GLYCOBIOLOGY

# The case of the missing base

Post-translational modification of proteins by *N*-acetylglucosamine (*O*-GlcNAc) is carried out by a single glycosyltransferase, OGT. Two independent groups have generated structures of ternary complexes that elegantly illuminate substrate and product binding modes, and thus the overall reaction coordinate, but the respective authors differ in their choice of catalytic base.

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The dynamic *O*-GlcNAc post-translational modification, ubiquitous in multicellular eukaryotes, is rapidly emerging as one of the most important cellular glycosylations. The installation of the β-linked *N*-acetylglucosamine to the target serine and threonine side chains of both nuclear and cytoplasmic proteins is catalyzed by a single enzyme: the O-GlcNAc transferase OGT. *O*-GlcNAc removal is achieved through the action of the O-GlcNAc hydrolase, OGA. Both OGA and OGT have been much studied, a reflection of their fundamental cellular importance in stability, cellular signaling and transcriptional control and of the implication of *O*-GlcNAc in diseases (with recent examples including neurodegeneration[1] and cancer[2]). Major outstanding questions in regard to the function of OGT include how the specificity of the enzyme is determined, as OGT has numerous diverse substrates, and how the UDP-sugar binds and is transferred. Furthermore, the catalytic base thought to be necessary to deprotonate, and thus activate, the serine residue of the protein substrate has eluded identification.

Efforts to study OGT had been hindered by the lack of a suitable three-dimensional structure. But in 2011, the three-dimensional structure of a truncated human OGT, featuring 4.5 of the 13.5 tricotetrapeptide repeats and in complex with peptide and UDP, was unveiled[3]. This structure, coupled with the recent development of a slowly transferred UDP-GlcNAc analog (UDP-5-thioGlcNAc[4]) provided the foundations that have now enabled two independent studies published in this issue of *Nature Chemical Biology* to report ternary structures including the sugar residue, providing insights into the reaction coordinate and mechanism of OGT[5,6].

The two papers describe similar strategies to access the ternary complexes of enzyme plus UDP-sugar and peptide acceptor in the absence of either donor hydrolysis or normal turnover. Both settle upon the use of UDP-5-thioGlcNAc as the severely catalytically compromised donor substrate, and both show that turnover can indeed still occur if a normal serine-containing peptide is employed. Lazarus *et al.*[5] provide a revised measure for the rate of 5-thio-GlcNAc transfer as being approximately 0.03% the rate of GlcNAc transfer, as opposed to their previous estimate of 7% (ref. 4), as is quoted by Schimpl *et al.*[6]. The papers differ in their choice of serine substitution in their respective peptide acceptor substrates (each used the respective authors' favorite peptide), with Lazarus *et al.*[5] opting for an alanine substitution and Schimpl *et al.*[6] electing to use an aminoalanine on the basis of their demonstration that it functions as only a very slow substrate with the UDP-GlcNAc donor. Despite the solved structures being rather similar, with the donor sugar adopting equivalent conformations, the two papers arrive at quite different mechanisms, especially with regard to the identity of the base catalyst that may be needed to accept the proton from the serine hydroxyl. Direct interaction of the acceptor with histidine residues, which had been proposed previously, was rejected on the basis of distance by both sets of authors, as well as on the basis of kinetic analysis of enzyme variants by Schimpl *et al.*[6], and neither paper identifies an appropriate nearby OGT side chain that could serve this purpose.

So what information do the authors obtain in their efforts to solve the case of the missing base? Schimpl *et al.*[6] observe interactions between the amine moiety of the aminoalanine and the pro-*R* oxygen of the α-phosphate of the donor analog, and on this basis the authors propose a mechanism in which the α-phosphate serves as the base catalyst (**Fig. 1a**). Considering the widely assumed p$K_a$ of ~2–3 for the conjugate acid of this phosphate in both the UDP-sugar and the UDP product and the p$K_a$ of ~16 for the serine hydroxyl, it would seem to be a rather unattractive candidate. However, the authors offer supporting evidence in the form of the demonstration that the chiral α-phosphorothioate analog of UDP-GlcNAc in which the sulfur is in the pro-*R* position does not serve as a donor substrate (but binds with a similar affinity to UDP-GlcNAc), whereas the other diastereomer is a good substrate. Further, they offer surprising $^{31}$P-NMR titration data on UDP-GlcNAc itself, expanded upon by one of the authors in a separate publication[7], implying that the p$K_a$ could be much higher than presumed, at 6.4. Further research is certainly needed to clarify this point.

