, Tables 1 and 2 contain sufficient information for the rates to be related to fresh weight The protein contents of the clubs [10] are also available Fisher's *P* values for the differences between the estimates for the ADPglucose enzymes at α, β and γ stage of *Arum* are 0 05 or less No significant difference between the stages was found if the rates were related to fresh weight except that the activity of ADPglucose pyrophosphorylase increased from α to β stage For example, the values for ADPglucose starch synthase are 574, 588 and 643 nmol per min/g fresh weight for α, β and γ stages, respectively For comparison, the activities of this enzyme per g fresh weight in 4, 7 and 11-day-old soybean cultures were 27, 22 and 9 nmol per min

We optimized the assay for UDPglucose starch synthase, as described for ADPglucose starch synthase for soybean cells, in extracts of β to γ stage clubs, and in

Table 2 Activities of enzymes of starch metabolism in extracts of developing clubs of the spadix of *Arum maculatum*

Stage of development	Fresh weight (mg/club)	Enzyme activity (nmol per min/club) ADPglucose starch synthase	ADPglucose pyrophosphorylase	UDPglucose pyrophosphorylase
α	263 ± 10 (10)	151 ± 9 (5)	612 ± 67 (5)	N D
β	553 ± 30 (17)	325 ± 48 (5)	899 ± 97 (6)	2372 ± 521 (6)
γ	1053 ± 80 (11)	677 ± 103 (5)	1383 ± 125 (6)	N D

Extracts were made from single clubs Each assay for each enzyme was done with a different extract Values are means ± s e m of estimates from the number of clubs shown in parenthesis The values for fresh weight are based on the total number of clubs analysed at each stage of development

N D, Not determined

Table 3 Activities of enzymes of starch metabolism in extracts of suspension cultures of soybean

Age of culture (days)	Enzyme activity (nmol per min/ml culture)		
	ADPglucose starch synthase*	ADPglucose pyrophosphorylase†	UDPglucose pyrophosphorylase†
Initial	0 50 ± 0 05	1 3, 2 2	38, 63
4	1 69 ± 0 26	17 5, 17 3	261, 284
7	3 07 ± 0 74	6 4, 6 7	136, 167
11	1 53 ± 0 43	11 7, 8 6	199, 161
14	0 87 ± 0 34	3 7, 4 2	207, 160

*For this enzyme four different but replicate cultures were harvested at each time, extracted and assayed values are means ± s e m of the four estimates

†Duplicate cultures were harvested at each time, three extracts were prepared and assayed from each culture values are means of the triplicate assays for each culture

extracts of 6-day-old cultures of soybean The observed activity was linearly related to time and the amount of extract The results of recovery experiments were as described for the ADPglucose enzymes However, we found very considerable variation in the activities obtained for comparable samples, 5–6 fold in clubs at the same stage of development. We used extracts of β and γ clubs to investigate this variation, and found that the activity observed for UDPglucose starch synthase varied with the preparations of UDP[^{14}C]glucose used in the assay Paper chromatography of the latter revealed labelled material that coincided with glucose 1-phosphate Preparations that gave high values for UDPglucose starch synthase, 1 μmol/min per g fresh weight at 12 mM UDP[^{14}C]glucose, contained 9% of their total ^{14}C in material that migrated with glucose 1-phosphate This figure was near 2% in the preparations that gave lower values for the enzyme The ^{14}C that was incorporated into starch during the assay for UDPglucose starch synthase amounted to 14% of that which chromatographed with glucose 1-phosphate in preparations of UDP[^{14}C]glucose that gave high activities

The above observations suggested that much of the measured activity of UDPglucose starch synthase was an artefact caused by incorporation into glucan of labelled glucose from [^{14}C]glucose 1-phosphate by α-glucan phosphorylase known to be present in the extracts of the clubs [11] Accordingly, we pretreated the UDP[^{14}C]glucose used in the assay in order to remove contaminating hexose phosphates Two pretreatments were used incubation with alkaline phosphatase that would hydrolyse hexose phosphates but not UDPglucose, incubation with a mixture of phosphoglucomutase, glucosephosphate isomerase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP that would convert hexose phosphates to pentose phosphates We prepared assay mixtures for UDPglucose starch synthase that contained UDPglucose plus 0 85 μM [U-^{14}C]glucose 1-phosphate (sp act 235 Ci/mol) Even at this low concentration of [^{14}C]glucose 1-phosphate, extracts of *Arum* clubs incorporated 1 6% (1440 dpm/assay) of the supplied ^{14}C into glucan under the conditions of the assay for UDPglucose starch synthase Pretreatment of the assay mixture that contained [^{14}C]glucose 1-phosphate with either alkaline phosphatase or the mixture of enzymes abolished incorporation of ^{14}C into glucan The pretreatments were then applied to the normal reaction mixtures that contained UDP[^{14}C]glucose but no added [^{14}C]glucose 1-phosphate At 5 mM UDP[^{14}C]glucose pretreatment with phosphatase reduced the activity of UDPglucose starch synthase to 13 nmol per min/g fresh weight for β clubs the corresponding value for pretreatment with the mixture of enzymes was 4 With assay mixtures that contained 0 7 mM UDP[^{14}C]glucose both pretreatments reduced the rate to less than 1 5 nmol per min/g fresh weight

