A Second UDP-glucose Pyrophosphorylase Is Required for Differentiation and Development in *Dictyostelium discoideum*

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Uridine diphosphoglucose pyrophosphorylase (UDPGP) is a developmentally regulated enzyme in *Dictyostelium discoideum*, which is involved in trehalose, cellulose, and glycogen synthesis. Two independent UDPGP proteins are believed to be responsible for this activity. To determine the relative contributions of each protein, the genes encoding them were disrupted individually. Cells lacking the *udppg1* gene exhibit normal growth and development and make normal levels of cellulose. In agreement with these phenotypes, *udppg1−* cells still have UDPGP activity, although at a reduced level. This supports the importance of the second UDPGP gene. This newly identified gene, *ugpb*, encodes an active UDPGP as determined by complementation in *Escherichia coli*. When this gene is disrupted, cells undergo aberrant differentiation and development ending with small, gnarled fruiting bodies. These cells also have decreased spore viability and decreased levels of glycogen, whose production requires UDPGP activity. These phenotypes suggest that UgpB constitutes the major UDPGP activity produced during development. Sequence analysis of the two UDPGP genes shows that UgpB has higher homology to other eukaryotic UDPGPs than does UDPGP1. This includes the presence of 5 conserved lysine residues. UdpP1 only has 1 of these lysines.

*Dictyostelium discoideum* is one of the simplest eukaryotic organisms in which the initiation and regulation of differentiation and development can be investigated. *Dictyostelium* cells normally exist as individual amoebae that feed on bacteria and divide by fission. When they overgrow their food source and begin to starve, they stop dividing and initiate a developmental program (1, 2). By using cAMP as a chemoattractant, up to 10^5 cells/ml can be attracted to the center of a mass of spore cells situated upon a column of stalk cells. During this process, cAMP regulates the expression of a large number of genes required for development by binding to cell surface receptors and activating several signal transduction pathways (1, 3–9). One such regulated gene is the *ugpg1* gene.

UDGP1 catalyzes the creation of UDP-glucose from glucose 1-phosphate and UTP. UDP-glucose is the energetically activated form of glucose and is required for the synthesis of trehalose, cellulose, and glycogen. Previous work (10, 11) has shown that the enzyme activity in vegetative cells is low, but detectable, and rises 5-fold during development. To understand better the role of UDPGP in development, cells expressing *udppg1* antisense RNA were created. However, these transformants grew and developed normally (12). This suggested that either low levels of UdpP1 activity were still present, a second gene existed, or UDPGP activity was not required for development in *Dictyostelium*. Consistent with the second hypothesis, Manrow and Dottin (13) and Fishel et al. (14) found that there appear to be two isoforms of UDPGP.

In this paper we describe the phenotype associated with disruption of the *udppg1* gene by homologous recombination. In addition, we describe the identification and disruption of a second UDPGP gene, *ugpb*.

**EXPERIMENTAL PROCEDURES**

Growth and Transformation of *D. discoideum*—*D. discoideum* strain Ax3 was grown axenically in HL5 medium to a density of 2 × 10^6 cells/ml or on SM plates in association with *Klebsiella* bacteria. DH1 wild-type cells were grown in shaking culture in HL5 medium as described by Gomer et al. (15) except that the medium was supplemented with 20 μg/liter biotin, 5 μg/liter vitamin B12, 200 μg/liter folic acid, 400 μg/liter lipoic acid, 500 μg/liter riboflavin, 600 μg/liter thiamine supplement, and uracil. A mixture of 0.3 g/liter streptomycin sulfate and 0.1 g/liter ampicillin was used as antibiotics. The procedure of Wood et al. (16) was used to determine the percentages of prestalk and prespore cells at low cell density. For development, cells at mid-log phase (2 × 10^6 cells/ml) were washed in buffer and plated on filter pads following Jain et al. (17).

