The mammalian α1,6-fucosyltransferase (FUT8) catalyzes the core fucosylation of N-glycans in the biosynthesis of glycoproteins. Previously, intensive in vitro studies with crude extract or purified enzyme concluded that the attachment of a GlcNAc on the α1,3 mannose arm of N-glycan is essential for FUT8-catalyzed core fucosylation. In contrast, we have recently shown that expression of erythropoietin in a GnTI knock-out, FUT8-overexpressing cell line results in the production of fully core-fucosylated glycoforms of the oligomannose substrate Man$_5$GlcNAc$_2$, suggesting that FUT8 can catalyze core fucosylation of N-glycans lacking an α1,3-arm GlcNAc in cells. Here, we revisited the substrate specificity of FUT8 by examining its in vitro activity toward an array of selected N-glycans, glycopeptides, and glycoproteins. Consistent with previous studies, we found that free N-glycans lacking an unmasked α1,3-arm GlcNAc moiety are not FUT8 substrates. However, Man$_5$GlcNAc$_2$ glycan could be efficiently core-fucosylated by FUT8 in an appropriate protein/peptide context, such as with the erythropoietin peptide, a V3 polypeptide derived from HIV-1 gp120, or a simple 9-fluorenylmethyl chloroformate-protected Asn moiety. Interestingly, when placed in the V3 polypeptide context, a mature bi-antennary complex-type N-glycan also could be core-fucosylated by FUT8, albeit at much lower efficiency than the Man$_5$GlcNAc$_2$ peptide. This study represents the first report of in vitro FUT8-catalyzed core fucosylation of N-glycans lacking the α1,3-arm GlcNAc moiety. Our results suggest that an appropriate polypeptide context or other adequate structural elements in the acceptor substrate could facilitate the core fucosylation by FUT8.

The asparagine-linked (N-linked) glycosylation is a major posttranslational modification in mammalian systems, which profoundly affects the intrinsic properties and biological functions of proteins (1, 2). The biosynthesis of N-glycoproteins involves multiple steps, including the initial transfer of a large precursor N-glycan, Glc$_3$Man$_5$GlcNAc$_2$, to the nascent protein by the oligosaccharyltransferase at the endoplasmic reticulum, followed by the processing of the precursor N-glycan at the endoplasmic reticulum and then in the Golgi apparatus to high-mannose, complex, and/or hybrid glycoforms. Core fucosylation, the transfer of an α1,6-linked fucose to the innermost GlcNAc by an α1,6-fucosyltransferase (FUT8),$^2$ is an important step in this process. It regulates various cellular functions, including cell activation (3) and cell adhesion (4–6). Studies in mouse models have shown that knock-out of the FUT8 gene induces severe growth retardation and death during postnatal development (7). Enhanced core fucosylation is often associated with cancer progression (8). For example, AFP-L3, the core-fucosylated α-fetoprotein, is a Food and Drug Administration-approved biomarker for hepatocellular carcinoma (a major form of liver cancer) (9). Core fucosylation also directly modulates the biological activity of glycoproteins, such as the bioactivities of several tyrosine kinase receptors (3, 7), and the antibody-dependent cellular cytotoxicity of IgG antibodies (10). In the mammalian N-glycosylation-processing pathway, core fucosylation is preceded by the transfer of an N-acetylglucosamine (GlcNAc), in an α1,2-glycosidic linkage, to the 3-arm mannose residue of the oligomannose substrate, Man$_5$GlcNAc$_2$, by N-acetylglucosaminyltransferase I (GnTI). Previously, this step was considered a prerequisite for the core fucosylation (Fig. 1A). A number of previous in vitro studies, using either crude extract (11–14) or purified enzyme (15–19), have shown that FUT8 could not transfer the fucose to high-mannose type N-glycan lacking the 3-arm GlcNAc moiety. Recently, Wang and co-workers (19) performed a comprehensive analysis using a library of 77 N-glycans, and the study demonstrated that FUT8 strictly requires the presence of a free GlcNAc moiety to the α1,3-arm in the N-glycan for the fucose transfer, whereas the requirement for the 1,6-arm is relaxed. Nevertheless, in contrast to those in vitro analysis, several previous reports have implicated a GnTI-independent fucosylation pathway in mammalian cells. Haltiwanger and co-workers (20) first reported the identification of core-fucosylated Man$_5$GlcNAc$_2$ glycoform from natural proteins in a GnTI knock-out CHO cell line. Later, $^2$The abbreviations used are: FUT8, α1,6-fucosyltransferase; GnTI, α1,3-mannosyl-glycoprotein 2-β-N-acetylglucosaminyltransferase; M5, Man$_5$GlcNAc$_2$; GnMs, GlcNAcMan$_5$GlcNAc$_2$; EPO, erythropoietin; Rb, ribonuclease B; Fmoc, N-(9-fluorenylmethoxycarbonyl); V3, third variable loop of HIV-1 gp120; EPO, erythropoietin; CT, complex type N-glycan; Endo-F3, endoglycosidase F3; PNGase F, peptide-N-glycosidase F; Man, mannose; Fuc, fucose.

