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Cloning and GST-fused expression in *E. coli* of mouse β-1,4-galactosyltransferase*

GONG Xing-guo(龚兴国)†, ZHONG Wen-tao (钟文涛), WU Wen-ying (吴文英)

(*Institute of Biomacromolecule & Enzyme Engineering, College of Life Sciences, Zhejiang University, Hangzhou 310027, China*)

†E-mail: Gongxg@cls.zju.edu.cn

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Abstract: β-1,4-galactosyltransferase (β4Gal-T) (EC 2.4.1.38) plays a multifunctional role in many aspects of normal cell physiology. By now, several dozens of β4Gal-T genes have been cloned, separated from mouse, chick, bovine, human, etc. This paper presents the cloning and GST-fused expression of mouse β4Gal-T gene in *Escherichia coli* (*E. coli*). The target gene was cloned by PCR, followed by identification by DNA sequencing and expression in *E.coli* with isopropyl-β-D-thiogalactoside (IPTG) gradient concentrations, products of which were separated on SDS-PAGE showing that the target protein had the same molecular weight as that of mouse β4Gal-T. The transcriptional product of β4Gal-T gene was proved by Western hybridization analysis to be due to GST-fusion.

Key words: β-1,4-galactosyltransferase (β4Gal-T), Cloning, GST-fusion
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INTRODUCTION

β-1,4-galactosyltransferase (β4Gal-T) (EC 2.4.1.38), as one of the most researched glycosyltransferases in recent years, produces a β-1,4-linked galactosylated glycan by the transferation of galactose (Gal) from an uridine diphosphate-galactose (UDP-Gal) to a monosaccharide N-acetylglucosamine (GlcNAc) or a GlcNAc residue located in the non-reducing terminal of glycans of glycoproteins or glycolipids. In addition to GlcNAc, the enzyme can also use other reducing sugars, such as N-acyl substituted glucosamines and N-acetyl-D-mannosamine, as acceptors.

As a Type II membrane protein, β4Gal-T exists not only in the Golgi complex, but also on the cytomembrane; and functions differently compared to other intracellular glycotransferases. β4Gal-T has multiple functions in many aspects of normal cell physiology, and seems to be associated with spermegg binding (Macek *et al.*, 1991; Youakim *et al.*, 1994), cell-cell recognition (Hathaway and Shur, 1996; Wassler and Shur, 2000; Wassler *et al.*, 2001; Shi *et al.*, 2001), cell migration on basal lamina (Maillet and Shur, 1993; Eckstein and Shur, 1992; Appeddu and Shur, 1994) and neurite extension (Begovac and Shur, 1990; Hathaway and Shur, 1992; Huang *et al.*, 1995), embryonic maturation (Bayna *et al.*, 1988), rheumatoid arthritis (Yang *et al.*, 1996; Axford, 1999), and cell development (Maillet and Shur, 1994; Asano *et al.*, 1997; Akimoto *et al.*, 1995).

By now, several dozens of β4Gal-T genes have been cloned from many vertebrates, such as mouse, chick, bovine and human. Since human β4Gal-T, as a house-keeping gene, was cloned in 1997 for the first time (Almeida *et al.*, 1997), seven members of

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this human gene family have been found (Vadaie *et al.*, 2002; Fan *et al.*, 2002).

This paper presents molecular cloning and high efficiency expression induced by isopropyl-β-D-thiogalactoside (IPTG) gradient concentrations, which yielded the first GST-fused mouse β4Gal-T gene in *Escherichia coli*.

MATERIAL AND METHODES

Bacteria and plasmids

E. coli strain of BL21 (DE3) and DH5α were conserved in our lab, and a strain of TOP10 was presented by the Biochemistry and Cell Institute of the Chinese Academy of Sciences (CAS).

pMGT-239/2615 vector, kindly provided by Professor Shur N.D. of Emory University School of Medicine, was exstracted as described (Sambrook *et al.*, 1989). pMD18-T vector was the cloning and sequencing vector and pGEX-4T-2 vector was the plasmid expression vector.