Transformation was performed by electroporation following the procedure of Dynes and Firtel (18) with some modifications. Cells were grown axenically to 2 × 10^6 cells/ml, and 5 ml were collected by centrifugation at 450 × g and washed in 2 volumes of electroporation buffer (EB, 1 mM sodium phosphate, pH 6.1, and 500 μM sucrose). The pellet was resuspended in 0.3 ml of EB. The cell suspension was mixed with 10 μg of DNA. The mixture was transferred to an electroporation cuvette (0.2 cm, Bio-Rad) in an ice water bath. After 3–4 min, the cells were electroporated using a 1.5-KV pulse from a 3-microfarad capacitor. The cells were immediately returned to an ice water bath for 10 min. The cells were then transferred to a 100-mm tissue culture plate and then treated with 3 μl of 100 mM CaCl2 and 100 mM MgCl2. After 15 min, 12 ml of HL5 was added. 24 h later, the medium was replaced with 2 volumes of HL5 containing 10 μg/ml G418. 200 μl was plated into each well of a 96-well plate. After 10 days, aliquots of the culture were plated

* This work was supported by NIGMS Grant S06-GM606564 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF150929.

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The abbreviations used are: UDPGP, uridine diphosphoglucose pyrophosphorylase; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine.

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on SM plates with bacteria to isolate individual clones for further analysis.

**Northern Blots**—Total cellular RNA was isolated from vegetative or developed cells as described by Alton and Lodish (19). Northern blots were prepared as described by Haribabu and Dottin (20). Equal amounts of RNA were loaded into each lane of an agarose gel as determined by ethidium bromide staining of the ribosomal RNA bands.

**Assays for UDPGP Activity and Cellulose**—The assays were performed as described previously (11) with modifications. Cell extracts were prepared by lysing the cells at a density of 2 × 10⁸ cells/ml in 0.1% Nonidet P-40. The assay mixture contained the following: 1 mM UDPGP, 2 mM sodium pyrophosphate, 1.6 mM NADP, 10 μM glucose 1,6-diphosphate, 1 U of glucose-6-phosphate dehydrogenase, 85 mM Tricine, pH 7.6, and cell extract to a total volume of 0.5 ml. The increase in absorbance at 340 nm was measured at 37 °C. One unit of enzyme activity is the amount of enzyme necessary to produce 1 nmol of NADPH per min at 37 °C. Total cellular protein was measured using the Bio-Rad protein determination kit. Specific activity is defined as units of enzyme activity per mg of cellular protein.

Cellulose was extracted first in a mixture of 80% acetic acid and 10% nitric acid and then measured as described previously (21). PCR Cloning and Library Screening—*D. discoideum* DNA was prepared as described previously (22). To identify the second UDPGP gene, PCR was performed on genomic DNA using degenerate oligonucleotide primers. The forward primer (F1) was 5'-AGAAGCTTG(A/G)(G/C/A/T)TG-3', and the reverse primer (R1) was 5'-GAGATTCAA(T/A/G)A(T/C)(A/G)TA(C/T)TC(C/T)TT(G/C/A/T)CC-3'. PCR was carried out for 35 cycles (94 °C for 60 s, 45 °C for 60 s, 72 °C for 3 min) with Taq DNA polymerase. The amplified PCR products were cloned in pBluescript (Stratagene, La Jolla, CA). This PCR product was then used to screen a ZAP library of *D. discoideum* genomic DNA (kindly provided by Dr. Herbert Ennis) and phagenid were rescued following the manufacturer's directions (Stratagene, La Jolla, CA).

**REMI Mutagenesis**—REMI mutagenesis of the *Dictyostelium* uracilprototrophic cell line DH1 was performed using DpnII-linearized DIV2 plasmid essentially as described by Kuspa and Loomis (23). After selection, mutated transformants were screened for developmental phenotypes. The DIV2 plasmid along with flanking genomic DNA of the disrupted gene was rescued from developmental mutants and used to recapitulate the mutant phenotypes by homologous recombination of the rescued plasmid into *Dictyostelium* strain Ax4. Mutants that recapitulated the phenotype were isolated, and the DIV2 plasmid along with flanking genomic DNA was rescued. The genomic DNA was then sequenced at the Baylor College of Medicine Sequencing Core (Houston, TX) to identify the disrupted gene.

