**Streptococcus pneumoniae** Serotype 11D Has a Bispecific Glycosyltransferase and Expresses Two Different Capsular Polysaccharide Repeating Units*

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**Background:** *Streptococcus pneumoniae* serotype 11D capsular polysaccharide (CPS) structure is unknown.

**Results:** Serotype 11D PS contains two different repeating units; one has αGlcNAc, and the other contains αGlc.

**Conclusion:** The 11D CPS is due to the bispecific glycosyltransferase WcrL. Based on codon 112, WcrL can transfer αGlc, αGlcNAc, or both.

**Significance:** Minimal genetic changes can make bacteria produce different polysaccharides.

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‡ The abbreviations used are: PS, polysaccharide; pneumococcus, S. pneumoniae; CPS, capsular polysaccharide; PGN, peptidoglycan; MurNAc, N-acetylmuramic acid; Gro, glycerol; do, de-O-acetylated; c112, codon 112 of wcrL; FCSEA, flow cytometric serotyping analysis; RU, repeating unit; SSI, Statens Serum Institute; HSQC, heteronuclear single quantum correlation; HMBC, heteronuclear multiple-bond correlation.
in other essential cellular functions (e.g. peptidoglycan synthesis, etc.), cps gene mutations that disrupt the completion of the synthesis cycle are theoretically lethal to pneumococci. Thus, the synthetic cycle may be largely inflexible to changes that affect later steps of the pathway. This stringency limits capsule type diversity to the finite number of cps loci encoding successful biosynthetic machinery.

Serogroup 11 is among the most extensively characterized pneumococcal serogroups. The six antigenically distinct serotypes in serogroup 11 (i.e. serotypes 11A–11F) have highly homologous cps loci (5, 6). To investigate the molecular sources of antigenic diversity in this serogroup, we previously examined the structures of serotype 11A, 11B, 11C, 11E, and 11F CPSs (7). Their CPS structures share a similar tetrasaccharide RU but differ in their acetyl and polyalcohol content. These structural modifications can be correlated to their antigenic properties in conventional serotyping assays. For instance, serotypes 11A and 11E do not react with serotyping factor serum 11b, whereas serotypes 11B, 11C, and 11F do (7). Because α-N-acetylglucosamine (αGlcNAc), as the fourth residue of the tetrasaccharide RU, is the only carbohydrate feature shared by serotype 11B, 11C, and 11F CPS but is not present in 11A and 11E CPS, factor serum 11b reactivity is strongly linked to the presence of αGlcNAc (7).

The structure of serotype 11D CPS has not been reported. Because of its reactivity with factor serum 11b, we hypothesized that serotype 11D CPS also contains GlcNAc. However, the published serotype 11D cps locus (5) differs from previously published 11A cps sequences (2, 8, 9) by only one base pair in the cps gene wcrL. Whether change of this codon (named codon 112, for its position in the gene) could mediate antigenic differences between the serotypes is unclear. Here, we demonstrate that serotype 11D CPS surprisingly contains two distinct RUs. Furthermore, we show that alteration of codon 112 in wcrL dictates expression of serotype 11A, serotype 11D, or a novel capsule serotype, 11X3.

**EXPERIMENTAL PROCEDURES**

**Capsular PSs, Bacterial Strains, and Culture Conditions—** Capsular PSs from serotypes 11A, 11D, and 11F were obtained from ATCC (Manassas, VA) or Staten Serum Institute (SSI, Copenhagen, Denmark). Reference strains SSISP 11A/2, SSISP 11D/1, and SSISP 11F/2, which express serotypes 11A, 11D, and 11F, respectively, were obtained from SSI. The previously characterized serotype 11A clinical isolate MNZ272 and the non-encapsulated strain TIGR-JS were from our collection (2, 7, 10). All strains were derived from a single colony. Unless otherwise noted, bacterial strains were grown on tryptic soy agar plates supplemented with 5% sheep blood or Todd Hewitt medium (BD Biosciences) plus 0.5% yeast extract (THY) broth. All cultures were incubated at 37 °C in 5% CO₂. THY cultures were harvested at an A₁₀₀₀ of 0.4–0.6, and aliquots in 15% glycerol (Gro) were kept at −80 °C until needed.