PCR amplification

The target gene was obtained from pMGT-239/2615 vector through Polymer Chained Reaction (PCR), primered with up-primer (5'-ATCGGATCCATGAGGTTTCGTGAGCAG-3') and down-primer (5'-ACGGAATTCCTATCTCGGTGTCCCGATG-3'). PCR amplification was conducted in total volume of 300 μl with 30 μl reaction buffer, 24 μl of 2.5 mmol/L deoxynucleoside triphosphates (dNTPs), 12 μl of 10 mmol/L primers mixture and 1.5 μl of 5 μg/μl Taq polymerase. Cool-starting method was applied to the PCR product, followed by thermo-cycling at 94 ºC for 30 seconds, 50 ºC for 50 seconds, and 72 ºC for 80 seconds for 32 cycles. DNA Sequencing of the PCR product was performed by the Sequencing Ward, College of Life Sciences, Zhejiang University.

Cloning of the target gene

Plasmids were extracted with WizardR Purification Kit by the method of alkaline lysis. Subcloning of the target gene was done with pMD18-T, the vector of which contains Glutathione S-transferase (GST) gene that expresses a fusion protein with potential for further protein detection and separation.

The nucleotide acid sequence of the target gene was compiled and analyzed on the basis of Genbank databases (http://www.ncbi.nlm.nih.gov).

Detection of target protein

IPTG gradient concentrations were applied to induce high efficiency expression of the target protein. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described (Sambrook *et al.*, 1989). Hybridization was proceeded at the presence of 0.6 mg/ml 3,3'-diaminobenzidine (DAB), goat anti-GST antibody as the primary antibody, and mouse anti-goat IgG marked by horse radish peroxidase (HRP) as the secondary antibody. The reaction temperature was held at 25 ºC.

RESULTS

Cloning of the β4Gal-T gene

On the basis of the conserved constituents of the amino acids sequence of homologues of β4Gal-T gene and taking into consideration of the guanosine (G) and cytidine (C) percentage of the β4Gal-T gene, the primers, including the up-primer containing a cleavage site for BamH I, and the down-primer containing a cleavage site for EcoR I, were designed.

PCR amplification carried out with pMGT-239/2615 vector as a template was detected by 1% agarose gel electrophoresis (Fig.1).

Detection of target gene by gel electrophoresis

pMD18-T vectors probably containing an extra sequence of the target gene were extracted from *E. coli* and cleaved by BamH I, followed by detection by 1% agarose gel electrophoresis (Fig.2). The digested products of the positive plasmids were detected by comparison with the 200 bp ladder of the DNA marker (Fig.3), and identified by PCR-gel electrohoresis (Fig.4).

Sequencing of PCR amplification product

The DNA sequence of the PCR amplification

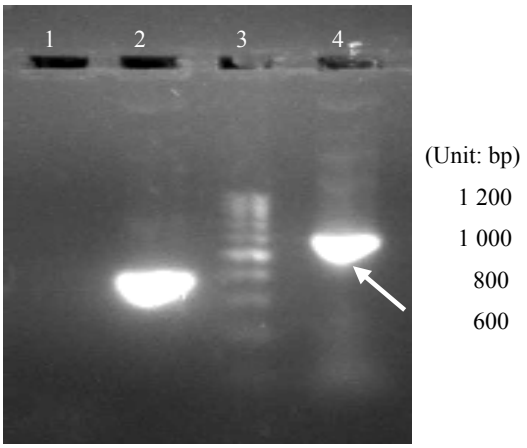

Fig.1 1% agarose gel electrophoresis of PCR amplification product of β4Gal-T

Lane 1 was negtive control; Lane 3 shows the 200 bp ladder of the DNA marker, the brightest band of which contained DNA of 1000 bp; Lane 2 shows the product of PCR (Lβ4Gal-T); and Lane 4 shows the Sβ4Gal-T gene (not from PCR)