$^1$To whom correspondence should be addressed: Dept. of Chemistry and Biochemistry, University of Maryland, 8051 Regents Dr., College Park, MD 20742. Tel.: 301-405-7527; E-mail: wang518@umd.edu.

This work was supported by the National Institutes of Health Grant R01 GM080374. The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
Crispin et al. (21) reported the detection of minor fractions (~5%) of core-fucosylated high-mannose glycoforms in recombinant glycoproteins produced in GnTI knock-out CHO and human HEK293S cell lines, confirming the presence of a GnTI-independent fucosylation pathway. Recently, our group has reported that expression of several glycoproteins, including ectodomain of FcγIIIa receptor, GM-CSF, and erythropoietin (EPO), in a GnTI knock-out HEK293T cell line in suspension culture produced a significant amount (3–50%) of the corresponding core-fucosylated Man₅GlcNAc₂ (M₅) glycoforms (6).

Overexpression of the FUT8 gene in this cell line led to full core fucosylation of EPO-M₅, whereas knockdown of this gene resulted in production of EPO glycoforms without core fucosylation, confirming that FUT8 is the sole enzyme responsible for core fucosylation (6). The apparently contradictory in vitro and in vivo results prompted us to revisit the substrate specificity of FUT8. We noticed that the acceptors used in those in vitro assays were either free glycans (with or without simple derivatization) or very small glycopeptide (produced from glycoproteins by intensive Pronase treatment). In contrast, all of the core fucosylations of oligomannose glycan detected in vivo are from glycoproteins. This led us to hypothesize that in addition to the N-glycan part, the peptide portion of certain glycoprotein (such as EPO) may also interact with FUT8 to facilitate the catalysis of core fucosylation of the N-glycans that otherwise have only low activity toward FUT8 (Fig. 1B). To test this hypothesis, we examined different substrates, including the Man₅GlcNAc₂ glycoforms of EPO and ribonuclease B (EPO-M₅ and Rb-M₅), HIV-1 V3 glycopeptides carrying a Man₅GlcNAc₂ or a complex type N-glycan (V3-M₅ and V3-CT), free M₅, and Fmoc-labeled Man₅GlcNAc₂-Asn (M₅-Asn-Fmoc). We found that FUT8 could efficiently core-fucosylate the M₅ in the context of EPO and the HIV-1 V3 polypeptide, without the need of an α1,3-arm GlcNAc moiety. FUT8 was also found to fucosylate the Man₅GlcNAc₂-Asn-Fmoc and even the complex type N-glycan in the context of the V3 peptide, albeit at relatively low efficiency.

Results

**FUT8-catalyzed fucosylation of EPO-M₅**

To test our hypothesis that the peptide portion of EPO may enhance the fucosylation by FUT8, we performed the in vitro reaction with EPO-M₅, following the procedures described by Wang and co-workers (Fig. 2) (19), with a human FUT8 (Thermo Fisher). We prepared the EPO-M₅ by α1,2-mannosidase treatment of the high-mannose glycoform of EPO that is produced from the HEK293T cell line. As shown in Fig. 3A, LC-MS analysis of EPO-M₅, the starting material, displayed a major peak of 23,008 Da (calculated mass = 23,011 Da), with a minor peak of 23,153 (addition of one fucose) that is derived from in vivo core fucosylation. After a 5-h reaction (Fig. 3B), three new major species emerged, with a molecular mass of 23,153, 23,300, and 23,447 Da, respectively (deconvolution data). The difference between the three species was 146 Da (a fucose moiety, suggesting the addition of 1–3 fucose moieties to the EPO glycoforms). The most abundant species corresponded to the addition of three fucose moieties to the Man₅GlcNAc₂-EPO (measured 23,447 Da, calculated mass = 23,449 Da). An extended incubation (24 h) of the reaction mixture with FUT8 (at a low concentration of 0.05 mg/ml), without replenishing any enzyme or GDP-fucose, converted the intermediates into this fully fucosylated glycoform of EPO (estimated 90%). (Fig. 3C). This result provided direct evidence that FUT8 was able to core-fucosylate Man₅GlcNAc₂ glycan in vitro in the context of EPO, lacking an unmodified GlcNAc moiety at the α1,3-arm. This experiment also confirms our previous in vitro
Revisiting substrate specificity of FUT8