**Antibodies and Flow Cytometric Serotyping Analysis (FCSA)—** Mouse hybridomas expressing monoclonal antibodies (mAbs) labeled Hyp11AAM1 and Hyp11AM7 were derived from mice immunized with serotype 11A CPS, as described (11). Rabbit polyclonal factor sera 11b, 11c, and 11g were purchased from SSI. Flow cytometry was used to detect capsule epitope expression on bacteria using polyclonal antisera or mAb, as described (7). Briefly, frozen bacterial stocks were thawed and washed, and bacterial density was adjusted to A₆₃₀ = 0.2 before diluting 100-fold. Test strains were then incubated with factor sera at a 1:1000 dilution or hybridoma supernatants containing mAb at a 1:100 dilution for 30 min at 4 °C. After washing, bound antibodies were detected with goat anti-rabbit Ig fluorescein isothiocyanate-conjugated antibodies or with rabbit anti-mouse IgM phycoerythrin-Cy7-conjugated antibodies (Southern Biotechnology Associates, Inc., Birmingham, AL). Stained bacteria were analyzed using a FACSCalibur (BD Biosciences), and data analysis was done with FCS Express version 3.0 (De Novo Software, Los Angeles, CA). Bacteria stained with the secondary antibody alone were used as negative controls.

**Polysaccharide Purification—** Capsular PS was purified from pneumococcal strains MNZ272 (denoted 11A PS), SSISP 11F/2 (denoted 11F PS), and SSISP 11D/1 (denoted 11D PS) and recombinant strains MBO129, MBO128, and MBO130 (see below), as described (7). Strains were grown overnight at 37 °C in a chemically defined medium (12) from JRH Biosciences (Lenexa, KS) supplemented with choline chloride (1 g/liter), sodium bicarbonate (2.5 g/liter), and cysteine-HCl (0.73 g/liter). Deoxycholic acid (1 g/liter) was then added to the culture, and the pH was adjusted to 7.0. The culture was incubated at 37 °C for 20 min to induce lysis. The pH of the lysate was adjusted to 6.0, and the lysate was centrifuged at 18,000 × g for 30 min to remove cell debris and precipitate the deoxycholic acid. The supernatant was collected and incubated in 30%, 50%, and then 75% ethanol, each step at 4 °C for 2 days. Between steps, lysates were centrifuged to remove precipitate. After the final incubation at 75% ethanol, the supernatant was decanted, and CPS precipitates were dissolved in 0.2 M NaCl and then desalted by dialysis against water. The solution containing the CPS was loaded onto a column (45 ml of DEAE-Sepharose, GE Healthcare), and the CPS was eluted with a linear NaCl gradient from 0 to 1 M. Fractions containing CPS detected by a multibead inhibition assay (13) were pooled, desalted, lyophilized, and redissolved in 10 mM Tris-HCl (pH 7.4) buffer containing 100 mM NaCl, to a concentration of ∼20 mg/ml. The sample was separated by a size exclusion chromatography column (120 ml of Sephacryl S-300 HR, Amersham Biosciences). High molecular weight fractions containing CPS were pooled, desalted, lyophilized, and stored at −20 °C until analyzed.

**Monosaccharide Analysis—** A 40-μg sample of lyophilized CPS was subjected to methanolysis in 3 N methanolic HCl at 80 °C for 16 h (19). Following evaporation of the methanolic HCl under vacuum, the residue was washed and dried several times with methanol. Re-N-acetylation of amino sugars in the samples was achieved by dissolving in 200 μl of methanol and adding 20 μl of pyridine and 20 μl of acetic anhydride. After 30 min at room temperature, the samples were dried under vacuum, dissolved in 100 μl of methanol, and transferred to glass conical inserts in sample vials. After evaporation to dryness, samples were trimethylsilylated by dissolving in 100 μl of Tri-Sil (Pierce) under argon for 20 min at 80 °C.

The reaction products were analyzed on a gas chromatograph/mass spectrometer (Varian 4000, Agilent Technologies, VOLUME 288 • NUMBER 30 • JULY 26, 2013 21946 JOURNAL OF BIOLOGICAL CHEMISTRY
MNZ272 was generated by transforming TIGR-JS, which contains a Janus cassette in place of a \( cps \) locus, with lysate from strain MNZ272 and by streptomycin (300 µg/ml) selection as described (10). Two additional strains (MBO128 and MBO130) putatively expressing \( WcrL \) variants were derived using site-directed mutagenesis of MBO129 (Fig. 4 and Table 2). The entire \( cps \) loci of MBO128-130 were sequenced (GenBank™ accession no. JX102570–JX102572) and were found to have \( wcrL \) genes encoding Ser-112, Asn-112, and Ala-112 for MBO128, MBO129, and MBO130, respectively.

