植物蛋白质*O*-糖基化修饰的研究进展

张月倩[1,2] 苏晓[1,2] 张耿[1,2] 张雪萍[1,2] 李晓娟[1,2]*

([1]北京林业大学生物科学与技术学院, 林木花卉遗传育种教育部重点实验室, 北京 100083;
[2]北京林业大学树木发育与基因编辑研究院, 北京 100083)

摘要 糖基化是最主要的蛋白质翻译后修饰方式之一, 主要有*N*-糖基化、*O*-糖基化和糖基磷脂酰肌醇锚定修饰三种类型。在植物细胞中, *O*-糖基化修饰广泛发生, 它不仅参与蛋白质转录调节、信号转导, 还与细胞壁合成等生物学过程紧密相关。在多种*O*-糖基化修饰类型中, *O*-N-乙酰氨基葡萄糖(*O*-GlcNAc)糖基化修饰结构独特、易于检测和表征, 因此已经有许多相关技术实现了对其的表征。然而, 其他类型*O*-糖基化修饰蛋白的结构和功能仍有待更全面的研究。该文综述了植物蛋白中不同类型*O*-糖基化修饰的相关研究进展, 总结了植物*O*-糖基化修饰蛋白检测技术的优缺点, 最后展望了这些技术在植物蛋白质*O*-糖基化修饰研究中的应用前景。

关键词 *O*-糖基化修饰; 植物蛋白; 生物学功能; 蛋白修饰表征

Recent Progress on Protein *O*-Glycosylation in Plants

ZHANG Yueqian[1,2], SU Xiao[1,2], ZHANG Geng[1,2], ZHANG Xueping[1,2], LI Xiaojuan[1,2]*

([1]*Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants, Ministry of Education, College of Bioscience and Bioengineering, Beijing Forestry University, Beijing 100083, China*;
[2]*Institute of Tree Development and Genome Editing, Beijing Forestry University, Beijing 100083, China*)

Abstract Glycosylation, including *N*-glycosylation, *O*-glycosylation, and glycosylphosphatidylinositol anchoring, is one of the most important post-translational modifications of proteins. *O*-glycosylation occurs widely in plant cells. It is not only involved in regulating protein transcription and signal transduction, but also closely related to cell wall synthesis. Among various kinds of *O*-glycosylated modifications, *O*-N-acetylglucosamine (*O*-GlcNAc) glycosylation which has specific structure is easy to be detected and characterized. Therefore, many techniques have been developed to detect and characterize these proteins. However, the structure and function of other *O*-glycosylated proteins still remain to be further elucidated. Here, this article reviews the research progresses of different types of *O*-glycosylated modifications in plants. Moreover, it summarizes the advantages and disadvantages of different technologies. Finally, the challenges and future directions in studying *O*-glycosylated protein in plant biology are discussed.

Keywords *O*-glycosylation; plant proteins; biological functions; protein modification characterization

蛋白质是生命体的基础物质, 其具有多种重要的翻译后修饰(protein post-translational modification, PTMs)。PTM是指对翻译后蛋白质的一个或多个氨基酸残基进行修饰的过程, 包括甲基化、磷酸化、糖基化、乙酰化、泛素化、硫酸化等不同的修饰类型。蛋白质翻译后修饰与其在机体中的功能紧密相关,

收稿日期: 2022-09-01 接受日期: 2022-09-29
国家自然科学基金(批准号: 91954202)和北京林业大学“杰出青年人才”培育计划(批准号: 2019JQ03003)资助的课题
*通讯作者。Tel: 13501323181, E-mail: lixj@bjfu.edu.cn
Received: September 1, 2022 Accepted: September 29, 2022
This work was supported by the National Natural Science Foundation of China (Grant No.91954202) and Beijing Forestry University Outstanding Young Talent Cultivation Project (Grant No.2019JQ03003)
*Corresponding author. Tel: +86-13501323181, E-mail: lixj@bjfu.edu.cn

其中, 糖基化(glycosylation)修饰是最主要的蛋白质翻译后修饰类型之一, 大多数分泌蛋白(>85%)都会发生糖基化修饰[1], 它在真核生物中普遍存在且修饰机制高度保守[2]。蛋白质的糖基化修饰主要发生在高尔基体和内质网中, 在多种糖基转移酶的作用下, 糖链与多肽链上的氨基酸残基结合并形成糖苷键。新产生的糖基化蛋白被进一步转运、剪接、修饰, 最终在特定位点发挥功能[3]。蛋白质的糖基化修饰主要有三种类型: *N*-糖基化(*N*-glycosylation)、糖基磷脂酰肌醇(glycosyl phosphatidyl inositol, GPI)锚定, 以及*O*-糖基化(*O*-glycosylation)[4]。在植物细胞中, *N*-糖基化修饰广泛发生在细胞内膜系统的大部分分泌蛋白中[5], 它可以通过影响蛋白质的折叠、定位、丰度和活性来调节糖蛋白的功能, 例如*N*-糖基化修饰参与了内质网腔内的蛋白质折叠和蛋白质质量控制的调节[4]。此外, *N*-糖基化修饰还在植物器官发育[6]、免疫防御中起调控作用[7]。GPI锚定修饰常发生在植物细胞的质膜与细胞壁连接处, 它能够将蛋白质靶向质膜的外小叶, 还能促进细胞表面的信号转导和细胞代谢[5]。*O*-糖基化修饰发生在大多数核蛋白和细胞质蛋白上, 参与了基因转录调控[8]、胞内信号转导[9]和植物细胞免疫[10]等多种细胞生物学过程。除了以单一形式调控蛋白功能外, 翻译后修饰还可以通过协同互作的方式发挥作用, 如*O*-糖基化修饰、磷酸化修饰、泛素化修饰之间常发生相互作用、相互影响, 在时间上和空间上改变蛋白质的功能[11-12]。

在真核生物中, 糖类化合物结构复杂, 糖基的组成和序列, 以及与糖基供体的连接方式各不相同。尽管存在众多差异, 糖缀合物也具有一些共同的特征, 例如相似的结构支架和末端修饰[13]。糖基化修饰蛋白的表征, 通常是进行其功能分析的第一步。在过去的几十年里, 有机化学、分析化学、化学生物学和生物化学的发展, 已经极大地促进了糖基化修饰蛋白表征技术的发展进步[14]。传统的分析方法遵循“自下而上”原则, 即先水解糖蛋白, 再单独分析单糖、聚糖以及水解产生的肽段, 最后再将单个片段的结构信息拼凑在一起, 以解析整个糖蛋白的结构特征[15]。传统分析方法已经取得了大量研究进展, 但仍存在对样品质量要求高、工作量大等局限性。近年来发展了一系列新技术, 例如蛋白质微阵列和基于质谱的蛋白质组学等。基于这些技术, 人们实现了大规模对*O*-*N*-乙酰氨基葡萄糖(*O*-*N*-acetylglucosamine, *O*-GlcNAc)修饰蛋白的表征, 为在完整蛋白质水平和特异性蛋白质位点水平上分析蛋白质*O*-糖基化修饰提供了可能[13]。

近年来已经有综述论文对*N*-糖基化修饰的结构和功能进行了总结[16], 然而对*O*-糖基化修饰的相关研究和总结较少, 尤其是植物蛋白*O*-糖基化修饰的研究仍落后于动物。在蛋白表征方式上, *O*-糖基化修饰在聚糖类型、聚糖数量及其连接位点等方面具都有高度多样性, 因而对表征技术的要求更加复杂。因此, 本文以植物蛋白*O*-糖基化修饰为重点, 综述了相关研究进展, 并讨论了目前存在的问题。

