CugP Is a Novel Ubiquitous Non-GalU-Type Bacterial UDP-Glucose Pyrophosphorylase Found in Cyanobacteria

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UDP-glucose pyrophosphorylase synthesizes UDP-glucose from UTP and glucose 1-phosphate and exists in almost all species. Most bacteria possess a GalU-type UDP-glucose pyrophosphorylase, whereas many cyanobacteria species do not. In certain cyanobacteria, UDP-glucose is used as a substrate for synthesis of exopolysaccharide cellulose in spite of the absence of GalU-type UDP-glucose pyrophosphorylase. Therefore, there should be an uncharacterized UDP-glucose pyrophosphorylase in cyanobacteria. Here, we show that all cyanobacteria possess a non-GalU-type bacterial UDP-glucose pyrophosphorylase, i.e., CugP, a novel family in the nucleotide triphosphate transferase superfamily. The expressed recombinant *Synechocystis* sp. strain PCC 6803 CugP had pyrophosphorylase activity that was highly specific for UTP and glucose 1-phosphate. The fact that the CugP gene cannot be deleted completely in *Synechocystis* sp. PCC 6803 suggests its central role as the substrate supplier for galactolipid synthesis. Galactolipids are major constituents of the photosynthetic thylakoid membrane and important for photosynthetic activity. Based on phylogenetic analysis, this CugP-type UDP-glucose pyrophosphorylase may have recently been horizontally transferred to certain noncyanobacteria.

Glycosylation, which is catalyzed by various types of glycosyl transferases, is important for the biosynthesis of many biological molecules, e.g., polysaccharides, glycoproteins, and glycolipids. Nucleotide diphosphate (NDP)-sugars, which are the substrates for glycosylation, are mostly synthesized by nucleotidyl transferases that use a nucleotide triphosphate (NTP) and a sugar 1-phosphate (sugar-1P) as substrates. Various NDPs and sugars are combined into NDP-sugars *in vivo*. Of these, UDP-glucose (UDP-Glc) is used for the biosynthesis of cellulose (1) and glycogen (2), galactose metabolism (3), and addition of the glucose moiety in heteroglycans, glycoproteins (4–6), and glycolipids (7). Other NDP-sugars are utilized in the biosynthesis of other polysaccharides and in various glycosylation reactions.

UDP-Glc and the by-product inorganic pyrophosphate (PP_i) are produced from UTP and Glc-1P by the UDP-Glc pyrophosphorylases (PPases) (EC 2.7.7.9) in the reversible reaction UTP + Glc-1P ↔ UDP-Glc + PPi. This enzyme is ubiquitous although its typical bacterial form, the GalU-type, is widely distributed throughout *Bacteria* including *Proteobacteria* (8), *Firmicutes* (9), and *Actinobacteria* (10, 11) and is distinct from its eukaryotic enzyme in amino acid sequence and three-dimensional structure (12–14). The best-known bacterial UDP-Glc PPase is GalU from *Escherichia coli* (15, 16). The crystal structures and the reaction mechanism of various GalU-type homologs have been elucidated (17–19). These bacterial GalU-type UDP-Glc PPases belong to a large superfamily of nucleotidyl transferases that also includes UDP-*N*-acetylglucosamine (GlcNAc) PPase, ADP-Glc PPase, CDP-Glc PPase, dTDP-Glc PPase, GDP-mannose (GDP-Man) PPase, and some yet uncharacterized enzymes. These enzymes probably diverged from a common ancestor in evolution (20).

There are many Glc-containing polysaccharides, such as glycogen and Glc-enriched exopolysaccharides, in cyanobacteria (21). Certain cyanobacteria also accumulate cellulose (β-1,4-glucan) (22, 23). Consistently, cyanobacterial genomes have been shown to encode many putative nucleotidyl transferases that might supply NDP-sugars (24, 25). Recently the GalU-type protein All3274

from the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 was biochemically revealed to be a UDP-Glc PPase (20). However, such a GalU-type enzyme is not present in all cyanobacteria. Specifically, although *Thermosynechococcus* is reported to possess a cellulose synthase to produce cellulose under conditions of light and low temperature (23), a GalU-type gene that could supply UDP-Glc to this cellulose has not been found in its genome (24). Given these observations, we hypothesized that an uncharacterized non-GalU-type UDP-Glc PPase may exist in such cyanobacteria. To test this hypothesis, we first focused on a common sequence feature that is shared only by GalU-type UDP-Glc PPases, UDP-GlcNAc PPases, and a putative enzyme family that is present in every species of cyanobacteria. Here, we report that the members of this previously uncharacterized family are cyanobacterial UDP-Glc PPases (thus denoted CugP).

MATERIALS AND METHODS

Sequence alignment and phylogram. The sequences of the cyanobacterial proteins categorized as belonging to the bacterial nucleotidyl transferase superfamily (Pfam PF00483, NTP_transferase) (http://pfam.sanger.ac.uk) were obtained from NCBI (http://www.ncbi.nlm.nih.gov/) and CyanoBase (http://genome.microbedb.jp/cyanobase) databases. Sequence alignment was performed by the neighbor-joining method using Clustal X (26).

Cloning of *sll1558* and single-amino acid substitution. *sll1558* from *Synechocystis* sp. strain PCC 6803, which encodes the hypothetical UDP-Glc PPase Sll1558 was PCR amplified using PrimeSTAR Max DNA Poly-

merase (TaKaRa Bio, Otsu, Japan) and then directly cloned into the expression vector pET-28a(+) (Merck, Darmstadt, Germany) using In-Fusion HD Cloning kit reagents (TaKaRa) to produce *sll1558*-pET28a. The primers were pET28a-1F (5′-GGA TCC GAA TTC GAG CTC CGT C-3′), pET28a-2R (5′-CAT ATG GCT GCC GCG CGG CAC-3′), sll1558-1F28a (5′-CGC GGC AGC CAT ATG AAA GCC ATG ATT TTG GCC-3′), and sll1558-2R28a (5′-CTC GAA TTC GGA TCC TTA TTC CGG CTG GAG AAG-3′). To generate a single amino acid substitution within the Sll1558 protein product, the plasmid was amplified by two sets of primers (sll1558-3FA8G, 5′-GCC GGT GGC AAG GGC ACT CGG GTC AGA CCA ATC-3′; sll1558-4RA8G, 5′-GCC CTT GCC ACC GGC CAA AAT CAT GGC TTT CAT-3′) and self-combined using the aforementioned cloning reagents. These proteins were His tagged from the pET28a vector. The resulting DNA constructs were verified by dideoxy sequencing. These proteins were N-terminally His tagged, derived from the pET28a vector.

Expression, purification, and SDS-PAGE analysis of recombinant proteins. *E. coli* strain C41 (DE3) carrying *sll1558*-pET28a or *sll1558*(A8G)-pET28a, which carries the *sll1558* gene encoding the amino acid change A to G at residue 8, was cultured in 1 liter of LB medium at 37°C. When each culture reached an optical density at 600 nm (OD_{600}) of 0.4 to 0.8, 1 mM isopropyl β-D-1-thiogalactopyranoside was added (final concentration, 100 μM), and the cells were grown at 18°C for 16 h to achieve induction. The cells from each culture were then collected by centrifugation at $4,220 \times g$ for 15 min, suspended in 20 mM HEPES (pH 7.5) containing 100 mM NaCl and 10% (wt/vol) glycerol, and homogenized three times with a French press at 1,500 kg/cm^{-2}. The soluble proteins were collected by centrifugation at $50,000 \times g$ for 30 min. His-tagged Sll1558 and Sll1558 with the A8G substitution [Sll1558(A8G)] were purified by Ni-affinity column chromatography (HisTrap HP; GE Healthcare, Little Chalfont, United Kingdom), with the eluent consisting of a gradient of 30 to 430 mM imidazole in the aforementioned buffer system. Proteins in each fraction were subjected to SDS-PAGE, followed by Coomassie brilliant blue R-250 staining. Low-molecular-mass calibration kit standards (GE Healthcare) served as the molecular mass markers. The fraction enriched in each targeted protein was dialyzed against the aforementioned buffer to remove the imidazole. Protein concentration was assayed using the Bradford method (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard.