**Glycogen Assay**—To determine cellular glycogen content, cells were harvested as vegetative or 20-h developed cultures, washed in PB, resuspended to 5 × 10⁸ cells/ml in 10% acetic acid, and lysed through a Cameo 25N 5-micron filter (MSI, Westboro, MA). The lysates were clarified by centrifugation and the supernatant titrated to pH 5.0 with KOH, and 200 μl of the neutralized supernatant was incubated with 100 μl of a 70 units/μl solution of α-amylglucosidase (in 0.4 M sodium acetate, pH 4.8) at 50 °C for 3 h to hydrolyze glycogen to free glucose. After 3 h, the glucose content of 5 and 15 μl of each culture was measured with the Trinder Glucose Assay Kit (Sigma) following the protocol of the manufacturer. Glucose concentrations of the cells were calculated from a standard curve generated from purified glucose. Glucose liberated from glycogen was determined by normalizing cellular glycogen concentrations to glucose concentrations in lysates incubated in the absence of α-amylglucosidase. Protein concentration of the clarified lysates was determined with the Bio-Rad Protein Assay Reagent (Bio-Rad) using a bovine serum albumin standard, following the protocol of the manufacturer.

**Spore Viability Assay**—The spore viability assay was performed essentially as described by Dynes et al. (24). To determine the number of viable spores or cells in a culture of *Dictyostelium*, 1 × 10⁷ cells were plated onto filters and allowed to develop. At various time points, cells were removed from the filters, washed in PB, and either serially diluted directly in a Klobisella aerogenes suspension and plated on SM/5 plates (for cell viability assay), or pelleted, resuspended in 10 mM EDTA/0.1% Nonidet P-40 in PB, incubated at 42 °C for 45 min, washed three times in PB, and then serially diluted in *K. aerogenes* and plated on SM/5 plates (spore viability assay).

**RESULTS**

**Insertion in the udppg1 Locus Results in Lack of Expression**—We constructed a 6.4-kb fragment containing the *udppg1* genomic locus in which 1.7 kb in the central region of the gene was replaced by a 2.1-kb neomycin resistance cassette (neo) (Fig. 1A). The modified UDPGP sequence with the neo cassette was excised from the vector DNA by digestion with *HinII* and *SacI* and then introduced into Ax3 cells by electroporation. Transformants were selected in the presence of G418. Solid box, gene; open box, flanking region. Restriction sites: N, *NsiI*; H, *HindIII*; E, *EcoRI*. B, genomic DNA from Ax3 (WT) and *udppg1* (Δ) cells was isolated and digested with *EcoRI*, *HindIII*, and *NsiI*. The resulting blot was probed with part of the coding region of the *udppg1* gene illustrated in A.

Fig. 1. Construction of *udppg1* deletion mutant. A, after homologous pairing between the linearized targeting vector, *udppg1−* construct, and the genomic *udppg1* locus, homologous recombination in the regions labeled X replaces the endogenous *udppg1* sequence with an exogenous sequence containing the neo cassette and a deletion of the *udppg1* gene. The cell lines produced by this recombination event will be resistant to G418. Solid box, gene; open box, flanking region. Restriction sites: N, *NsiI*; H, *HindIII*; E, *EcoRI*. B, genomic DNA from Ax3 (WT) and *udppg1* (Δ) cells was isolated and digested with *EcoRI*, *HindIII*, and *NsiI*. The resulting blot was probed with part of the coding region of the *udppg1* gene illustrated in A.
the udpgp1 gene is replaced with the altered deleted construct by double crossing over as indicated in Fig. 1A.

udpgp1 Cells Grow and Develop Normally—To determine what effect loss of udpgp1 had on vegetative growth, wild-type and udpgp1- cells were grown in suspension and their densities measured. Cells lacking udpgp1 grow at the same rate as wild-type cells (data not shown). To examine the effect of disrupting the udpgp1 gene on development, wild-type and udpgp1- cells were allowed to starve and develop on filter pads for 24 h. udpgp1- cells developed fruiting bodies at the same rate and with the same size as wild-type cells (data not shown). This is in agreement with previous reports (12) that antisense inactivation of udpgp1 had no obvious effect on growth or development. Thus, the udpgp1 gene is not required for vegetative growth or fruiting body formation.