1 糖基化的分类

1.1 *N*-糖基化

N-糖基化修饰是*N*-聚糖通过*N*-糖苷键以共价方式连接到多肽链的共有序列Asn-X-Ser/Thr/Cys(其中X代表除脯氨酸之外的任何氨基酸)中的天冬酰胺残基上的过程, 且所有连接的聚糖链都有共同的五碳糖核心[17-18]。根据结构分类, *N*-聚糖通常有三种类型: 高甘露糖型、复杂型和杂合型, 三者结构复杂程度由低到高[9]。研究表明, 复杂型*N*-聚糖的结构在不同物种之间存在差异, 例如仅存在于植物的特异性*N*-聚糖。HORIUCHI等[19]利用植物中的特异性*N*-聚糖转化合成了高甘露糖型*N*-聚糖, 并成功将其构建成复杂型*N*-聚糖。杂合型*N*-聚糖的结构和组成较为复杂, 有待于进一步研究。目前已经有大量研究对不同植物物种, 包括拟南芥[20]、番茄[21]、棉花[22]、水稻[23]等的*N*-糖基化修饰蛋白组进行了分析并通过这些研究确定了大量重要的*N*-糖基化修饰蛋白。通常情况下, 与*N*-聚糖连接的蛋白质在糙面内质网和高尔基体中被剪接修饰, 最终被运输到质膜和细胞外基质等作用部位[24]。这些*N*-糖基化修饰的蛋白质在细胞壁结构修饰[25]、糖代谢[26]、信号转导[27]和病原体抵御[28]等方面发挥了重要作用。

1.2 糖基磷脂酰肌醇锚定

糖基磷脂酰肌醇锚定连接是一种在真核生物中广泛存在的连接方式, 由多糖链通过磷酸乙醇胺与蛋白质的羧基端的氨基酸连接, 肌醇上的甘油磷脂长脂肪链能够嵌入脂质双分子层, 从而将蛋白质锚定到细胞质膜上[29]。糖基磷脂酰肌醇结构保守, 包括磷酸乙醇胺桥、聚糖核心结构和由磷脂酰肌醇组成的脂质部分[30]。在植物中, 大部分GPI锚定的

蛋白质会被特异性阿拉伯半乳聚糖(arabinogalactan, AG)进一步修饰, 例如拟南芥的类成束蛋白(fasciclin-like arabinogalactan proteins, FLAs)家族已知的21个成员中, 有14个FLA蛋白具有GPI锚定结构域[31-32]。GPI锚定与某些蛋白质的最终定位有关, 许多多肽通过GPI锚定到质膜的外小叶上[16], XUE等[33]研究发现, GPI锚定序列的缺失会导致AtFLA4蛋白的定位由质膜转移到胞内。此外, GPI锚定修饰作为一种重要的糖基化修饰, 还会影响信号转导[34]、细胞壁代谢[35]和胞间连丝运输[36]等过程。

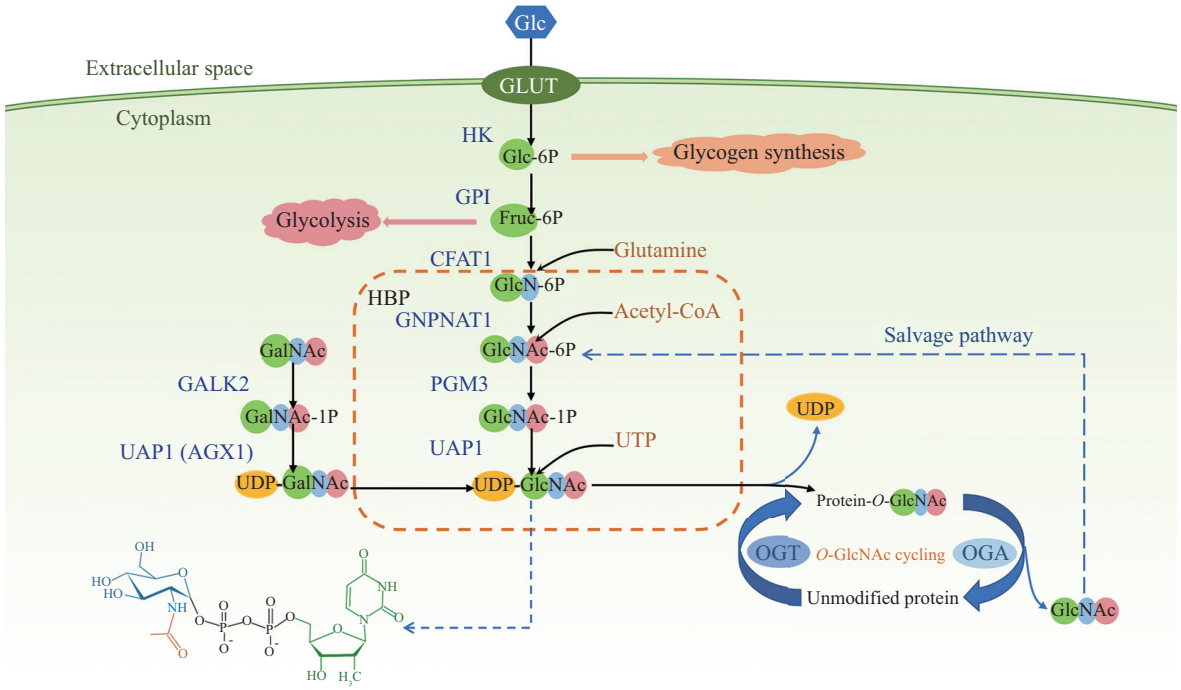
1.3 *O*-糖基化

O-糖基化是常见的翻译后修饰, 通过蛋白质肽链中的丝氨酸或苏氨酸残基上的羟基末端与糖基形成*O*-糖苷键进行修饰[37]。根据与丝氨酸或苏氨酸连接的不同类型单糖残基, *O*-糖基化修饰分为黏蛋白型和非黏蛋白型。在黏蛋白型*O*-糖基化修饰中, 与蛋白连接的单糖残基是*N*-乙酰半乳糖胺(*N*-acetylgalactosamine, GalNAc); 而在非黏蛋白型*O*-糖基化修饰中, 与蛋白连接的单糖残基可以是*N*-乙酰氨基葡萄糖(*N*-acetylglucosamine, GlcNAc)、岩藻糖(fucose, Fuc)、甘露糖(mannose, Man)、葡萄糖(glucose, Glu)、木糖(xylose, Xyl)、半乳糖(galactose, Gal)、阿拉伯糖(arabinose, Ara)等[38]。黏蛋白型*O*-糖基化修饰具有八个从单糖侧链连接到GalNAc的核心结构, 其表征具有一定复杂性, 且其在哺乳动物中的相关研究较多[39]。在非黏蛋白型*O*-糖基化修饰中, *O*-岩藻糖基化修饰在植物蛋白中时有发生, 它主要通过岩藻糖基转移酶将岩藻糖残基添加到蛋白质的丝氨酸或苏氨酸残基上进行修饰[40]。*O*-GlcNAc修饰是一种独特的非黏蛋白型*O*-糖基化修饰类型, 它仅由GlcNAc单糖修饰, 且不存在糖链的延伸[41]。目前已经发现上千种*O*-GlcNAc修饰蛋白, 由于其结构简单而独特, 相关蛋白的检测和鉴定易于进行, 因此其结构和功能已经有很多研究报道[41-43]。此外, 还有一些非黏蛋白型*O*-糖基化修饰在植物中含量极少或未被发现, 如*O*-甘露糖基化修饰最初在酵母中被发现, 后来也被发现存在于细菌和人类蛋白中, 但在植物中未被发现[44]。

