Dynamic Glycosylation of Nuclear and Cytosolic Proteins: Interplay Between O-GlcNAc and O-Phosphate in Regulatory Pathways.

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Our laboratory continues to elucidate the functions of the dynamic glycosylation of nuclear and cytoskeletal proteins by O-linked N-acetylglucosamine (O-GlcNAc). O-GlcNAc is an abundant protein modification that is as dynamic as protein phosphorylation in virtually all higher eukaryotes. **Some Recent Highlights: 1)** The O-GlcNAc Transferase (OGT) tightly associates with phosphatases, consistent with its proposed 'yin-yang' relationship to O-phosphorylation on some proteins. **2)** The major O-GlcNAc site (Ser^{16}) on the estrogen receptor ß (ER-ß) is reciprocally phosphorylated. We have shown that modification of this O-GlcNAc/O-phosphate site regulates ER-ß transcriptional activity, as well as its turnover rate. **3)** Yeast two-hybrid analyses have identified many proteins that interact with the tetratricopeptide (TPR) repeat domain of the O-GlcNAc Transferase. One of these proteins, OIP98, binds to the TPR domain via its coiled coil domain, and is highly enriched in brain. OIP98 translocates into the nucleus upon neuronal differentiation. Data suggest that OIP98 is a transcriptional coactivator, which serves to target OGT to transcriptional complexes. **4)** Many of the basal transcription factors, including the TATA-binding protein, are multiply O-GlcNAcylated. **5)** SR RNA splicing proteins are reciprocally O-GlcNAcylated/O-phosphorylated. The glyco-SR proteins are localized to the cytoplasm and the phosphoforms are localized to the nucleus. One major glyco-SR protein associates with ribosomes and appears to play a role in RNA export. **6)** Knock-out studies of OGT, in collaboration with the Marth group at UCSD, have shown that *OGT* is an X-linked gene, and that O-GlcNAc is required for life at the level of a single cell. **7)** OGT activity and selectivity are exquisitely regulated by its multimerization (mediated by the TPR domains), by its post-translational modification, and by the UDP-GlcNAc/UDP levels in the cell throughout a large concentration range (micromolar to millimolar). **8)** Facile mass spectrometric methods for mapping sites of O-GlcNAcylation/ phosphorylation have been developed and applied to synaptic vesicle regulatory proteins. **8)** Insulin promoter factor –1 (IPF-1), the glucose-sensitive transcription factor regulating insulin synthesis, and PPAR-γ, a nuclear receptor important to insulin signalling are both multiply O-GlcNAcylated. **9)** The tau microtubule-associated protein associates with OGT, perhaps bridging it to tubulin. **10)** The retinoblastoma, Rb proteins are reciprocally O-GlcNAcylated/O-phosphorylated, suggesting a role for O-GlcNAc in the cell cycle. Supported by NIH Grants HD13563, CA42486, a grant from the Juvenile Diabetes Fdn, and a grant from American Health Assistance Fdn. GWH is required to disclose that he serves on the scientific advisory board of Oxford Glycosciences.