Nucleotidyl transferase activity assay. Nucleotidyl transferase activities were assayed at 37°C in the direction of NDP-sugar formation from NTP and sugar-1P. The reaction rate was determined as the change in the PP$_i$ concentration with time and measured using an EnzChek Pyrophosphate Assay kit (Molecular Probes, Life Technologies, Carlsbad, CA). The basic reaction medium contained 50 mM Tris-HCl (pH 7.5), 8 mM MgCl$_2$, 200 μM 2-amino-6-mercapto-7-methylpurine ribonucleoside, 1 U of purine nucleoside phosphorylase, 30 mU of inorganic pyrophosphatase, and substrates. To reduce contaminating inorganic phosphate found in the substrate preparations, each reaction solution was preincubated for 20 min at 22°C and for 10 min at 37°C prior to the addition of the protein. For measurements of substrate specificity, all substrates were included in the reaction mixtures at a 200 μM concentration, and the purified enzymes were included at 30 μg ml^{-1}. For measurements of kinetics, purified enzymes were included in the reaction mixtures at 4.5 to 18.0 μg ml^{-1}.

Structure prediction based on previous studies. The available Protein Data Bank (PDB) data of GalU-type UDP-Glc PPase (PDB 2PA4) (18), CDP-Glc PPase (PDB 1TZF) (27), and dTDP-Glc PPase (PDB 1H5T) (28) were used to compare the structures and substrate-binding regions of Sll1558.

Gene disruption and mutant analysis. The *sll1558* gene was inactivated by replacement with the chloramphenicol resistance cassette in a pPCRscript-derived DNA by the In-Fusion method using primers sll1558-5F (5′-CCG GGG GAT CCG CCC GAT GCG GAG CTG TGG GAC-3′), sll1558-6R (5′-CCG GAC ATC AGC GCT CAA CAA TTT TCC

TCA AAC-3′), sll1558-7F (5′-ACG GTT AGC AGG CCT TCA CAG AAG AGA ACT GGG-3′), and sll1558-8R (5′-CGG CCG CTC TAG CCC GCA GGG CCT TTT CCC GTA-3′). Mutants were generated by natural transformation of *Synechocystis* cells with this DNA and selected on BG11 plates containing 20 μg ml^{-1} chloramphenicol. The segregation was examined by PCR with primers sll1558-9F (5′-GTG TGA TTG AGT TTG AGG-3′) and sll1558-10R (5′-TTT CCC CCA GTT CTC TTC-3′).

Chlorophyll (Chl) content was determined after extraction with 100% methanol as described previously (29). Total photosynthetic activity of cells was measured as oxygen evolution supported by 0.2 mM Na$_2$CO$_3$ in BG11 medium using a Clark-type electrode (Oxygraph, Hansatech, United Kingdom) at a chlorophyll concentration of 2.5 μg ml^{-1} (30).

RESULTS AND DISCUSSION

Sequence features of nucleotidyl transferases. The phylogeny of the nucleotidyl transferase superfamily (protein family [Pfam], NTP_transferase) in cyanobacterial species was analyzed based on alignment of full-length amino acid sequences of the transferases (Fig. 1). It is of note that GalU-type UDP-Glc PPase is found in only a few species (*Anabaena* sp. PCC 7120, *Arthrospira platensis* NIES-39, and *Nostoc punctiforme* ATCC 29133). On the other hand, many other enzymes such as dTDP-Glc PPase, CDP-Glc PPase, ADP-Glc PPase, UDP-GlcNAc PPase, and as yet uncharacterized nucleotidyl transferases are found in most species of cyanobacteria. We searched for common residues in the GalU-type UDP-Glc PPase and UDP-GlcNAc PPase sequences. As reported previously, the G-X-G-T-R motif is highly conserved in all sequences as a part of the (d)NTP-binding site. We found that one residue just prior to the motif is characteristically conserved: Ala is present in GalU-type UDP-Glc PPase, UDP-GlcNAc PPase, and one uncharacterized family (provisionally annotated GDP-Man PPase), whereas Gly is present in all of the others (Fig. 2). Since this residue was the only candidate that might be responsible for substrate discrimination, we expressed and characterized the activities of Sll1558, the putative *Synechocystis* sp. PCC 6803 GDP-Man PPase, and its Ala-8-Gly mutant Sll1558(A8G), which we chose because that cyanobacterium is a well-studied model for cyanobacteria in general.

Purified Sll1558 has a specific UDP-Glc PPase activity. We expressed Sll1558 as a His-tagged protein, which we purified by nickel-affinity chromatography (see Fig. S1 in the supplemental material). According to our SDS-PAGE study, the molecular mass of Sll1558 is that predicted by the translated sequence of *sll1558* (45 kDa). The enzyme activity of this product was measured through the liberation of a by-product PPi using a combination of various NTPs (UTP, dTTP, CTP, ATP, and GTP) and sugar-1Ps (Glc-1P, GlcNAc-1P, Gal-1P, and Man-1P). We found that Sll1558 showed activity only in the presence of the combination of UTP and Glc-1P. Notably, it showed practically no activity toward any other substrates or combinations, including Man-1P, even though Sll1558 and related homologs had provisionally been considered to be GDP-Man PPases, as annotated in CyanoBase. dTTP does not substitute for UTP in reactions with Sll1558, a finding that contrasts with the ability of dTTP to act as a substrate of the GalU-type UDP-Glc PPase from *Xanthomonas campestris* (8) and *Sphingomonas elodea* ATCC 31461 (31).