With extracts of 4 to 8-day-old cultures of soybean, pretreatment of the UDP[^{14}C]glucose with phosphatase almost abolished starch synthase activity when the concentration of UDP[^{14}C]glucose was 2 1 mM If the latter was raised to 12 5 mM, the pretreatments removed only about half of the observed activity In the latter assays the residual activities after pretreatment with phosphatase, and the mixture of enzymes, were 7 4 ± 0 8 and 10 3 ± 1 9 (mean ± s e m of five estimates), respectively When the ADP[^{14}C]glucose, used to assay ADPglucose starch synthase, was treated either with the phosphatase or the mixture of enzymes, the observed activity of starch synthase was not diminished in extracts of either *Arum* clubs or soybean cells

Content of ADPglucose and UDPglucose

Samples were freeze-clamped, killed and extracted in cold $HClO_4$, and the sugar nucleotides were isolated and measured by HPLC (Table 4) We investigated whether significant losses occurred during extraction and analysis For each test we made duplicate samples One was killed and extracted in the usual way the other was treated similarly except that measured amounts of ADPglucose and UDPglucose were added to the freeze-clamped sample before it was added to the $HClO_4$ Comparison of the amounts of nucleotide sugars found in the two samples allowed calculation of the extent to which the added compounds had survived the complete process of

Table 4 Amounts of UDPglucose and ADPglucose in γ-stage clubs of the spadix of *Arum maculatum* and 6-day-old cultures of soybean

Measurement*	UDPglucose	ADPglucose
Arum spadix		
Content (nmol/g fr wt)	292 ± 19 (8)	28 8 ± 4 9 (8)
Recovery (%)†	99 ± 5 (5)	99 ± 8 (8)
Soybean culture		
Content (nmol/ml culture)	6 1 ± 0 9 (8)	0 43 ± 0 11 (6)
Recovery (%)†	72 ± 6 (7)	109 ± 24 (5)

*All values are means ± s e m of estimates from the number of samples shown in parenthesis

†The amounts recovered are expressed as percentages of those added

killing, extraction and assay The difference between the amounts found in the two samples is expressed as a percentage of the amount added to give the recovery We stress that the amounts that were added in these experiments were comparable to the amounts found in the samples of tissue The recoveries were good except for UDPglucose from soybean where there is evidence of a 30% loss (Table 4)

The nucleotide sugars isolated by HPLC were identified by co-chromatography, and comparison of their retention times, with pure samples of these compounds In addition we took the HPLC fractions, hydrolysed them and determined whether the amounts of sugar and base released were equivalent to the amount of nucleotide sugar said to be present For extracts of *Arum* the amounts of glucose and uridine from UDPglucose were 77% and 89%, and of glucose and adenine from ADPglucose 73% and 84% of those expected, respectively For extracts of soybean the corresponding figures were glucose 92%, uridine 84% and glucose 75%, adenine 65%

DISCUSSION

Our estimates of the nucleotide sugars in *Arum* clubs are authenticated by the good recoveries and the adequate characterization of the material isolated by HPLC The estimates for soybean cells, where the total content of nucleotide sugars was very much lower than in the clubs, may be less good The recoveries suggest a loss of up to 30% of the UDPglucose, the characterization suggests that not all of the material designated as ADPglucose after HPLC was so, and thus the value may be an overestimate by 30% Nonetheless, our data are reliable enough to establish that in these two non-photosynthetic tissues, there is at least ten times as much UDPglucose as ADPglucose

