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Finding the Missing Link between the Unfolded Protein Response and O-GlcNAcylation in the Heart

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Abstract

The ER stress-inducible transcription factor, x-box binding protein 1 (XBP1), which enhances protein glycosylation in the endoplasmic reticulum (ER), was shown to also enhance protein glycosylation outside the ER, via a process called O-GlcNAcylation, which protected the heart from ischemia/reperfusion damage.

Keywords

heart; cardiac; ischemia/reperfusion; ER stress; unfolded protein response; XBP1; O-GlcNAcylation

O-GlcNAcylation is a reversible post-translational modification (PTM) that takes place outside of the ER and affects the functions of target proteins. In cardiac myocytes, O-GlcNAcylation increases acutely in response to a variety of conditions, including hypoxia, ischemia, ischemia/reperfusion, and oxidative stress, during which O-GlcNAcylation is generally protective. However, chronic increases in O-GlcNAcylation during diseases, such as diabetes exacerbate cardiac dysfunction and damage. In contrast to O-GlcNAcylation, N-linked glycosylation (N-glycosylation) of proteins occurs in the ER, is relatively permanent, and is required for folding and trafficking of proteins in the ER and Golgi. N-glycosylation can be impaired by many of the same stresses that affect O-GlcNAcylation in the heart. Impaired N-glycosylation causes ER stress, subsequent activation of the unfolded protein response (UPR), and activation of the transcription factor, X-box binding protein 1 (XBP1), which induces genes that restore N-glycosylation in the ER and promote adaptation to ER stress. A recent study, published in the journal *Cell*, showed that XBP1 also enhances O-GlcNAcylation, which protects the heart from ischemia/reperfusion damage¹. Thus, in response to potentially damaging stress, XBP1 coordinates glycosylation inside and outside of the ER to confer protection.

The study by Wang et al showed that XBP1 is the missing link between protein O-GlcNAcylation and the UPR¹ (**Fig. A**). In the heart, ischemia/reperfusion leads to ER stress, activation of the UPR and XBP1, which, Wang et al determined to be a direct transcriptional activator of the gene encoding the rate-limiting step in the hexosamine biosynthetic

pathway, which supplies the substrate required for O-GlcNAcylation, i.e. uridine diphosphate N-acetyl glucosamine (UDP-GlcNAc). These findings reveal previously unappreciated links between the UPR, hexosamine biosynthesis, and O-GlcNAcylation that may serve protective roles in several tissues other than the heart.

The UPR is a conserved intracellular signaling system that is activated in response to an imbalance in ER protein homeostasis, or ER proteostasis². Conditions that impair protein folding in the ER, such as the lack of oxygen and nutrients in the ischemic heart, result in ER stress, which can lead to an imbalance in proteostasis due to the accumulation of potentially toxic misfolded proteins. One of the major ER stress signaling pathways is mediated by the ER-transmembrane protein, inositol-requiring protein-1 (IRE1). By way of mRNA splicing, IRE1 converts the XBP1 mRNA from a transcript that encodes a protein that does not have transcriptional activity, XBP1 unspliced (XBP1_u), to a transcript that encodes a form of XBP1 that is a potent transcription factor, XBP1 spliced (XBP1_s). XBP1_s-responsive genes encode proteins that, among other things, augment ER protein-folding in various ways, including restoration and fortification of protein glycosylation in the ER lumen.

Protein glycosylation is a widespread PTM that has substantial impact on the function of the heart. It can take place in the ER lumen, as well as in the cytosol, mitochondria, and nucleus (**Fig. B**)³. The O-linked protein glycosylation that takes place outside the ER is responsible for the post-translational modification of over 1,000 different proteins. O-linked glycosylation involves the post-translational addition of the monosaccharide, N-acetylglucosamine (GlcNAc), to a serine or threonine in target proteins. In contrast to N-linked glycosylation, there is no known consensus sequence on target proteins that are modified by O-linked glycosylation outside the ER. O-linked glycosylation in the cytosol, nucleus, and mitochondria, also called O-GlcNAcylation, is mediated by O-GlcNAc transferase (OGT), which uses uridine diphospho-β-N-acetylglucosamine (UDP-GlcNAc) as the glycosyl donor (**Fig. C**). N-glycosylation and O-GlcNAcylation share the intermediate, UDP-GlcNAc, which is generated from glucose by the hexosamine biosynthetic pathway (**Fig. D**). The rate-limiting step in UDP-GlcNAc formation is the conversion of fructose-6-phosphate to glucosamine-6-phosphate by the enzyme, glutamine:fructose amidotransferase (GFAT).