2 植物蛋白*O*-糖基化修饰途径

在真核生物中, 大多数类型的*O*-糖基化修饰是在内质网中启动的, 只有*O*-GalNAc修饰和*O*-蛋白聚糖(proteoglycan, PG)糖基化修饰是在高尔基体中启动的。几乎所有类型的*O*-糖基化修饰都有多肽糖基转移酶的参与, 大多数聚糖被运转到高尔基体, 随后在不同酶的催化作用下依次添加单糖, 进一步拉长糖链或进行末端分支[16,45]。大多数*O*-糖基化修饰途径是复杂的, 如在*O*-GalNAc修饰途径中有多达20种同工酶参与*O*-聚糖修饰位点和结构模式的调控; *O*-蛋白聚糖糖基化修饰途径中具有一条或多条复杂的线性聚糖长链, 因而难以对其发生途径进行分析[45]。相比而言, *O*-GlcNAc仅由单糖修饰, 并且几乎只作用于细胞核、细胞质和线粒体中的蛋白质, 因此目前对*O*-GlcNAc修饰的研究相对较深入。在植物中, *O*-岩藻糖转移酶SPINDLY(SPY)和*O*-GlcNAc转移酶SECRET AGENT(SEC)是两种重要的*O*-糖基化相关酶, 它们在植物生长发育中的功能既有特异性也有冗余性[46-47]。从进化发育的角度来看, SPY和SEC是高等植物中特有的两个*O*-糖基化酶的分支, 目前已经在小麦[48]和拟南芥[49]等植物中得到证明。SPY作用于特定底物蛋白能够使该蛋白发生*O*-岩藻糖基化修饰并促进糖链的延伸, 糖链长度的差异也在一定程度上影响着*O*-岩藻糖基化修饰的功能。SPY和SEC均被报道有*O*-GlcNAc转移酶活性, 但两者的生物学功能不完全相同。SPY和SEC通过作用于不同的靶蛋白及途径介导了不同的生物过程, 研究发现, SPY介导的*O*-GlcNAc修饰途径在拟南芥的开花时间、生物周期调控中发挥了更重要的作用[50]。下文以*O*-GlcNAc修饰为例, 介绍了植物蛋白*O*-GlcNAc修饰途径及其关键酶。

细胞从胞外环境摄取的葡萄糖大部分将用于糖酵解和糖原合成途径, 但还有小部分(2%~5%)的葡萄糖会被导入己糖胺生物合成途径(hexosamine-biosynthesis pathway, HBP)[51]。HBP是UDP-GlcNAc的从头合成途径, 也是葡萄糖代谢中的一种非常规途径, 经典的HBP由四个关键的连续酶促反应组成[3]。在这四个酶促反应中, 首先是果糖-6-磷酸(Fru-6P)在谷氨酰胺–果糖-6-磷酸氨基转移酶1(GFAT1)的催化下转化为葡萄糖胺-6-磷酸(GlcN-6P)。随后GlcN-6P在乙酰辅酶A的催化下产生*N*-乙酰氨基葡萄糖-6-磷酸(GlcNAc-6P), GlcNAc-6P受到异构酶的催化转化为GlcN-1P。GlcN-1P发生尿苷化修饰, 产生蛋白质*O*-GlcNAc糖基化的关键底物: 尿苷二磷酸GlcNAc(UDP-GlcNAc)[52]。UDP-GlcNAc是*O*-GlcNAc修饰过程中的唯一糖供体, 也是一种几乎参与动植物中每个代谢途径的生物合成前体。UDP-GlcNAc介导了葡萄糖、氨基酸、脂肪



葡萄糖进入己糖胺生物合成途径(HBP)后, 经HBP合成尿苷二磷酸GlcNAc(UDP-GlcNAc), UDP-GlcNAc作为底物进入*O*-GlcNAc循环, 分别在OGT和OGA的作用下实现蛋白质的*O*-GlcNAc修饰以及去糖基化修饰。HK: 己糖激酶; GPI: 葡萄糖-6-磷酸异构酶; GFPT1(也称GFAT): 谷氨酰胺-果糖-6磷酸酰胺转移酶; GNPNAT1(也称GNA1): 氨基葡萄糖-6-磷酸*N*-乙酰转移酶; PGM3(也称AGM1): 磷酸乙酰葡萄糖胺变位酶; UAP1: UDP-*N*-乙酰己糖胺焦磷酸化酶; GALK2: *N*-乙酰半乳糖胺激酶; GALE: UDPG半乳糖4'-差向异构酶。

When glucose enters the hexosamine biosynthetic pathway (HBP), uridine diphosphate GlcNAc (UDP-GlcNAC) is produced through the HBP pathway. Subsequently, UDP-GlcNAC enters the *O*-GlcNAc cycle as a substrate. Under the catalysis of OGT or OGA, *O*-GlcNAc or deglycosylation modification of protein occurs. HK: hexokinase; GPI: glucose-6-phosphate isomerase; GFPT1 (also known as GFAT): glutamine-fructose-6 phosphoamide-transferase; GNPNAT1 (also known as GNA1): glucosamine 6-phosphate *N*-acetyltransferase; PGM3 (also known as AGM1): acetylglucosamine phosphate mutase; UAP1: UDP-*N*-acetylhexosamine pyrophosphorylase; GALK2: *N*-acetylgalactosamine kinase; GALE: UDPG galactose 4'-differential isomerase.

图1 己糖胺生物合成途径(HBP)和蛋白质*O*-GlcNAc循环(根据参考文献[13]修改)

Fig.1 Hexosamine biosynthesis pathway and protein *O*-GlcNAc cycle (modified from the references [13])

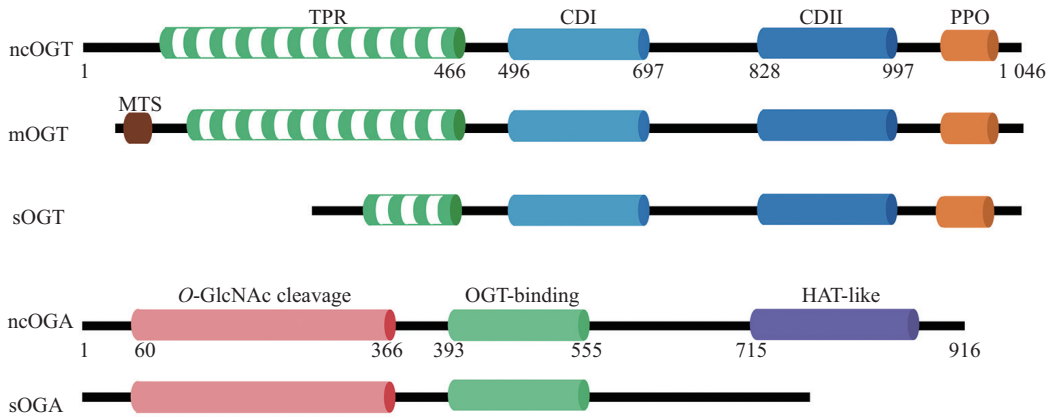
酸和核苷等大部分胞内产物的代谢反应, 因此也被称之为葡萄糖传感器[53]。 最后, 在乙酰氨基转移酶(*O*-GlcNAc transferase, OGT)的作用下, *O*-GlcNAc被连接到细胞内底物蛋白质的丝氨酸或苏氨酸残基上。*O*-GlcNAc糖基化修饰是一种动态、可逆的过程, β-*N*-乙酰氨基己糖苷酶(*O*-GlcNAcase/OGA)可以催化去除蛋白质*O*-GlcNAc修饰[54]。而细胞内游离的GlcNAc可通过GlcNAc回收途径重新转化为GlcNAc-6P, 从而再次进入HBP循环(图1)。