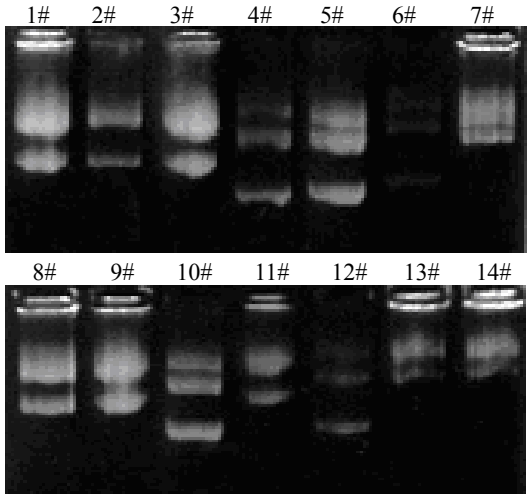

Fig.2 1% agarose gel electrophoresis

Containing an extra gene, the recombinant plasmids (1#, 2#, 3#, 7#, 8#, 9#, 11#) ran slower than the origin plasmids

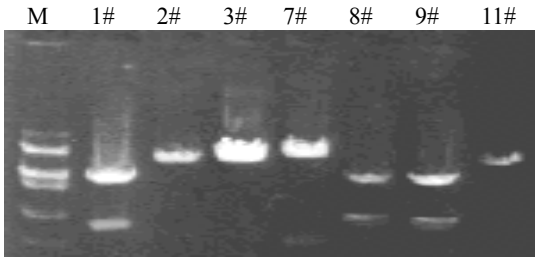

Fig.3 Digest analysis of the recombinant plasmids

Plasmids 1#, 8# and 9# were the candidates, containing DNA band of 1200 bp, while the DNA marker shows the ladder of 6050, 2690, 2160, 1100, 840, 710 and 300 bp

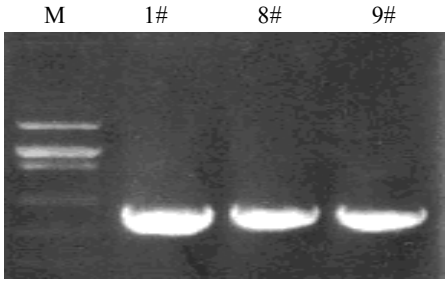

Fig.4 1% agarose gel electrophoresis of PCR amplification products

Plasmids 1#, 8# and 9# showed are positive clones. The DNA marker ladder is the same as that in Fig.3

product is shown in Fig.5. The β4Gal-T gene of the sequence of 1 216 bp DNA, sub- cloned in the cloning and sequencing vector of pMD18-T, was the same as reported (Nomura *et al.*, 1988), except for guanosine (G) of the +12 site, which was mutated from thymine (T) but did not modify the anticipant sequence of the transcriptional product, because the original codon (CGT) and the mutant codon (CGG) both encoded the amino acid of arginine (Q).

Mouse β4Gal-T gene encodes two isoforms of mRNA in somatic cells, differentiating in the transcription promoter of the long isoform (Lβ4Gal-T, 4.1 kb) located upstream the first exon (about 200 bp) while the short one's (Sβ4Gal-T, 3.9 kb) located downstream. As a result, the long isoform of mouse β4Gal-T gene containing 13 more amino acids in the N terminal had transcription pattern different from that of the short isoform. The same happens with the isoforms of bovine and human gene (Masri *et al.*, 1988; Mengle *et al.*, 1991).

