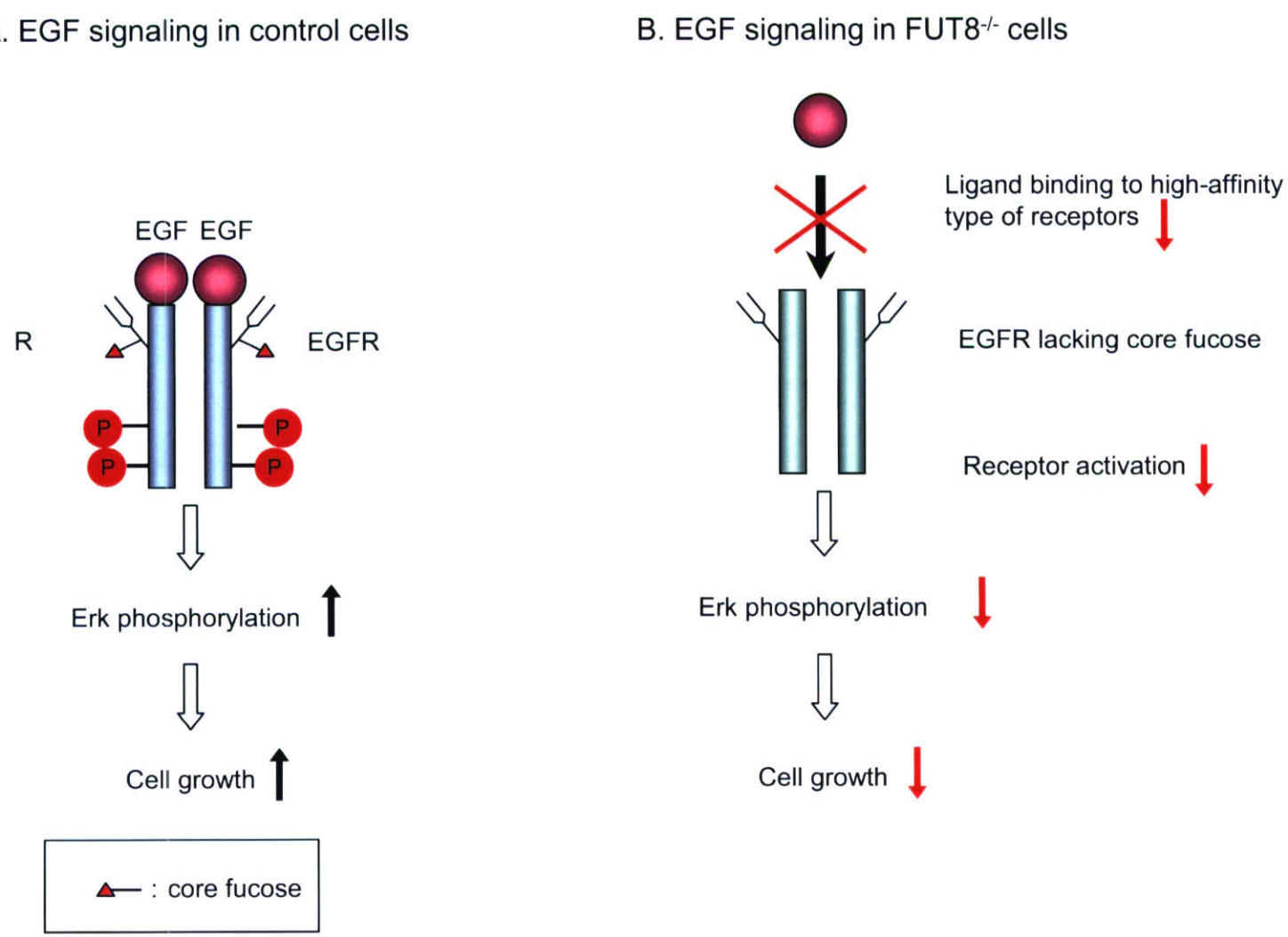
Fig. 3 Taniguchi N. et al.



