News Release

Discovery of a Novel Substrate Recognition Motif in an Evolutionarily Conserved Glycosyltransferase

Key Points

- EGF domain-specific *O*-GlcNAc transferase (EOGT) is an enzyme that adds sugar molecules (*O*-GlcNAc) to proteins, and mutations in the *EOGT* gene have been reported in the congenital disorder Adams-Oliver syndrome. In this study, the researchers determined the atomic-level three-dimensional structure of EOGT and clarified how these genetic mutations impair its function.
- Based on the crystal structure of the complex formed by EOGT and the sugar donor uridine diphosphate (UDP), the researchers demonstrated that the spatial arrangement of three amino acids (Asn-Arg-Arg) among the residues interacting with UDP is critical for UDP recognition and enzymatic activity.
- The study also revealed that this structural arrangement—termed the "N-R-R constellation"—is conserved across the GT61 family, to which EOGT belongs, and has been evolutionarily preserved in enzymes across both plants and animals, serving as a fundamental structural basis for UDP interaction.

Summary

A research group led by Professor Tetsuya Okajima and Lecturer Yuko Tashima of the Graduate School of Medicine at Nagoya University, in collaboration with Lecturer Masamichi Nagae of Osaka University (currently at Tohoku Medical and Pharmaceutical University) and Professor Jiaoyang Jiang of the University of Wisconsin–Madison, has determined the crystal structure of the glycosyltransferase EOGT and discovered a novel substrate recognition motif.

Within cells, many proteins function properly only after being modified by the addition of molecules known as sugars. One of the enzymes responsible for this process is EGF domain-specific *O*-GlcNAc transferase (EOGT), which adds a sugar molecule known as *O*-GlcNAc to specific protein regions called EGF domains. Mutations in EOGT have been identified in Adams-Oliver syndrome, a congenital disorder that affects the development of limbs and skin, but the underlying molecular mechanism has remained unclear.

In this study, the researchers revealed the three-dimensional structure of EOGT in its bound state with uridine diphosphate (UDP), a molecule that carries sugar molecules. The researchers found that among the three EOGT mutations previously reported in Adams-Oliver syndrome, one affects an amino acid that is critical for UDP binding, while the other two alter amino acids essential for maintaining EOGT's structural integrity.

They also identified a distinctive spatial arrangement composed of three amino acids—one asparagine and two arginines—that functions like a keyhole in recognizing UDP. This newly identified structural motif has been named the N-R-R constellation.

The study further revealed that this N-R-R constellation is conserved across the GT61 family, to which EOGT belongs, in both plants and animals. This finding suggests that organisms have evolutionarily preserved a common mechanism for transferring sugars to glycosyltransferases via UDP.

These findings are expected not only to provide new insights into the molecular pathology of Adams-Oliver syndrome, but also to serve as a foundation for understanding the evolution and function of glycosyltransferases.

The study was published in PNAS Nexus, a journal launched by the National Academy of Sciences and published by Oxford University Press, on April 15, 2026.

Research Background

Protein function within cells is regulated through glycosylation, a process in which sugar molecules are attached to proteins. Among these modifications, *O*-GlcNAcylation is one of the most well-known forms of glycosylation and has traditionally been attributed primarily to the intracellular enzyme *O*-GlcNAc transferase (OGT). In contrast, EGF domain-specific *O*-GlcNAc transferase (EOGT) is an enzyme that catalyzes a similar modification on extracellular and secreted proteins and has attracted attention as a newly identified pathway (Figure 1). EOGT was previously identified by Professor Okajima and colleagues in this laboratory and functions within the endoplasmic reticulum.

EOGT modifies proteins containing a specific structural motif known as the EGF domain and is also involved in the function of the NOTCH receptor. Reduced EOGT activity causes Adams-Oliver syndrome, a congenital disorder characterized by abnormalities of the scalp and limbs; however, the detailed molecular mechanism underlying this condition remains unclear.