Expression pattern of β4Gal-T

GST-fused expression of β4Gal-T in *E. coli* in the presence of IPTG gradient concentrations, was examined on SDS-PAGE by Western hybridization analysis. The holoproteins on SDS-PAGE showed that the optimum culture condition was 3 hours at 37 ºC with 0.4 mmol/L of IPTG (Fig.6). Density Scanning of β4Gal-T protein on SDS-PAGE (Fig.7) showed that the weight of this protein is 10% that of

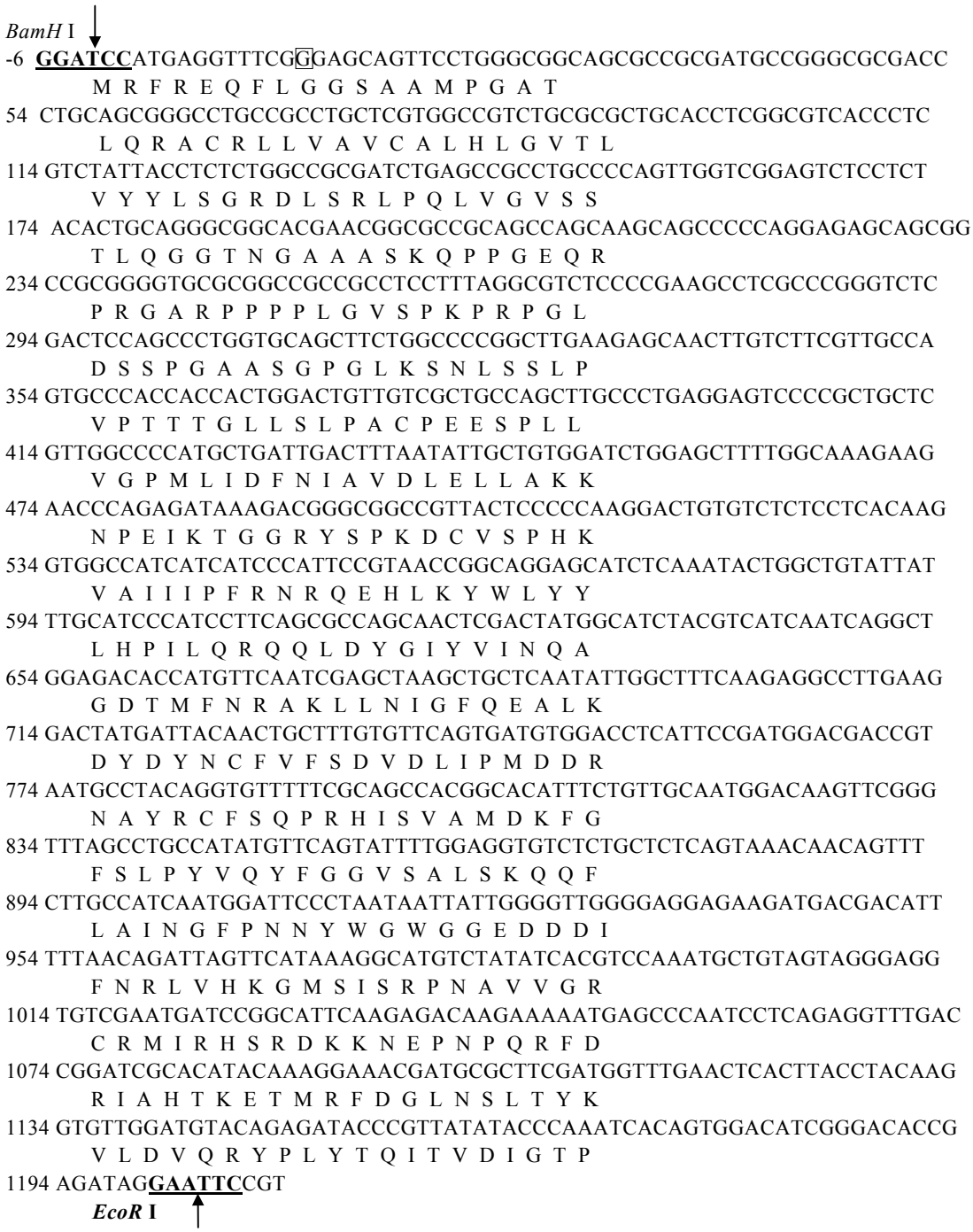

Fig.5 DNA sequence of the PCR product (mouse β4Gal-T gene) and the amino acid sequence transcript hypothesized from the former DNA sequence

the holoproteins. Western hybridization analysis showed that positive GST-fusion proteins were of the molecular weight of 70 kD (Fig.8). As the molecular weight of β4Gal-T is about 45 kD and that of GST is 26 kD, the 70 kD band was confirmed as the β4Gal-T-GST fusion proteins.