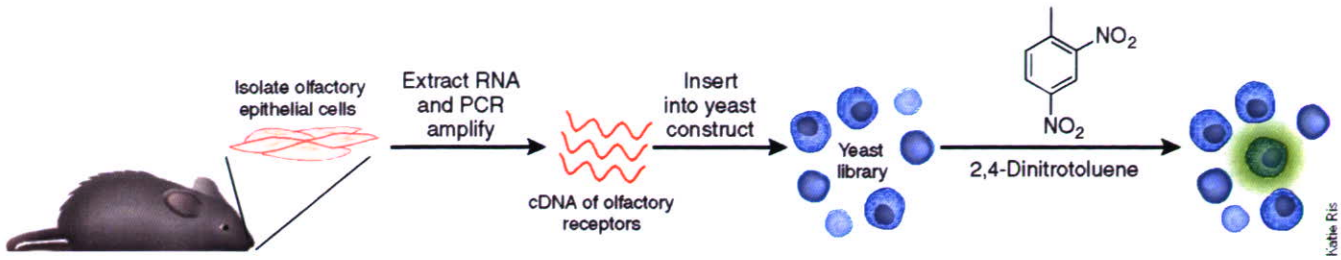


Figure 1 Radhika *et al.* isolated rat olfactory epithelial cells, extracted mRNA from the cells and created a complementary DNA library of olfactory receptor genes. These genes were then inserted into yeast cells to create a library. The yeast library was then exposed to DNT, and cells containing DNT receptors emitted green fluorescence as a result of GFP expression.

The comprehensiveness of the yeast olfactory receptor library is also not clear. Though it is known that mammalian systems have approximately 1,000 putative receptors, the number of functional receptors in the yeast library has not been determined. This determination will require that individual clones be isolated, their genes sequenced and their specificities ascertained to demonstrate that they respond to odorants. Regardless of whether DNT is a physiological ligand for the identified receptor, the identification of a yeast cell that is responsive to DNT is an important step toward developing a DNT detection method. For the yeast cells to be used as biosensors, however, it will be essential to engineer the cells such that they respond much more rapidly than the GFP-expressing cells.

The results are significant on a number of fronts. First, this yeast bioassay system should enable the specificities of a wide range of olfactory receptors to be determined. Olfactory receptors are known to be promiscuous. By examining the response of many different receptors to many different odorants, it should be possible to develop structure-activity relationships for individual receptors and get a better handle on the determinants of chemical specificity. In addition to simply matching the odorants with receptors, the system should allow dose-response relationships to be determined for various odorant-receptor pairs. Such information may help clarify why we can smell some odors better than others. Is sensitivity encoded in receptor affinity, or must we look elsewhere? Perhaps more important will be the opportunity to elucidate the combinatorial code in which the response patterns of many receptors are used to recognize both pure odorants and complex odor mixtures[5]. Second, the availability of a functional signaling pathway coupled to olfactory receptors may also enable scientists to deconstruct the various contributions of the different steps between odorant binding and signal generation. This complex cascade is postulated to provide the sensitivity that cannot be simply due to binding affinity.

With explosive-sniffing yeast in hand, one can imagine the day when an abandoned minefield will be sprayed with a slurry of yeast and nutrient broth. Wherever DNT is present in the soil, the yeast will give off a green fluorescence, indicating that a land mine is buried there.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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A sugar-coated switch for cellular growth and arrest

Naoyuki Taniguchi

The reversible attachment of an activated form of *N*-acetylglucosamine (UDP-GlcNAc) acts as a molecular switch between the growth and arrest of cells, establishing a new role for cell surface glycans.

Protein glycosylation has an important role in many cellular processes, including cell growth, cell–cell interactions, cancer metastasis, differentiation and development. Using a systems-level approach to investigate the connection between glycosylation and cellular function, Lau *et al.*[1] present a fine-tuning mechanism for switching from growth to arrest in cells based on the flux of UDP-GlcNAc through the Golgi and the extent of N-glycan branching of growth factor receptors.

Most cell surface proteins, such as growth factor receptors, are N-glycosylated. Biosynthesis of N-linked glycans is initiated in the lumen of the endoplasmic reticulum (ER) with the transfer of a precursor oligosaccharide to an asparagine residue in the Asn-X-Ser/Thr sequence within a nascent polypeptide chain. Mature N-linked glycans are generated from the differential and sequential action of glycosidases and glycosyltransferases in the ER and the Golgi apparatus, and typically fall into either high- (8–16 N-glycan sites) or low-*n* (very few N-glycan sites) classes based on the total number of sugar substituents attached (**Fig. 1**). The availability and localization of donor sugars influences the biosynthesis of N-linked glycans. UDP-GlcNAc acts as a donor sugar and is generated through the hexosamine biosynthetic pathway, a nutrient-sensing pathway. Intracellular pools of the donor sugar UDP-GlcNAc are themselves regulated by the nutrient status of the cell, as its precursors are controlled by the nucleotide, glucose, amino acid and fatty acid metabolic pathways.