Activity and substrate affinity comparison between Sll1558 and Sll1558(A8G). To further examine our prediction about nucleoside discrimination at the Ala residue, we evaluated the possible role of the Ala-8 residue of Sll1558 by site-directed replacement of this residue with Gly. After purification, the substrate
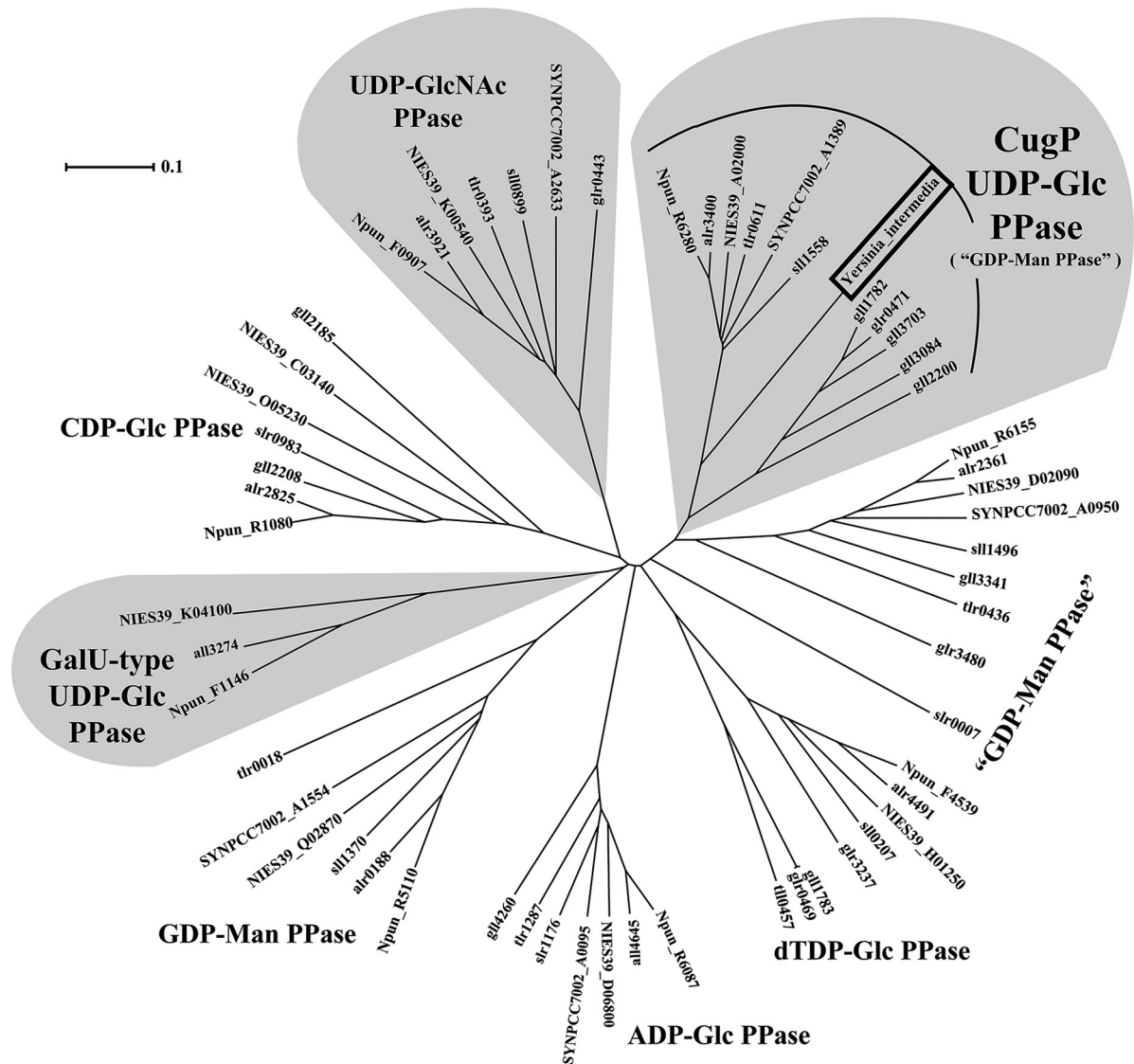
FIG 1 Phylogram and sequence alignment of cyanobacterial nucleotidyl transferases. The phylogram of the nucleotidyl transferases from *Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120, *Arthrospira platensis* NIES-39, *Synechococcus* sp. PCC 7002, *Thermosynechococcus elongatus* BP-1, *Nostoc punctiforme* ATCC 29133, and *Gloeobacter violaceus* PCC 7421 and the proteobacterium *Yersinia intermedia* (boxed) nucleotidyl transferase sequence. Families of UDP-Glc PPase and UDP-GlcNAc PPase are highlighted with a gray background.

specificity and enzyme kinetics of Sll1558(A8G) were measured as described above for Sll1558. Sll1558(A8G) also exhibited the PPase activity only for a combination of UTP and Glc-1P, as with wild-type Sll1558.

According to Lineweaver-Burk plots (Fig. 3), the K_m for UTP and K_m for Glc-1P for Sll1558(A8G) were 0.35 mM and 0.088 mM, and for Sll1558 they were 0.18 mM and 0.085 mM, respectively (Table 1). Thus, the K_m for UTP was found to be approximately 2-fold higher than that of the wild type, and the K_m for Glc-1P was comparable to the wild-type level. This finding suggests that the Ala-8 residue plays a slight role in the UTP binding. Notably, the V_{max} and k_{cat} values for Sll1558(A8G) were comparable to those of the wild-type enzyme (Table 1). The K_m values for Sll1558 were similar to those of GalU-type enzymes from bacteria other than cyanobacteria, whereas the V_{max} and k_{cat} were about 60- to 200-fold lower than those of these counterpart enzymes

(8–10). On the other hand, the kinetics parameters of Sll1558 were found to be similar to those of All3274, the GalU-type UDP-Glc PPase from *Anabaena* sp. PCC 7120 (20).

Distribution of the GalU-type and CugP-type enzymes, two types of UDP-Glc PPase. Herein, we designated Sll1558 and its homologs as CugP-type UDP-Glc PPase after cyanobacterial UDP-Glc PPase. Because all cyanobacterial genomes examined to date encode a CugP-type UDP-Glc PPase, this enzyme appears to be present in all cyanobacteria (Fig. 1), whereas the GalU-type UDP-Glc PPase is present in only certain species (20). As shown in Fig. 1, a phylogram of all related enzymes in the cyanobacterial nucleotidyl transferase superfamily confirmed such a distribution in cyanobacteria. It must be mentioned also that the cyanobacterial species that possess the GalU-type also possess the CugP-type UDP-Glc PPase.