在细胞中, OGT和OGA协同合作, 确保了*O*-GlcNAc循环的正常发生(图1)。OGT有三种亚型: 核质型OGT(nuclear-cytoplasmic OGT, ncOGT)、线粒体型OGT(mitochondrial OGT, mOGT)和短链型OGT(short-chain OGT, sOGT)[3]。它们具有相同的羧基末端催化结构域和磷酸肌醇结合结构域, 但是氨基末端四肽的排列方式各不相同, 序列长度也有差异[42,55]。此外, 这三种亚型的亚细胞定位并不相同, ncOGT和sOGT定位于细胞质和细胞核, 而mOGT因为有独特的线粒体靶向序列, 所以只存在于线粒体中[55-56]。目前, 对于OGT的底物识别机制最认可的假设是: OGT的三十四肽重复序列(tetratricopeptide repeat, TPR)结构域负责底物的选择和结合[57]。OGA有两种亚型: 核质型OGA(nuclear-cytoplasmic OGA, ncOGA)和短链型OGA(short-chain OGA, sOGA)[3]。ncOGA同时拥有氨基端*O*-GlcNAc水解酶结构域和羧基端组蛋白乙酰转移酶样(帽状)结构域, 而sOGA缺乏帽状结构域[58]。此外, ncOGA主要定位于细胞质和细胞核中, 而sOGA主要定位于内质网中[42](图2)。

3 植物蛋白*O*-糖基化的功能

3.1 *O*-糖基化修饰调节转录和细胞内信号转导

在植物中广泛存在*O*-糖基化修饰蛋白, 不同类



OGT的三种亚型都具有两个羧基末端催化结构域(carboxyl terminal domains I and II, CDI/II)和一个磷酸肌醇结合结构域(phosphatidylinositol-3,4,5-trisphosphate-binding domain, PPO), 但三十四肽重复序列(tetratricopeptide repeat sequence, TPR)的数量差异导致它们的长度不同。此外, mOGT氨基末端具有线粒体靶向序列(mitochondria targeting sequence, MTS)。OGA的两种亚型在氨基端具有相同的*O*-GlcNAc水解酶结构域, 序列中段有OGT结合区, 但sOGA缺乏羧基末端类组蛋白乙酰转移酶结构域。

The three isoforms of OGT all have two carboxy-terminal domains (CDI and II) and one phosphatidylinositol-3,4,5-trisphosphate-binding domain (PPO), but the different numbers of the tetratricopeptide repeat sequence (TPR) resulting in the varying lengths. Furthermore, the mOGT has mitochondria targeting sequence (MTS) in the N-terminal. Both isoforms of OGA have the *O*-GlcNAc hydrolase domain in the N-terminal and OGT-binding domain in the central part of the sequence, but the sOGA lacks the C-terminal histone acetyltransferase-like domain.

图2 OGT和OGA亚型的蛋白序列示意图(根据参考文献[3]修改)

Fig.2 A schematic of OGT and OGA subtypes amino acid sequence (modified from the reference [3])

型的*O*-糖基化修饰在维持植物细胞正常的信号转导过程中发挥着重要作用。在拟南芥中, AtACINUS是哺乳动物剪接调控蛋白的同源物, AtACINUS蛋白普遍发生SEC和SPY介导的*O*-糖基化修饰, 包括*O*-GlcNAc修饰和*O*-岩藻糖基化修饰。*O*-糖基化修饰激活了AtACINUS介导胞内转录、选择性剪接等多种细胞过程的重要功能。此外, AtACINUS对开花抑制因子*FLC*转录的负调控和对ABA信号负调节因子*ABH1*和*HAB1*的转录调控都依赖其*O*-糖基化修饰的发生[14]。XING等[59]发现拟南芥H3K4组蛋白甲基转移酶ATX1(ARABIDOPSIS HOMOLOG OF TRITHORAX1)介导开花调控因子*FLC*基因座中H3K4me3的甲基化, 最终影响拟南芥的花期, 而ATX1功能的激活需要在SEC催化下才能发生*O*-GlcNAc修饰。DELLAs是赤霉素信号的负调控因子, 也是各种信号通路的中枢蛋白[60]。ZENTELLA等[61]发现DELLA蛋白普遍在SPY的作用下发生*O*-岩藻糖基化修饰, *O*-岩藻糖基化修饰激活了DELLA蛋白, 并促使了其与其他蛋白相互作用, 共同调控了某些关键基因的转录, 进而影响了细胞生长。

近年来, 许多研究表明*O*-糖基化对植物细胞内的信号转导也有重要影响。*O*-糖基化修饰在调节生物钟系统中发挥着十分重要的作用, 哺乳动物中主要是*O*-GlcNAc修饰起调控作用, 而在高等植物中, 主要是*O*-岩藻糖基化修饰参与调控[61-62]。WANG等[63]探究了*O*-岩藻糖化对拟南芥生物钟信号的调节机制, 发现了生物钟蛋白的核心成分PRR5(PSEUD*O*-RESPONSE REGULATOR5)在SPY的作用下普遍发生*O*-岩藻糖基化修饰, PRR5是SPY主要的下游靶点, *O*-岩藻糖基化通过调节PRR5的活性从而特异性地介导其对生物钟信号的调节。此外, *O*-糖基化修饰还能通过和其他翻译后修饰相互作用, 共同调控蛋白功能。磷酸化是真核生物中广泛存在的共价修饰, 其修饰位点与*O*-GlcNAc糖基化重合于蛋白质丝氨酸/苏氨酸残基的羟基末端, 二者之间广泛存在相互影响、相互作用的现象[5]。XU等[64]利用凝集素亲和色谱等方法从冬小麦胚芽中鉴定出168种*O*-GlcNAc修饰的春化相关蛋白, 其中有31种同时具有*O*-GlcNAc修饰和磷酸化修饰。春化相关蛋白TaGRP2具有*O*-GlcNAc(S87位点)和磷酸化(S152位点)修饰, 这两个位点的单一突变均影响了TaGRP2与春化关键蛋白的结合, 最终影响小麦开花。KAASIK等[12]发现, *O*-GlcNAc修饰通过调控生物钟蛋白的转录活性来影响生物钟, 当*O*-GlcNAc修饰位点被磷酸化修饰竞争性占据时, 生物钟的节律发生微调。

3.2 *O*-糖基化修饰影响细胞壁的合成

细胞壁由多糖和多种富含羟脯氨酸糖蛋白(hydroxyproline-rich glycoproteins, HRGPs)组成, 其中包括植物细胞壁伸展蛋白(extensin, EXTs)和阿拉伯半乳聚糖蛋白(arabinogalactan proteins, AGPs)[65]。羟脯氨酸(Hyp)-*O*-阿拉伯糖基化修饰通常以线性低聚阿拉伯糖链的形式作用于植物细胞糖蛋白的Hyp残基, 并通过促进蛋白的构象稳定发挥作用[66]。在自然界中, EXTs常发生高频率的Hyp-*O*-阿拉伯糖基化修饰, 有研究发现, Hyp-*O*-阿拉伯糖基化修饰的EXTs能够与III型过氧化物酶在酪氨酸残基上发生活性连接, 构成EXT连接网络。EXTs构建成的糖蛋白网络对根毛细胞的发育有着至关重要的作用, VELASQUEZ等[67]发现, Hyp-*O*-阿拉伯糖基化和苏氨酸(Ser)-*O*-半乳糖基化修饰是EXTs和EXT相关蛋白质发挥正常功能的重要保障, 当使用抑制剂破坏HRGPs和相关蛋白的Hyp-*O*-阿拉伯糖基化或Ser-*O*-半乳糖基化修饰时, 细胞壁无法正常扩张, 根毛的生长也会受损。进一步研究发现, Hyp-*O*-阿拉伯糖基化修饰在很大程度上影响着EXTs的二级结构稳定性, 当EXTs的Hyp-*O*-阿拉伯糖基化修饰缺失或遭到破坏时, EXTs无法正确折叠, 异常的构象阻碍了EXTs与其他蛋白的相互作用, 进而影响了细胞壁的生长[65,67]。此外, Hyp-*O*-阿拉伯糖基化修饰的EXTs还能与果胶等细胞壁组分发生相互作用, 共同介导细胞壁防御反应[68-69]。由此可见, *O*-糖基化修饰在很大程度上影响着包括伸展蛋白在内的HRGPs的活性和功能, 在细胞壁正常结构和功能的维持中扮演着重要角色。