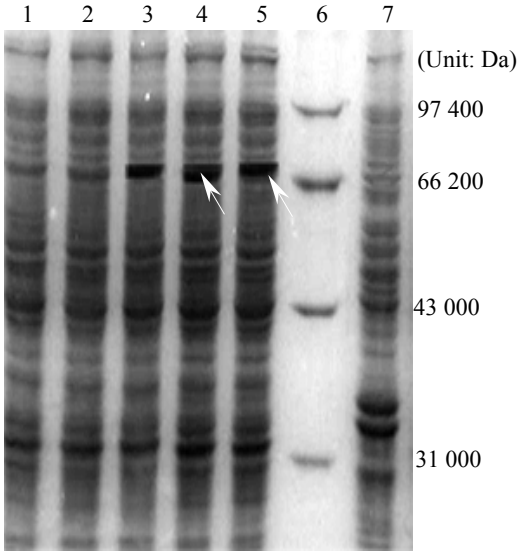

Fig.6 SDS-PAGE analysis for the expression product of β4Gal-T with ladder of IPTG concentration

Lane 6 contains low molecular weight marker, consisting of protein ladder of 97.4, 66.2, 43 and 31 kD. Lane 7 is the negative control of IPTG-induction. Lane 3, 4 and 5, respectively, show a strip on 70 kD, having the same molecular weight as β4Gal-T as reported

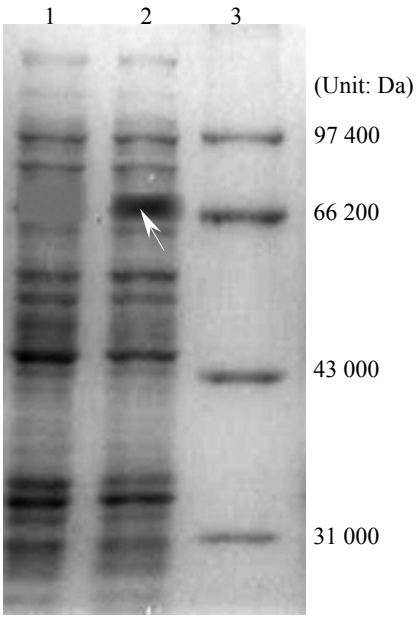

Fig.7 SDS-PAGE analysis for the expression product of β4Gal-T from *E. coli* (BL21)

Lane 3 contains low molecular weight marker, consist of protein ladder of 97.4, 66.2, 43.0 and 31.0 kD. Lane 7 is the negative control of IPTG-induction. Lane 2 shows an apparent strip on about 70 kD in comparison with the marker

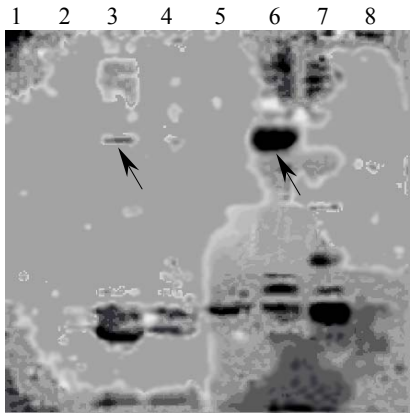

Fig.8 Western blotting of product of pGEX-4T-2-GT

Lane 1 and 2 contain the products of IPTG-free expression. Lane 3 and 6 contain the product of expression with induced by IPTG of 0.4 mmol/L, showing the β4Gal-T-GST fused gene. Lane 4, 5 and 7 are the negative controls. Lane 8 is the low molecular weight marker

DISCUSSION

Hyperstructure of β4Gal-T

The secondary structure of β4Gal-T protein was measured by DNA Star program (Fig.9).