According to the structure of the phylogram, the GalU-type

↓ **G-x-G-t-R motif** ↓

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CugP UDP-Glc PPase
("GDP-Man PPase")
  sll1558          2  KAMILAAGKGTRVPITHTIPKPMIPILQ-----KPVMEFLLELLRQHGFDQIMVNVSHLAEEIESYFRDGQ--------------------RFGVQIAYSFEGNIVGDLVGKALGSA  95
  tlr0611          2  KAMILAAGKGTRVRPITYTIPKPMIPILQ-----KPVMEFLLDLLRQHGFDQIMVNVSHLAHEIEGYFKDGQ--------------------RFGVEIAYSFEGYIKDGELVGKALGSA  95
  SYNPCC7002_A1389 2  KAMILAAGKGTRVRPITYTIPKPLIPILQ-----KPVMEFLLDLLAQHGFDQIMVNVSHLAHEIEGYFKDGQ--------------------RYGVHLAYSFEGSIQDGELQGAALGSA  95
GalU-type UDP-Glc PPase
  all3274          8  KAVIPAAGFGTRLFPATKVVKKELFPIIDQDGRAKPVILAIVEEAISAGITEVGIVVQPDDIGIFADLFKNPPAPELLQKLSPQNQEYSQYLQDLGQKVVLLTQ---------SEQEGYG  118
  Npun_F1146       8  KAVIPVAGFGTRLFPATKVVKKELFPIIDRDGRAKPVILAIIEEAISAGIAEVGIVVQPDDREIFEDLLKNPPKKELFKKLSPQNQEYSRYLEDLGSKVTTLLQ---------EEQLGYG  118
  NIES39_K04100   11  KAVITAAGFGTRMFPASKMIKKEFFPIVDAEGIAKPVIQVVVEEAINAGIEEVGIIVQSGDRSLFEDYFQGSPKPEFWRKLSPSSRDYSHYLQTLGERITILTQ---------EQQEGFG  121
UDP-GlcNAc PPase
  sll0899          5  --AVLAAGKGTRMKSD----LPKVLHQLGG----YSLVERVLQSCRPLAPERQILVGYQAAAVESSL----AGRSDVEF--------------------VEQ--------REQLGTG  80
  alr3921          5  --AVLAAGKGTRMKSD----LPKVLHSLGG----RSLVERVIDSVEPLSPSRRLVIVGYQAEQVKTGL-----QSPNLEF--------------------VEQ--------TVQLGTG  79
CDP-Glc PPase
  Npun_R1080       2  KAVILAGGLGTRLSEETSIRPKPMVEIGG----KPILWHIMKTYSSAHGINDFIICCGYKGYIIKEYFANYFLHMSDVTFDMRFNQMNVHSGYAEPWRVTLVNT---------GDNTMT  106
  alr2825          2  KAVILAGGLGTRLSEETSIKPKPMVEIGG----KPILWHIMKTYSSHGINDFIICCGYKGYVIKEYFANYFLHMSDVTFDMRFNQMNIHSGYAEPWRVTLVNT---------GDNTMT  106
  slr0983          2  KAVILAGGLGTRISEETHLRPKPMIDIGG----RPILWHILKLYSAYGVNDFIICCGYKGYVIKEYFANYFLHMSDVTFDMVSNEMIVHQRKAEPWKVTLVDT---------GENSLT  106
dTDP-Glc PPase
  alr4491          2  KALILSGGKGTRLRPLTYTGAKQLVPVAN----KPILWYGIEEMVAAGITDIGIIISPETGAEVQSKTGDG--------------------KLFGANITYILQ--------EQPAGLA  87
  sll0207         30  KALILSGGKGTRLRPLTYTGAKQLVPVAN----KPILWYGIEAIAKAGITDIGIIISPETGEEIKTITGNG--------------------EKFGIQITYILQ--------SEPLGLA  115
  tll0457         36  -GIILAGGSGTRLYPLTQVISKQLMPIYD----KPMIYYPLSVLMLAGIREILIISTPEHLYLFEKLLGDG--------------------HQWGLSLSYCVQ--------PQPNGLA  120
ADP-Glc PPase
  slr1176         16  -AIILGGGAGTRLYPLTKLRAKPAVPLAG----KYRLIDIPVSNCINSEIVKIYVLTQFNSASLNRHISRAYNFSGFQEG---------FVEVLAAQQTKDNP--------DWFQGTA  111
  all4645          6  -AIILGGGAGTRLYPLTKLRAKPAVPVAG----KYRLIDIPVSNCINSEIFKIYVLTQFNSASLNRHIARTYNFSGFSEG---------FVEVLAAQQTPENP--------NWFQGTA  101
  tlr1287         14  -AIILGGGAGTRLYPLTKRRAKPAVPLAG----KYRLIDIPVSNCINSEIHNIYVLTQFNSASLNRHIARTYTFPGLTGG---------FVEVLAAQQTPENP--------NWFQGTA  109
GDP-Man PPase
  tlr0018          4  IPVILSGGVGSRLWPLSRQAHPKQFIRLTPEG-KSLLQQTWQRVQGLPQLQPPLVANEAHRFLVAEQFQELG---------------VTPTR-ILLEPVGR---------NTAPAIT  95
  sll1370         20  IPVILAGGKGERFWPLSRRQRPKQFLSLDGSG-VSLLQATAQRLLSLAGDWENLWVITAAP--IAEGVFEQLP---------------SLPKENCLVEPEG--------DTAPAVA  110
"GDP-Man PPase"
  slr0007         10  -VVILAGGFGTRIKQQLPDLPKPLAPVAG----QPFLDWQLRYLQQQGFRRSLLSTGYLAEKVAAYAQDAD--------------------IVDMAIACVAE--------MEPLGTA  93
  sll1496          3  -AVLMAGGAGTRLRPLTCDLPKPMVIVN-----QPIAAHIIHLLRRHNITEVVATLHYLPDAMRDYFLDGS-----------------EFGVQMTYAVE--------DEHPLGTA  87
  SYNPCC7002_A0950 3  -AVLMAGGAGTRLRPLTCDLPKPMVPVLN----RPIAEHIINLLKRHRITEVIATLHYVPDIMRDYFQDGH----------------EFGVKMHYAVE--------EEQPLGTA  87
  tlr0436          4  -VVMAGGSGTRLPLTCDLPKPMVPVVN-----RPIAEHILNLLRRHNLDDVVMTLHYLPDVVRDYFGDGN----------------EFGVHLSYVVE--------EEQPLGTA  87
```
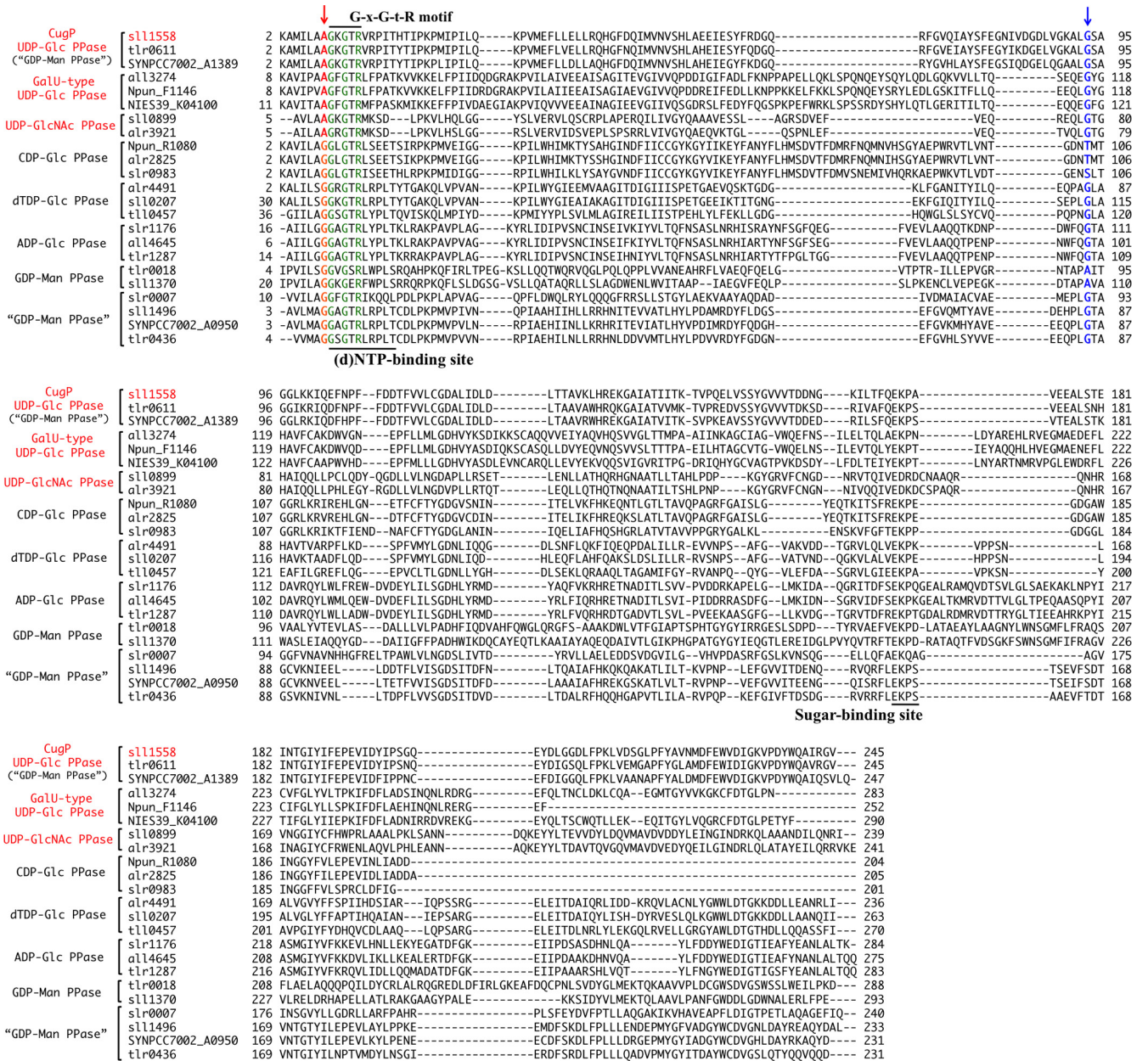
(d)NTP-binding site

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  sll1558          96  GGLKKIQEFNPF--FDDTFVVLCGDALIDLD--------LTTAVKLHREKGAIATIITK-TVPQELVSSYGVVVTDDNG----KILTFQEKPA-----------------VEEALSTE  181
  tlr0611          96  GGIKRIQDFNPF--FDDTFVVLCGDALIDLD--------LTAAVAWHRQKGAIATVVMK-TVPREDVSSYGVVVTDKSD----RIVAFQEKPS-----------------VEEALSNH  181
  SYNPCC7002_A1389 96  GGLRKIQDFHPF--FDDTFVVLCGDALIDLD--------LTAAVRWHREKGAIATVITK-SVPKEAVSSYGVVVTDDED----RILSFQEKPS-----------------VTEALSTK  181
  all3274         119  HAVFCAKDWVGN----EPFLLMLGDHVYKSDIKKKSCAQQVVEIYAQVHQSVVGLTTMPA-AIINKAGCIAG-VWQEFNS---ILELTQLAEKPN--------LDYAREHLRVEGMAEDEFL  222
  Npun_F1146      119  HAVFCAKDWVQD----EPFLLMLGDHVYASDIQKSCASQLLDVYEQVNQSVVSLTTTPA-EILTTAGCVTG-VWQELNS---ILEVTQLYEKPT--------IEYAQQHLHVEGMAENEFL  222
  NIES39_K04100   122  HAVFCAAPWVHD----EPFMLLLGDHVYASDLEVNCARQLLEVYEKVQQSVVGVRITPG-DRIQHYGCVAGTPVKDSDY--LFDLTEIYEKPT--------LNYARTNMRVPGLEWDRFL  226
  sll0899          81  HAIQQLLPCLQDY-QGDLLVLNGDAPLLRSET--------LERLLATHQRHGNAATLLTAHLPDP----KGYGRVFCNGD--NRVTQIVEDRDCNAAQR---------------QNHR  168
  alr3921          80  HAIQQLLPHLEGY-RGDLLVLNGDVPLLRTQT--------LEQLLQTHQTNQNAATILTSHLPNP----KGYGRVFCNGN--NIVQQIVEDKDCSPAQR---------------QNHR  167
  Npun_R1080      107  GGRLKRIREHLGN----ETFCFTYGDGVSNIN--------ITELVKFHKEQNTLGTLTAVQPAGRFGAISLG--------YEQTKITSFREKPE-----------------GDGAW  185
  alr2825         107  GGRLKRVREHLGN----DTFCFTYGDGVCDIN--------ITELIKFHREQKSLATLTAVQPAGRFGAISLG--------YEQTKITSFREKPE-----------------GDGAW  185
  slr0983         107  GGRLKRIKTFIEND--NAFCFTYGDGLANIN--------IQELIAFHQSHGRLATVTAVVPPGRGYGALKL----------ENSKVFGFTEKPP-----------------GDGGL  184