Our assays for ADPglucose pyrophosphorylase and starch synthase, and for UDPglucose pyrophosphorylase, were carefully optimized and characterized The recovery experiments argue against significant losses during extraction Consequently, we suggest that our measurements reflect the maximum catalytic activities of the tissues The assays for UDPglucose starch synthase were also thoroughly investigated in both tissues, and in both the bulk of the observed activity may be ascribed to contaminating labelled hexose phosphate in the UDP[^{14}C]glucose In extracts of *Arum* the highest activity, that resisted both pretreatments designed to remove hexose phosphates, required 5 mM UDPglucose and was only 4 nmol/min per club in comparison to a rate of starch synthesis of 93 nmol/min This difference is even greater if we remember that the enzyme was assayed at 30° and starch accumulated at 12° In soybean the highest activity of UDPglucose starch synthase that resisted the pretreatments was 10 nmol/min per g fresh weight (1 4 nmol/min per ml of 6-day-old culture) This is higher than the rate of starch accumulation, 0 47 nmol/min per ml of culture However, the above activity required 12 mM UDPglucose at 2 mM no activity at all was found In order to be at 12 mM, the UDPglucose in the soybean cultures would have to be restricted to 0 06% of the total volume of the samples taken for analysis This seems most unlikely, particularly as both enzymes known to be capable of forming UDPglucose are present in the cytosol of soybean cultures [12] From these results and arguments, we suggest that in *Arum* clubs and soybean suspension cultures UDPglucose starch synthase does not make a quantitatively significant contribution to starch synthesis

During the period of maximum starch accumulation in soybean cultures, days 4–11, the maximum catalytic activities of ADPglucose pyrophosphorylase and starch synthase are at least three times greater than the rate of starch accumulation This suggests that the whole of starch synthesis could proceed via ADPglucose This view is strongly supported by the observations that the above enzymes are confined to the amyloplasts, and those responsible for the synthesis of UDPglucose to the cytosol [12] As starch accumulation in *Arum* was measured at 12°, we determined the effects of lowering the temperature on the enzyme with the lowest maximum catalytic activity in Table 2 Measurements of ADPglucose starch synthase in extracts of *Arum* clubs at 30° and 12° showed that activity at 12° was 37% of that at 30° Thus at 12° the activities of this enzyme at β and γ stage, when the accumulation of starch occurred, would have been about 120 and 250 nmol/min per club, respectively These exceed the rate of starch accumulation Consequently, we suggest that in *Arum* clubs and soybean cultures starch synthesis proceeds almost entirely via ADPglucose

The above conclusion raises two apparent anomalies that require explanation before the hypothesis is established First, the concentrations of ADPglucose used to assay starch synthase *in vitro* are high relative to the amount of ADPglucose found *in vivo* We used 10 mM for the assay with extracts of *Arum* and 0 7 mM for those of soybean Murata *et al* [7] used 14 6 mM, Ozbun *et al* [13] 0 7 mM, and Cardini and Frydman [14], 2 25 mM In our work the high concentrations were chosen to ensure optimum activity However, very considerable reduction in the concentration of ADPglucose used in the assays still gave appreciable activity For extracts of *Arum* reduction to 1–0 5 mM still gave almost half of the activity at 10–20 mM (Fig 1) To reach 0 5 mM the ADPglucose in *Arum* clubs would have to be concentrated into about 6% of the volume of the club As both ADPglucose pyrophosphorylase and starch synthase are confined to amyloplasts [12], it is highly probable that ADPglucose is confined to the stroma of the amyloplasts Such plastids are packed with starch and the stromal volume will be a very small proportion of the total volume of the club figures of 2–6% seem quite likely The ADPglucose

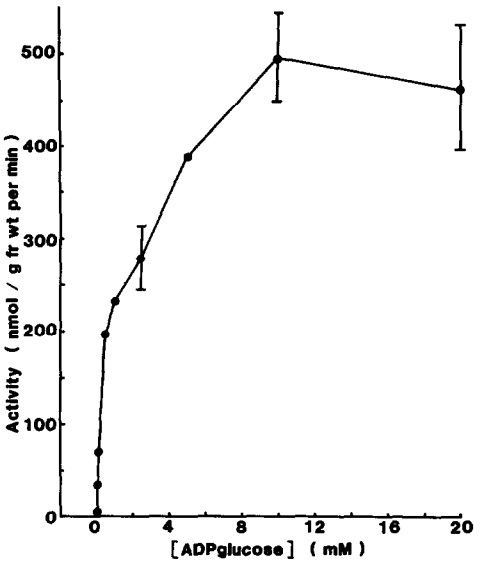

Fig 1 Activity of ADPglucose starch synthase in unfractionated extracts of clubs of β-stage spadices of *Arum maculatum* at different concentrations of ADPglucose Four clubs were extracted, separately, and each extract was assayed at each concentration of ADPglucose Each point is the mean of the estimates from the four extracts, where s e m exceeds 10% of the mean, the former is shown by the bars

content of the soybean cultures would have to be concentrated to a greater extent than that of the clubs Two factors may explain this One is that there are likely to be fewer amyloplasts in the soybean cells than in the club The other is that the soybean cells are highly vacuolate and were only loosely packed where they were harvested to give the material analysed Thus an unusually high proportion of the fresh weight will have been water In 7-day-old cultures the dry weight was only 5% of the fresh weight