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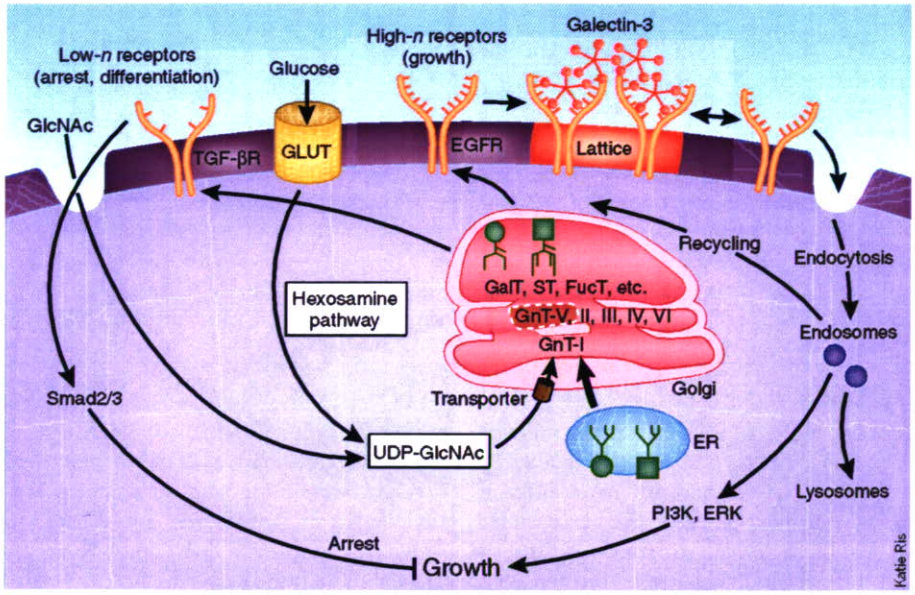

Figure 1 Once β-1,6-GlcNAc branching is added to cell surface receptors, they can associate with galectin-3 to form a molecular lattice that opposes glycoprotein endocytosis, leaving the activated receptors to reside on the cell surface where they promote signaling and growth. Sensitivity toward increasing UDP-GlcNAc levels, however, differs between high-*n* and low-*n* receptors, with high-*n* receptors showing a hyperbolic response and low-*n* receptors giving a switch-like or sigmoid response. Therefore, UDP-GlcNAc produced by the hexosamine pathway (boxes) is able to regulate the switch from growth to arrest and vice versa. ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol-3-OH kinase; FucT, fucosyltransferase; GalT, galactosyltransferase; Smad2,3, Smad-family transcription factors 2 and 3; ST, sialyltransferase.

Even though there is much heterogeneity in N-glycan structures, even within the same protein, grossly inappropriate protein glycosylation may lead to changes in protein function. For instance, unglycosylated epidermal growth factor receptor (EGFR) has been shown to undergo spontaneous oligomerization that results in constitutive activation of the receptor[2]. Conversely, overexpression of the glycosyltransferase GnT-III leads to an enhancement of EGFR internalization (reviewed in ref. 3). Mutation of other glycosyltransferase genes (*Fut8* and *Mgat4*) in mice causes dysfunction of the transforming growth factor-β receptor (TGF-βR)[4], EGFR[5] and a glucose transporter (GLUT-2)[6].