Lazarus *et al.*[5] provide a series of snapshots including the binary complex UDP-sugar and the product complex

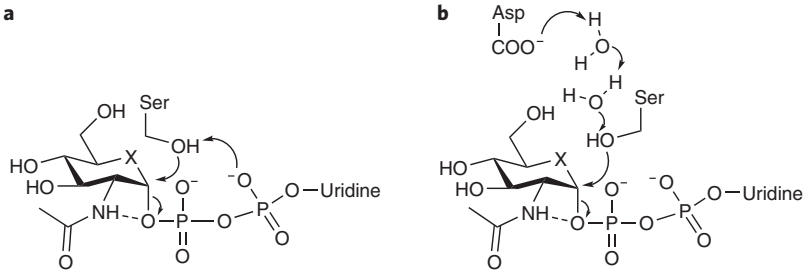


**Figure 1 |** Two proposed mechanisms for O-GlcNAc transfer by OGT. (**a**) The α-phosphate as base mechanism proposed by Schimpl *et al.*[6]. (**b**) The Grotthuss water 'wire' shunting a proton out of the active site following transfer with a late transition-state, suggested by Lazarus *et al.*[5]. Both mechanisms for *O*-GlcNAc transfer from UDP-GlcNAc (where X = O) were enabled through the elegant use of UDP-5-thio-GlcNAc (where X = S).

with UDP and GlcNAcylated product that together highlight the electrophilic migration mechanism. In contrast to Schimpl *et al.*[6], the authors explicitly reject the α-phosphate as a candidate base on the basis of their structure wherein the (modeled) serine rotamer that could interact at that position would both be incorrectly aligned for the displacement reaction and receive an unfavorable hydrogen bond from a peptide amide moiety. Rather, Lazarus *et al.*[5] favor a mechanism in which the proton is relayed out of the site through a chain of intervening water residues in a Grotthuss type of mechanism, possibly to Asp554 (**Fig. 1b**). Such a mechanism is analogous to that proposed previously for some inverting glycosidases that also seem to lack a base catalyst, exemplified by family GH6 (ref. 8). However, a more direct role of Asp554 is rendered unlikely by the report by Schimpl *et al.*[6] that the D554N mutant is active.

So what have we learned? The structures in both papers beautifully exemplify the key species along the reaction coordinate for an inverting glycosyltransferase and highlight how, just as has been seen for glycosidases, the chemical step involves a nominally static protein with the movement required for bond breaking and formation being associated with an electrophilic migration of the anomeric carbon between the donor and acceptor oxygens, as best illustrated by Lazarus *et al.*[5] in Figure 2a of their paper. Both groups argue in favor of a role for the substrate acetamide in the reaction, as the nitrogen is seen to coordinate with the terminal phosphate of UDP in product complexes. Schimpl *et al.*[6] also posit an additional role of the acetamide carbonyl in orienting the 'serine' nucleophile, but this may be obscured by the choice of aminoalanine, as may well also be the close interaction of the α-phosphate oxygen. Interestingly, a recent computational paper[9] also suggests a role for the *N*-acetyl NH moiety in stabilizing the diphosphate leaving group at the transition state by hydrogen bonding to the glycosidic oxygen. However, the computational study is based on His498 functioning as the base, which these new structural and mutational studies refute. Thus, the identity of the general base is not resolved. It is quite probable that, just as is seen to be the case for some inverting glycosidases, general base catalysis may not be essential for reactions with such late transition states. Time, and more examples, will tell.

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## Competing financial interests

The authors declare no competing financial interests.



CHANNELS

# Rotamers affect ion conductance

A major determinant of the ion flux rate through acetylcholine receptors is a ring of five residues, four glutamates and a glutamine, at the channel's cytoplasmic mouth. The glutamates adopt alternate rotamer conformations so that only two directly affect channel conductance.

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The single channel conductance, a measure of the ease of ion translocation through a channel, is an important characteristic of an ion channel. The conductance is largely determined by the electrostatic and steric interactions of ions with the residues lining the ion translocation pathway. This includes residues lining the transmembrane channel and those lining the vestibules at the extracellular and cytoplasmic ends of the channel. Nicotinic acetylcholine receptors (AChRs), members of the Cys loop receptor superfamily of neurotransmitter-gated channels, are formed by the pseudosymmetrical assembly of five homologous or identical subunits around the central axis (**Fig. 1a**,**b**)[1,2]. The subunits have similar transmembrane topologies with an ~200 amino acid extracellular N-terminal domain, four membrane-spanning segments (M1, M2, M3 and M4) and a large intracellular loop connecting M3 and M4 (**Fig. 1b**)[1,3–5]. The transmembrane channel is lined by rings of aligned residues, one from each subunit's M2 segment (**Fig. 1c**,**d**)[6]. In the cation-selective AChR, previous work identified three rings of mostly negatively charged residues in and flanking M2, named the extracellular, intermediate and cytoplasmic rings[7]. Altering the number of charged residues in the intermediate ring had the greatest impact on conductance and raised questions about the ionization state of the four intermediate ring glutamates[7]. In an elegant set of experiments using mutagenesis and single-channel patch clamp recordings, Cymes and Grosman[8] demonstrate that all four glutamates are ionized but that only two seem to face into the channel lumen and have a considerable effect on channel conductance. They speculate that the side chains of the other two adopt an alternate rotamer conformation pointing away from the channel axis and have minimal electrostatic interactions with permeating ions.

Using voltage-clamped patch recordings, the current amplitude through individual channels was measured at several voltages, and the single-channel conductance for wild-type AChRs was calculated to be ~140 pS. Mutating all intermediate ring residues to alanine reduced the conductance to 30 pS[8]. The conductance increased in ~50-pS steps as the number of intermediate ring glutamates increased. With one glutamate and four alanines the conductance was ~80 pS; with two glutamates it was 140 pS. Surprisingly, with two, three or four

CORRIGENDUM

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In the version of this article initially published online, the wrong article was listed as reference 4. The error has been corrected for the PDF and HTML versions of this article.