3.3 *O*-糖基化修饰影响植物的生长发育

O-糖基化在维持植物的正常生长发育和抵御病害等方面也发挥着重要作用。在植物中, 由AGP半乳糖基转移酶(galactosyltransferases, GALTs)介导的Hyp-*O*-半乳糖基化修饰, 发生在大多数AGPs上[70]。当GALTs的活性被抑制或破坏时, Hyp-*O*-半乳糖基化修饰不能正常发生。BASU等[70-71]研究了五种Hyp-*O*-半乳糖基转移酶(*Hyp-O-GALT*)基因敲除突变体, 发现突变体植株中AGP的蛋白丰度均明显降低, 突变体植株表现出根毛数量减少、根毛长度变短、种皮黏液减少、结实率降低、叶片衰老加快等现象。ZHANG等[72]发现*glcat14a glcat14b*双重突变体和*glcat14a glcat14b glcat14c*三重突变体普遍表现出种子萌发延迟、毛状体分支减少、缺陷花粉数量增多等现象。最近, KAUR等[73]研究了拟南芥的*galt2 galt5 galt7 galt8 galt9*五重突变体, 发现突变体植株的花药大小和花粉活力都受到了严重损害, 花粉的内部结构缺失, 减数分裂未能正常进行。*O*-GlcNAc修饰也被证明在拟南芥[59]和小麦[64]开花过程中发挥重要作用。此外, *O*-糖基化修饰还与植物免疫防御密切相关, *O*-糖基化修饰的破坏促使植物内与假单胞菌PA-IIL同源的凝集素水平增加, 降低了青枯菌的致病性[10]。*O*-糖基化修饰是多种植物蛋白正常发挥功能的必要保障, 随着相关研究的不断深入, *O*-糖基化修饰对植物生长发育的影响也会更加明确。

4 *O*-GlcNAc修饰蛋白的表征

自糖基化蛋白质被发现以来, 人们一直希望能准确地描述糖蛋白的结构。被分离出的含量较高的糖蛋白(>picomolar)的表征已经可以通过多种分析技术实现(表1)[74]。这些技术大多基于所谓的“自下而上”原则, 即先将糖蛋白水解成肽链, 再单独分析多肽的组成和结构, 最后将信息整理汇总[75]。以高效液相色谱法(high performance liquid chromatography, HPLC)、荧光标记法或完整糖肽的液相色谱与质谱法(liquid chromatograph mass spectrometer, LC-MS)联用为中心, 并适当结合其他分析技术, 已有研究报道了多种糖基化蛋白的整体结构[38,76]。然而, 许多*O*-糖基化修饰蛋白的含量较低, 其化学计量比也很低, 因而难以对其进行检测和分析。*O*-GlcNAc修饰由于其结果简单(仅由GlcNAc单糖构成, 无糖链延伸), 而且具有重要的生物学功能, 因而大量技术被开发用于对其进行表征。随着糖基化蛋白表征技术的发展和进步, 研究人员逐步实现了从完整蛋白水平和特异性蛋白位点水平上对*O*-GlcNAc修饰蛋白的分析鉴定。此外, 已有研究对非黏蛋白型糖基化蛋白进行表征[38], 而对除*O*-GlcNAc修饰蛋白之外的非黏蛋白型*O*-糖基化修饰蛋白的表征还有待于进一步研究。

在完整*O*-GlcNAc蛋白的表征中, 化学酶标记法和代谢标记法是两个被广泛应用的方法。近年来, 以叠氮化物为代表的生物正交反应−炔烃[3+2]环加成反应(也称点击化学)迅速发展[3]。四个主要的生物正交反应, 包括Staudinger连接、铜催化的

叠氮–烷烃环化加成反应(copper-catalyzed azide–alkyne cycloaddition, CuAAC)、无铜催化的叠氮–烷烃的环化加成反应(strain-promoted alkyne-azide cycloaddition, SPAAC), 以及逆电子需求Diels-Alder反应(inverse electron demand Diels-Alder reaction, IEDDA或DAINV)。科研人员通过对糖蛋白进行叠氮标记、追踪显影与FLAG标签发生特异性反应的叠氮基团来确定糖蛋白的定位, 这在很大程度上扩展了用于探测多种聚糖(包括*O*-GlcNAc)的技术方法[77-78]。荧光标记法也越来越广泛地被应用于蛋白的检测中, WU等[79]利用荧光基团偶联唾液酸残基实现了对多种*O*-GlcNAc修饰蛋白的检测。基于荧光标记稳定、灵敏度高等特点, 一些高通量荧光检测技术被不断开发和应用[80]。对于特异性位点*O*-GlcNAc的表征常用质谱电离技术和质谱碎片技术。质谱是一个强大的工具, 能从复杂样品中获取蛋白质/肽的准确信息(如分子量、氨基酸序列, 甚至样本数量), 在质谱电离技术中, 基质辅助激光

表1 *O*-GlcNAc修饰蛋白的表征技术

Table 1 Characterization techniques of *O*-GlcNAc protein

类型 Type	方法 Method	应用 Application	优缺点 Advantages and disadvantages	参考文献 References
Characterization of intact *O*-GlcNAc proteins	Radiometric detection	Stoichiometric analysis of *O*-GlcNAc proteins, and profiling of modification sites	Although claimed to be more sensitive, this approach might result in nonspecific detection	[81-82]
	Western blot	Quantification and site localization of *O*-GlcNAcylated proteins	This method is simple, but the specificity depends on the antibody	[83]
	Lectin blot	With appropriate sample pretreatment and controls, lectins labeled with biotin or HRP can be used to probe *O*-GlcNAcylation	Although it has strong affinity, new natural lectins or synthetic lectins with higher sensitivity and specificity should be developed	[84]
	Chemoenzymatic labeling detection	Chemoenzymatic labeling and enrichment of effector proteins in host cells, and analyzing the specific-site with specific enzymes	Comparing with the traditional antibody-based immunohistochemistry, this technique, which has high-resolution, can visualize the distribution of *O*-GlcNAc proteins in cell septum or organs	[85]
	Metabolic labeling detection	It can be indirectly tagged on *O*-GlcNAc proteins for detecting dynamic modification	Despite the successful labeling of *O*-GlcNAcylation by some metabolic probes, the labeling efficiency is to be assessed	[86]
Site-specific characterization of *O*-GlcNAc protein	MALDI and ESI	Identify *O*-GlcNAc proteins, *O*-glycan types, branching structures and O-GlcNAc sites occupancy	It has been widely used to analyze complex samples, but the complicated operation and high-quality sample requirements should be taken into consideration	[87-88]
	MS fragmentation techniques	Detecting the localization of *O*-GlcNAc proteins	Although the mass spectra results are complicated to annotate, it is accurate and reliable	[89]
High-throughput characterization of *O*-GlcNAc proteins	Protein microarray	Identify hundreds even thousands of separately purified proteins at the same time, and detecting *O*-GlcNAc modification levels	As a high-throughput technique, it is widely used for large-scale characterization of proteins with high signal-to-noise ratio, but the arrays can't generate straightforward sequence information	[90]
	Proteomics	Identification the modification stie of *O*-GlcNAc and quantification of large-scale *O*-GlcNAc proteins	It can be combined with various methods in many areas. But the instrument stability and experimental operation requirements should be further improved	[91]

解吸电离(matrix-assisted laser desorption ionization, MALDI)和电喷雾电离(electrospray ionization, ESI)被广泛用于*O*-GlcNAc修饰蛋白的分析[38]。在高通量*O*-GlcNAc修饰蛋白的表征方法中, 蛋白质微阵列和基于质谱的蛋白质组学是两个高通量平台, 蛋白质微阵列能够同时对成百上千个纯化蛋白进行表征, 鉴定*O*-GlcNAc修饰蛋白; 而蛋白质组学不仅能够确定*O*-GlcNAc修饰蛋白的位点信息, 还能鉴定*O*-聚糖的类型和大小信息(表1)。