**Experimental observation** that FUT8 is the sole enzyme responsible for core fucosylation of high-mannose N-glycans of glycoproteins. To confirm that fucose is specifically transferred to M5 glycan, the whole glycan was released from the transferred product and analyzed with MALDI-TOF MS. As shown in Fig. 3D, the major N-glycan (>90% in relative intensity), with a molecular mass of 1403.6 Da, matches well with the expected molecular mass of Man5GlcNAc2Fuc (M5F; calculated molecular mass = 1403.4 Da).

**Fucosylation of other M5 compounds without α1,3-arm GlcNAc (Rb-M5, V3-M5 glycopeptide, M5-Asn-Fmoc, and M5)**

In addition to EPO-M5, we examined the Man5GlcNAc2 glycoform of ribonuclease B (Rb-M5). The Rb-M5 was prepared by trimming of the commercially available bovine ribonuclease B (mixtures of Man5–9GlcNAc2 glycoforms) with 1,2-mannosidase to give the pure Man5GlcNAc2-RNase glycoform (found, 14,898 Da, calculated mass = 14,899 Da) (Fig. 4A). Interestingly, incubation of Rb-M5 with FUT8 (0.05 mg/ml) under the same conditions as for EPO-M5, resulted in almost no core fucosylation of the Rb-M5 (Fig. 4B), which was in sharp contrast to that of EPO-M5. This result suggests that an appropriate protein context is important to facilitate in vitro core fucosylation by FUT8. We have shown previously that different glycoproteins gave different levels of core fucosylation in 293GnTI−/− cells. Whereas expression of EPO-M5 resulted in >50% core fucosylation, expression of the FcγIIa receptor gave only 3% of the core-fucosylated glycoforms (6).

We next tested an HIV-1 V3 glycopeptide (33 amino acid residues) carrying a glycan (V3-M5) and also simple Man5GlcNAc2 derivatives. The results are summarized in Table 1. We observed significant core fucosylation of glycopeptide V3-M5 by FUT8 (0.05 mg/ml), giving ~30 and 50% core fucosylation within a 5- and 24-h incubation, respectively (Fig. 4D). On the other hand, we also found that an Fmoc-modified Man5GlcNAc2 glycan, M5-Asn-Fmoc, could also serve as a substrate of FUT8, albeit with lower reactivity than the V3-M5. 24-h incubation of M5-Asn-Fmoc and M5-V3 did not give a detectable fucosylated product (Fig. 4F). This result is consistent with previous reports.

**Fucosylation of M5 compounds with an unmodified GlcNAc moiety at the α1,3-arm**

To compare the contribution of the α1,3-arm GlcNAc for core fucosylation, we synthesized the corresponding α1,3-arm GlcNAc derivatives by GnTI-catalyzed transfer of a GlcNAc moiety to V3-M5, M5-Asn-Fmoc, and M5, respectively, and tested their reactivity to FUT8. We found that all of the α1,3-arm GlcNAc modified derivatives were excellent substrates of FUT8. Under the same enzymatic reaction conditions as above, 100% core fucosylation of the α1,3-arm GlcNAc modified derivatives were achieved within a 2-h incubation (Fig. 5 and Table 1). This result verified the previous conclusion that N-glycans with an unmodified GlcNAc attached to the α1,3 mannose arm are preferred substrates for FUT8.