udpgp1 Cells Make Wild-type Levels of Cellulose—Because UDPGP is a key enzyme in the production of various carbohydrates, we examined the ability of udpgp1- cells to make cellulose. Cellulose is almost undetectable in vegetative cells. However, cellulose is required for fruiting body formation, consisting of 4% of the dry weight of mature fruiting bodies, and is a major component of stalk and spore cells (1, 11, 25, 26). From 0 to 15 h of development, the total amount of cellulose in wild-type cells remains fairly low at 10 μg/10⁸ cells. It then rapidly increases to 80 μg/10⁸ cells by 25 h (Fig. 3). The udpgp1- cells show slightly less cellulose than wild-type cells during development, but in the fully developed organism cellulose levels are normal. Thus, udpgp1 is not the major protein involved in cellulose generation.

udpgp1 Cells Have Decreased but Detectable UDPGP Activity—Given that the udpgp1- cells developed normally and have normal levels of cellulose, we decided to examine whether these cells had any UDPGP activity. The developmental profile of UDPGP enzyme activity has been well established (10, 11). Fig. 4 shows the expected increase in activity that was previously observed during development for wild-type cells. The enzyme activity declines slightly at 5–10 h into development and then rises to a peak at 20 h (10, 14). The udpgp1- strain does not show any enzyme activity in vegetative cells. However, starting at 15 h into development, enzyme activity appears and increases up to about one-third of the total specific activity of wild-type cells. This suggests the existence of at least one other gene that encodes a protein with UDPGP activity.

Identification of a Second UDPGP Gene, ugpB—Previously, we reported (14) that immunoprecipitation of the products of in vitro translated total mRNA from wild-type cells with antiserum to UDPGP enzyme identified two polypeptides of 55 and 53 kDa. However, hybrid selection with cloned udpgp1 cDNA rescued mRNA encoding only the higher molecular weight in vitro translated product (14). In accordance with the presence of UDPGP activity in the udpgp1- strain, this suggests the presence of a second UDPGP gene.

To identify this second gene, termed ugpB, degenerate PCR was performed on wild-type and udpgp1- genomic DNA using primers matching potentially conserved regions. As expected, PCR products from both reactions measured 112 bp. If the degenerate primers hybridized to the second UDPGP gene, the PCR products from the wild-type DNA would be a mixture amplified from both the udpgp1 and ugpB genes, although that from the udpgp1- DNA should only have originated from the ugpB gene. To test this, the two PCR products were digested with BglII, because a BglII site occurs in the middle of the amplified region of the udpgp1 gene. Such a site would most likely not be present in the ugpB gene. Indeed, the 112-bp PCR product from wild-type DNA was reduced by more than 70% on digestion with BglII, whereas the 112-bp PCR product from udpgp1 DNA was unaffected. This suggested that the majority of the PCR product from the udpgp1- genomic DNA was amplified from the ugpB gene.

The 112-bp PCR product from the udpgp1- DNA was subcloned into a vector and sequenced. This sequence was then used to screen a genomic library for the second gene. A genomic clone was isolated that contained the partial sequence of the ugpB gene. To recover the ugpB cDNA, a search of the Dictyostelium EST data base project revealed several clones. These clones were ordered from the data base project, and the two largest were entirely sequenced. These two clones proved to contain full-length ugpB cDNAs (Fig. 5).

UgpB is 42.7% identical and 57.1% similar to Udpgp1 at the amino acid level. However UgpB actually possesses greater homology to UDPGPs from several species of animals and plants than it does to Udpgp1 or Udpgp1 does to any of these.
other orthologues (Table I). The molecular mass of the UgpB protein as derived from the amino acid sequence is 56,153 Da, which is 1734 Da less than the derived molecular weight of Udpgp1. Furthermore, UgpB has a net charge of \( +1001 \) whereas Udpgp1 has a net charge of \( +1002 \). The differences in calculated charge and size between Udpgp1 and UgpB are consistent with the analyses of Manrow and Dottin (13) and Fishel et al. (14), in which they demonstrated that Udpgp1 is the larger and more acidic of the two UDPGP isozymes.