EOGT belongs to the glycosyltransferase family GT61 and is classified in the same family as POMGNT2, an enzyme that uses UDP-GlcNAc as a sugar donor, based on similarities in amino acid sequences. However, the detailed similarities between these enzymes have not yet been fully understood.

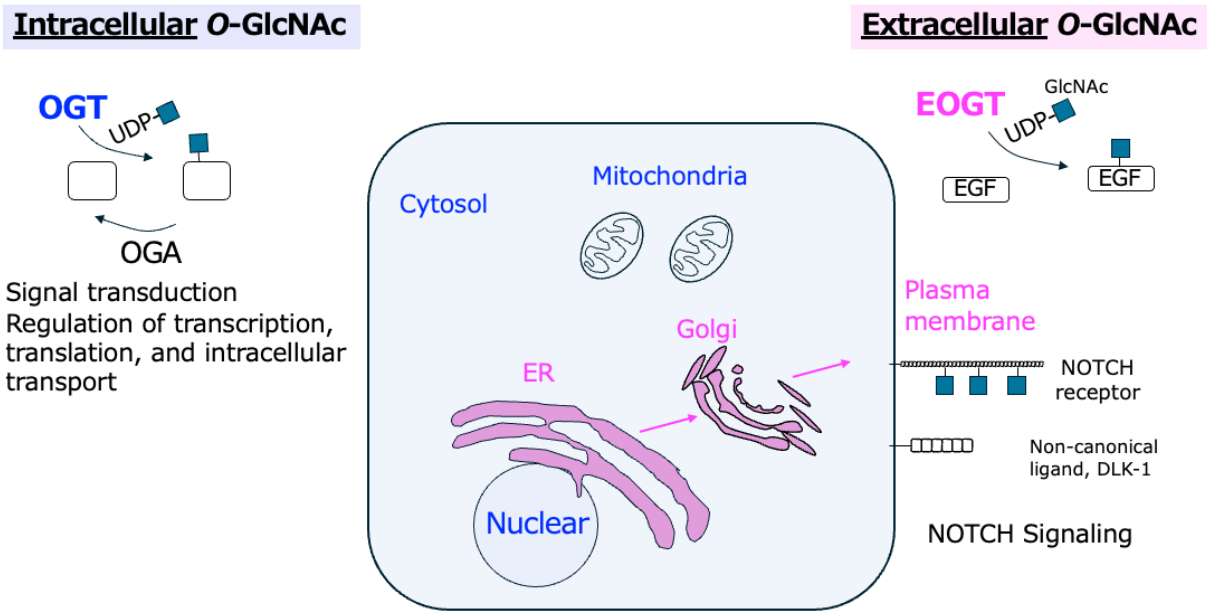

Figure 1. Intracellular and extracellular *O*-GlcNAc modification and their glycosyltransferases.

Research Results

In this study, we comprehensively elucidated the three-dimensional structure and function of EOGT, a glycosylation enzyme involved in protein modification (Figure 2).

We performed crystallographic analysis of a complex consisting of purified EOGT protein and UDP (a sugar donor) prepared using cultured cells. As a result, we successfully determined the crystal structure at a high resolution of 1.8 Å (*8). The analysis revealed that EOGT adopts a GT-B fold structure composed of two domains, with UDP binding within the cleft between them. Structural and mutational analyses further demonstrated that specific amino acid residues, including N245, R372, and R377, play critical roles in UDP binding.

Based on the crystal structure of the EOGT-UDP complex identified in this study, we also clarified the structural effects of mutations associated with Adams-Oliver syndrome. The R377Q mutation was found to be located in the UDP-binding site and significantly reduces enzymatic activity by weakening UDP binding. In addition, the C135Y and W207S mutations were shown to affect the structural stability of the protein.

One of the most significant findings of this study was the identification of a

spatial arrangement essential for UDP binding, consisting of one asparagine residue and two arginine residues, which we named the N-R-R constellation. This structural feature was found to be conserved not only in EOGT but also in other enzymes belonging to the GT61 family.

Specifically, this motif is widely conserved across organisms ranging from fruit flies to primitive species such as sponges. Similar spatial arrangements were also identified in the animal enzyme POMGNT2 and in plant enzymes (core β1,2-XylT, XYXT, XAXT, and XAT).