**RESULTS**

**MNZ272 Has Serologic Properties of Serotype 11A**—The \( cps \) locus of strain MNZ272 (GenBank™ accession number GU074952), the isolate used to elucidate the CPS structure and antigenicity of serotype 11A (7), is genetically almost identical to the reported serotype 11D \( cps \) locus (GenBank™ accession number CR931656) but is notably different from the reported \( cps \) locus of a serotype 11A reference strain (GenBank™ accession number CR939653) (2, 5). This discrepancy raised concerns that strain MNZ272 may actually express CPS of serotype 11D. Therefore, we reinvestigated the ability of factor serum 11b, which reacts with all serogroup 11 serotypes except serotypes 11A and 11E, to react with strain MNZ272 and reference strains expressing serotypes 11A (SSISP 11A/2), 11D (SSISP 11D/1), and 11F (SSISP 11F/2). Factor serum 11b induced slide agglutination of strains SSISP 11D/1 and SSISP 11F/2 but not SSISP 11A/2 and MNZ272 (data not shown). FCSA also showed that antibodies in factor serum 11c, which reacts only with serotypes 11A, 11C, 11D, and 11E (7), bound to strains SSISP 11A/2, SSISP 11D/1, and MNZ272 but not SSISP 11F/2 (data not shown). Despite these
serologic differences, all four strains expressed similar amounts of capsule because they were equally stained by Hyp11AM1 (Fig. 1), an mAb that reacts with serotype 11A, 11D, 11E, and 11F CPS (7). Thus, the serotype of MNZ272 is 11A, not 11D, although its cps locus is almost identical to the published 11A cps locus.

**Serotype 11D CPS Contains GlcNAc**—Because serotype 11D reacts with factor serum 11b, we hypothesized serotype 11D capsule contains N-acetylglucosamine (GlcNAc). To examine this possibility, we used gas chromatography and mass spectroscopy (GC/MS) to determine the carbohydrate content of CPS purified from strains SSISP 11D/1, SSISP 11F/2, and MNZ272 (herein referred to as 11D CPS, 11F CPS, and 11A CPS, respectively) and serotype 11A CPS purchased from ATCC. Because all characterized serogroup 11 CPSs contain 2 mol of galactose (Gal) per RU (7), the GC/MS spectra of CPS demonstrated an additional weak anomeric signal at 4.96/100.7 ppm, which was identified to be H1 of the glycosidic backbone of the CPS sample solution. Thus, αGlcNAc is integral to the glycosidic backbone of 11D PS.

In addition to identifying the H1 and H2 signals of GlcNAc, standard homonuclear and heteronuclear experiments were performed to assign all resonance peaks in do 11D CPS to Gro and 4 carbohydrate residues: αGlc, αGal, βGlc, and βGal (Table 1). These studies also assigned a small but distinct peak at 4.55/99.1 ppm that is present in the dO 11D PS spectrum but not the dO 11A PS spectrum (Fig. 3C). A diffusion-ordered spectroscopy experiment (16) revealed no evidence of free αGlcNAc in the CPS samples tested. Thus, αGlcNAc is integral to the glycosidic backbone of 11D PS.
(4.07/79.12) did not associate with any other peaks of the αGlcNAc in the dO 11D PS ¹H-¹³C HMBC spectrum (data not shown). Thus, the 11D CPS glycosidic backbone contains a αGlcNAc(1→4)αGal linkage similar to 11F CPS (7). In addition, the dO 11D ¹H-¹³C HSQC spectrum also contains strong ¹H signals at 4.98/100.70 and 4.13/79.89 ppm characteristic of the
**WcrL Mediates Pneumococcal Capsular PS Diversity**

