protein acceptors passing through the ER, or under circumstances where the N-glycosylation consensus sequence (Asn-X-Ser/Thre motif) is not glycosylated. Although this site is consistently glycosylated in many individual proteins, the local protein environment may dictate incomplete glycosylation. Currently, a clear understanding of this mechanism is lacking, but in either case, the resultant dolichyl oligosaccharide is cleaved and free oligosaccharide is generated. This oligosaccharide is eventually cleared from the lumen of the ER to the lysosome, after limited digestion with cytosolic mannosidases [9].

In this issue, Imperiali's group have attempted to rationalize the mechanism that allows the constitutive rate of expression of OT to be regulated at the level of substrate interactions with the active site [10]. Using neoglycopeptides, the authors propose that the oligosaccharide is transferred to the peptide, in a *cis*-amide conformation, which generates an energetically favorable *trans*-amide linkage with weaker affinity for the active site. This allows the N-linked glycoprotein to dissociate from the OT complex and prevent potential product inhibition. An interesting possibility now exists to explain the presence of free oligosaccharides in the ER. If the native glycopeptide does not isomerize, the amide bond could be weakened, resulting in water-assisted hydrolysis to produce an oligosaccharide with a free reducing group. This could be mediated through unfavorable interactions between the oligosaccharide and the protein secondary structure [11], supporting the role for the primary amino acid sequence in determining glycosylation. Could this oligosaccharide be the source of the free $Glc_3Man_9GlcNAc_2$ in the ER lumen, and the reason why many consensus N-glycosylation sites are never occupied? Previous hypotheses have invoked a dolichyl oligosaccharide hydrolase activity of OT that is responsible for preferential transfer to water [12] under peptide acceptor reducing conditions. This would be an inefficient process and would not be regulated by protein determinants. However, if isomerization goes to completion and the product is released, the active site for transfer would now be accessible for dolichyl oligosaccharide binding to incoming peptide, ensuring a continuous and efficient supply of N-linked glycosylated protein, as specified by the protein sequence.

The Imperiali group also shows that the use of product mimics of OT reactants allows further definition of the mechanism of action of this enigmatic enzyme and demonstrates the exquisite means that enzymes use to control biologically significant processes in eukaryotic cells.

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Selected Reading

1. Lennarz, W.J. (1983). CRC Crit. Rev. Biochem. *14*, 257–272.
2. Petrescu, A.J., Butters, T.D., Reinkensmeier, G., Petrescu, S., Platt, F.M., Dwek, R.A., and Wormald, M.R. (1997). EMBO J. *16*, 4302–4310.
3. Marek, K.W., Vijay, I.K., and Marth, J.D. (1999). Glycobiology *9*, 1263–1271.
4. Ioffe, E., and Stanley, P. (1994). Proc. Natl. Acad. Sci. USA *91*, 728–732.
5. Metzler, M., Gertz, A., Sarkar, M., Schachter, H., Schrader, J.W., and Marth, J.D. (1994). EMBO J. *13*, 2056–2065.
6. Ellgaard, L., and Helenius, A. (2001). Curr. Opin. Cell Biol. *13*, 431–437.
7. Dwek, R.A., Butters, T.D., Platt, F.M., and Zitzmann, N. (2002). Nature Rev. Drug Discovery *1*, 65–75.
8. Ufret, M.L., and Imperiali, B. (2000). Bioorg. Med. Chem. Lett. *10*, 281–284.
9. Moore, S.E.H. (1999). Tr Cell Biol *9*, 441–446.
10. Peluso, S. de L. Ufret, M., O'Reilly, M.K., and Imperiali, B. (2002). Chem. Biol. *9*, this issue, 1323–1328.
11. Mingarro, I., Nilsson, I., Whitley, P., and von Heijne, G. (2000). BMC Cell Biol. *1*, 3.
12. Spiro, M.J., and Spiro, R.G. (1991). J. Biol. Chem. *266*, 5311–5317.

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Combinatorial Glycosylation of Glycopeptide Antibiotics

The glycosyltransferases GtfE and GtfD from the vancomycin producer *Amycolatopsis orientalis* have promiscuous substrate and NDP-sugar specificities. They have been used to generate novel glycopeptide antibiotics containing the heptapeptide scaffolds of vancomycin and teichoplanin [1].