Masibay *et al.*(1993) found that the secondary structure of β4Gal-T contained 20% β-sheets, 23% α-helices and 2% α-3_{10}-helices. The catalytic site (Leu131−Ser402) is a 52×50×42 Å tri-dimensional structure that consists of 11 β-sheets (β1−β11), 6α-helices (α1−α6) and 2 α-3_{10}-helices, the central part of which is a twist of 8 β-sheets [Code: 7(81)32465] enclosed by 2 α-helices and 4 α-helices on each side respectively. The central β-sheets (β2) structure locating in the N terminus follows an unaffirmatory amino acids terminus and a nude α-helix (α1β1) hairpin structure. The C terminal domain that begins with a β-sheet (β8), consists of α-helix (α4, α5) and a short β-sheet (β9). β9 trifolds parallelly in reverse upon the central β-sheets are enclosed by an α-helix (3_{10}-α6) and a long β-hairpin (β10β11) respectively. The tapered hyperstructure of β4Gal-T has a big and deep capacious pocket with a diameter of 13 Å (Vrielink *et al.*, 1994); and shows no structural homology with T4 DNA-modified β-glucotransferase (Holm and Sander, 1983).

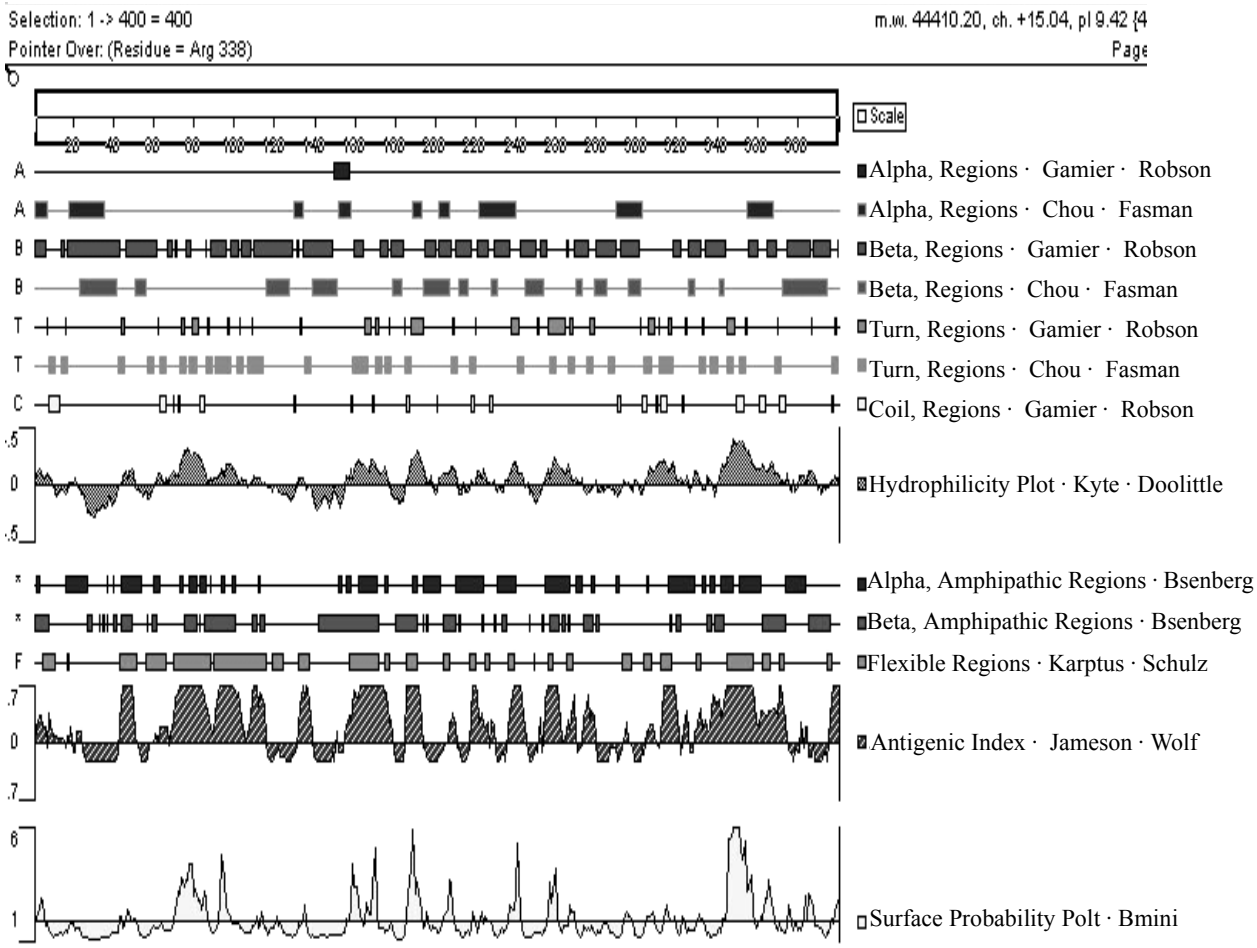

Fig. 9 The secondary structure of the β4Gal-T protein

Phylongenetic tree of Gal-T homologues from vertebrates

β4Gal-T encoded by a DNA fragment of 1 200 bases contains 399 amino acids. The cloned gene of β4Gal-T originally separated from cellular plasma contained 13 more amino acids than the β4Gal-T gene in the Golgi complex; the short sequence functions like a leader sequence to the former gene while transiting through the cellular membrane (Lopez *et al.*, 1991; Evans *et al.*, 1995).

Originating from mouse, the expression product of the cloned gene of β4Gal-T shares structural homology with β4Gal-Ts from other vertebrates, such as human, bovine and chick. But Uehara (1998) found that mouse testis β4Gal-T gene had some apparent phylongenetic difference with from genes of β4Gal-T and other glycosyltransferases.

