α1,4-Linkage Glycosyltransferases

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Introduction

Glycosyltransferases that transfer GlcNAc and Gal to βGal residues with α1,4-links on glycoprotein and glycolipid templates, respectively, are categorized as α1,4-linkage glycosyltransferases. At present, two enzymes, namely, α1,4-N-acetylglucosaminyltransferase (α4GnT) and α1,4-galactosyltransferase (α4GalT, Gb3/CD77 synthase) are assigned to this family. α4GnT is responsible for biosynthesis of GlcNAcα1 → 4Galβ → R attached to O-glycans, whereas α4GalT is a key enzyme in the formation of Gb3/CD77 (Fig. 1). Since cDNAs encoding both enzymes were cloned by expression cloning by the authors (Nakayama et al. 1999; Kojima et al. 2000), we describe here the procedure for expression cloning of α1,4-linkage glycosyltransferases, focusing primarily on α4GnT.

Procedure for Expression Cloning of α4GnT

1. Prepare COS-1 cells (1.2 × 10⁷ cells) cultured in 15-cm dishes as recipient cells because they express core 2 β1,6-N-acetylglucosaminyltransferase-I but not GlcNAcα1 → 4Galβ → R itself.
2. Cotransfect COS-1 cells with 30 μg of a human stomach cDNA library constructed in pcDNAI and the same amount of leukosialin vector, pRcCMV-leu using LipofectAmine (Invitrogen, Carlsbad, CA, USA), as leukosialin has 80 O-glycosylation sites in the extracellular domain.
3. Sixty hours following transfection, collect COS-1 cells expressing GlcNAcα1 → 4Galβ → R by cell sorting using a mixture of HIK1083, PGM36, and PGM37 antibodies, which are specific for terminal α1,4-linked GlcNAc.
4. Rescue plasmid DNA from sorted cells using the Hirt method (Hirt 1967), and in the presence of ampicillin and tetracycline transform host E. coli MC1061/P3 cells with the rescued plasmid by electroporation. Note that bacteria transformed by pcDNAI become resistant to both antibiotics because pcDNAI vector contains the sup F gene, which corrects the defect of both ampicillin- and tetracycline-resistant genes in the P3 episome. By contrast, bacteria transformed by the leukosialin vector only are resistant.

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to ampicillin but not to tetracycline. Thus, due to this differential selection, only plasmids derived from the library are amplified.

5. Make a replica of the bacteria plate by placing a nitrocellulose membrane on the aforementioned E. coli plate for 4 h, prepare a pool of plasmid DNA from the replica, and cotransfect COS-1 cells cultured in 6-well dishes with 1 μg of the rescued plasmid DNA and the same amount of pReCMV-leu in each well using LipofectAmine. After 60 h, assay for expression of GlcNAcα1 → 4Galβ → R on transfected COS-1 cells using the antibody mixture specific for terminal α1,4-linked GlcNAc and an FITC-conjugated anti-mouse IgM antibody as secondary antibody under immunofluorescent microscopy.

6. Once positive COS-1 cells are identified, make a replica again as described in step 5, cut the replica into 10 pieces, place them on LB plates containing ampicillin and tetracycline for 4 h to make smaller replicas of each piece, and prepare plasmid DNA from each replica.

7. Cotransfect COS-1 cells cultured in 6-well dishes with 1 μg of each prepared plasmid DNA and the same amount of leukosialin cDNA. After 60 h, check for expression of GlcNAcα1 → 4Galβ → R on transfected COS-1 cells under an immunofluorescent microscope in a manner similar to that described above.

8. Once a section of the replica is identified that enables COS-1 cells to express GlcNAcα1 → 4Galβ → R by transfection, divide bacteria on that piece into smaller pools, prepare plasmid DNA from the each bacteria pool, and repeat step 7.

9. Repeat steps 8 and 7 (so-called sibling selection) until a single plasmid encoding human α4GnT is isolated.

For expression cloning of α4GalT (Kojima et al. 2000), mouse fibroblast L cells were used as recipient cells, because they express a precursor ofGb3, LacCer. The cDNA library was prepared from human melanoma SK-MEL-37 cells constructed with pCDM8. L cells were then cotransfected with that library and pdl3027 encoding polyoma T antigen. After transfection, L cells expressingGb3 were enriched by panning with mAb 38.13 specific forGb3, and then E. coli MC1061/P3 cells were transformed with plasmid DNA rescued from the panned cells. Sibling selection was repeated until a single plasmid encoding human α4GalT was isolated.

Results

Both human α4GnT and α4GalT are typical type II membrane-bound proteins of 340 and 353 amino acid residues, respectively (Nakayama et al. 1999; Kojima et al. 2000), showing 35% overall sequence similarity. In normal human tissues, expression of α4GnT is exclusively limited to gland mucous cells of the gastric mucosa, Brunner’s gland of the duodenal mucosa, and accessory glands of the pancreatobiliary tract. Because α4GnT is also expressed in gastric and pancreatic cancer cells, quantitative RT-PCR for