The glycopeptide antibiotics vancomycin and teicoplanin are secondary metabolites produced by soil actinomycetes, *A. orientalis* and *Actinoplanes teichomyceticus,* respectively. Both are clinically important for the treatment of infections due to gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) [2, 3]. With the emergence of vancomycin-resistant enterococci (VRE) and *S. aureus* (VRSA), the development of new derivatives of vancomycin [4] and the development of other antibiotics with new modes of action (e.g., daptomycin [5]) are important for the treatment of life-threatening gram-positive infections.

Like vancomycin, chloroeremomycin is produced by a strain of *A. orientalis* [6]. Chloroeremomycin differs from vancomycin in its pattern of glycosylation. Vancomycin has the disaccacharide D-glucose-L-vancosamine attached to the phenolic group of hydroxyphenylglycine at amino acid 4 of the heptapeptide. Chloeremomycin has the disaccharide D-glucose-L-4-*epi*-vancosamine

attached at the same position, but also has a second L-4-*epi*-vancosamine attached to the 2-hydroxyl group of 2-hydroxytyrosine at amino acid 6. The difference in glycosylation patterns apparently accounts for the increased activity of chloroeremomycin against VRE [4]. Oritavancin (LY333328) is an *N*-alkyl derivative of chloroeremomycin containing a *p*-chloro-biphenyl group attached to the amino group of the disaccharide D-glucose-4-*epi*-vancosamine. This compound has substantially improved antibacterial activity against VRE [4, 7]. This type of modification apparently shifts the mechanism of action of glycopeptides from being predominantly transpeptidase inhibitors to being predominantly transglycosylase inhibitors [8, 9], thus reducing the influence of the D-ala-D-lac stem peptide terminus in VanR strains.

The heptapeptide backbones of glycopeptides related to vancomycin and teicoplanin are assembled by giant multidomain multisubunit nonribosomal peptide synthetases (NRPSs) [10, 11]. NRPSs and polyketide synthases (PKSs) are involved in the biosynthesis of a vast array of secondary metabolites produced by *Streptomyces* species and other actinomycetes. These versatile enzymes use simple fatty acid (PKSs) or amino acid (NRPSs) building blocks to synthesize very complex scaffolds that can be further modified by "tailoring" enzymes (e.g., hydroxylases, methyltransferases, halogenases/haloperoxidases, and glycosyltransferases). Many gene clusters for polyketide and peptide biosynthesis have been cloned and analyzed, and advancements in methodologies for the genetic manipulation of actinomycetes have paved the way to modify the genetic blueprints of PKS pathways to carry out "combinatorial biosynthesis" of the polyketide core structures [12, 13]. The modular organization of the NRPS genes, which is similar to the modular organization of type I PKS genes, also suggests that combinatorial biosynthesis of novel peptides will be extended to actinomycetes. Examples of molecular genetic manipulation of NRPS genes in *Bacillus* have been reported [14].

Many polyketides and NRPS-derived peptides (such as vancomycin and chloroeremomycin) are glycosylated, primarily by 6-deoxysugars. Over 100 different deoxyhexoses have been identified in secondary metabolites [15]. It is not surprising, therefore, that sugar residues are often required for the exquisite biological activities of many secondary metabolites [16]. This appears to be true for glycopeptides: some nonglycosylated glycopeptides have excellent in vitro antibacterial activities but lack in vivo efficacy [17]. Many genes for deoxyhexose biosynthesis have been cloned and sequenced, and a number of them are organized in discrete clusters that can be genetically manipulated [18]. Some glycosyltransferases have relatively broad substrate specificities for type I or type II polyketides and can be used to generate novel structures not amenable to chemical syntheses [18, 19].

Solenberg et al. [6] cloned three glycosyltransferase genes from the chloroeremomycin producer and two from the vancomycin producer. They showed by expression studies in *E. coli* that the glucosyltransferase enzymes encoded by the *gtfE* gene from the vancomycin producer, and the *gtfB* gene from the chloroeremomycin producer, could attach glucose to the vancomycin aglycone using TDP- or UDP-D-glucose as sugar donors. The GtfE enzyme (but not GtfB) could also glycosylate A47934, an antibiotic with a teicoplanin-like heptapeptide, but lacking sugar residues. Insertion of the *gtfE* gene into the chromosome of *S. toyocaensis* under the control of the *ermEp** promoter caused the production of novel antibiotic glucosyl-A47934.