The second apparent anomaly between our conclusions and data is the high activity of UDPglucose pyrophosphorylase relative to that of ADPglucose pyrophosphorylase in β-stage *Arum* clubs where starch accumulation is particularly marked A similar situation has been reported for other non-photosynthetic tissues in which starch synthesis is a major metabolic activity [15–17] UDPglucose is regarded as a precursor for structural polysaccharides In *Arum* clubs starch synthesis far outweighs the synthesis of structural polysaccharides over 90% of ^{14}C incorporated into insoluble material, when clubs were fed [^{14}C]sucrose, was recovered in starch [Hargreaves, personal communication] Thus the high content of UDPglucose and the high activity of UDPglucose pyrophosphorylase suggest that the latter may play some role other than merely providing UDPglucose for polymer synthesis

EXPERIMENTAL

Material Isotopes were from the Radiochemical Centre, Amersham, U K Substrates, co-factors and enzymes were from Boehringer except that 3-phosphoglycerate, glucose 1,6-bisphosphate, alkaline phosphatase (Type VII) and inorganic pyrophosphatase (Type III) were from Sigma Clubs of *Arum maculatum* L were taken from plants in local natural sites and were recognized and treated as in ref [10] Cultures of soybean (*Glycine max* L cv Acme) derived from and grown as those in ref [18] were harvested by filtration through glass-fibre paper The filtered cells were weighed at once to give the measurements of fr wt Unless we say otherwise, the experiments were done with 3 to 7-day-old cultures

Enzyme assays Clubs were homogenized as in ref [10] Soybean cells were ground with PVP (100 mg/g fr wt) and sand in a pestle and mortar Examination of the homogenates revealed very few unbroken cells For ADPglucose starch synthase the extraction media were *Arum*, 0 1 M Bicine (pH 8 4), 10 mM EDTA, 0 1% (w/v) bovine serum albumin, soybean, as above plus 20 mM cysteine-HCl, 20 mM mercaptoethanol, 20 mM diethyldithiocarbamate Assay was as in ref [13] Reaction mixtures, which were incubated at 30° for 30 min, contained in 200 μl *Arum*, 20 μmol Bicine (pH 8 4), 5 μmol potassium acetate, 1 mg amylopectin, 2 μmol ADP[U-^{14}C]glucose (0 025 Ci/mol), extract equivalent to 1 5 mg fr wt, soybean, as for *Arum* except 4 μmol potassium acetate, 2 mg amylopectin, 0 14 μmol ADP[U-^{14}C]glucose (0 09 Ci/mol), 1 μmol dithiothreitol, extract from 20 mg fr wt Extraction media for UDPglucose starch synthase were *Arum*, 50 mM Tris–acetate buffer (pH 8 0), 10 mM EDTA, 2 mM dithiothreitol, 0 1% (w/v) albumin, soybean, as for ADPglucose starch synthase Assay was as for the ADPglucose enzyme reaction mixtures contained in 200 μl, *Arum*, 5 μmol Bicine (pH 7 7), 2 5 μmol potassium acetate, 4 μmol EDTA, 1 mg amylopectin, 2 5–4 μmol UDP[U-^{14}C]glucose (0 006–0 04 Ci/mol), soybean, as for ADPglucose starch synthase except that UDP[U-^{14}C]glucose was used and the dithiothreitol was omitted Pretreatment of UDP[^{14}C]glucose to remove contaminants was either by adding 30 μl (2 units) alkaline phosphatase (EC 3 1 3 1) to the reaction mixture minus tissue extract and incubating at 25° for 45 min, or by carrying out a similar incubation after adding 30 μl of a mixture that contained 0 7 unit glucose-6-phosphate dehydrogenase, 0 024 unit 6-phosphogluconate dehydrogenase, 0 6 unit phosphoglucomutase, 66 mM $MgCl_2$, 5 3 mM NADP and 0 16 mM glucose 1,6-bisphosphate After the above incubation the reaction mixtures were placed in boiling water for 2 min, cooled and then used to assay UDPglucose starch synthase To confirm that the product of the starch synthase assays was starch, we pooled, in 0 5 ml, the water-soluble components of the methanol-insoluble products from four identical assays This solution was incubated, in 1 0 ml, with 2 units amyloglucosidase and 0 2 unit pig pancreas α-amylase in 0 1 M sodium acetate buffer (pH 4 8) for 12 hr at 37° The mixture was then centrifuged at 9000 *g* for 6 min and [^{14}C]glucose was isolated from the supernatant by PC with EtOAc–pyridine–H_2O (8 2 1)