Characterization of pMD 18-T vector

Modified from pUC18 vector, pMD 18-T vector is an ampicillin selective marker with a new cleavage site for EcoR V added between the cleavage sites of Xba I and Sal I in the polycloning site of pUC18 vector. After cleaved by EcoR V, thymines (T) were added to both of the 3'-ends of the linear fragment respectively.

pMD18-T vector was specially designed for cloning of PCR products, which helped to promote the efficiency of linkage of PCR products and subcloning.

DNA recovery from agarose gel

Among methods for recovery of DNA from agarose gel, DNA recovery kits are highly efficient but expensive. We developed an easy, economical

but efficient and stable method similar to low melting point gel processing but had quicker response time, which is suitable for subcloning of PCR products into pMD18-T vector.

Expression and characterization of β4Gal-T

As reported, the expressions of human β4Gal-T I in *E. coli* and yeast (Malissard *et al.*, 2000), and the expressions of human β4Gal-T II in a transformed insect cell line (Tomiya *et al.*, 2003), had been successful.

This paper presents a GST-fused expression vector of mouse β4Gal-T in *E. coli*. As an expression vector, pGEX-4T-2 engenders a GST fusion protein stabler than natural protein and with larger molecular weight than other natural proteins of *E. coli*; and have potential for detection and purification of target protein. Gradients of temperature, IPTG concentration and time were designed for the highly efficiency expression of proteins in the recombinant pGEX-4T-2 vector (pGEX-4T-2β4-Gal-T).

Studies on the structure and biophysics of β4Gal-T were prevented by the propensity of recombinant human β4Gal-T I proteins to aggregate when their concentrations were larger than 1 mg/ml. Malissard and Berger (2001) developed a mutated form of the catalytic domain of human β4Gal-T I, which was proved much more soluble in aqueous solutions.