### TABLE 1

| Residue | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | C-6′ |
|---------|-----|-----|-----|-----|-----|-----|------|
| βGlc    | 4.56/103.13* | 3.39/73.67 | 3.60/75.46 | 3.67/79.50 | 3.67/75.07 | 3.98/61.01 | 3.81 |
| βGlc (variant) | 4.55/103.28 | ND | ND | ND | ND | ND | ND |
| βGal    | 4.51/103.56 | 3.68/70.33 | 3.77/78.57 | 4.17/65.83 | 3.72/75.75 | 3.82/61.63 | 3.78 |
| αGlc    | 4.98/100.70 | 3.64/72.82 | 3.91/72.82 | 4.11/75.07 | 4.33/70.80 | 4.18/68.99 | 3.88 |
| αGal    | 5.17/99.76 | 3.91/69.16 | 4.03/69.78 | 4.13/79.89 | 4.26/71.81 | 3.85/60.93 | 3.85 |
| αGal (variant) | ND | ND | ND | ND | ND | ND | ND |
| αGlcNAc | 4.96/99.11 | 4.02/54.47 | ND | ND | ND | ND | ND |
| Glycero | 3.68/62.95 | 3.91/71.60 | 3.98/67.50 | 3.61/62.95 | 4.05/67.50 | ND | ND |

* Chemical shifts (1H/13C) are in ppm and are referenced to 4,4-dimethyl-4-silapentane-1-sulfonic acid-d4 and DMSO-d6.

**αGlc H1 and αGal H4, respectively, of the βGlc(1→4)αGal linkage characteristic of 11A CPS** (14). These observations led to the model of 11D CPS with two different RUs (Fig. 3B): the major RU contains βGlc as the fourth glycosyl residue, whereas the minor RU contains αGlcNAc. This model is also consistent with the presence of the H1 of variant βGlc (that theoretically would be 1→6-linked to the αGlcNAc in the adjacent RU) in the 11D CPS spectrum (Table 1).

This structural model of 11D CPS predicts the presence of three distinct phosphate bonds (i.e., βGlc-4P, αGlcNAc-4P, and Gro-3P). To confirm the model, we examined their presence in native 11D CPS with 31P HMBC. The 31P HMBC is ideally suited for identifying long range correlations in PSs (17). As expected, the 11D 31P CPS spectrum (Fig. 3C) contained a 31P signal at 4.12/−0.15 ppm and a diastereotopic signal at 3.98,4.045/−0.13 ppm, assigned to αGlc H4 and Gro H3, respectively (14). The spectrum contains a signal at 4.18 ppm, which is assigned to Glc H6. However, overlays of the 31P HMBC spectra of 11A CPS reveal no serotype differences in the αGal H6 and βGal H5 proton signals, and these residues of 11D CPS have no geminal or vicinal phosphate substitutions.

To investigate if our findings are not limited to our own bacterial strains, we obtained and de-O-acetylated 11A, 11D, and 11F CPS from a commercial source and examined the anomeric region of their 1H NMR spectra. As shown in Fig. 3D, the 11A spectrum contains anomeric peaks corresponding to αGlc and the 11F spectrum contains an αGlcNAc peak. In contrast, the 11D CPS spectrum shows both αGlc and αGlcNAc peaks. Integration of 1H NMR peaks arising from αGlc and αGlcNAc calculated that ~75% of the 11D RUs contained αGlc and ~25% contained αGlcNAc, consistent with observations made in the GC/MS spectra of 11D CPS. We concluded that the 11D CPS glycosidic backbone is a heteropolymer, in contrast to the homopolymer backbones of 11A and 11F PSs.

Polymorphism at Codon 112 (c112) of wcrL Is Sufficient for Serotype Switching—Along with previous research (7), our structural findings support that the identity of the fourth glycosyl residue in the CPS RU correlates with factor serum 11b reactivity. To examine the molecular basis of serum 11b reactivity, we performed a comparative analysis of serogroup 11 wcrL, which putatively encodes the glycosyltransferase that mediates the addition of the fourth glycosyl residue (18). Alignment of deduced WcrL amino acid sequences revealed a correlation between c112 and the identity of the fourth glycosyl residue (Fig. 5A). Namely, wcrL alleles from serotypes that contain αGlc as the fourth glycosyl residue (11A and 11E) encode Asn-112, wcrL alleles from serotypes that contain αGlcNAc (11B, 11C, and 11F) encode Ala-112, and the wcrL gene from serotype 11D, which contains both αGlc and αGlcNAc, encodes Ser-112.