For assay of ADPglucose pyrophosphorylase extraction media were *Arum*, 50 mM Tris–HCl (pH 7 5), 2 mM EDTA, 2 mM GSH, soybean, 25 mM glycylglycine (pH 7 5) Soybean extracts were assayed unfractionated, those of *Arum* after centrifugation at 100 000 *g* for 30 min Assay was as in ref [19] Reaction mixtures, 200 μl incubated for 10 min at 37°, contained *Arum*, 100 mM Hepes (pH 7 0), 1 2 mM ATP, 7 mM 3-phosphoglycerate, 10 mM $MgCl_2$, 50 μg albumin, 0 33 μg inorganic pyrophosphatase, 1 mM [U-^{14}C]glucose 1-phosphate (0 5 Ci/mol), extract from 0 5 mg fr wt, soybean, 100 mM Hepes (pH 7 5), 1 mM ATP, 3 5 mM 3-phosphoglycerate, 5 mM $MgCl_2$, 50 μg albumin, 0 17 μg pyrophosphatase, 0 5 mM [U-^{14}C]glucose 1-phosphate (0 5 Ci/mol), extract from 1 3 mg of cells For assay of UDPglucose pyrophosphorylase *Arum* and soybean were extracted in 25 mM glycylglycine buffer (pH 7 5), the homogenates were centrifuged at 100 000 *g* for 30 min and the supernatants

assayed at 25° as in ref [20] Reaction mixtures (1 0 ml) contained *Arum*, 100 mM glycylglycine buffer (pH 7 7), 1 mM $Na_4P_2O_7$, 0 2 mM NADP, 1 mM $MgCl_2$, 2 4 μM glucose 1,6-bisphosphate, 1 4 units glucose-6-phosphate dehydrogenase, 2 units phosphoglucomutase, 1 2 units 6-phosphogluconate dehydrogenase, 0 2 mM UDPglucose, extract from 1 5 mg fr wt, soybean, 100 mM glycylglycine (pH 7 8), 0 2 mM NADP, 0 5 mM $Na_4P_2O_7$, 0 5 mM $MgCl_2$, 4 8 μM glucose 1,6-bisphosphate, 2 units phosphoglucomutase, 3 units glucose-6-phosphate dehydrogenase, 0 5 unit 6-phosphogluconate dehydrogenase, 0 4 mM UDPglucose, extract from 2 6 mg cells

Measurement of ADPglucose and UDPglucose Individual clubs, and samples (2 g fr wt) of soybean cells were freeze-clamped and killed as in ref [21] The resulting frozen mass was kept at 0° for 30 min, broken up with a glass rod, allowed to thaw and centrifuged at 27 000 *g* for 2 min The pellet was re-extracted with 1 41 M $HClO_4$ neutralized with 5 M K_2CO_3 The two extracts were combined, neutralized with 5 M K_2CO_3, and centrifuged as above The resulting pellet was washed with 1 41 M $HClO_4$ neutralized with 5 M K_2CO_3 and the washings were added to the neutralized supernatant The complete extract was passed through a Millipore membrane (0 45 μm) and assayed by HPLC as in ref [21] except that the elution program was altered to give complete separation of the two nucleotide sugars The program was A is 10 mM $NH_4H_2PO_4$ (pH 3), B is 450 mM $NH_4H_2PO_4$ (pH 4 3), 0–15 min, A at 0 8 ml/min, 15–40 min, linear gradient from 100% A 0% B to 70% A 30% B at 0 8 ml/min, 40–44 min, linear gradient from 70% A 30% B to 100% B at 0 8 ml/min, 44–51 min, 100% B at 1 6 ml/min, 51–53 min, linear gradient from 100% B to 100% A at 1 6 ml/min, 53–60 min, 100% A at 1 6 ml/min The composition of the fractions isolated by HPLC was determined as in ref [21]

Miscellaneous The starch content of soybean cells was determined by extracting samples of 0 3–1 0 g fr wt with boiling 80% (v/v) aq EtOH and measuring the starch in the insoluble material as in ref [11]. ^{14}C was measured as in ref [21]

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