5 总结与展望

O-糖基化修饰是受到广泛关注的翻译后修饰之一, 自1984年TORRES等[92]首次发现*O*-糖基化修饰以来, *O*-糖基化修饰的相关研究已经取得了较大的进步, 目前已经鉴定出4 000多种*O*-糖基化修饰的蛋白质[93]。已有研究发现, *O*-糖基化修饰与许多人类疾病的发生密切相关[94-95]。在植物中, 蛋白糖基化修饰与植物的生长息息相关, *O*-糖基化修饰参与调控了植物生长发育的诸多过程, 同时在表观遗传上也发挥了重要作用。许多植物蛋白的活性有赖于*O*-糖基化修饰的激活, 而且一些植物糖蛋白的二级构象稳定性也需要*O*-糖基化修饰来维持。然而, 目前的研究主要是利用生化和遗传的方法, 通过对糖基化修饰过程中的关键酶的研究, 来揭示蛋白质糖基化的功能。但是对于如何表征糖基化修饰的蛋白质, 仍面临诸多难题, 特别是*O*-糖基化修饰在聚糖类型、聚糖数量及其连接位点等方面具都有高度多样性, *O*-糖基化修饰蛋白普遍难以富集。值得庆幸的是, *O*-GlcNAc修饰作为*O*-糖基化修饰中较早被深入研究的类型, 目前已经在植物的相关研究中取得了突破性进展。为了深入了解不同类型*O*-糖基化修饰蛋白在植物中的功能和应用, 特别是在活细胞中对*O*-糖基化修饰发生的过程以及其对蛋白功能的调控进行实时动态的研究, 仍需要不断开发新的技术和方法。深入探究*O*-糖基化修饰的功能和生理意义将会在一定程度上推动植物学和细胞生物学等多个领域的发展和进步。

参考文献 (References)

[1] STEENTOFT C, VAKHRUSHEV S Y, JOSHI H J. Precision mapping of the human *O*-GalNAc glycoproteome through simple cell technology [J]. EMBO J, 2013, 32(10): 1478-88.

[2] LIU W, TRIPLETT L, CHEN X L. Emerging roles of posttranslational modifications in plant-pathogenic fungi and bacteria [J]. Annu Rev Phytopathol, 2021, 59(1): 99-124.

[3] YANG X, QIAN K. Protein *O*-GlcNAcylation: emerging mechanisms and functions [J]. Nat Rev Mol Cell Biol, 2017, 18(7): 452-65.

[4] LIU C, TALBOT N J, CHEN X. Protein glycosylation during infection by plant pathogenic fungi [J]. New Phytol, 2021, 230(4): 1329-35.

[5] YEATS T H, BACIC A, JOHNSON K L. Plant glycosyl-phosphatidylinositol anchored proteins at the plasma membrane-cell wall nexus [J]. J Integr Plant Biol, 2018, 60(8): 649-69.

[6] WANG X, DENG X, ZHU D, et al. *N*-linked glycoproteome analysis reveals central glycosylated proteins involved in wheat early seedling growth [J]. Plant Physiol Biochem, 2021, 163: 327-37.

[7] CHANG Y, ZHU D, DUAN W, et al. Plasma membrane *N*-glycoproteome analysis of wheat seedling leaves under drought stress [J]. Int J Biol Macromol, 2021, 193(Pt B): 1541-50.

[8] YAN J, KIM Y J, SOMERS D E. Post-translational mechanisms of plant circadian regulation [J]. Genes, 2021, 12(3): 325.

[9] ESTEVEZ J M, STRASSER R, SEIFERT G, et al. Cracking the "sugar code": a snapshot of *N*-and *O*-glycosylation pathways and functions in plants cells [J]. Front Plant Sci, 2021, 12: 640919.

[10] ELHENAWY W, SCOTT N E, TONDO M L, et al. Protein *O*-linked glycosylation in the plant pathogen *Ralstonia solanacearum* [J]. Glycobiology, 2016, 26(3): 301-11.

[11] VU L D, GEVAERT K, DE SMET I. Protein language: post-translational modifications talking to each other [J]. Trends Plant Sci, 2018, 23(12): 1068-80.

[12] KAASIK K, KIVIM E S, ALLEN J, et al. Glucose sensor *O*-GlcNAcylation coordinates with phosphorylation to regulate circadian clock [J]. Cell Metab, 2013, 17(2): 291-302.

[13] MA J, WU C, HART G W. Analytical and biochemical perspectives of protein *O*-GlcNAcylation [J]. Chem Rev, 2021, 121(3): 1513-81.

[14] WILKINSON H, SALDOVA R. Current methods for the characterization of *O*-Glycans [J]. J Proteome Res, 2020, 19(10): 3890-905.

[15] THAYSEN-ANDERSEN M, PACKER N H. Advances in LC-MS/MS-based glycoproteomics: getting closer to system-wide site-specific mapping of the *N*-and *O*-glycoproteome [J]. Biochim Biophys Acta, 2014, 1844(9): 1437-52.

[16] NAGASHIMA Y, VON SCHAEWEN A, KOIWA H. Function of *N*-glycosylation in plants [J]. Plant Sci, 2018, 274: 70-9.

[17] VANIER G, LUCAS P L, LOUTELIER-BOURHIS C, et al. Heterologous expression of the *N*-acetylglucosaminyltransferase I dictates a reinvestigation of the *N*-glycosylation pathway in *Chlamydomonas reinhardtii* [J]. Sci Rep, 2017, 7(1): 10156.

[18] ZHANG S, LI W, LU H, et al. Quantification of *N*-glycosylation site occupancy status based on labeling/label-free strategies with LC-MS/MS [J]. Talanta, 2017, 170: 509-13.

[19] HORIUCHI R, HIROTSU N, MIYANISHI N. *N*-glycan transition of the early developmental stage in *Oryza sativa* [J]. Biochem Biophys Res Commun, 2016, 477(3): 426-32.

[20] XU S L, MEDZIHRADSZKY K F, WANG Z Y, et al. *N*-glycopeptide profiling in *Arabidopsis* inflorescence [J]. Mol Cell Proteomics, 2016, 15(6): 2048-54.

[21] CATALÁ C, HOWE K J, HUCKO S, et al. Towards characterization of the glycoproteome of tomato (*Solanum lycopersicum*) fruit

using Concanavalin A lectin affinity chromato-graphy and LC-MALDI-MS/MS analysis [J]. Proteomics, 2011, 11(8): 1530-44.

[22] KUMAR S, KUMAR K, PANDEY P, et al. Glycoproteome of elongating cotton fiber cells [J]. Mol Cell Proteomics, 2013, 12(12): 3677-89.

[23] WANG J, WEN H, LI M, et al. *N*-glycoproteome reveals that *N*-glycosylation plays crucial roles in photosynthesis and carbon metabolism in young rice leaves [J]. J Plant Biol, 2020, 63(3): 165-75.

[24] CHEN X, LIU C, TANG B, et al. Quantitative proteomics analysis reveals important roles of *N*-glycosylation on ER quality control system for development and pathogenesis in *Magnaporthe oryzae* [J]. PLoS Pathog, 2020, 16(2): e1008355.

[25] ALMEIDA F, ANTONIÊTO A C, PESSONI A M, et al. Influence of *N*-glycans on expression of cell wall remodeling related genes in *Paracoccidioides brasiliensis* yeast cells [J]. Curr Genomics, 2016, 17(2): 112-8.

[26] HARADA Y, NAKAJIMA K, SUZUKI T, et al. Glycometabolic regulation of the biogenesis of small extracellular vesicles [J]. Cell Rep, 2020, 33(2): 108261.

