REGULATION OF TERMINAL GLYCOSYLATION

Eric C. Svensson, Eryn Ujita Lee, Brian Livingston, Xiao-hong Wen, Jasminder Weinstein, and James C. Paulson.

Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024.

Mammalian cell lines used for production of recombinant glycoproteins elaborate terminal glycosylation structures on N-linked and O-linked carbohydrate groups that are determined by the glycosyltransferases expressed by these cells. As many as twelve glycosyltransferase cDNAs have now been cloned by a variety of strategies (1). By expressing these glycosyltransferase cDNAs in cells not normally expressing them, it is now possible to alter the cellular glycosylation machinery to produce new terminal glycosylation sequences (2,3). This principle was demonstrated by expressing the rat β-galactoside α2,6 sialyltransferase (α2,6ST) cDNA in CHO cells, which are known not to express the product of this sialyltransferase. After selection for stable expression, these cells were shown to produce N-linked carbohydrate groups with terminal α2,6 linked sialic acid, demonstrating an altered glycosylation machinery (2).

Mis-expression of glycosyltransferases can also be useful in assessing some of the biological roles of the carbohydrate structures within an organism. Our recent work in this area has focused on expressing α2,6 ST in developing Xenopus embryos to block the action of the endogenous α2,8 polysialyltransferase. By injection of α2,6 ST mRNA into Xenopus embryos, the glycosylation pattern throughout the organism was altered, and expression of α2,8 linked sialic acid in the neural tube was decreased (4). This technique of altering the glycosylation pattern of an entire organism may prove useful in studying the developmental roles of glycosylation.

As more biological roles for carbohydrates are discovered, understanding the mechanisms by which their expression is regulated becomes more important. One mechanism of regulating expression occurs at the genetic level as illustrated by the ABO blood group system. The A, B, and O blood group antigens are known to be cell surface carbohydrate groups formed by the action or inaction of two different glycosyltransferases which are encoded as alleles of one gene. As recently reported by Yamamoto *et al.*, cell lines containing the A, B, or O alleles all express similar amounts of mRNA from this gene, but point mutations and insertions within the coding region of the alleles determine the specificity of the donor substrate used (A and B) or if the specific glycosyltransferase is active at all (O) (5,6). Thus, alterations in the genetic background can determine which carbohydrate structures an organism expresses.

It is also known, however, that terminal carbohydrate structures vary between individual tissues of an organism. This diversity is believed to be generated by the expression of various glycosyltransferase genes in a tissue specific and developmentally regulated manner. Previous work has demonstrated that α2,6 ST is expressed in a tissue specific manner, with highest levels being found in the liver. Our recent cloning and characterization of the gene coding for α2,6 ST has revealed that it appears to be regulated by three promotors, two of which produce tissue specific transcripts. The most active promotor contains transcription factor binding sites for DBP and HNF-1, which have been shown to confer liver specificity to various promotors and appear to account for the high level of expression of α2,6 ST in this tissue. The results provide direct evidence that glycosyltransferase genes may be subject to cell type specific regulation at the transcriptional level by virtue of containing promotors regulated by tissue specific transcription factors.

References:

1. K. J. Colley and J. C. Paulson, J. Biol. Chem. 263, (1989) 17615-17618.
2. E. Ujita Lee, J. Roth and J. C. Paulson, J. Biol. Chem. 263, (1989) 13848-13855.
3. R. D. Larsen, V. P. Rajan, M. M. Ruff, J. Kukowska-Latallo, R. D. Cummings and J. B. Lowe, Proc. Nat. Acad. Sci. 86, (1989) 8221-8231.
4. B. Livingston, E. De Robertis and J. C. Paulson, Glycobiology, (1990) (in press).
5. F. Yamamoto, J. Marken, T. Tsuji, T. White, H. Clausen and S. Hakomori, J. Biol. Chem. 265, (1990) 1146-1151.
6. F. Yamamoto, H. Clausen, T. White, J. Marken and S. Hakomori, Nature 345, (1990) 229-233.