To confirm that c112 of wcrL mediates the identity of the fourth glycosyl residue, we performed site-directed mutagenesis to create three strains that are isogenic except for c112 of WcrL (Fig. 4 and Table 2). Strains MBO128, MBO129, and MBO130 contain wcrL alleles that encode Ser-112, Asn-112, and Ala-112, respectively. According to FCSA, Hyp11AM1 mAb comparably bound all three strains, revealing that all isogenic strains express similar levels of capsule (Fig. 5B). Despite this, factor serum 11b antibodies comparably bound strains MBO128 and MBO130 but did not bind strain MBO129 (Fig. 5B). In contrast, another mAb specific for serogroup 11 epitopes, Hyp11AM7, bound MBO128 and MBO129 but not MBO130. We concluded that the three strains are serologically distinct and that the polymorphism at c112 is sufficient to mediate serotype switching among three different serotypes.

N112A Mutation Results in Exclusive Addition of αGlcNAc to the Serogroup 11 Glycosidic Backbone—Factor serum 11b reactivity provides only indirect evidence for the presence of αGlcNAc in the RU. To obtain direct evidence, we performed GC/MS analysis of CPS purified from strains MBO128, MBO129, and MBO130 (Fig. 6, A—D). c112 of wcrL encodes Ala in both MBO130 and SSI 11F. The GC/MS spectra spanning 7.5–22.5 min of elution time were comparable for 11F CPS and MBO130 CPS (Fig. 6, A and B). Both spectra contained strong, GlcNAc-associated signals eluting between 15.5 and 16.5 min. In contrast, the spectrum for MBO129 CPS contained prominent Gal and Glc signals with no signals between 15.5 and 16.5 min (Fig. 6C). This spectrum was indistinguishable from the 11A CPS spectrum (Fig. 2). The spectrum for MBO128 (Fig. 6D), whose wcrL c112 encodes Ser as in SSIP 11D/1, was similar to that of 11D CPS (Fig. 2), including a small GlcNAc-associated signal (identified with an arrow). Taken together, these findings indicate that MBO130 CPS contains only αGlcNAc.

**DISCUSSION**

We have elucidated the structure of 11D CPS and shown it to be a novel CPS containing two different RUs; about 25% of its RUs have αGlcNAc as the fourth residue, whereas about 75% have αGlc (Fig. 4C). The presence of αGlcNAc in the 11D CPS can readily explain the reactivity of serotype 11D with factor...
serum 11b, which has been found to react with other serogroup 11 serotypes containing GlcNAc (7). However, factor serum 11b reacts equally well with 11D and 11F, although GlcNAc RUs account for only about 25% of the total RUs of serotype 11D CPS (Fig. 4C). This similar reactivity may occur because small amounts of GlcNAc may significantly alter conformation of 11D CPS. For instance, a minor modification of type III CPS of Group B streptococcus has been shown to change its antigenicity significantly (20). Alternatively, GlcNAc RUs could be more common at the terminus of a CPS chain, and factor serum 11b may preferentially react with terminal GlcNAc. 11D CPS is predicted to be formed by linking the first (reducing end) residue of the growing chain to the fourth (non-reducing end) residue of a single RU by the polymerase (Wzy) (3). If Wzy has a higher affinity for RUs with Glc rather than GlcNAc in the 4th position, the 11D CPS chain may terminate more often with GlcNAc RU.

We also show by site-directed mutation that a non-synonymous change of one single nucleotide (c112 of WcrL) of 18 kb in the cps is sufficient to convert serotype 11A to serotype 11D. c112 of wcrL encodes asparagine in 11A but serine for 11D. This finding resolves a major conundrum, that serotypes 11A and 11D have almost identical cps loci despite their serologic differences (8), and should facilitate distinguishing serotypes 11A and 11D molecularly. We further demonstrate the importance of c112 by showing that an N112A mutation creates a viable pneumococcal strain producing a novel CPS; it differs from 11D CPS because it does not have Glc and should differ from 11F because its pendant is expected to be phosphoglycerol, not phosphoribitol. In addition, the N112A mutant has distinct serological properties, and therefore the N112A mutant represents a new serotype. We propose giving the new serotype a provisional name, 11X3, because it has not yet been found in nature. Nevertheless, this site-directed mutation experiment clearly demonstrates that c112 of wcrL is the basis for serotype 11D and the identity of the fourth glycosidic residue.