Although these plant enzymes also use UDP as a sugar donor, they transfer sugars such as xylose and arabinose, which differ from the *O*-GlcNAc modification catalyzed by EOGT. Because the crystal structures of these plant enzymes and those found in primitive organisms have not yet been determined, we conducted structural prediction analyses using AlphaFold2.

This study provides the first atomic-level understanding of the molecular mechanism of EOGT and proposes a universal principle by which GT61 family glycosylation enzymes interact with UDP.

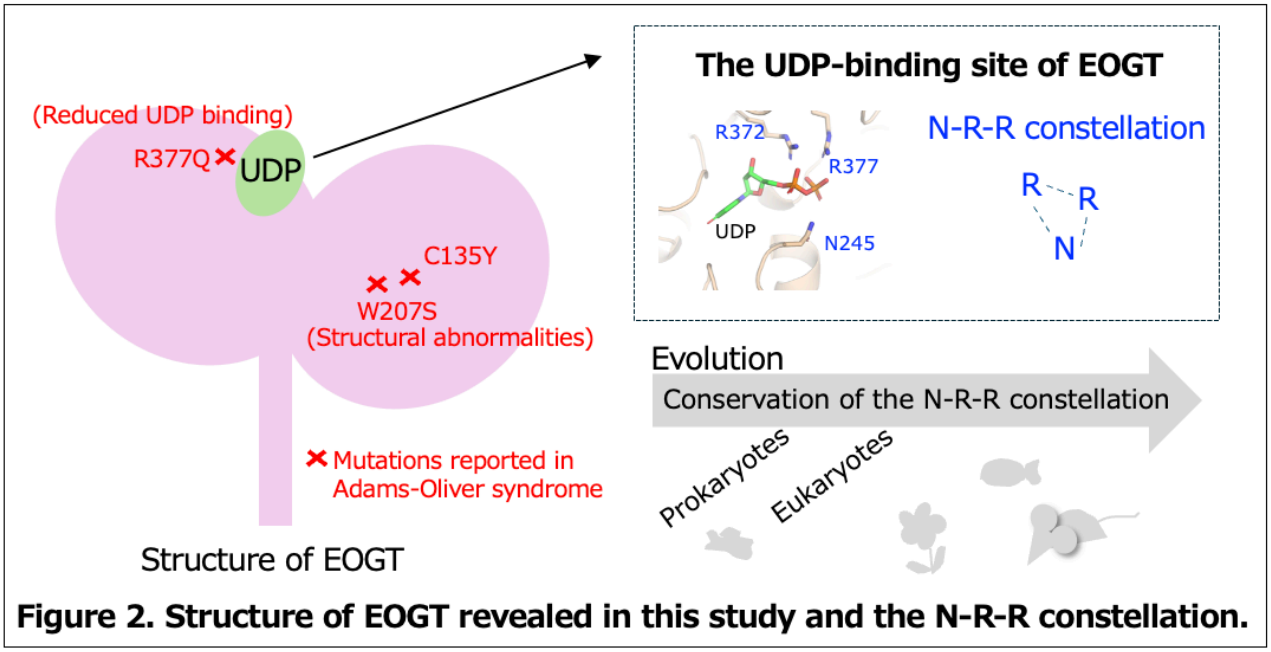

Figure 2. Structure of EOGT revealed in this study and the N-R-R constellation.

Research Summary and Future Perspective

Although this study has provided detailed insights into the structure of EOGT and its mechanism for recognizing UDP, further research is needed to fully elucidate its catalytic mechanism. In particular, the three-component complex structure consisting of EOGT, UDP, and its substrate receptor remains unresolved and represents an important future challenge for understanding the substrate specificity of EOGT.

In addition, similar to the GT61 family-specific structural feature identified in

this study, other glycosyltransferase (GT) families may also possess their own unique three-dimensional structural arrangements. Further advances in this area are expected to deepen our understanding of the evolution and functional mechanisms of glycosyltransferases.

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