Building on these observations, Losey et al. [20] cloned, expressed, and purified the products of the *gtfB* and *gtfC* genes from the chloroeremomycin producer and the *gtfD* and *gtfE* genes from the vancomycin producer. They confirmed that the GtfE glucosyltransferase had relaxed substrate specificity. They further demonstrated that GtfC and GtfD enzymes were proficient in attaching *epi*-vancosamine to the vancomycin psuedoaglycone and to the glucosylated teicoplanin aglycone when UDP-β-L-4-*epi*-vancosamine was used as the sugar donor. Importantly, the GtfD enzyme, which normally adds vancosamine to the vancomycin pseudoaglycone, was able to catalyze the reaction using an unnatural accepter substrate and an unnatural UDP-sugar donor.

In the study published in this issue of *Chemistry & Biology*, Losey et al. [1] have used a chemienzymatic approach to synthesize a number of NDP-glucose analogs, using a promiscuous thymidylyltransferase [21, 22]. The generation of NDP-sugar substrates has limited the in vitro exploration of glycosyltransferases involved in secondary metabolism in the past. They demonstrated that the GtfE enzyme from the vancomycin producer could use both UDP- and TDP-glucose analogs, including various deoxyglucose analogs and analogs with amino groups at the 2, 3, 4, or 6 position, as sugar donors for glycosylation of the vancomycin and teichoplanin aglycones. Remarkably, the GtfD enzyme, which normally adds vancosamine to the glucose moiety of the vancomycin pseudoaglycone, was able to add 4-*epi*-vancosamine to each of the derivatives generated by the GtfE enzyme (except those with 2-deoxy substitutions, the site of glycosylation). This indicates that both enzymes have relaxed specificity for acceptor substrate and NDP-sugar cofactor. The generation of several derivatives of vancomycin having two amino sugars, with the amino group of the first sugar distributed at three different positions, provides novel scaffolds for further *N*-alkylation to generate analogs related to oritavancin.

This is the first extensive demonstration of the feasibility to carry out combinatorial glycosylation of peptides using promiscuous glycosyltransferases. Coupled with the recent advances on combinatorial glycosylation of polyketides, this adds a new dimension to the possibilities for combinatorial biosynthesis to generate molecules with important pharmacological properties not readily obtainable by chemical syntheses. These in turn can be further modified chemically to generate candidates for clinical development.

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Selected Reading

1. Losey, H.C., Jiang, J., Biggens, J.B., Oberthur, M., Ye, J.-Y., Dong, S.D., Kahne, D., Thorson, J.S., and Walsh, C.T. (2002). Chem. Biol. *9*, 1305–1314.
2. Wood, M.J. (1996). J. Antimicrob. Chemother. *37*, 209–222.
3. Zeckel, M.L. (1997). J. Chemother. *9*, 311–331.
4. Cooper, R.D., Snyder, N.J., Zweifel, M.J., Staszak, M.A., Wilkie, S.C., Nicas, T.I., Mullen, D.L., Butler, T.F., Rodriquez, M.J., Huff, B.E., et al. (1996). J. Antibiot. *49*, 575–581.
5. Tally, F.P., Zeckel, M., Wasilewski, M.M., Carini, C., Berman, C.L., Drusano, G.L., and Oleson, F.B. (1999). Expert Opin. Investig. Drugs *8*, 1223–1238.
6. Solenberg, P.J., Matsushima, P., Stack, D.R., Wilkie, S.C., Thompson, R.C., and Baltz, R.H. (1997). Chem. Biol. *4*, 195–202.
7. Barrett, J.F. (2001). Curr. Opin. Investig. Drugs *2*, 1039–1044.
8. Ge, M., Chen, Z., Onishi, H.R., Kohler, J., Silver, L.L., Kerns, R., Fukuzawa, S., Thompson, C., and Kahne, D. (1999). Science *284*, 507–510.
9. Eggert, U.S., Ruiz, N., Falcone, B.V., Branstrom, A.A., Goldman, R.C., Silhavy, T.J., and Kahne, D. (2001). Science *294*, 361–364.
10. van Wageningen, A.M., Kirkpatrick, P.N., Williams, D.H., Harris, B.R., Kershaw, J.K., Lennard, N.J., Jones, M., Jones, S.J., and Solenberg, P.J. (1998). Chem. Biol. *5*, 155–162.
11. Pootoolal, J., Thomas, M.G., Marshall, C.G., Neu, J.M., Hubbard, B.K., Walsh, C.T., and Wright, G.D. (2002). Proc. Natl. Acad. Sci. USA *99*, 8962–8967.
12. Hopwood, D.A. (1997). Chem. Rev. *97*, 2465–2497.
13. Katz, L. (1997). Chem. Rev. *97*, 2557–2575.
14. Marahiel, M., Stachelhaus, T., and Mootz, H.D. (1997). Chem. Rev. *97*, 2651–2673.
15. Piepersberg, W., and Distler, J. (1997). In Biotechnology, 2nd ed., Volume 7, H.-J. Rehm, G. Reed, A. Pühler, and P. Stadler, eds. (Weinheim: Wiley-VCH), pp. 399–488.
16. Weymouth-Wilson, A.C. (1997). Nat. Prod. Rep. *14*, 99–110.
17. Lancini, G.C., and Cavalleri, B. (1990). In Biochemistry of Peptide Antibiotics, H. Kleinkauf and H. Von Dühren, eds. (Berlin: Walter de Gruyter), pp. 159–178.
18. Mendez, C., and Salas, J.A. (2001). Trends Biotechnol. *19*, 449–456.
19. Tang, L., and McDaniel, R. (2001). Chem. Biol. *8*, 547–555.
20. Losey, H.C., Peczuh, M.W., Chen, Z., Eggert, U.S., Dong, S.D., Pelczer, I., Kahne, D., and Walsh, C.T. (2001). Biochemistry *40*, 4745–4755.
21. Jiang, J., Biggens, J.B., and Thorson, J.S. (2001). Angew. Chem. Int. Ed. Engl. *40*, 1502–1505.
22. Barton, W.A., Lesniak, J., Biggens, J.B., Jeffrey, P.D., Jiang, J., Rajashankar, K.R., Thorson, J.S., and Nikolov, D.B. (2001). Nat. Struct. Biol. *8*, 545–551.