To determine whether distinct subgroups could be identified within the UDPGP family of genes, UDPGP amino acid sequences derived from all 21 of the eukaryotic cDNA sequences in GenBank™ were aligned using the multiple sequence alignment program PileUp provided in the Wisconsin Package (Gen.

**Fig. 5. Sequence of ugpB.** The open reading frame begins at nucleotide 88 and ends at nucleotide 1593. The five conserved lysines are labeled K1–K5 and are in **boldface type**. The **black arrowhead** identifies the site of insertion for the REMI mutant. The sequence is available at GenBank™ under accession number AF150929.
The amino acid sequences of UgpB and Udpgp1 were compared using Vector NTI, and the percentage similarity and identity were tabulated.

| Organism   | UgpB Sim | UgpB ID | Udpgp1 Sim | Udpgp1 ID |
|------------|----------|--------|------------|-----------|
| Yeast      | 60.8     | 48.2   | 54.2       | 37.8      |
| Potato     | 61.2     | 48.9   | 53.8       | 37.3      |
| Worm       | 61.3     | 49.4   | 55.6       | 40.2      |
| Human      | 65.1     | 53.9   | 57.6       | 41.7      |
| Udpgp1     | 57.1     | 42.7   | 100        | 100       |

The amino acid sequences of UgpB and Udpgp1 were compared using Vector NTI, and the percentage similarity and identity were tabulated.

**Fig. 6. Relatedness of UDPGP genes from different organisms.** The amino acid sequences of 21 UDPGP homologues contained in GenBank™ were aligned, and a dendrogram was produced displaying pairwise alignment relationships of the sequences. Distance on the horizontal axis is inversely proportional to homology. The amino acid at each of the five functionally important K sites for each of the sequences is displayed on the right, if different from lysine. The three bottom sequences that are the least homologous with the remainder of the UDPGP sequences possess the most mutations at these functional residues.

**Table I**

Sequence comparison of UgpB and Udpgp1 to homologues from different organisms

The amino acid sequences of UgpB and Udpgp1 were compared using Vector NTI, and the percentage similarity and identity were tabulated.

| Organism       | Sim | ID  | Sim | ID  |
|----------------|-----|-----|-----|-----|
| Yeast          | 60.8| 48.2| 54.2| 37.8|
| Potato         | 61.2| 48.9| 53.8| 37.3|
| Worm           | 61.3| 49.4| 55.6| 40.2|
| Human          | 65.1| 53.9| 57.6| 41.7|
| Udpgp1         | 57.1| 42.7| 100 | 100 |

NETICS (Computer Group, Inc., Madison, WI). The alignments are displayed as a distance tree (Fig. 6). From the tree it is readily apparent that Udpgp1, Saccharomyces cerevisiae2, and Caenorhabditis elegans4 are each on their own branch, whereas the remaining 19 sequences share a common branch. Within the branch containing these 19 sequences, all of the remaining yeast sequences cluster together; all of the plant sequences cluster together; all of the vertebrate sequences cluster together; and all of the nematode sequences cluster together. The Dictyostelium UgpB sequence is on its own branch but is more closely aligned with the animal sequences than the plant or yeast sequences and more closely aligned to all of these sequences than to Udpgp1.

Close inspection of the sequences revealed that the three proteins that fail to group with the remaining proteins (Udpgp1, C. elegans4, and S. cerevisiae2) all share the loss of at least two of five conserved lysine residues previously identified as being important for substrate binding or catalysis (27, 28). These five functionally important lysines are residues 263, 329, 367, 409, and 410 in Potato1 UDPGP (for clarity when discussing homologues of varying lengths these will hereafter be referred to as sites K1, K2, K3, K4, and K5). The residues at sites K1–K5 in the 21 aligned genes are detailed in Fig. 6. From Fig. 6 it is apparent that of the 21 UDPGP amino acid sequences, mutations in sites K1–K3 are restricted to only a small subset of the proteins; K1 is mutated in only Udpgp1 and S. cerevisiae2; K2 is mutated in only Udpgp1, Schizosaccharomyces pombe2, and S. cerevisiae2; K3 is mutated in only S. cerevisiae2; and only Udpgp1, S. cerevisiae2, and C. elegans4 possess mutations in both K4 and K5. This analysis confirms that these five lysine residues are tightly conserved across a wide range of taxa and suggests that Udpgp1, S. cerevisiae2, and C. elegans4, three proteins that segregate as outgroups from the main tree, form a subgroup of UDPGP proteins with possible differences in catalytic or substrate binding abilities from the majority of UDPGP proteins.