The Dennis group had previously shown that mice lacking the glycosyltransferase GnT-V (encoded by *Mgat5*) cannot add β-1,6-GlcNAc to N-glycans, so the most complex types of N-glycan, such as tetrantenna species and poly-*N*-lactosamine, cannot be formed. As a result, the mice failed to develop normally and showed a variety of phenotypes associated with altered susceptibility to autoimmune diseases, enhanced delayed-type hypersensitivity and lowered T-cell activation thresholds owing to direct enhancement of T-cell antigen-receptor clustering[7,8]. This hyperactivation of the receptors could be explained by their increased cell surface residence times caused by physical association with specific cross-linked lattices that were observed to form between poly-*N*-acetyllactosamine and galectin-3, a glycan-binding protein. The lattice thereby opposes endocytosis and subsequent receptor down-regulation (**Fig. 1**). Similarly, GnT-V–modified N-glycans are required on the receptors for the growth factors EGF, IGF, PDGF and bFGF to oppose their constitutive endocytosis[9].

To understand the role of branched N-glycan number in living cells and to rationalize the effects of altered branching on cell surface receptor function, Lau *et al.*[1] explored the enzymatic basis for biosynthesis of branched N-glycans and metabolic flux of its donor substrate, UDP-GlcNAc. They investigated the effect of UDP-GlcNAc concentration on receptors having various N-glycan status, including high-*n* receptors, such as those for the growth factors EGF, FGF and IGF, and low-*n* receptors, such as TGF-βR, CTLA-4 (cytotoxic T lymphocyte–associated protein-4) and GLUT-4 (glucose transporter-4). They found that increasing intracellular UDP-GlcNAc concentration generated a hyperbolic activation profile for high-*n* receptors but a sigmoid or switch-like profile for low-*n* receptors. This implies that N-glycan branching is extremely sensitive to increases in UDP-GlcNAc levels. Specifically, a small change in UDP-GlcNAc concentration leads to a large response from a low-*n* receptor, thereby promoting either a cell arrest or differentiation signal. These data were confirmed by structural analysis using mass spectrometry and computational modeling, and led to a model in which increased branching of N-glycans causes increased association with cell surface galectin-3 lattices and enhanced signaling. The model also invokes metabolic flux through the hexosamine pathway, which regulates UDP-GlcNAc concentration in the Golgi, as a regulator of the strength of the association with the galectin-3 lattice and thus a modulator of the choice between growth and arrest (**Fig. 1**).

Each of the numerous glycosyltransferases has a unique role, and the interplay and balance between the various glycosyltransferases is important in terms of N-glycan branching *in vivo*. In the Golgi apparatus there are numerous other glycosyltransferases beyond GnT-V, including GnT-I, GnT-II, GnT-III, GnT-IV and GnT-VI (encoded by *Mgat1*, *Mgat2*, *Mgat3*, *Mgat4* and *Mgat6*, respectively), that require UDP-GlcNAc as a donor substrate. GnT-III, for instance, catalyzes the addition of bisecting GlcNAc (β-1,4-GlcNAc branching) to biantennary sugar chains and prevents the subsequent actions of GnT-V and GnT-IV. Because it leads to the inhibition of further branch formation[3,5,10], GnT-III action has a regulatory role in the biosyntheses of N-linked oligosaccharides. Indeed, GnT-III has been shown to be an antagonist of GnT-V in terms of diminished metastatic potential and integrin-mediated cell migration[4]. Further, the phenotypic changes caused by the addition of bisecting GlcNAc would explain the deficiency of β-1,6-GlcNAc branching catalyzed by GnT-V.

Based on the kinetic parameters in the simulations used in this study, the order of actions of GnT-I, GnT-II, GnT-IV and GnT-V during biosynthesis corresponds to the order of magnitude of K_m values. The late-acting enzymes, GnT-IV and GnT-V, have much higher K_m values and so are capable of forming branches in proportion to the increase of UDP-GlcNAc level[11]. Early-acting, low-K_m enzymes yield fewer products. The higher concentration of UDP-GlcNAc generated through the hexosamine pathway and transported into the Golgi lumen is the key for generating branched glycan structures *in vivo*[11].

This enzyme system may be considered to serve as a sensor that converts information on metabolic status to extent of branching. This would further be reflected by cellular response through relative dominance of high-*n* and low-*n* glycoproteins. Interestingly, such a function of GnT-IV and GnT-V seems analogous

to glucose-sensing by glucokinase, whose K_m is much higher compared to hexokinase[12].