**Fucosylation of complex type V3 glycopeptide**

A previous study showed that FUT8 was unable to transfer fucose to complex type N-glycan in which the α1,3-arm...
GlcNAc was capped by a galactose or was chemically modified (15, 19). In our study, we tested whether FUT8 could transfer fucose to Gal2GlcNA2Man3GlcNAc2 (CT) in the context of the V3 peptide (Fig. 6). We found that incubation of V3-CT with FUT8 (0.05 mg/ml) for 5 days did result in core fucosylation, giving the core-fucosylated V3 glycopeptide, albeit at a slow transformation (~30% conversion). A new species at \( m/z \) 5482.3, an addition of 146 Da to the starting V3-CT, appeared in LC-MS analysis, suggesting an addition of a fucose moiety to the V3-CT (Fig. 7, A and B). To verify that the fucose was attached to the innermost GlcNac moiety of the N-glycan, we performed Endo-F3 and PNGase F treatment of the reaction product. Endo-F3 specifically cleaves core-fucosylated N-glycans between the two GlcNac moieties, whereas PNGase F removes the whole N-glycan from the glycopeptide by hydrolyzing the glycosylamide linkage. As illustrated in Fig. 7C, treatment of the product with Endo-F3 revealed a new peak of 4261.9 Da in LC-MS analysis, which corresponds to V3 peptide plus a GlcNac and a fucose moiety. The V3-CT peak remained intact. This result indicated that the fucose was attached specifically at the innermost GlcNac of the glycopeptide. Treatment of the product with PNGase F showed a single peak of 3913.8 Da, matching well the V3 peptide backbone where the Asn is converted to Asp after removal of the whole N-glycan (Fig. 7D). This result indicated that the fucose is specifically transferred to the N-glycan, not the peptide portion. Taken together, the data clearly confirmed that fucose was specifically transferred to the innermost GlcNac of the complex type V3 glycopeptide by the FUT8.

**Discussion**

In 1968, Bosmann et al. (11) first reported a fucosyltransferase from crude extract of human HeLa cells that specifically
transfers fucose to asialoagalacto-fetuin, but not to mono- or disaccharide acceptors. Later, Wilson et al. (12) observed that rat liver microsomes contained a fucosyltransferase that catalyzed transfer of fucose to asparagine-linked GlcNAc on asialoagalactoglycopeptide (prepared from α1-acid glycoprotein), with the attachment of a GlcNAc to α1,3-mannose branch. When no GlcNAc was added to the α1,3-mannose arm, no transfer of fucose was detected, suggesting a requirement of a free GlcNAc moiety on the α1,3-mannose arm for FUT8-catalyzed fucosylation.

### TABLE 1
The structures of various substrates/products and the estimated efficiency of FUT8-catalyzed core fucosylation

| Compounds       | Starting Material | Product          | Yield |
|-----------------|-------------------|------------------|-------|
| EPO-M5          | ![Structure](structure1.png) | ![Structure](structure2.png) | 90% a |
| Rb-M5           | ![Structure](structure3.png) | Not detected     | 0%    |
| V3-M5           | ![Structure](structure4.png) | ![Structure](structure5.png) | 50% b |
| M5-Asn-Fmoc     | ![Structure](structure6.png) | ![Structure](structure7.png) | 30% b |
| M5              | ![Structure](structure8.png) | Not detected     | 0%    |
| V3-GnM5         | ![Structure](structure9.png) | ![Structure](structure10.png) | 100% c |
| GnM5-Asn-Fmoc   | ![Structure](structure11.png) | ![Structure](structure12.png) | 100% c |

* Calculated on the basis of HPLC quantification of the released N-glycans after tagging with Fmoc.
* Calculated by HPLC monitoring and quantification.
* 2-h incubation.
Serna et al. (17), and, more recently, Wang and co-workers (18, 19), have further tested the substrate specificity of FUT8 using either crude extract or purified enzyme from various resource on different N-glycan substrates. All of these studies have reached the same conclusion that FUT8 is unable to perform in vitro core fucosylation of N-glycans lacking the \( /H9251 \) 1,3-arm GlcNAc or the N-glycans in which the \( /H9251 \) 1,3-arm GlcNAc is masked or modified. These in vitro studies appeared to be contradictory to the detection of core-fucosylated high-mannose type glycoforms in GnTI knock-out CHO cell lines (20, 21). More recently, we have shown that FUT8 is the sole enzyme responsible for the full core fucosylation of the \( \text{Man}_n \text{GlcNAc}_2 \) glycoform of EPO in a GnTI knock-out and FUT8-overexpressing HEK293T cell line (6).
Revisiting substrate specificity of FUT8

The apparently contradictory results from the previous in vitro and in vivo studies prompted us to test a hypothesis that in addition to the α1,3-arm GlcNAc moiety, the protein context of the substrate could also play a role in promoting FUT8-catalyzed core fucosylation. Indeed, except for the first report in 1968, all of the in vitro characterization of FUT8 used either free glycans, glycans with simple labeling, or glycans linked to very short peptides. We notice that all in vivo core fucosylation occurred in the context of proteins. In the present study, we provided the first examples indicating that FUT8 was able to core-fucosylate Man₅GlcNAc₂ glycan in vitro when it was placed in the context of an appropriate protein, such as the EPO, a peptide, such as the V3 domain peptide, and even an Fmoc-tagged Asn moiety. We also found that the glycosylation efficiency was dependent on the context, with the EPO peptide being the most effective to promote the FUT-catalyzed fucosylation. Interestingly, we also found that the FUT8 could also fucosylate a full-length complex type N-glycan in the context of the V3 peptide, although at a much lower efficiency compared with the Man₅GlcNAc₂-V3 glycopeptide.