The Expression of ugpB Parallels That of udpgp1—To examine the developmental expression pattern of ugpB, Northern blots were performed using total RNA from wild-type cells. The udpgp1 mRNA is ~1.8 kb, whereas the ugpB mRNA is ~1.6 kb. For both transcripts, expression does not become strong until 12 h into development and peaks at 16 h into development (Fig. 7). It is important to note that the accumulation of udpgp1 and ugpB transcripts is consistent with the accumulation of UDPGP enzyme activity. The udpgp1 and ugpB mRNAs were expressed in both prestalk and prespore cell types (data not shown).

The cDNA Encoding ugpB Complements the Escherichia coli galU Mutant—It has been shown that clones encoding a UDPGP from different organisms can functionally complement a bacterial galU mutant that lacks UDPGP activity (29, 30). To determine whether the cDNA coding for ugpB is functional, we subjected it to this complementation test. E. coli mutant strains CA10 and CA198 were able to grow on minimal M9 plates containing 0.2% glucose but not on plates supplemented with 0.2% galactose because of deficient UDPGP activity. We transformed these strains with the Dictyostelium cDNA SSA730 encoding ugpB, and we observed rescued growth on galactose-containing plates. These observations demonstrate that this ugpB codes for a functional UDPGP.

Disruption of the ugpB Locus Causes Loss of Expression and Abnormal Development—An REMI mutant of ugpB was identified and analyzed. The gene was determined to be disrupted between codons 333 and 334, which is upstream of the regions coding 4 of the 5 conserved lysine residues (Fig. 5). We examined the expression of ugpB in the disruptant by Northern blot, and we found that the ugpB− strain fails to make a detectable transcript (data not shown).

To characterize the phenotype of ugpB− mutants, parental DH1 and ugpB− cells were starved on filter pads at densities between 1 × 105 and 1 × 106 cells/ml, and the extent of
Dictyostelium Development Requires UDPGP

The importance of UDPGP activity has long been implicated in Dictyostelium development (10). Previous work (11) has shown that randomly generated mutants that lack UDPGP activity are unable to complete development. The study of these mutants, however, has been hampered by the inability to identify the exact genetic lesion producing loss of UDPGP activity; in fact, many of these strains are likely to possess genetic mutations in addition to those in the genes encoding UDPGP.

Until now, a udpgp1 knockout had not been generated; however, a strain carrying an antisense udpgp1 construct had been made (12). This udpgp1 antisense strain failed to produce detectable amounts of either Udpgp1 or UgpB, yet in contrast to the mutants of Dimond et al. (11), which lack UDPGP activity, the UDPGP antisense strain displayed no overt mutant phenotype. The authors concluded that either UDPGP activity is not required for growth and development or that the antisense mRNA failed to give a complete suppression of translation and yielded an undetectable amount of Udpgp1 and/or UgpB. Subsequently, UDPGP antisense mutants have been produced in plants (31) and yeast (32). In both cases, the antisense transcript produced a greater than 95% reduction in UDPGP activity without creating any obvious mutant phenotype. However, total loss of UDPGP activity by genetic disruption is lethal in yeast (30). Thus, the data indicate that just a small fraction of the wild-type level of protein is sufficient to preserve viability and maintain an overtly wild-type phenotype. The data also suggest that the udpgp1 antisense strain in Dictyostelium maintains a wild-type phenotype due to insufficient suppression of the UDPGP activity.