Although Lau *et al.* have provided convincing evidence for this switching effect in cells, its effect within whole organisms is not yet known. The switching hypothesis may open a new avenue for the understanding of growth factor receptor signaling by metabolic flux by way of the hexosamine pathway. The lattice model provided by Lau *et al.*[1] is also intriguing in that extracellular interactions can control the rate of endocytosis of cell surface receptors. Although endocytosis of receptors has been studied only with regard to cytoplasmic events such as interactions between cytoplasmic tails of receptors and adaptor proteins, this work clearly shows an extra level of control executed by the extracellular environment. Direct visualization of the lattice under several different conditions should yield fruitful indications for understanding the switch between cellular growth and arrest.

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COMPETING INTERESTS STATEMENT

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Sugars synthesized in a snap

Peng George Wang

The chemical synthesis of natural oligosaccharides by sequentially stitching monosaccharides together remains a major challenge because of the complexity of carbohydrate structures. A recent paper reports a versatile technology for creating selectively protected synthetic intermediates, thus providing easy access to complex oligosaccharides.

Understanding the biological roles of polysaccharides has been a significant challenge because of several factors, including the heterogeneity of sugar substituents, the molecular redundancy of many sugars, and their structural diversity. Accordingly, one important part of saccharide research has been the chemical synthesis of carbohydrates to confirm presumed structures or to better understand the molecular interactions driving a biological process. Because of these complex structures, however, synthetic methods for obtaining complex carbohydrates are often just as unwieldy as deciphering the original biological system. This is particularly apparent in the cumbersome and laborious preparation necessary to create selectively functionalized raw materials on a large scale. This difficulty in preparing synthetic carbohydrates is reflected in our minimal understanding of the major biological roles played by these very important biomolecules. Recent work by Wang *et al.* offers new hope for advances in carbohydrate synthesis, as they report a versatile combinatorial and regioselective one-pot methodology for the synthesis of orthogonally protected monosaccharide units[1].

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Selectively protected building blocks are necessary in the chemical synthesis of biopolymers. However, unlike proteins and nucleotides, which are linear biopolymers, the branching in carbohydrates makes their preparation more tedious because (i) more functional groups must be protected and (ii) the diversity of desired structures requires that the protecting groups not only be orthogonal (like the protecting groups on amino acid side chains and nucleotide bases) but also be amenable to further reactions. Problems are further encountered in achieving high-yielding and stereoselective couplings for multifunctional carbohydrate donors and acceptors, but these can also be traced back to the protection groups on saccharide units. To circumvent this problem, a number of different methods for selective protection and deprotection of hydroxyls on monosaccharides have been developed. For example, Wong *et al.* developed an efficient orthogonal protection-deprotection strategy to achieve combinational carbohydrate synthesis[2]. Even with this and similar advances, the arduous reactions and workup procedures that are required continue to make carbohydrate research a difficult field.

In the recent report by Wang *et al.*[1], the authors cleverly designed a new method for distinguishing the reactivity of all non-anomeric hydroxyls on glucose by preforming a *per*-trimethylsilylated glucoside (**Fig. 1**). The resultant intermediate serves as the basis for the remainder of the synthetic strategy, as it is a precursor to a panel of useful and well-characterized carbohydrate derivatives. The beauty of this one-pot method is that the orthogonal protection and selective deprotection to furnish any of the required donors can be accomplished by the sequential addition of reagents in one reaction flask without isolating any intermediates, thereby sidestepping many of the shortcomings of previous synthetic methods.

To obtain the orthogonally protected monosaccharide, trimethylsilyl trifluoromethanesulfonate (TMSOTf) was used as an ideal reagent to catalyze protecting group exchange in the one-pot strategy. Substituted and unsubstituted benzyl ethers were selected as optimal protecting groups because they can be deprotected under distinctive reaction conditions, and can thus be installed in a tandem process. In particular, the authors made judicious use of known chemistry in the initial reaction of C6-*O*-TMS acetal with an aryl aldehyde followed by the TMSOTf-catalyzed cyclization with O4 to a thermodynamically more stable six-membered cyclic arylidine. TMSOTf similarly catalyzed the formation of a second TMS acetal at the C3 position of the sugar with another aryl aldehyde. The last step of the initial sequence highlights the innovative design of this one-pot orthogonal protection strategy: the increased stability of the C2-*O*-TMS relative to the rest of the TMS ethers on the monosaccharide allowed a final conversion to an ester using catalytic TMSOTf and acid anhydride.