These results suggest that in addition to an α1,3-arm GlcNAc moiety in the glycan, FUT8 could also interact favorably with a suitable peptide portion, as in the case of EPO, or maybe even a simple hydrophobic motif, as exemplified by the Fmoc-tagged M5, to promote the fucosylation. More studies, including detailed structural analysis of enzyme FUT8 or of FUT8 in complex with selected acceptor substrates, as well as testing of additional, specifically modified N-glycan substrates, should be performed to further characterize the nature of the enzyme–substrate interactions for enhanced catalytic efficiency.

Materials and methods

Preparation of various substrates containing M5 glycan

EPO-M5 was produced by trimming EPO with high mannose glycan (EPO-HM) with α1,2-mannosidase from Bacteroides thetaiotaomicron (22). The EPO-HM was produced from the HEK293T cell line in the presence of kifunensine, the mannosidase inhibitor (23), and purified according to the protocol reported previously (6). Rb-M5 was purified by trimming ribonuclease B (Sigma-Aldrich) with the same α1,2-mannosidase. In the experiments, 100 μg of the EPO-HM or ribonuclease B was mixed with 0.5 μg of α1,2-mannosidase in 10 μl of 50 mM sodium citrate buffer, pH 5.6, containing 5 mM of calcium chloride and incubated at 37 °C for 1 h. The biotinylated V3-M5 glycopeptide with one M5 glycan on position Asn-332 was synthesized as reported (24); it was originally designed for antibody binding study in an HIV vaccine design. The M5-Asn-Fmoc was prepared from soybean flour according to a reported protocol (25). The M5 glycan with a reducing end was prepared by trimming EPO-M5 with PNGase F (6). The released M5 glycan was purified with HyperSep™ Hypercarb™ SPE cartridges (Thermo Fisher Scientific) (26). All substrates were dialyzed to MES buffer (Sigma-Aldrich), pH 7, before carrying out in vitro fucosylation.

Preparation of various substrates containing GlcNAcMan₅GlcNAc₂ (GnM5)

Each substrate (V3-M5, M5-Asn-Fmoc, and M5) was mixed with 0.5 μg/μl human GnTI (27), 3 molar eq of GlcNAc-UDP (Sigma-Aldrich) in 0.1 M Tris-HCl buffer (pH 8) containing 20 mM manganese chloride. The reaction mixture was incubated at 37 °C overnight to reach completion. All substrate was dialedyzed to MES buffer, pH 7.

In vitro substrate specificity study of FUT8

All reactions were carried out on a 10-μl scale at 37 °C. For glycoprotein substrate EPO-M5 or Rb-M5, ~0.6 nmol of substrate was mixed with 1 mM GDP-fucose (Carbosynth USA, San Diego, CA) and 0.05 μg/μl human FUT8 enzyme (Thermo Fisher Scientific) in the presence of 0.1 M MES buffer, pH 7. For other substrates (V3-M5, M5-Asn-Fmoc, M5, V3-GnM5, GnM5-AsnF-Fmoc, GnM5, and V3-CT), 3 nmol of material was used. The reaction was monitored by LC-MS analysis of reaction fractions collected at certain time points.

LC-MS analysis

To characterize the processes, each enzymatic reaction was analyzed by an ExactivePlus Orbitrap LC-MS (Thermo Fisher Scientific). The evaluation of protein reactions was performed with an Xbridge™ BEH300 C4 column (3.5 μm, 2.1 × 50 mm, Waters), under a 6-min linear gradient of 5–90% acetonitrile with 0.1% formic acid at a flow rate of 0.4 ml/min. For glycans, Fmoc-Asn-glycans, and glycopeptide, assessments were carried out by an Xbridge™ C18 column (3.5 μm, 2.1 × 50 mm; Waters) under the same gradient and flow rate as for the C4 column.

