
# Glycosylation to Adapt to Stress

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increased liquid causes swelling of the mobile gel layer; such swelling is generally well tolerated (*8*). With decreasing hydration, the periciliary layer draws liquid from the gel layer because of the higher osmotic modulus of the periciliary layer, attenuating dehydration of the periciliary layer until the gel layer becomes too dehydrated to resist further liquid transfer. At this point, mucus clearance fails catastrophically because compression of cilia prevents their propulsive action (*3*) and probably also as a result of adhesion between the two layers (*9*).

Underhydration may be caused by a primary defect in the volume of liquid within the airway lumen in cystic fibrosis or by acquired defects in chronic obstructive pulmonary disease (COPD) and airway infections (*2*). Indirect underhydration of the gel layer may occur when polymeric mucins are produced, stockpiled, and then suddenly released in asthma, overwhelming the normal liquid volume (*10*). Another cause of underhydration in cystic fibrosis is the failure of polymeric mucins to fully expand after exocytosis due to defective bicarbonate secretion, resulting in inadequate calcium ion sequestration and excessive mucin cross-linking (*11*–*13*).

One issue not addressed by Button *et al*. is that the absence of cilia and their grafted mucins would seem to leave gaps in the periciliary macromolecular network overlying secretory cells (see the figure, panels B and C). Some space may be needed for mucin polymers to flow from secretory granules to the mobile gel layer (*14*). The gap is partially filled by the outward bulging of secretory cells (see the figure, panel C). In addition, one of the largest proteins in the mammalian genome, the mucin MUC16, is tethered to the surface of secretory cells (*4*), where it may form an effective glycoconjugate brush together with tethered mucopolysaccharides.

The gel-on-brush model proposed by Button *et al*. has the capacity to provide a common underlying mechanism to explain the progression of human airway diseases that have mucus stasis, inflammation, and infection in common. It has immediate implications for understanding how contact between pathogens and the underlying epithelial cells is prevented and how airway surface liquid is allocated between the two layers. The gel-on-brush model and the pioneering measurement methods of Button *et al*. should be used in the future to see how factors such as mesh size, osmotic modulus, rate of mucus clearance, and degree of microbial colonization change in mutant animals (for example, tethered mucin or ion channel deletants) or in conditions of challenge (for example, asthma or infection models). Eventually, these insights should help to yield novel therapeutic strategies for airway diseases.

**References and Notes**


1. A gel is a dilute macromolecular network that does not flow. In mucus, the solvent is water and the network is composed of mucin glycoproteins.
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CANCER

# Glycosylation to Adapt to Stress

Cancer cells may cope with oxidative stress through the glycosylation of a metabolic enzyme.

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The metabolism of cancer cells differs from that of nontransformed cells (*1*), yet the mechanism for regulating metabolic pathways in cancer cells is incompletely understood. On page 975 of this issue, Yi *et al*. (*2*) report that modification of the enzyme phosphofructokinase-1 (PFK1) by O-linked β-*N*-acetylglucosamine (O-GlcNAc) controls its catalytic activity in cancer cells and affects carbon distribution, redox balance, and tumor formation. Unlike most metabolic changes reported in cancer, this mode of regulation appears to be tumor specific.

PFK1 catalyzes the third step of glycolysis—the phosphorylation of fructose-6-phosphate (F6P) by adenosine 5′-triphosphate (ATP) to generate fructose-1,6-bisphosphate (FBP) and adenosine 5′-diphosphate (ADP) (see the figure). This reaction is the rate-limiting step of glycolysis under some physiological conditions (*3*), and as such, PFK1 is subject to complex allosteric regulation. PFK1 is directly inhibited by ATP, citrate, lactate, and long-chain acyl–coenzyme As (Acyl-CoAs) (*4*), likely because accumulation of these metabolites is a sign of adequate cellular nutrition. Conversely, PFK1 can be activated by adenosine 5′-monophosphate (AMP) and fructose-2,6-bisphosphate (F2,6BP) (*4*). F2,6BP is a critical determinant of physiological PFK1 activity, and F2,6BP concentration is controlled by a bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFK2), that both produces and degrades F2,6BP. Selective expression of particular PFK2 isoforms can bias PFK1 toward a more or less active state. For example, the PFKFB3 isoform of PFK2 favors production of F2,6BP and increases glycolysis (*5*), whereas the PFKFB4 isoform of PFK2 favors degradation of F2,6BP and is important for prostate cancer cell survival (*6*). Additional cell signaling events important in cancer also converge on the regulation of PFK1 activity. For instance, the gene *TIGAR*, whose expression is induced by the tumor suppressor protein p53, encodes a phosphatase that converts F2,6BP to F6P and thereby decreases glucose metabolism through PFK1. This can redirect carbon flow into the oxidative pentose phosphate pathway to produce the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), which helps cells adapt to oxidative stress (*7*).