  alr4491          88  HAVTVARPFLKD----SPFVMYLGDNLIQQG-------DLSNFLQKFIQEQPDALILLR-EVVNPS--AFG--VAKVDD--TGRVLQLVEKPK-------VPPSN------------L  168
  sll0207         116  HAVKTAADFLQD----SPFVMYLGDNLIQD--------HLEQFLAHFQAKSLDSLILLR-RVSNPS--AFG--VATVND--QGKVLALVEKPE--------HPPSN------------L  194
  tll0457         121  EAFILGREFLQG----EPVCLTLGDNLLYGH-------DLSEKLQRAAQLTAGAMIFGY-RVANPQ--QYG--VLEFDA--SGRVLGIEEKPA--------VPKSN------------Y  200
  slr1176         112  DAVRQYLWLFREW--DVDEYLILSGDHLYRMD--------YAQFVKRHRETNADITLSVV-PVDDRKAPELG--LMKIDA--QGRITDFSEKPQGEALRAMQVDTSVLGLSAEKAKLNPYI  217
  all4645         102  DAVRQYLWMLQEW--DVDEFLILSGDHLYRMD--------YRLFIQRHRETNADITLSVI-PIDDRRASDFG--LMKIDN--SGRVIDFSEKPKGEALTKMRVDTTVLGLTPEQAASQPYI  207
  tlr1287         110  DAVRQYLWLADW-DVDEYLILSGDHLYRMD--------YRLFVQRHRDTNADITLTVV-PVEEKAASGFG--LLKVDG--TGRVTDFREKPTGDALRDMRVDTTRYGLTIEEAHRKPYI  215
  tlr0018          96  VAALYVTEVLAS---DALLLVLPADHFIQDVAHFQWGLQRGFS--AAAKDWLVTFGIAPTSPHTGYGYIRRGESLSDPD----TYRVAEFVEKPD-LATAEAYLAAGNYLWNSGMFLFRAQS  207
  sll1370         111  WASLEIAQQYGD---DAIIGFFPADHWIKDQCAYEQTLKAAIAYAQEQDAIVTLGIKPHGPATGYGYIEQGTLEREIDGLPVYQVTRFTEKPD-RATAQTFVDSGKFSWNSGMFIFRAGV  226
  slr0007          94  GGFVNAVNHHGFRELTPAWLVLNGDSLIVTD--------YRVLLAELEDDSVDGVILG--VHVPDASRFGSLKVNSQG-----ELLQFAEKQAG---------------AGV  175
  sll1496          88  GCVKNIEEL-----LDDTFLVISGDSITDFN--------LTQAIAFHKQKQAKATLILT-KVPNP--LEFGVVITDENG---RVQRFLEKPS-----------------TSEVFSDT  168
  SYNPCC7002_A0950 88  GCVKNVEEL------LTETFVVISGDSITDFD--------LAAAIAFHREKGSKATLVLT-RVPNP--VEFGVVITEENG---QISRFLEKPS-----------------TSEIFSDT  168
  tlr0436          88  GSVKNIVNL-----LTDPFLVVSGDSITDVD--------LTDALRFHQQHGAPVTLILA-RVPQP--KEFGIVFTDSDG----RVRRFLEKPS-----------------AAEVFTDT  168
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Sugar-binding site

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  sll1558          182  INTGIYIFEPEVIDYIPSGQ---------------EYDLGGDLFPKLVDSGLPFYAVNMDFEWVDIGKVPDYWQAIRGV---  245
  tlr0611          182  INTGIYIFEPEVIDYIPSNQ---------------EYDIGSQLFPKLVEMGAPYFGLAMDFEWIDIGKVPDYWQAVRGV---  245
  SYNPCC7002_A1389 182  INTGIYIFEPEVIDFIPPNC---------------EFDIGGQLFPKLVANAPFYALDMDFEWVDIGKVPDYWQAIQSVLQ-  247
  all3274          223  CVFGLYVLTPKIFDFLADSINQNLRDRG---------EFQLTNCLDKLCQA--EGMTGYVVKGKCFDTGLPN-----------  283
  Npun_F1146       223  CIFGLYLLSPKIFDFLAEHINQNLRERG---------EF-----------------------  252
  NIES39_K04100    227  TIFGLYIIEPKIFDFLADNIRRDVREKG---------EYQLTSCWQTLLEK--EQITGYLVQGRCFDTGLPETYF---------  290
  sll0899          169  VNGGIYCFHWPRLAAALPKLSANN----------DQKEYYLTEVVDYLDQVMAVDVDDYLEINGINDRKQLAAANDILQNRI--  239
  alr3921          168  INAGIYCFRWENLAQVLPHLEANN----------AQKEYYLTDAVTQVGQVMAVDVEDYQEILGINDRLQLATAYEILQRRVKE  241
  Npun_R1080       186  INGGYFVLEPEVINLIADD-----------------------------------------  204
  alr2825          186  INGGYFILEPEVIDLIADDA-----------------------------------------  205
  slr0983          185  INGGFFVLSPRCLDFIG-------------------------------------------  201
  alr4491          169  ALVGVYFFSPIIHDSIAR---IQPSSRG---------ELEITDAIQRLIDD-KRQVLACNLYGWWLDTGKKDDLLEANRLI---  236
  sll0207          195  ALVGLYFFAPTIHQAIAN---IEPSARG---------ELEITDAIQYLISH-DYRVESLQLKGWWLDTGKKDDLLAANQII---  263
  tll0457          201  AVPGIYFYDHQVCDLAAQ----LQPSARG---------ELEITDLNRLYLEKGQLRVELLGRGYAWMLDTGTHDLLQQASSFI---  270
  slr1176          218  ASMGIYVFKKEVLHNLLEKYEGATDFGK---------EIIPDSASDHNLQA--------YLFDDYWEDIGTIEAFYEANLALTK-  284
  all4645          208  ASMGIYVFKKDVLIKLLKEALERTDFGK---------EIIPDAAKDHNVQA-------YLFDDYWEDIGTIEAFYNANLALTQQ  275
  tlr1287          216  ASMGIYVFKRQVLIDLLQQMADATDFGK---------EIIPAAARSHLVQT-------YLFNGYWEDIGTIGSFYEANLALTQQ  283
  tlr0018          208  FLAELAQQQPQILDYCRLALRQGREDLDFIRLGKEAFDQCPNLSVDYGLMEKTQKAAVVPLDCGWSDVGSWSSLWEILPKD---  288
  sll1370          227  VLRELDRHAPELLATLRAKGAAGYPALE-------------KKSIDYVLMEKTQLAAVLPANFGWDDLGNWANELRLFPE-----  293
  slr0007          176  INSGVYLLGDRLLARFPAHR-----------------PLSFEYDVFPTLLAQGAKIKVHAVEAPFLDIGTPETLAQAGEFIQ---  240
  sll1496          169  VNTGTYILEPEVLAYLPPKE-----------------EMDFSKDLFPLLLENDEPMYGFVADGYWCDVGNLDAYREAQYDAL---  233
  SYNPCC7002_A0950 169  VNTGTYILEPEVLKYLPENE-----------------ECDFSKDLFPLLIDRGEPMYGYIADGYWCDVGHLDAYRKAQYD----  231
  tlr0436          169  VNTGIYILNPTVMDYLNSGI-----------------ERDFSRDLFPLLLQADVPMYGYITDAYWCDVGSLQTYQQVQQD----  231
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FIG 2 Sequence alignment of the NTP transferase domain of the nucleotidyl transferases shown in Fig. 1. The highly conserved residues of the G-X-G-T-R motif are indicated in green. The residues that correspond to position 8 in Sll1558 are highlighted in red (Ala) and orange (Gly). The residues that are aligned with Gly-93 in Sll1558 are in blue. Gly-93 in Sll1558 corresponds to Gly-117 in the GalU-type UDP-Glc PPase from *Corynebacterium glutamicum*, as shown in Fig. S2 in the supplemental material.