MALDI-TOF mass spectrometry analysis

To confirm that fucose was specifically transferred to M5 glycan on EPO, glycan was released from EPO-M5 fucosylation product by PNGase F. Purified glycans were then analyzed by a Bruker UltraflexXtreme MALDI-TOF/TOF mass spectrometer in positive reflector mode. 100 μg/ml 2,5-dihydroxybenzoic acid (Sigma-Aldrich) matrix was prepared in a 1:1 H₂O/ACN solution, with an additional 20 μl of N,N-dimethylaniline (Sigma-Aldrich) (28).

Author contributions—Q. Y. and L.-X. W. designed the experiments; Q. Y., R. Z., and L.-X. W. performed the experiments; Q. Y., R. Z., and L.-X. W. analyzed the experimental data; Q. Y., R. Z., and L.-X. W. wrote and edited the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgments—We thank other members of the Lai-Xi Wang laboratory for helpful discussions and technical assistance.

References
1. Dwek, R. A. (1996) Glycobiology: Toward understanding the function of sugars. Chem. Rev. 96, 683–720
2. Helenius, A., and Aebi, M. (2001) Intracellular functions of N-linked glycans. Science 291, 2364–2369
3. Li, W., Yu, R., Ma, B., Yang, Y., Jiao, X., Liu, Y., Cao, H., Dong, W., Liu, L., Ma, K., Fukuda, T., Liu, Q., Ma, T., Wang, Z., Gu, J., et al. (2015) Core...
fucosylation of IgG B cell receptor is required for antigen recognition and antibody production. *J. Immunol.* **194**, 2596–2606

4. Osumi, D., Takahashi, M., Miyoshi, E., Yokoe, S., Lee, S. H., Noda, K., Nakamori, S., Gu, J., Ikeda, Y., Kuroki, Y., Sengoku, K., Ishikawa, M., and Taniguchi, N. (2009) Core fucosylation of E-cadherin enhances cell-cell adhesion in human colon carcinoma WiDr cells. *Cancer Sci.* **100**, 888–895

5. Miyoshi, E., Noda, K., Yamaguchi, Y., Inoue, S., Ikeda, Y., Wang, W., Ko, J. H., Uozumi, N., Li, W., and Taniguchi, N. (1999) The α1–6 fucosyltransferase gene and its biological significance. *Biochim. Biophys. Acta* **1473**, 9–20

6. Yang, Q., and Wang, L. X. (2016) Mammalian α1,6-fucosyltransferase (FUT8) is the sole enzyme responsible for the N-acetylgalactosaminyltransferase I-independent core fucosylation of high-mannose N-glycans. *J. Biol. Chem.* **291**, 11064–11071

7. Wang, X., Inoue, S., Gu, J., Miyoshi, E., Noda, K., Li, W., Mizunohirikawa, Y., Nakano, M., Asahi, M., Takahashi, M., Uozumi, N., Ihara, S., Lee, S. H., Ikeda, Y., Yamaguchi, Y., *et al.* (2005) Dysregulation of TGF-β1 receptor activation leads to abnormal lung development and emphysemalike phenotype in core fucose-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 15791–15796

8. Pinho, S. S., and Reis, C. A. (2015) Glycosylation in cancer: mechanisms and clinical implications. *Nat. Rev. Cancer* **15**, 540–555

9. Sato, Y., Nakata, K., Kato, Y., Shima, M., Ishii, N., Koji, T., Takekata, K., Endo, Y., and Nagatani, S. (1993) Early recognition of hepatocellular carcinoma based on altered profiles of α-fetoprotein. *N. Engl. J. Med.* **328**, 1802–1806

10. Wang, X., Inoue, S., Gu, J., Miyoshi, E., Noda, K., Li, W., Mizunohirikawa, Y., Nakano, M., Asahi, M., Takahashi, M., Uozumi, N., Ihara, S., Lee, S. H., Ikeda, Y., Yamaguchi, Y., *et al.* (2005) Dysregulation of TGF-β1 receptor activation leads to abnormal lung development and emphysemalike phenotype in core fucose-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 15791–15796

11. Bosmann, H. B., Hagopian, A., and Eylar, E. H. (1968) Glycoprotein biosynthesis: the characterization of two glycoprotein fucosyltransferases in HeLa cells. *Arch. Biochem. Biophys.* **128**, 470–481

12. Wilson, J. R., Williams, D., and Schachter, H. (1976) The control of glyco-acet-glycoprotein and quantitative analysis of sialylated glycans by MALDI-TOF mass spectrometry. *J. Am. Soc. Mass Spectrom.* **9**, 1138–1146