Chemistry & Biology *13*, 923–928, September 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.chembiol.2006.09.001

Fine-Tuning ER-β Structure with PTMs

Estrogen receptor β is differentially regulated by alternative O-GlcNAcylation/O-phosphorylation at Ser^{16}. NMR, CD, and molecular dynamics analyses of model peptides [1] show that these alternative modifications induce different peptide conformations, providing a molecular basis for their differential regulation of protein function.

Protein function is regulated by over two hundred known posttranslational modifications [2]. In multicellular organisms, two of the most common and dynamic modifications of nuclear and cytosolic proteins are Ser(Thr)-O-phosphorylation and Ser(Thr)-O-β-GlcNAcylation (attachment of N-acetylglucosamine in a β-glycosidic linkage to the hydroxyl moiety of serine or threonine). These two modifications occur on hundreds to thousands of signaling and regulatory proteins. O-GlcNAc and O-phosphate not only have similar cycling rates, but also generally occur on the same proteins. Several site mapping studies have also shown that O-GlcNAc and O-phosphate can be alternatively attached to the *same* serine or threonine residue within a polypeptide [3]. The reciprocal site occupancy by either O-GlcNAc or O-phosphate leads to different functional properties or activities of proteins that have been studied thus far, including the c-*myc* oncogene, endothelial nitric oxide synthetase, RNA polymerase II, and estrogen receptor β [3, 4]. The competitive interplay between O-GlcNAc and O-phosphate cycling is important in nutrient/stress sensing, transcription, and signaling, and the balance between O-GlcNAc and O-phosphate on signaling proteins is key to normal cellular metabolism and functions. For example, tipping the balance of this interplay toward prolonged increased O-GlcNAcylation underlies glucose toxicity and insulin resistance [5], the hallmark of type II diabetes.

Estrogen receptors (ER-α and ER-β) are ligand-activated members of the nuclear receptor family of transcription factors [6]. Upon binding estrogen, the receptors bind to *cis*-regulatory DNA sequences in the promoters of target genes and activate specific transcription by physical associations with cofactors and the transcriptional machinery. Activation of estrogen receptors also involves phosphorylation at multiple sites by different kinases, and both types of ER are O-GlcNAcylated as well [4, 7]. ER-β shares many structural and functional features in common with ER-α. However, ER-β has a different tissue distribution, developmental profile, and ligand responsiveness. Prior work established that ER-β is alternately O-GlcNAcylated or O-phosphorylated at Ser^{16} within the N-terminal intrinsically disordered region of the molecule [4]. Interestingly, when O-GlcNAcylated at Ser^{16}, ER-β is less active in stimulating target gene expression, but is more stable within the cell (Figure 1). In contrast, when ER-β is O-phosphorylated at Ser^{16}, it is more active in stimulating target gene expression, but paradoxically is rapidly degraded.

In this issue of *Chemistry & Biology*, Chen and coworkers have probed the possible molecular basis for the reciprocal functions of the O-GlcNAcylation and O-phosphorylation at Ser^{16} on ER-β by examining the solution structures of model ER-β-based peptides [1]. By performing nuclear magnetic resonance (NMR), circular dichroism (CD), and molecular dynamics simulation experiments on 17-mer unmodified, O-GlcNAcylated, or O-phosphorylated peptides, they found that O-phosphorylation induced a more extended structure, discouraging the formation of a β-turn in the peptide, whereas O-GlcNAcylation promoted type II β-turn formation with a tighter turn than found in the unmodified peptide (Figure 1). In addition, the physical measurements and molecular dynamics analyses revealed subtle differences in the structures of the peptides that are dependent upon the type of modification at Ser^{16}. Chen and coworkers' findings [1] are consistent with those of Wong et al. [8], who showed that O-GlcNAcylation promoted turn-like structures in model peptides based upon the sequence of the C-terminal domain of RNA polymerase II, which is also extensively O-GlcNAcylated or O-phosphorylated in an alternate fashion [9].

The types of structural comparisons described by Chen and coworkers [1] are key to elucidating the molecular bases for the functional significance of the highly dynamic and regulated interplay between these two ubiquitous protein modifications. The distinct polypeptide conformations induced by each modification not only could account for different effects on ER-β activity, but also more broadly could regulate protein:protein interactions, influence subcellular localization and trafficking, and also influence the subsequent decoration of polypeptides with other types of posttranslational modifications (PTMs).

While studies with model peptides provide important insights, we will eventually need to extend both the structural and functional work to examine posttranslational modification-induced changes in full-length proteins. When modifications like O-GlcNAc and O-phosphate alternately occupy the same Ser(Thr) residue, site-directed mutagenesis of the site to a nonhydroxy amino acid, as is often done in the functional analysis of phosphorylation, cannot reveal the respective functions of each modification. Clearly, what is needed are facile methods to stoichiometrically modify full-length polypeptides with either O-GlcNAc or O-phosphate at the specific Ser(Thr) residues that are alternatively modified in vivo. Availability of these full-length modified proteins would allow the structure and the biological functions of each molecular species to be directly assessed both in vitro and in vivo by protein transfection into living cells that lack the native protein. Although Schultz and colleagues [10] have made advances in the development of these types of site-specific protein modification methods, the current approaches have limitations and are not yet readily