and CugP-type enzyme sequences diverged early on from each other or from other related enzymes during the evolution of the nucleotidyl transferase superfamily. It is thus possible that CugP-type UDP-Glc PPases evolved independently within this super-family. When a BLAST search was performed for the whole NCBI database, the GalU-type PPases were found to be widely distributed throughout the bacterial kingdom, whereas the CugP-type could be found in only very limited species other than cyanobacteria. These limited species included certain, but not all, of the *Beta-* and *Gammaproteobacteria*. The sequence similarity among the cyanobacterial and proteobacterial CugP-type enzymes is much greater (E value, 1e−122 to 1e−147) than that of their GalU-type counterparts (E value, 1e−28 to 1e−32). Notably, the proteobacterial sequences have clearly diverged from the inside of the cyanobacterial CugP cluster (e.g., *Yersinia intermedia*, *Methylotenera mobilis*, and *Vibrio ichthyoenteri*) (Fig. 1), suggesting that the proteobacterial CugP-type enzymes have been acquired by horizontal gene transfer from cyanobacteria rather recently. Conversely, although the GalU-type enzymes were, in general, widely distributed in *Bacteria*, they were found in only some filamentous cyanobacteria and may, therefore, have also been acquired through another horizontal transfer from bacteria (e.g., *Poribac-*
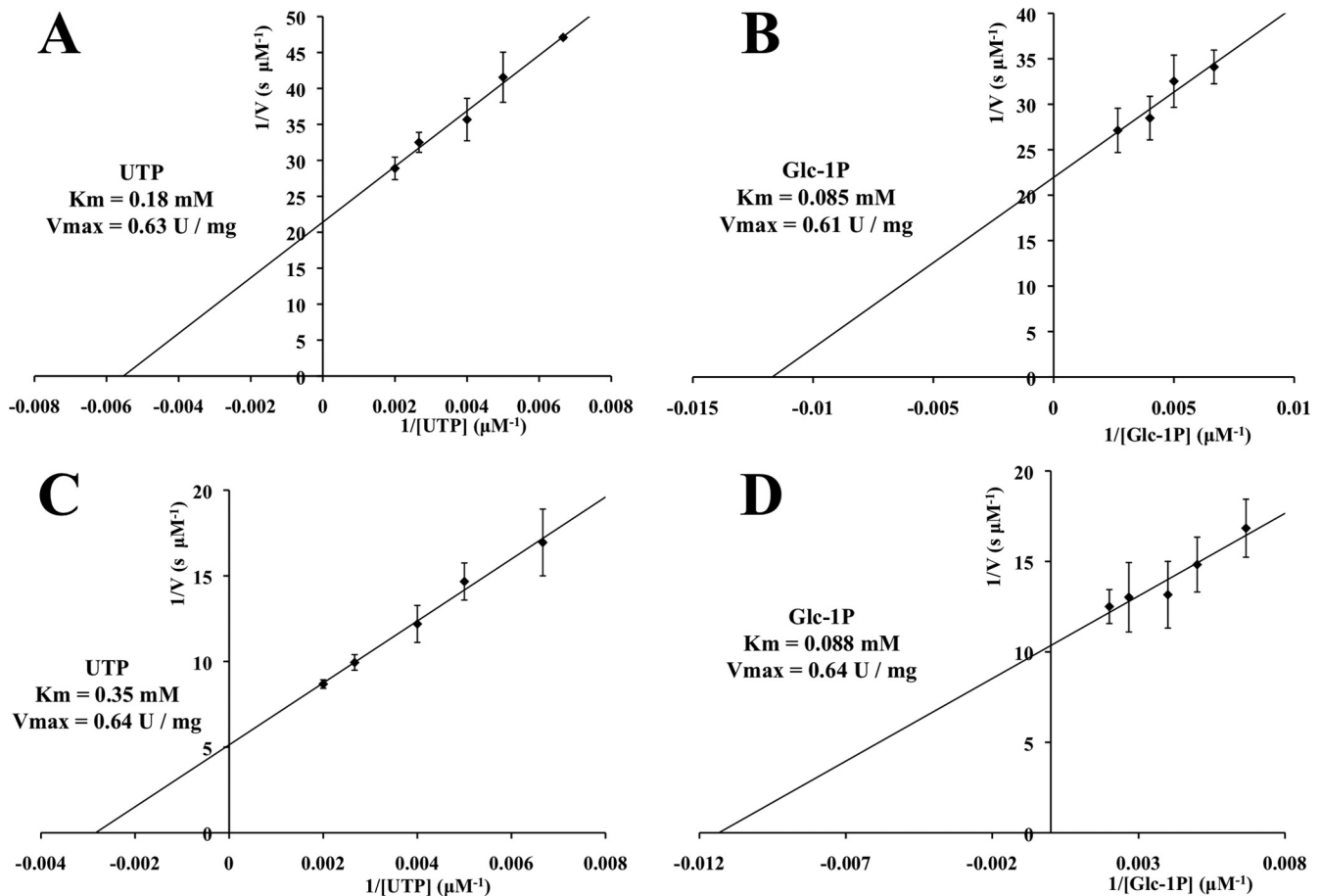
FIG 3 Lineweaver-Burk plots of the enzyme activity of Sll1558 (A and B) and A8G-Sll1558 (C and D). As a fixed substrate, 1 mM Glc-1P was included in the experiments for panels A and C, or 2 mM UTP was included in the experiments for panels B and D. Protein concentrations: 4.5 µg ml^{-1} (A and B), 18.0 µg ml^{-1} (C), and 9.0 µg ml^{-1} (D). The error bars indicate standard deviations (for A, B, and C, $n = 3$; for D, $n = 6$).

teria and *Anaerolinea thermophila*) to the limited species of cyanobacteria or may have been lost in many cyanobacterial lineages. We speculate that the GalU-type enzyme may supply UDP-Glc to certain glycosyltransferases specific for cell differentiation in those filamentous cyanobacteria.

Structural implications of the motif for substrate specificity. According to the crystal structure and sequence similarities, the overall structure of the whole catalytic domain seems to be conserved between GalU-type UDP-Glc PPase, CDP-Glc PPase, dTDP-Glc PPase, and other subfamilies of the nucleotidyl transferases. Because CugP-type UDP-Glc PPase is also included in this superfamily, we discuss structural implications of the motif for

TABLE 1 Kinetic parameters for Sll1558 and Sll1558(A8G)

Protein and substrate	V_{max} (U mg^{-1})	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})
Sll1558				
UTP	0.63	0.18	28	160
Glc-1P	0.61	0.085	27	320
Sll1558(A8G)				
UTP	0.64	0.35	29	83
Glc-1P	0.64	0.088	29	330

substrate specificities based on the known structures. These enzymes bind (d)NTP and sugar-1P sequentially in the substrate-binding pocket, which can be divided into a (d)NTP-binding site and a sugar-binding site (18, 31). In the former site, three highly conserved residues directly interact with the pyrimidine/purine base of (d)NTP by hydrogen bonds (Fig. 4; see also Fig. S2 in the supplemental material). However, these hydrogen bonds are mostly derived from the main-chain amide of an Ala or Gly residue, and, therefore, the specificity determination for (d)NTP has not clearly been elucidated. For example, the amide NH of Gly-117 appears to discriminate uracil of UTP in UDP-Glc PPase from cytosine of CTP (see Fig. S2) but is also conserved in other nucleotidyl transferases. The Ala residue that is highlighted in this study as a possible marker for the UTP-utilizing enzymes directly interacts by hydrogen bonding with the C-2 carbonyl of uracil, but very similar hydrogen bonding is found between the Gly-8 and C-2 carbonyl of cytosine in CDP-Glc PPase (see Fig. S2). Thus, the precise mechanism to determine the specificity for UTP still remains elusive. However, it must be mentioned that the spatial arrangement of the Gly residue (Gly-21/Gly-9/Gly-9 in GalU/CugP/CDP-Glc PPase) next to the mutated Ala position is markedly different, resulting in distinct hydrogen bonding to cytosine in CDP-Glc PPase or to ribose in GalU-type UDP-Glc PPase

FIG 4 A predicted model of substrate binding in Sll1558 (CugP-type UDP-Glc PPase). This model is based on the crystal structure of GalU-type UDP-Glc PPase from *Corynebacterium glutamicum* in complex with magnesium and UDP-Glc (2PA4) (18). Only a few key residues and substrates are shown (green, carbon; blue, nitrogen; red, oxygen; and orange, phosphorus). The broken lines denote hypothetical hydrogen bonds.

(Fig. 4; see also Fig. S2 in the supplemental material). This may be the reason why the K_m of Sll1558(A8G) for UTP increased slightly. Thus, it is still possible that the highlighted Ala residue, which is exclusively conserved in the UTP-utilizing transferases, is somehow involved in discrimination of the uracil of UTP as a substrate. To test this hypothesis, it will be necessary to study the substrate discrimination of the UTP-utilizing transferases by a thorough mutagenesis survey.