Posttranslational modifications play a key role in controlling enzyme activity. Although PFK1 is regulated by phosphorylation (*8*), Yi *et al*. demonstrate that O-GlcNAcylation also controls its activity. An alternative product of glucose metabolism, β-*N*-acetylglucosamine (GlcNAc), can be transferred to the hydroxyl group of serine and threonine residues on proteins to generate the O-GlcNAcylated product. The degree of protein O-GlcNAcylation is determined by differential activities of the enzymes O-GlcNAc transferase and

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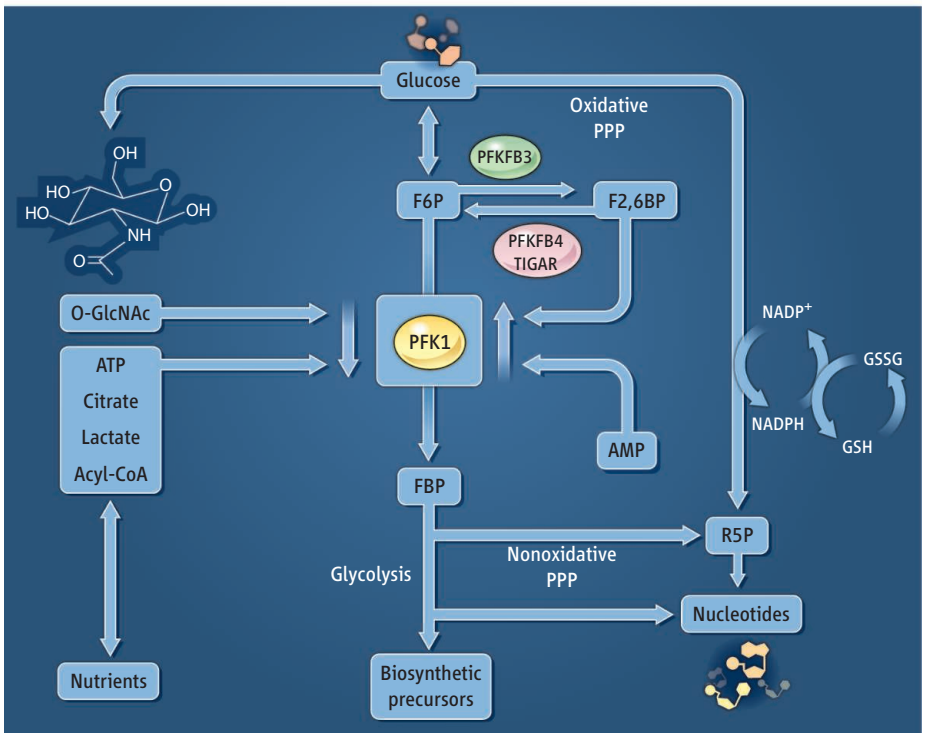


**Converging on PFK1.** PFK1 catalyzes the formation of FBP from F6P in glycolysis. Decreased PFK1 activity diverts glucose carbon from glycolysis and downstream biosynthetic pathways to the oxidative pentose phosphate pathway (PPP). This allows production of NADPH to maintain reduced glutathione pools (GSH) and allow cells to counteract oxidative stress. R5P, ribose-5-phosphate; GSH/GSSG, reduced/oxidized glutathione.