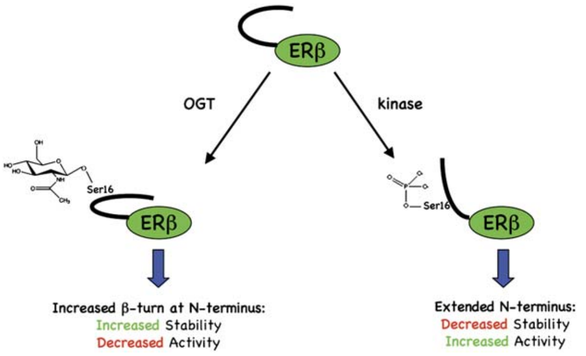

Figure 1. Regulation of Estrogen Receptor β by Alternate O-GlcNAcylation or O-Phosphorylation at Ser^{16}

O-GlcNAcylation by O-linked N-acetylglucosamine transferase (OGT) at Ser^{16} enhances the β-turn conformation at the N terminus, leading to decreased ER-β activity, but it also results in stabilization of the protein, increasing its half-life within the cell. In contrast, O-phosphorylation at Ser^{16} causes the N terminus to adopt a more extended conformation, leading to increased transcriptional activity, but also causing the protein to become rapidly degraded within the cell.

amenable to widespread use. Despite the considerable chemical and methodological challenges remaining, sorting out the distinctive functions of O-GlcNAcylated or O-phosphorylated forms of regulatory molecules will be key to understanding the regulation of signaling and transcription and the assembly of the cytoskeleton, as well as being important for developing treatments for diabetes, neurodegenerative disorders, and cancer.

Gerald W. Hart[1] and Kaoru Sakabe[1]
[1] Department of Biological Chemistry
School of Medicine
Johns Hopkins University
725 North Wolfe Street
Baltimore, Maryland 21205

Selected Reading

1. Chen, Y.-X., Du, J.-T., Zhou, L.-S., Liu, X.-H., Zhao, Y.-F., Nakanishi, H., and Li, Y.-M. (2006). Chem. Biol. *13*, this issue, 937–944.
2. Walsh, C. (2006). Posttranslational Modification of Proteins (Englewood, CO: Roberts and Company).
3. Zachara, N.E., and Hart, G.W. (2006). Biochim. Biophys. Acta *1761*, 599–617.
4. Cheng, X., and Hart, G.W. (2001). J. Biol. Chem. *276*, 10570–10575.
5. Slawson, C., Housley, M.P., and Hart, G.W. (2006). J. Cell. Biochem. *97*, 71–83.
6. Hart, L.L., and Davie, J.R. (2002). Biochem. Cell Biol. *80*, 335–341.
7. Jiang, M.-S., and Hart, G.W. (1997). J. Biol. Chem. *272*, 2421–2428.
8. Simanek, E.E., Huang, D.-H., Pasternack, L., Machajewski, T.D., Seitz, O., Millar, D.S., Dyson, H.J., and Wong, C.-H. (1998). J. Am. Chem. Soc. *120*, 11567–11575.
9. Comer, F.J., and Hart, G.W. (2001). Biochemistry *40*, 7845–7852.
10. Zhang, Z., Gildersleeve, J., Yang, Y.-Y., Xu, R., Loo, J.A., Uryu, S., Wong, C.-H., and Schultz, P.G. (2004). Science *303*, 371–373.

Chemistry & Biology *13*, September 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.chembiol.2006.09.002

A Noncanonical Path to Mechanism of Action

Improved methods for discovering small-molecule mechanisms of action are needed. In this issue of *Chemistry & Biology*, Zhang et al. [1] make clever use of the zebrafish to study the mechanism of the angiogenesis inhibitor fumagillin and reveal that it targets the noncanonical Wnt pathway.

Few things are more intriguing in the world of chemical biology than a small molecule with pronounced biological activity and therapeutic importance for which little is known of its mechanism of action. Deciphering these molecules' mechanisms of action can provide surprising biological insights and avenues for novel therapeutic approaches. One such small molecule is fumagillin, a natural product serendipitously discovered to inhibit endothelial cell proliferation. Fumagillin and its derivative TNP-470 are potent inhibitors of angiogenesis and have been the focus of numerous basic and clinical studies [2]. Although fumagillin has been shown to target methionine aminopeptidase 2 (MetAP-2) [3], little is known about how MetAP-2 inhibition exerts its antiangiogenic influence.

In this issue of *Chemistry & Biology*, Zhang et al. [1] employ a clever combination of in vivo and in vitro experiments to uncover an unexpected connection between MetAP-2 and noncanonical Wnt signaling. Their approach takes advantage of the zebrafish, a genetic model organism for which thousands of mutations and their phenotypes have been characterized and cataloged [4, 5]. Each mutant zebrafish embryo can be thought of as providing a three-dimensional readout of a gene's biological activity. The rich variety of zebrafish phenotypes makes it possible to characterize in detail the effects of disrupting a single gene product and to link a specific embryonic phenotype with the molecular disruption that causes it. The authors reasoned that treating zebrafish embryos with TNP-470 might also produce a distinct phenotype, and that by comparing the resultant phenotype with those cataloged for zebrafish gene mutations, they might be able to learn what pathway is targeted by TNP-470 (Figure 1).

In many cases, zebrafish embryos can be treated with small molecules simply by adding the molecules to the water in which the embryos are developing [6]. When the authors exposed developing zebrafish embryos to fumagillin or TNP-470, the embryos developed a defect in the anterior-posterior axis that resulted in a severely truncated tail. They also observed the same developmental defect in zebrafish embryos in which MetAP-2