Role of CugP-type UDP-Glc PPase in cyanobacteria. To better understand the role of cyanobacterial CugP-type UDP-Glc PPases, we attempted to disrupt *sll1558* in the *Synechocystis* sp. PCC 6803 genome. However, we could not produce such a mutant because cyanobacteria typically contain multiple copies of their chromosomes, and, consequently, the mutated *Synechocystis* sp. PCC 6803 did not cleanly segregate, suggesting that the CugP-type UDP-Glc is essential for survival of *Synechocystis* (see Fig. S3 in the supplemental material). In this context, it must be mentioned that a galactolipid, monogalactosyldiacylglycerol (MGDG), which is essential in *Synechocystis*, is supplied from UDP-Glc via monoglucosyldiacylglycerol (32). The galactolipids MGDG and digalactosyldiacylglycerol are major constituents of the photosynthetic thylakoid membrane in cyanobacteria and in plant chloroplasts. They bind to the membrane-spanning photosynthetic complexes and thereby maintain them in a functional state (33). Hence, the role of UDP-Glc PPases in providing UDP-Glc must be essential for growth and survival in cyanobacteria.

As a preliminary survey, we compared this nonsegregated mutant with the wild type. The chlorophyll (Chl) content was lower in the mutant than in the wild type (mutant, 3.47 ± 0.51 μg Chl/10^8 cells; wild type, 4.22 ± 0.04 μg Chl/10^8 cells; $n = 3$). The whole photosynthetic activity from water to CO_2 was higher in the mutant than in the wild type (mutant, 263 ± 40 μmol O_2/mg Chl/h; wild type, 138 ± 7 μmol O_2/mg Chl/h; $n = 4$). These facts may reflect modified membrane biogenesis in the partially disrupted mutant (see Fig. S3 in the supplemental material). To fur-

ther characterize the phenotype, it is essential to achieve the complete segregation of the mutant.

Because galactolipids are specific to photosynthetic membranes, the unique CugP-type enzyme, found mainly in cyanobacteria, may be the result of an evolutionary event in oxygenic photosynthesis. Certain exopolysaccharides secreted by cyanobacteria, including *Synechocystis*, often contain glucose as the major constituent (21). However, it is unknown if they are essential for survival. Cellulose is selectively deposited in a thermophilic cyanobacterium, *Thermosynechococcus*, under conditions of low temperature and light (23). So, a CugP-type Tlr0611 appears to be the sole enzyme to provide UDP-Glc for biosynthesis of cellulose and might be induced under such conditions in this cyanobacterium. Regional distribution of GalU-type PPases in certain cyanobacteria may account for variation in the requirement of exopolysaccharides. Further functional studies of the CugP-type and GalU-type enzymes are needed to understand the role of UDP-Glc in cyanobacteria.

REFERENCES

1. **Romling U.** 2002. Molecular biology of cellulose production in bacteria. Res. Microbiol. **153**:205–212. http://dx.doi.org/10.1016/S0923-2508(02)01316-5.
2. **Alonso MD, Lomako J, Lomako WM, Whelan WJ.** 1995. A new look at the biogenesis of glycogen. FASEB. J. **9**:1126–1137.
3. **Holden HM, Rayment I, Thoden JB.** 2003. Structure and function of enzymes of the Leloir pathway for galactose metabolism. J. Biol. Chem. **278**:43885–43888. http://dx.doi.org/10.1074/jbc.R300025200.
4. **Roth J.** 1995. Compartmentation of glycoprotein biosynthesis, p 237–312. *In* Montreuil J, Vliegenthart JFG, Schachter H (ed), Glycoproteins. Elsevier Science, Amsterdam, The Netherlands.
5. **Verbert A.** 1995. From Glc3MangGlcNAc2-protein to Man5GlcNAc2-protein: transfer en bloc, p 145–152. *In* Montreuil J, Vliegenthart JFG, Shachter H (ed), Glycoproteins. Elsevier Science, Amsterdam, The Netherlands.
6. **Silbert JE, Sugumaran G.** 1995. Intracellular membranes in the synthesis, transport, and metabolism of proteoglycans. Biochim. Biophys. Acta **1241**:371–384. http://dx.doi.org/10.1016/0304-4157(95)00011-9.
7. **Sandhoff K, van Echten G, Schroder M, Schnabel D, Suzuki K.** 1992. Metabolism of glycolipids: the role of glycolipid-binding proteins in the function and pathobiochemistry of lysosomes. Biochem. Soc. Trans. **20**:695–699.
8. **Bosco M, Machtey M, Iglesias A, Aleanzi M.** 2009. UDPglucose pyrophosphorylase from *Xanthomonas* spp. Characterization of the enzyme kinetics, structure and inactivation related to oligomeric dissociation. Biochimie **91**:204–213. http://dx.doi.org/10.1016/j.biochi.2008.09.001.
9. **Ma Z, Fan H-j, Lu C-p.** 2011. Molecular cloning and analysis of the UDP-Glucose Pyrophosphorylase in *Streptococcus equi* subsp. *zooepidemicus*. Mol. Biol. Rep. **38**:2751–2760. http://dx.doi.org/10.1007/s11033-010-0420-8.
10. **Lai X, Wu J, Chen S, Zhang X, Wang H.** 2008. Expression, purification, and characterization of a functionally active *Mycobacterium tuberculosis* UDP-glucose pyrophosphorylase. Protein Expr. Purif. **61**:50–56. http://dx.doi.org/10.1016/j.pep.2008.05.015.
11. **Asencion Diez MD, Peiru S, Demonte AM, Gramajo H, Iglesias AA.** 2012. Characterization of recombinant UDP-and ADP-glucose pyrophosphorylases and glycogen synthase to elucidate glucose-1-phosphate partitioning into oligo- and polysaccharides in *Streptomyces coelicolor*. J. Bacteriol. **194**:1485–1493. http://dx.doi.org/10.1128/JB.06377-11.
12. **Mollerach M, Lopez R, Garcia E.** 1998. Characterization of the *galU* gene of *Streptococcus pneumoniae* encoding a uridine diphosphoglucose pyrophosphorylase: a gene essential for capsular polysaccharide biosynthesis. J. Exp. Med. **188**:2047–2056. http://dx.doi.org/10.1084/jem.188.11.2047.