[27] KAJIURA H, WASAI M, KASAHARA S, et al. *N*-glycosylation and *N*-glycan moieties of CTB expressed in rice seeds [J]. Mol Biotechnol, 2013, 54(3): 784-94.

[28] SAIJO Y. ER quality control of immune receptors and regulators in plants [J]. Cell Microbiol, 2010, 12(6): 716-24.

[29] YADAV U, KHAN M A. Targeting the GPI biosynthetic pathway [J]. Pathog Global Health, 2018, 112(3): 115-22.

[30] BERNAT-SILVESTRE C, MA Y, JOHNSON K, et al. Characterization of *Arabidopsis* post-glycosylphosphatidylinositol attachment to proteins phospholipase 3 like genes [J]. Front Plant Sci, 2022, 13: 817915.

[31] SILVA J, FERRAZ R, DUPREE P, et al. Three decades of advances in arabinogalactan-protein biosynthesis [J]. Front Plant Sci, 2020, 11: 610377.

[32] HE J, ZHAO H, CHENG Z, et al. Evolution analysis of the Fasciclin-like arabinogalactan proteins in plants shows variable Fasciclin-AGP domain constitutions [J]. Int J Mol Sci, 2019, 20(8): 1945.

[33] XUE H, VEIT C, ABAS L, et al. *Arabidopsis thaliana* FLA4 functions as a glycan-stabilized soluble factor via its carboxy-proximal Fasciclin 1 domain [J]. Plant J, 2017, 91(4): 613-30.

[34] ZHOU K. Glycosylphosphatidylinositol-Anchored proteins in *Arabidopsis* and one of their common roles in signaling transduction [J]. Front Plant Sci, 2019, 10: 1022.

[35] ZHOU K. GPI-anchored SKS proteins regulate root development through controlling cell polar expansion and cell wall synthesis [J]. Biochem Biophys Res Commun, 2019, 509(1): 119-24.

[36] ZAVALIEV R, DONG X, EPEL B L. Glycosylphosphati-dylinositol (GPI) modification serves as a primary plasmodesmal targeting signal [J]. Plant Physiol, 2016, 172(2): 1061-73.

[37] MOON S P, JAVED A, HARD E R, et al. Methods for studying site-specific *O*-GlcNAc modifications: successes, limitations, and important future goals [J]. JACS Au, 2021, 2(1): 74-83.

[38] MULAGAPATI S, KOPPOLU V, RAJU T S. Decoding of *O*-linked glycosylation by mass spectrometry [J]. Biochemistry, 2017, 56(9): 1218-26.

[39] MORMANN M, PAULSEN H, PETER-KATALINIĆ J. Electron capture dissociation of *O*-glycosylated peptides: radical site-induced fragmentation of glycosidic bonds [J]. Eur J Mass Spectrom, 2005, 11(5): 497-511.

[40] HOLDENER B C, HALTIWANGER R S. Protein *O*-fucosylation: structure and function [J]. Curr Opin Struct Biol, 2019, 56: 78-86.

[41] BUTKINAREE C, PARK K, HART G W. *O*-linked β-N-acetylglucosamine (*O*-GlcNAc): extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress [J]. Biochim Biophys Acta, 2010, 1800(2): 96-106.

[42] LIU Y, YAO R, LIAN S, et al. *O*-GlcNAcylation: the “stress and nutrition receptor” in cell stress response [J]. Cell Stress Chaperon, 2021, 26(2): 297-309.

[43] CHO H J, MOOK-JUNG I. *O*-GlcNAcylation regulates endoplasmic reticulum exit sites through Sec31A modification in conventional secretory pathway [J]. FASEB J, 2018, 32(9): 4641-57.

[44] LARSEN I S B, NARIMATSU Y, CLAUSEN H, et al. Multiple distinct *O*-Mannosylation pathways in eukaryotes [J]. Curr Opin Struct Biol, 2019, 56: 171-8.

[45] JOSHI H J, NARIMATSU Y, SCHJOLDAGER K T, et al. Snapshot: *O*-Glycosylation pathways across kingdoms [J]. Cell, 2018, 172(3): 632.

[46] BI Y, DENG Z, NI W, et al. *Arabidopsis* ACINUS is *O*-Glycosylated and regulates transcription and alternative splicing of regulators of reproductive transitions [J]. Nat Commun, 2021, 12(1): 1-13.

[47] LIANG L, WANG Q, SONG Z, et al. *O*-fucosylation of CPN20 by SPINDLY derepresses abscisic acid signaling during seed germination and seedling development [J]. Front Plant Sci, 2021, 12: 724144.

[48] FAN M, MIAO F, JIA H, et al. *O*-linked N-acetylglucosamine transferase is involved in fine regulation of flowering time in winter wheat [J]. Nat Commun, 2021, 12(1): 2303.

[49] SHIMADA A, UEGUCHI-TANAKA M, SAKAMOTO T, et al. The rice *SPINDLY* gene functions as a negative regulator of gibberellin signaling by controlling the suppressive function of the DELLA protein, SLR1 and modulating brassinosteroid synthesis [J]. Plant J, 2006, 48(3): 390-402.

[50] HARTWECK L M, GENGER R K, GREY W M, et al. SECRET AGENT and SPINDLY have overlapping roles in the development of *Arabidopsis thaliana* L. Heyn [J]. J Exp Bot, 2006, 57(4): 865-75.

[51] YANG X, QIAN K. Protein *O*-GlcNAcylation: emerging mechanisms and functions [J]. Nat Rev Mol Cell Biol, 2017, 18(7): 452-65.

[52] CORK G K, THOMPSON J, SLAWSON C. Real talk: the interplay between the mTOR, AMPK, and hexosamine biosynthetic pathways in cell signaling [J]. Front Endocrinol, 2018, 9: 522.

[53] LUCENA M C, CARVALHO-CRUZ P, DONADIO J L, et al. Epithelial mesenchymal transition induces aberrant glycosylation through hexosamine biosynthetic pathway activation [J]. J Biol Chem, 2016, 291(25): 12917-29.

[54] MARTINEZ M, RENUSE S, KREIMER S, et al. Quantitative proteomics reveals that the OGT interactome is remodeled in response to oxidative stress [J]. Mol Cell Proteomics, 2021, 20: 100069.

[55] JOINER C M, LI H, JIANG J, et al. Structural characterization of the *O*-GlcNAc cycling enzymes: insights into substrate recognition and catalytic mechanisms [J]. Curr Opin Struct Biol, 2019, 56: 97-106.

[56] JANETZKO J, WALKER S. The making of a sweet modification: structure and function of *O*-GlcNAc transferase [J]. J Biol Chem, 2014, 289(50): 34424-32.

[57] STEPHEN H M, ADAMS T M, WELLS L. Regulating the regulators: mechanisms of substrate selection of the *O*-GlcNAc cycling enzymes OGT and OGA [J]. Glycobiology, 2021, 31(7): 724-33.

[58] ELSEN N L, PATEL S B, FORD R E, et al. Insights into activity and inhibition from the crystal structure of human *O*-GlcNAcase [J]. Nat Chem Biol, 2017, 13(6): 613-5.

[59] XING L, LIU Y, XU S, et al. *Arabidopsis O*-GlcNAc transferase SEC activates histone methyltransferase ATX1 to regulate flowering [J]. EMBO J, 2018, 37(19): e98115.

[60] BLANCO-TOURIÑÁN N, SERRANO-MISLATA A, ALABADí D. Regulation of DELLA proteins by post-translational modifications [J]. Plant Cell Physiol, 2020, 61(11): 1891-901.

[61] ZENTELLA R, SUI N, BARNHILL B, et al. The *Arabidopsis O*-fucosyltransferase SPINDLY activates nuclear growth repressor DELLA [J]. Nat Chem Biol, 2017, 13(5): 479-85.