We have now created a genetic knockout of the udpgp1 gene. As was seen with the udpgp1 antisense strain, these cells are normal for growth and development. In addition, these cells make normal levels of cellulose, a product that requires UDPGP activity. The udpgp1 strain is the first strain of any organism to retain viability despite disruption of a UDPGP gene. This lack of phenotype is most likely explained by the presence of UDPGP activity encoded by the second UDPGP gene, ugpB.

Like the udpgp1 null strain, the ugpB null strain is viable. However, unlike the udpgp1 null cells, the ugpB null cells do have a mutant phenotype late in development. This demonstrates that expression of ugpB is dispensable for vegetative growth and early development but is required for completion of the developmental cycle as is evidenced by the small, gnarled phenotype of the fruiting bodies formed by the ugpB cells. Furthermore, the expression of ugpB is not required for establishing the proper ratio of differentiated pre-spore and pre-stalk cells, although the efficiency of differentiating into either of these cell types is decreased when ugpB is not expressed.

We show here that the expression pattern of ugpB is developmentally regulated and follows very closely the pattern of expression of udpgp1 (14). This synchronized pattern of expression is consistent with the synchronized accumulation of the two UDPGP proteins during development as demonstrated by Haribabu et al. (33). The steady increase in expression levels...
with a peak late in development is also consistent with the appearance of the mutant phenotype late in development. It is important to note that the higher intensity of the ugpB bands in Fig. 7 does not reflect a heightened level of expression over Northern blots. The authors concluded that the UDPG null mutation was important to note that the higher intensity of the probes used for the Northern blots.

The ugpB Phenotype May be Caused by Trehalose and Glycogen Deficiencies—UDP-glucose, produced by UDPGP, is an essential substrate in the synthesis of trehalose and glycogen. We show here that glycogen stores in both vegetative and starved ugpB null cells are significantly lower than in the parental cell line. These results indicate that UgpB is required for proper glycogen metabolism and suggest that a glycogen deficiency might contribute to the mutant phenotype.

One aspect of the mutant phenotype is an inability to produce viable spores. UDP-glucose is required for the synthesis of a number of compounds in spores, including cellulose and trehalose, as well as glycogen. The ugpB null cells fail to differentiate into viable spores, yet the cells themselves remain viable long after cells of the parental strain have sporulated. This suggests that the inability of ugpB null cells to differentiate into viable spores is not caused by the death of the starving cells due to insufficient energy stores (glycogen levels). The glycogen deficiency is further unlikely to directly prevent at least the initiation of spor differentiation because ugpB null cells are able to differentiate into pre-spore cells.

Examination of UDPGP mutants in other organisms may lend insight into the cause of ugpB spore non-viability. S. cerevisiae UDPGP underexpressors displayed a cell wall β-glucan deficiency and were more sensitive to treatments that interfere with cell wall assembly than were wild-type cells (30). The authors concluded that the UDPGP null mutation was most likely lethal in yeast due to disruption of cell wall formation. The inability of ugpB null Dictyostelium cells to form viable spores might be due to an inability to produce the structural components of the cell wall required to withstand the harsh treatments of the viability assay. Alternatively, the lack of viable spores may be due to low levels of trehalose. Dictyostelium spores possess high concentrations of trehalose (34), a disaccharide that in a number of organisms, including Dictyostelium, has been shown to confer protection against desiccation and heat shock (35, 36). UDPGP antisense S. cerevisiae strains have trehalose deficiencies (30), raising the possibility that in Dictyostelium disruption of ugpB could lead to alterations in trehalose metabolism and a decrease in spore viability.

**Udpgp1 and UgpB Are Most Likely Enzymatically Distinct—** Extensive work has been done on understanding the enzymology of UDPGPs. Analysis of the amino acids that are important for substrate binding and catalysis in potato UDPGP was done (28) using reactive substrate analogues to label the residues associated with substrate binding and catalysis. From these experiments a set of 5 lysine residues, all of which were specifically labeled by reactive analogues of both UTP and UDP-glucose, were identified. Katsube et al. (27) subsequently used site-directed mutagenesis to substitute glutamines for the K site lysines and then assayed the $K_m$ and $V_{max}$ values of these mutants to determine the relative importance of these residues for substrate binding and for catalysis. Substitution of the lysine K3 essentially abolishes enzymatic activity without affecting substrate binding. Substitution of either K1 or K2 produces mutants with dramatically altered substrate binding abilities and hence lowered $V_{max}$ values. Substitution of either K4 or K5 produces mutants with only small alterations in $V_{max}$ and $K_m$. The authors concluded that K3 is an essential residue in the catalytic active site of the enzyme, whereas K1 and K2 are important for substrate binding, and K4 and K5 play less important substrate binding roles.