13. **Flores-Diaz M, Alape-Giron A, Persson B, Pollesello P, Moos M, von Eichel-Streiber C, Thelestam M, Florin I.** 1997. Cellular UDP-glucose deficiency caused by a single point mutation in the UDP-glucose pyrophosphorylase gene. J. Biol. Chem. **272:**23784–23791. http://dx.doi.org/10.1074/jbc.272.38.23784.

14. **Mollerach M, Garcia E.** 2000. The *galU* gene of *Streptococcus pneumonia*e that codes for a UDP-glucose pyrophosphorylase is highly polymorphic and suitable for molecular typing and phylogenetic studies. Gene **260:**77–86. http://dx.doi.org/10.1016/S0378-1119(00)00468-6.

15. **Weissborn AC, Liu Q, Rumley MK, Kennedy EP.** 1994. UTP: alpha-D-glucose-1-phosphate uridylyltransferase of *Escherichia coli*: isolation and DNA sequence of the *galU* gene and purification of the enzyme. J. Bacteriol. **176:**2611–2618.

16. **Hossain SA, Tanizawa K, Kazuta Y, Fukui T.** 1994. Overproduction and characterization of recombinant UDP-glucose pyrophosphorylase from *Escherichia coli* K-12. J. Biochem. **115:**965–972.

17. **Thoden JB, Holden HM.** 2007. The molecular architecture of glucose-1-phosphate uridylyltransferase. Protein Sci. **16:**432–440. http://dx.doi.org/10.1110/ps.062626007.

18. **Thoden JB, Holden HM.** 2007. Active site geometry of glucose-1-phosphate uridylyltransferase. Protein Sci. **16:**1379–1388. http://dx.doi.org/10.1110/ps.072864707.

19. **Kim H, Choi J, Kim T, Lokanath NK, Ha SC, Suh SW, Hwang HY, Kim KK.** 2010. Structural basis for the reaction mechanism of UDP-glucose pyrophosphorylase. Mol. Cells **29:**397–405. http://dx.doi.org/10.1007/s10059-010-0047-6.

20. **Kawano Y, Sekine M, Ihara M.** 2013. Identification and characterization of UDP-glucose pyrophosphorylase in cyanobacteria *Anabaena* sp. PCC 7120. J. Biosci. Bioeng. **117:**531–538. http://dx.doi.org/10.1016/j.jbiosc.2013.10.015.

21. **De Philippis R, Vincenzini M.** 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol. Rev. **22:**151–175. http://dx.doi.org/10.1111/j.1574-6976.1998.tb00365.x.

22. **Nobles DR, Romanovicz DK, Brown RM.** 2001. Cellulose in cyanobacteria. Origin of vascular plant cellulose synthase? Plant Physiol. **127:**529–542. http://dx.doi.org/10.1104/pp.010557.

23. **Kawano Y, Saotome T, Ochiai Y, Katayama M, Narikawa R, Ikeuchi M.** 2011. Cellulose accumulation and a cellulose synthase gene are responsible for cell aggregation in the cyanobacterium *Thermosynechococcus vulcanus* RKN. Plant Cell Physiol. **52:**957–966. http://dx.doi.org/10.1093/pcp/pcr047.

24. **Nakamura Y, Kaneko T, Sato S, Ikeuchi M, Katoh H, Sasamoto S, Watanabe A, Iriguchi M, Kawashima K, Kimura T, Kishida Y, Kiyokawa C, Kohara M, Matsumoto M, Matsuno A, Nakazaki N, Shimpo S, Sugimoto M, Takeuchi C, Yamada M, Tabata S.** 2002. Complete genome structure of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1. DNA Res. **9:**135–148. http://dx.doi.org/10.1093/dnares/9.4.135.

25. **Kaneko T, Nakamura Y, Wolk CP, Kuritz T, Sasamoto S, Watanabe A, Iriguchi M, Ishikawa A, Kawashima K, Kimura T.** 2001. Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. DNA Res. **8:**205–213. http://dx.doi.org/10.1093/dnares/8.5.205.

26. **Larkin M, Blackshields G, Brown N, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R.** 2007. Clustal W and Clustal X version 2.0. Bioinformatics **23:**2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404.

27. **Koropatkin NM, Holden HM.** 2004. Molecular structure of alpha-D-glucose-1-phosphate cytidylyltransferase from *Salmonella typhi*. J. Biol. Chem. **279:**44023–44029. http://dx.doi.org/10.1074/jbc.M407755200.

28. **Zuccotti S, Zanardi D, Rosano C, Sturla L, Tonetti M, Bolognesi M.** 2001. Kinetic and crystallographic analyses support a sequential-ordered bi bi catalytic mechanism for *Escherichia coli* glucose-1-phosphate thymidylyltransferase. J. Mol. Biol. **313:**831–843. http://dx.doi.org/10.1006/jmbi.2001.5073.

29. **Midorikawa T, Matsumoto K, Narikawa R, Ikeuchi M.** 2009. An Rrf2-type transcriptional regulator is required for expression of *psaAB* genes in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiol. **151:**882–892. http://dx.doi.org/10.1104/pp.109.141390.

30. **Hihara Y, Sonoike K, Ikeuchi M.** 1998. A novel gene, pmgA, specifically regulates photosystem stoichiometry in the cyanobacterium *Synechocystis* species PCC 6803 in response to high light. Plant Physiol. **117:**1205–1216. http://dx.doi.org/10.1104/pp.117.4.1205.

31. **Silva E, Marques AR, Fialho AM, Granja AT, Sá-Correia I.** 2005. Proteins encoded by *Sphingomonas elodea* ATCC 31461 *rmlA* and *ugpG* genes, involved in gellan gum biosynthesis, exhibit both dTDP-and UDP-glucose pyrophosphorylase activities. Appl. Environ. Microbiol. **71:**4703–4712. http://dx.doi.org/10.1128/AEM.71.8.4703-4712.2005.

32. **Awai K, Kakimoto T, Awai C, Kaneko T, Nakamura Y, Takamiya K-i, Wada H, Ohta H.** 2006. Comparative genomic analysis revealed a gene for monoglucosyldiacylglycerol synthase, an enzyme for photosynthetic membrane lipid synthesis in cyanobacteria. Plant Physiol. **141:**1120–1127. http://dx.doi.org/10.1104/pp.106.082859.

33. **Sakurai I, Mizusawa N, Wada H, Sato N.** 2007. Digalactosyldiacylglycerol is required for stabilization of the oxygen-evolving complex in photosystem II. Plant Physiol. **145:**1361–1370. http://dx.doi.org/10.1104/pp.107.106781.