Before the study by Wang et al, it had been shown that ischemia could activate the UPR and XBP1 in cultured cardiac myocytes and in infarcted mouse hearts, and that XBP1 served a protective role under these conditions (**Fig. A**)⁴. Moreover, it had been shown that ischemia/reperfusion can increase protein O-GlcNAcylation in cultured cardiac myocytes and in the mouse heart, *in vivo*, and that O-GlcNAcylation protected the heart from ischemia/reperfusion damage⁵. However, the link between the UPR and O-GlcNAcylation remained unknown (**Fig. A, black box**). In pursuit of finding this missing link, Wang et al observed that, in the mouse heart ischemia/reperfusion activated ER stress, XBP1, O-GlcNAcylation and GFAT1, as well as several other enzymes in the hexosamine biosynthetic pathway. They then postulated that enzymes responsible for hexosamine biosynthesis, in particular, GFAT1, which catalyzes the rate-limiting reaction, might be transcriptionally controlled by XBP1_s. This was a critical insight that led to the discovery of the missing link. A most

insightful leap was when Wang et al went on to show that the promoter-proximal 5'-flanking region of the GFAT1 gene has sequence through which XBP1_s enhanced GFAT1 transcription in cardiac myocytes. Moreover, using combinations of XBP1 gain- and loss-of-function in the heart, *in vivo*, and in cultured cardiac myocytes, coupled with GFAT1 gain- and loss-of-function maneuvers, Wang et al provided clear mechanistic evidence supporting the hypothesis that XBP1_s is the missing link between the UPR and protein O-GlcNAcylation. Moreover, they provided evidence supporting the idea that XBP1_s-mediated increases in O-GlcNAcylation can protect the heart from ischemia/reperfusion damage, demonstrated by a reduction in infarct size.

A few questions arise from the study by Wang et al, one of which concerns the other isoform of GFAT, GFAT2, which is considered to be the major isoform of GFAT in the heart⁶. Other studies have shown that it is GFAT2, and not GFAT1 that is regulated in the heart by pressure overload or exercise^{7, 8, 9}. This leads to the question of whether GFAT2 could be regulated by XBP1_s? There is a sequence at -273 to -265 in the mouse GFAT2 promoter (TCACGTCT), which is close to the sequence and location of the XBP1_s binding site that Wang et al found at -276 of the mouse GFAT1 promoter (CCACGTCA). Both elements have the core ACGT sequence, which was previously shown to be required for XBP1_s binding¹⁰. Thus, as with GFAT1, XBP1_s might bind to the GFAT2 promoter and increase GFAT2 transcription. Wang et al briefly investigated whether GFAT2 might also be regulated by XBP1, but found that, in contrast to GFAT1, GFAT2 expression was not increased by XBP1. These results are consistent with a previous study, which showed that GFAT1, but not GFAT2, was induced by XBP1¹¹. Thus, even though a putative XBP1_s binding site exists in GFAT2, it appears that, in contrast to GFAT1, GFAT2 does not serve as an XBP1 target.

Another question that arises is how does O-GlcNAcylation protect the heart from ischemia/reperfusion damage? Answering this question will require knowledge of the proteins that are O-GlcNAcylated, as well as an understanding of how O-GlcNAcylation alters their functions. Recently, the identities of many O-GlcNAcylated cardiac proteins were identified, but just how O-GlcNAcylation affects their functions remains to be determined¹².

Is O-GlcNAcylation always cardioprotective? Although O-GlcNAcylation protects the heart from ischemia/reperfusion damage, in other settings, including the diabetic heart, O-GlcNAcylation appears to contribute to cardiac dysfunction¹³. Driven mostly by elevated glucose and the resulting increase in flux through the hexosamine biosynthesis, O-GlcNAcylation of a number of proteins increases in the diabetic heart. For example, Ca²⁺/calmodulin-dependent kinase II (CaMKII) is O-GlcNAcylated in the diabetic heart, which leads to a hyperactivation of CaMKII, increased phosphorylation of the ryanodine receptor. Ryanodine receptor phosphorylation by CaMKII results in increased calcium leaks from the sarcoplasmic reticulum, which contributes to the arrhythmia observed in diabetic cardiomyopathy¹⁴.

What roles does O-GlcNAcylation play in other cardiac pathologies? O-GlcNAcylation is increased in mouse models of pathological cardiac hypertrophy and heart failure. In hypertrophy, NFAT activation, which is a key driver of hypertrophic growth, is inhibited by

blocking O-GlcNAcylation¹⁵. Moreover, NFAT is O-GlcNAcylated, leading to speculation that cardiac hypertrophy is evoked, at least partly, by the direct O-GlcNAcylation of NFAT¹⁶. In a mouse model of heart failure induced by myocardial infarction, O-GlcNAcylation increased during heart failure¹⁷. In this study, cardiac specific deletion of OGA decreased O-GlcNAcylation, increased infarct size, and decreased survival, suggesting that O-GlcNAcylation is protective in this model of heart disease.

In summary, O-GlcNAcylation has major effects in the healthy and diseased heart. However, unlike protein phosphorylation, which governs the structure and function of a wide spectrum of hundreds of protein kinase substrates, O-GlcNAcylation addition and removal require only two enzymes; thus, the molecular mechanisms regulating the extent and determining the targets of O-GlcNAcylation must be quite different than those that regulate protein phosphorylation. The study by Wang et al has contributed significantly to our understanding of how O-GlcNAcylation can be regulated by the UPR and the transcription factor, XBP1, and that XBP1 protects the heart from ischemia/reperfusion damage, partly by increasing O-GlcNAcylation. As a result of their study, XBP1 is now also recognized for its roles as a regulator of O-glycosylation outside of the ER. These findings suggest that, by coordinating N- and O-glycosylation, XBP1 plays a pivotal role in most glycosylation events and, thus, potentially regulates the functions of a vast number of proteins. Further underscoring the potentially beneficial functions of XBP1 on an organismal level was a study that appeared in the same issue of *Cell* as the paper by Wang et al., which demonstrated that XBP1-mediated increases in hexosamine biosynthesis in *C. elegans* extended lifespan¹⁸. Taken together, these paradigm-shifting studies significantly expand our understanding of the unfolded protein response, and specifically, XBP1 as central regulators of life and death decisions in cells.