It is known that S. pneumoniae can undergo serotype conversions when a missense mutation inactivates enzymatic activity of the involved gene (2, 21). We show here that a simple missense mutation that preserves the enzymatic activity can still cause serotype conversions. There are additional examples of simple missense mutations causing serotype conversions among other bacteria. Neisseria meningitidis serogroups Y and W-135, respectively, produce capsular PSs containing either Glc or Gal (22). Site-directed mutational analysis showed that amino acid residue 310 of SiaD determines both its substrate specificity and its serogroup association (22). Campylobacter jejuni produces lipo-oligosaccharide that mimics host gangliosides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23). Depending on the amino acid at position 51, Cst-II can produce lipo-oligosaccharides using a sialyltransferase Cst-II (23).
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ride with α2→3 linkages only or with both α2→3 and α2→8 linkages (24). Also, residue 51 has been associated with different human autoimmune diseases (23, 25). In view of these examples, it is likely that future studies would identify additional examples of simple missense mutation-induced serotype conversions among pneumococci. It is hard to recognize the functional significance of missense mutations causing no genetic inactivations without functional studies. Thus, identification of bacterial capsule or LPS types would be difficult with genetic information alone, as was shown with distinguishing serotypes 11A and 11D genetically.

Another interesting feature is that 11D WcrL is a bispecific transferase capable of catalyzing the transfer of two different substrates. WcrL may be useful for studying the structural basis for donor bispecificity. For instance, heparan sulfate synthesis in humans involves five EXT genes, which may encode bispecific transferases, including EXT12, that can transfer either GlcNAc or GalNAc (26–28). But molecular mechanisms for the bispecificity are not yet clear (27). A well studied example of a bispecific transferase is an animal β1,4-galactosyltransferase mutant, which can use both Gal and GalNAc as donors. However, this enzyme is not found in nature (29). Bacterial PSs provide additional examples. Serotype 3 pneumococci produce capsule by alternatively linking two different monosaccharides into a polymer with a synthase (3). But its donor specificity is determined by target substrate (3). Meningococcal strains expressing both serotypes Y and W135 were identified, and molecular analysis suggested that SiaD with serine at 310 may use both uridine diphosphate (UDP)-Glc and UDP-Gal as donor (30). However, the role of residue 310 could not be confirmed by a site-directed mutagenesis study (22). 11D WcrL not only putatively encodes a natural bispecific transferase, but also it can be compared with wcrL alleles of 11A and 11X3. Thus, 11D WcrL would be useful for studying the molecular basis of bispecific transferases.

The three different donor specificities of WcrL of 11A, 11D, and 11X3 may be explained at the atomic level with a steric hindrance model involving the 112th amino acid in WcrL (Fig. 7). The bulky side group of the Asn-112 in 11A WcrL may interact with the N-acetyl (NAc) of a UDP-αGlcNAc molecule.
and inhibit its function as a N-acetylglicosyltransferase but permit its function as a glucosyltransferase. In contrast, a small and hydrophobic Ala-112 in the 11X3 WcrL binding pocket would preferentially accommodate UDP-αGlcNAc to UDP-αGlc. The Ser-112 found in 11D WcrL has a side group of intermediate size and may offer a more relaxed donor site specificity than Asn-112 but still restrict the ability to use UDP-αGlcNAc as a donor substrate. Because both substrates are abundant in pneumococci, the observed ratio may reflect that 11D WcrL has a higher affinity for UDP-Glc than UDP-GlcNAc as its donor substrate. Interestingly, a similar model has been described for Gal/GalNAc specificity of β1,4-galactosyltransferases that distinguish vertebrates from invertebrates (31).

Briefly, in these galactosyltransferases, one specific amino acid residue (residue 289) largely determines the enzyme specificity, and the amino acid has bulky side groups for Gal-transferases that distinguish vertebrates from invertebrates (31).

Because CPS synthesis involves various transferases and other enzymes that must work together (34), protective CPSs have generally been considered to be not so malleable. However, to evade host immunity, many ways to produce different structures with different serologic properties have evolved in bacteria. The pneumococcus, which is a very successful commensal accidental pathogen, can express diverse capsule types by slightly altering transferases or inactivating acetyltransferases. It is clear that pneumococci may produce more capsule types than we have identified so far. For instance, we show here that serotype 11A can have two variants by altering codon 112 of WcrL. All three serotypes should be able to generate three additional variants by inactivating an acetyltransferase wcjE in its cps, as was shown for the 11A/11E pair (2). Thus, we need to view the bacterial PS as more malleable than we have previously recognized. Also bacteria would explore all of the variations in transferases to survive the host immune system, and therefore they should provide many examples of functional variations in transferases. Thus, studies of bacterial PS, such as pneumococcal capsule, should help us investigate structure-function relationships of glycosyltransferases.

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