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One Step Closer to a Sweet Conclusion

OtsA is required for the biosynthesis of trehalose, a nonreducing disaccharide that is important for bacterial survival and stress responses. In this issue of *Chemistry & Biology*, the structure of OtsA is uncovered and reveals an unexpected relationship between the enzyme's structure and function.

The synthesis and degradation of glycosidic bonds occurs via enzyme-catalyzed glycosyltransfer reactions. Much is known about the structures and mechanisms of the degradative enzymes, glycosidases. Over 80 sequence-derived families have been identified to date, and structural representatives are available for approximately 50 of these; revealing a large number of different folds. Mechanistically, they are divided into two classes. The inverting glycosidases function via an acid/base-catalyzed direct displacement mechanism, while the majority of the retaining glycosidases use a double-displacement mechanism in which a covalent glycosyl-enzyme intermediate involving an active-site carboxylic acid is formed and hydrolyzed with acid/base catalytic assistance. In an interesting variation found so far in hexosaminidases from families 18, 20, and 56, the substrate's own amide moiety functions as the nucleophile, and reaction occurs via a bound oxazoline intermediate [1].

By contrast, much less is known about the enzymes involved in glycoside synthesis, the sugar nucleotide-dependent glycosyltransferases. Some 60 sequence-derived families have been defined, but structures for only ten of these have been determined, of which seven are of the mechanistically predictable inverting transferases. A key distinction from the glycosidases is that only two fundamental protein folds (termed GT-A and GT-B; Figure 1) have been uncovered for transferases, and sequence analysis has suggested that this situation will hold true for a large number of the as yet structurally uncharacterized families [2]. The GT-A fold family comprises a single Rossman fold domain and has been identified in the structures of both inverting and retaining transferases (Figure 1). Enzymes of the GT-B fold have a twin Rossman fold structure, and up to this point all those identified have been inverting transferases, possibly suggesting a causal relationship. However, the paper in this issue by Gibson and coworkers [3] on the retaining glycosyltransferase, trehalose-6-phosphate synthetase (OtsA), also reveals a twin Rossman fold for this enzyme, showing clearly again that the fold does not dictate the mechanism. This provides a cautionary note on the overinterpretation of functional data from a predicted protein fold (a major premise of structural genomics).

The OtsA structural analysis also revealed a fascinating mechanistic story. Despite the absence of any detectable sequence similarity, the active site residues of OtsA are essentially superimposable on those of a nonnucleotide sugar glycosyltransferase of the GT-A class, glycogen phosphorylase. This enzyme catalyzes the reversible phosphorolysis of glycogen with net retention of configuration to produce glucose-1-phosphate. One of the more enigmatic aspects of the glycogen phosphorylase story has been its absolute requirement for the coenzyme pyridoxal phosphate, which is covalently bound at the active site as a Schiff's base. A reason for this requirement has eluded explanation. Structural and