The sequence alignment reveals that the catalytic residue K3 is conserved in all genes except for a conservative substitution to arginine in S. cerevisiae2. This mutation preserves a positively charged residue at K3, which is thought to be essential.
for the catalytic mechanism (27). Thus, this change is unlikely to abolish activity in the enzyme as did the non-conservative K3Q site-directed substitution. However, as inferred by comparison to the site-directed substitutions at K1 and K2, the substitutions at these loci in Udpp1 and S. cerevisiae2 probably produce enzymes with lowered substrate binding and thus lowered activity. Katsube et al. (27) did not see strong effects on the enzyme kinetics from substitutions at K4 or K5. From the sequence analysis, it is clear that differences at one or the other of these positions is common across species. Both lines of evidence thus suggest that changes at either K4 or K5 have little effect on the physiological role of the enzyme. However, three proteins Udpp1, C. elegans4, and S. cerevisiae2 possess substitutions in both K4 and K5, a condition likely to be more deleterious than either single change alone.

Enzymatic assays have demonstrated that the activity of udpp1 is 10-fold less than the activity of potato UDPGP (37). Konishi et al. (38) attributed this reduction in activity to the K site substitutions in udpp1. It is therefore likely that C. elegans4 and S. cerevisiae2, which also possess K site substitutions, also have reduced enzymatic activities. Intriguingly, although the three proteins possess changes that cause reductions in substrate binding, none of them possesses a severe mutation of the critical catalytic site K3 and are therefore likely to remain active, if at a level less than wild-type. It is therefore possible that these impaired UDPGP proteins represent a newly identified subgroup of UDPGP genes with distinct kinetic properties and hence distinct physiological roles. This interpretation is supported by the fact that all of the organisms that possess genes with K site alterations also possess normal UDPGP genes, which would maintain wild-type UDPGP function.

One possible role for multiple UDPGP isozymes could be in the control of tissue-specific or developmental stage-specific requirements for the direction of carbon flux. Although the direction of the UDPGP reaction is believed to be largely regulated by substrate concentrations, isozymes specific for the forward or reverse reaction have been speculated to occur in specific subcellular organelles, tissues, or at specific time points of development (39). Similarly, there is evidence of the ability of UDPGP protein from a variety of species to dimerize or oligomerize (39). Therefore, varying isozyme ratios might lead to the formation of varying hetero-oligomers, each of which might produce a distinct physiological effect.

In Dictostelium, although the UDPGP transcripts are not temporally segregated, Manrow and Dottin (13) and Fishel et al. (14) have reported the existence of UDPGP isozymes with distinct molecular weights and isoelectric points. These isozymes have differing patterns of activity in vegetative and stationary phase cells. Similarly, a requirement for differing UDPGP isozymes could arise between stalk and spore cells. Stalk cells must produce large amounts of cellulose and other structural polysaccharides but have no need to store glycogen because they undergo death during development. Spore cells also need to synthesize structural polysaccharides for a sporid oat, but they must also maintain glycogen reserves to nourish the germinating spore as well as produce trehalose to protect the spore from desiccation. In addition, the lowered efficiency of differentiation into pre-spore and pre-stalk cells seen in ugpB mutants might reflect a segregation of UDPGP isozyme activity between null cells and pre-stalk/pre-spore cells that is disrupted in the ugpB mutant.

Acknowledgment—The infrastructure and instrumentation of the Biological Sciences Department at Hunter are supported by Research Centers in Minority Institutions Award RR-03057 from the National Center for Research Resources of the National Institutes of Health.

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