O-GlcNAcase, as well as the abundance of the metabolites used in the biosynthesis of GlcNAc (*9*). O-GlcNAc transferase expression has been linked with cancer (*10*), as well as other diseases (*9*); however, because only one enzyme catalyzes this modification and the basis for substrate specificity is unknown, the identification of key substrates has been challenging.

Yi *et al*. report that O-GlcNAcylation of a key serine residue on PFK1 ($Ser^{529}$) decreases enzyme activity and regulates central metabolism. As a result, more glucose carbon enters the oxidative pentose phosphate pathway. Because this pathway boosts the production of NADPH and reduced glutathione (GSH), cells with higher PFK1 O-GlcNAcylation are resistant to reactive oxygen species–induced cell death and proliferate better under hypoxia. Furthermore, Yi *et al*. observed that glycosylation of PFK1 leads to larger xenograft tumors in mice. Although O-GlcNAcylation of $Ser^{529}$ decreases PFK1 activity, this residue is also partially responsible for the binding of F2,6BP to PFK1, which boosts the enzyme's activity (*11*). Nevertheless, Yi *et al*. show that under conditions when PFK1 is normally glycosylated, the S529A mutant (in which $Ser^{529}$ is replaced by alanine) is more active than wild-type PFK1, suggesting that the effect of O-GlcNAcylation can be dominant.

PFK1 glycosylation is stimulated by both hypoxia and glucose deprivation and is observed in multiple human cancer cell lines and tissue samples (*2*). Hypoxia increases glucose uptake (*1*) that in turn can increase GlcNAc concentrations (*12*), whereas glucose-deprived cells increase O-GlcNAc transferase expression (*12*). Therefore, it appears that different mechanisms increase PFK1 GlcNAcylation under different physiological conditions. Intriguingly, PFK1 glycosylation is not increased in highly proliferative normal T cells or epithelial cells, indicating that this mechanism of controlling PFK1 activity is not characteristic of all dividing cells. High glycolytic rates support anabolic metabolism in many cancer and normal proliferating cells (*1*), so it is puzzling that the modification of PFK1 to lower its activity is adaptive only for transformed cells. Because PFK1 is such a proximal step in glycolysis, a decrease in enzyme activity can only redirect carbon from glucose into a limited number of pathways, and for most cancer cells this will be the oxidative pentose phosphate pathway.

Why increase activity of the oxidative pentose phosphate pathway at the expense of all other biosynthetic pathways downstream of PFK1? The pentose phosphate pathway is important for nucleotide synthesis to support cell proliferation, but the nonoxidative branch produces ribose precursors for nucleotides in many tumors (*13*). The oxidative branch of the pentose phosphate pathway also produces NADPH, and because glycosylation is dynamic, exposing cells to oxidative stress may cause PFK1 to become O-GlcNAcylated, thereby allowing rapid NADPH production. A similar response is observed with TIGAR-mediated inhibition of PFK1 to increase NADPH production (*7*). However, because the latter is a transcriptional response, which is slower than a posttranslational modification, PFK1 O-GlcNAcylation may allow cancer cells to adapt to a rapidly changing microenvironment and to the higher amounts of reactive oxygen species characteristic of transformed cells (*1*). Although counterintuitive, physiological hypoxia also leads to increased production of cellular reactive oxygen species (*14*), and increased PFK1 glycosylation may help tumors adapt to growth in low-oxygen environments.

A better understanding of how O-GlcNAc transferase substrate specificity for PFK1 is determined would aid efforts to target this mode of regulation for therapeutic intervention. Nevertheless, if PFK1 glycosylation is a response to oxidative stress rather than a mechanism to promote anabolic metabolism, there is reason for optimism that this pathway could be tumor specific and therefore a good target. Interference with PFK1 O-GlcNAcylation might find particular efficacy as an adjuvant to other forms of therapy that promote reactive oxygen species formation. Many cancers already cope with increased oxidative stress, and thus, using an agent to block this adaptive response may exploit a preexisting weakness that is specific to transformed cells.

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