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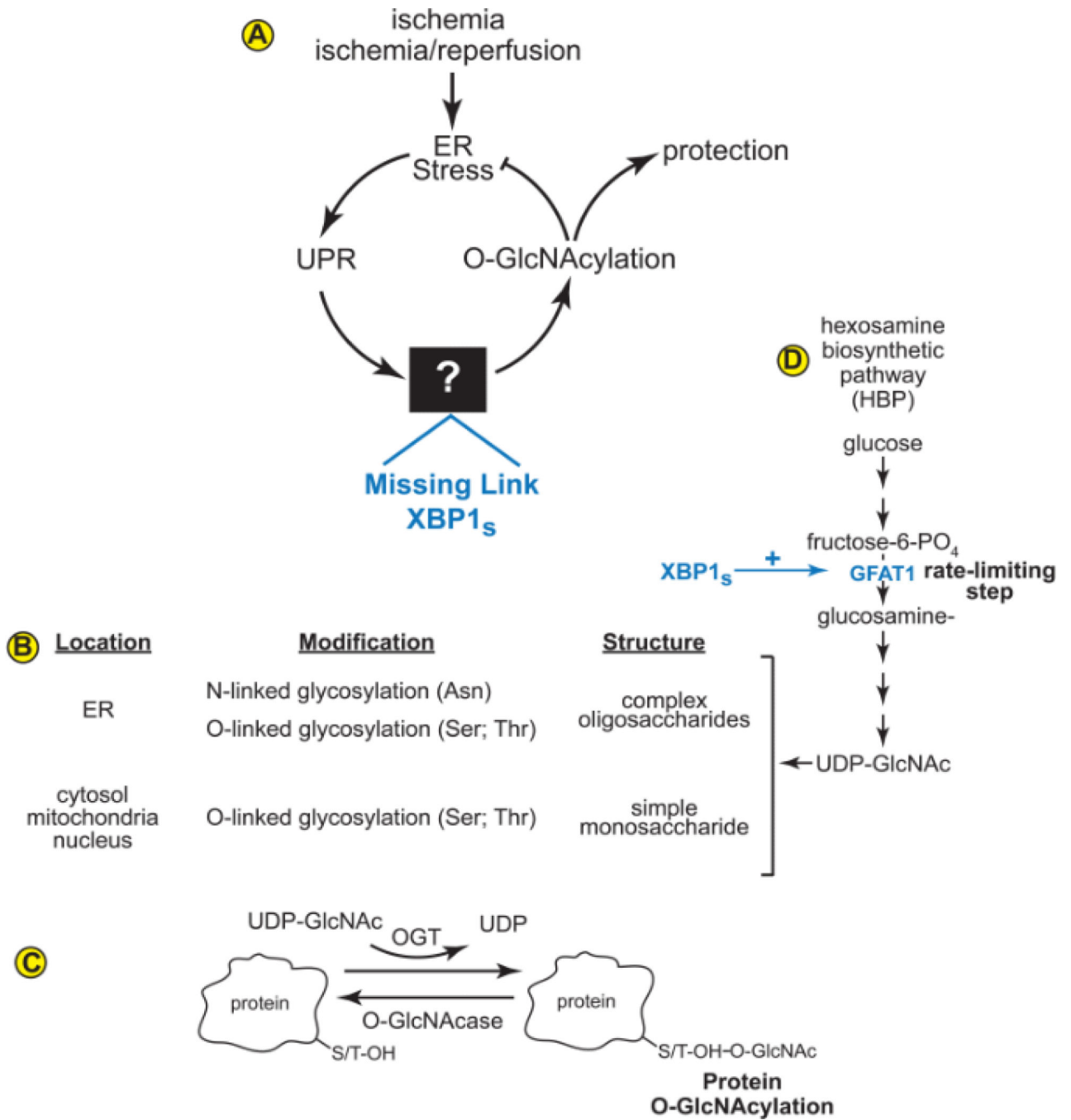


Figure. (A) XBP1 is the Missing Link between the UPR and O-GlcNAcylation. The contributions made by Wang et al are shown in blue. (B) Proteins are glycosylated in different cell compartments. (C) A key substrate for O-GlcNAcylation, which is used by the enzyme OGT (O-GlcNAc transferase), is UDP-GlcNAc (uridine diphosphate N-acetyl glucosamine). (D) UDP-GlcNAc is generated by the hexose biosynthetic pathway (HBP). O-GlcNAc groups

can be removed from proteins by OGA (O-GlcNAcase). The contributions made by Wang et al are shown in blue.