[62] LI B, WANG Y, ZHANG Y, et al. PRR5, 7 and 9 positively modulate TOR signaling-mediated root cell proliferation by repressing *TANDEM ZINC FINGER* 1 in *Arabidopsis* [J]. Nucleic Acids Res, 2019, 47(10): 5001-15.

[63] WANG Y, HE Y, SU C, et al. Nuclear iocalized *O*-fucosyltransferase SPY facilitates PRR5 proteolysis to fine-tune the pace of *Arabidopsis* circadian clock [J]. Mol Plant, 2019, 13(3): 446-58.

[64] XU S, XIAO J, YIN F, et al. The protein modifications of *O*-GlcNAcylation and phosphorylation mediate vernalization response for flowering in winter wheat [J]. Plant Physiol, 2019, 180(3): 1436-49.

[65] VELASQUEZ S M, RICARDI M M, DOROSZ J G, et al. *O*-Glycosylated cell wall proteins are essential in root hair growth [J]. Science, 2011, 332(6036): 1401-3.

[66] OGAWA-OHNISHI M, MATSUSHITA W, MATSUBAYASHI Y. Identification of three hydroxyproline *O*-arabinosyltransferases in *Arabidopsis thaliana* [J]. Nat Chem Biol, 2013, 9(11): 726-30.

[67] VELASQUEZ S M, ELIANA M, CECILIA B, et al. Low sugar is not always good: impact of specific *O*-Glycan defects on tip growth in *Arabidopsis* [J]. Plant Physiol, 2015, 168(3): 808-13.

[68] CASTILLEUX R, PLANCOT B, VICRÉ M, et al. Extensin, an underestimated key component of cell wall defence [J]? Ann Bot, 2021, 127(6): 709-13.

[69] CANNON M C, TERNEUS K, QI H, et al. Self-assembly of the plant cell wall requires an extensin scaffold [J]. Proc Natl Acad Sci USA, 2008, 105(6): 2226-31.

[70] BASU D, LIANG Y, LIU X, et al. Functional identification of a hydroxyproline-*O*-galactosyltransferase specific for arabinogalactan protein biosynthesis in *Arabidopsis* [J]. J Biol Chem, 2013, 288(14): 10132-43.

[71] BASU D, TIAN L, WANG W, et al. A small multigene hydroxyproline-*O*-galactosyltransferase family functions in arabinogalactan-protein glycosylation, growth and development in *Arabidopsis* [J]. BMC Plant Biol, 2015, 15: 295.

[72] ZHANG Y, HELD M A, SHOWALTER A M. Elucidating the roles of three β-glucuronosyltransferases (GLCATs) acting on arabinogalactan-proteins using a CRISPR-Cas9 multiplexing approach in *Arabidopsis* [J]. BMC Plant Biol, 2020, 20(1): 221.

[73] KAUR D, MOREIRA D, COIMBRA S, et al. Hydroxyproline-*O*-galactosyl transferases synthesizing type II arabinogalactans are essential for male gametophytic development in *Arabidopsis* [J]. Front Plant Sci, 2022, 13: 935413.

[74] GEYER H, GEYER R. Strategies for analysis of glycoprotein glycosylation [J]. Biochim Biophys Acta, 2006, 1764(12): 1853-69.

[75] STÖCKMANN H, ADAMCZYK B, HAYES J, et al. Automated, high-throughput IgG-antibody glycoprofiling platform [J]. Anal Chem, 2013, 85(18): 8841-9.

[76] HAGAN A K, WANG M, LIU L. Current approaches to glycoprotein analysis [J]. Protein Pept Lett, 2014, 21(10): 986-99.

[77] NING X, GUO J, WOLFERT M, et al. Visualizing metabolically labeled glycoconjugates of living cells by copper-free and fast huisgen cycloadditions [J]. Angew Chem, 2008, 120(12): 2285-7.

[78] BLACKMAN M L, ROYZEN M, FOX J M. Tetrazine ligation: fast bioconjugation based on inverse-electron-demand diels alder reactivity [J]. J Am Chem Soc, 2008, 130(41): 13518-9.

[79] WU Z L, LUO A, GRILL A, et al. Fluorescent detection of *O*-GlcNAc via tandem glycan labeling [J]. Bioconjugate Chem, 2020, 31(9): 2098-102.

[80] STEPANENKO O V, SULATSKY M I, MIKHAILOVA E V, et al. New findings on GFP-like protein application as fluorescent tags: Fibrillogenesis, oligomerization, and amorphous aggregation [J]. Int J Biol Macromol, 2021, 192: 1304-10.

[81] GREIS K D, HART G W. Analytical methods for the study of *O*-GlcNAc glycoproteins and glycopeptides [J]. Methods Mol Biol, 1998, 76: 19-33.

[82] WU Z L, ROBEY M T, TATGE T, et al. Detecting *O*-GlcNAc using *in vitro* sulfation [J]. Glycobiology, 2014, 24(8): 740-7.

[83] TEO C F, INGALE S, WOLFERT M A, et al. Glycopeptide-specific monoclonal antibodies suggest new roles for *O*-GlcNAc [J]. Nat Chem Biol, 2010, 6(5): 338-43.

[84] RIOS P, CARTER T S, MOOIBROEK T J, et al. Synthetic receptors for the high-affinity recognition of *O*-GlcNAc derivatives [J]. Angew Chem Int Edit, 2016, 55(10): 3387-92.

[85] GAO L, SONG Q, LIANG H, et al. Legionella effector SetA as a general *O*-glucosyltransferase for eukaryotic proteins [J]. Nat Chem Biol, 2019, 15(3): 213-6.

[86] WALTER L A, BATT A R, DARABEDIAN N, et al. Azide-and alkyne-bearing metabolic chemical reporters of glycosylation show structure-dependent feedback inhibition of the hexosamine biosynthetic pathway [J]. Chem Bio Chem, 2018, 19(18): 1918-21.

[87] MA J, WANG W, LI Z, et al. *O*-GlcNAc site mapping by using a combination of chemoenzymatic labeling, copper-free click chemistry, reductive cleavage, and electron-transfer dissociation mass spectrometry [J]. Anal Chem, 2019, 91(4): 2620-5.

[88] LENEY A C, RAFIE K, VAN AALTEN D M F, et al. Direct monitoring of protein *O*-GlcNAcylation by high-resolution native mass spectrometry [J]. ACS Chem Biol, 2017, 12(8): 2078-84.

[89] HARDIVILLÉ S, BANERJEE P S, SELEN ALPERGIN E S, et al. TATA-box binding protein *O*-GlcNAcylation at T114 regulates formation of the B-TFIID complex and is critical for metabolic

gene regulation [J]. Mol Cell, 2020, 77(5): 1143-52.

[90] ORTIZ-MEOZ R F, MERBL Y, KIRSCHNER M W, et al. Microarray discovery of new OGT substrates: the medullo-blastoma oncogene OTX2 is *O*-GlcNAcylated [J]. J Am Chem Soc, 2014, 136(13): 4845-8.

[91] GAO J, YANG Y, QIU R, et al. Proteomic analysis of the OGT interactome: novel links to epithelial-mesenchymal transition and metastasis of cervical cancer [J]. Carcinogenesis, 2018, 39(10): 1222-34.

[92] TORRES C R, HART G W. Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surfaces of intact lymphocytes. Evidence for *O*-linked GlcNAc [J]. J Biol Chem, 1984, 259(5): 3308-17.

[93] MA J, HART G W. *O*-GlcNAc profiling: from proteins to proteomes [J]. Clin Proteom, 2014, 11(1): 8.

[94] MAGALHÃES A, DUARTE H O, REIS C A. The role of *O*-glycosylation in human disease [J]. Mol Aspects Med, 2021, 79: 100964.

[95] VAN TOL W, WESSELS H, LEFEBER D J. *O*-glycosylation disorders pave the road for understanding the complex human *O*-glycosylation machinery [J]. Curr Opin Struct Biol, 2019, 56: 107-18.