Park *et al.* (2002) got an expression of β4Gal-T from *Neisseria meningitidis* and *Neisseria gonorrhoeae*, showing that most of the β4Gal-Ts were insoluble and proteolysed into inactive enzymes that lacked C terminus residues (29.5 kD and 28 kD) when purified. The β4Gal-Ts exhibited optimum activity at pH 6.5−7.0 in the presence of the Mn^{2+} ions. But in the presence of Fe^{2+}, Zn^{2+} or Cu^{2+} ions, Mn^{2+} ions could not show activity, so the β4Gal-T activity did not increase any more. Interestingly, the β4Gal-T activity could be stimulated to 150 per cent in the presence of non-ionic detergent Triton X-100 at the concentration of 0.1%−5 %.

Function of GST fusion protein

Glutathione S-transferase (GST; EC 2.5.1.18), encoded by the parasitic helminth *Schistosoma japonicum*, was a 26 kD protein with C terminal of Sj26. GST-fusion proteins are soluble in aqueous solutions and can be easily purified by affinity chromatography on immobilised glutathione. The GST part of the fusion protein can be cleaved off by digestion with site-specific proteases, such as thrombin or blood coagulation factor X, followed by abstersion by glutathione-agarose, which can absorb the cleaved-off GST part and the uncleaved fusion protein (Smith and Johnson, 1988).

GST-fusion proteins can be applied as probes for qualitative detection in Western hybridization (Posern *et al.*, 1998). After separation on SDS-PAGE, GST-fusion proteins can be transformed to the detection membrane directly, followed by reaction of sealant, such as 5% bovine plasma albumin (BPA) or phosphate buffer solution (PBS) (Gillespie and Hudspeth, 1991). Goat anti-GST antibody as the primary antibody, and mouse anti-goat IgG marked with horse radish peroxidase (HRP) as the secondary antibody, were applied to the succeeding hybridization with GST-fusion proteins.

In addition, GST-fusion protein can be applied for sensitive quantitative detection or other complicated molecular analysis (Posern *et al.*, 1998; McDonald *et al.*, 1999).

CONCLUSION

As β4Gal-T has important functions in many physiological aspects of different kinds of cells, especially in cell-cell recognition (Hathaway and Shur, 1996; Wassler and Shur, 2000; Wassler *et al.*, 2001) and rheumatoid arthritis (Yang *et al.*, 1996; Axford, 1999), further experimental research on this protein must be conducted. But it is complex and separating β4Gal-T with classical biochemical approaches is hampered by low efficiency. We present the cloning and GST-fused expression of β4Gal-T gene in *E. coli*, which have potential for further studies, and subsequently facilitating the medical application of β4Gal-T.

References

Akimoto, Y., Obinata, A., Endo, H., Furukawa, K., Aoki, D., Nozawa, S., Hirano, H., 1995. Immunocytochemical

localization of the protein reactive to human beta-1,4-galactosyltransferase antibodies during chick embryonic skin differentiation. *Anat. Rec*., **243**(1):109-119.

Almeida, R., Amado, M., David, D., Levery, S.B., Holmes, E.H., Merkx, G., van Kessel, A.G., Rygaard, E., Hassan, H., Bennett, E., Clausen, H., 1997. A Family of human β4-galactosyltransferases: cloning and expression of two novel UDP-galactose β-N-acetyl-glucosamine β-1,4-gal-actosyltransferases: β4-Gal-T2 and β4Gal-T3. *J. Biol. Chem.*, **272**(51):31979-31991.

Appeddu, P.A., Shur, B.D., 1994. Molecular analysis of cell surface beta-1,4-galactosyltransferase function during cell migration. *Proc. Natl. Acad. Sci. U.S.A*, **91**(6): 2095-2099.

Asano, M., Furukawa, K., Kido, M., Matsumoto, S., Umesaki, Y., Kochibe, N., Iwakura, Y., 1997. Growth retardation and early death of β-1,4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. *EMBO J.*, **16**(8):1850-1857.

Axford, J.S., 1999. Glycosylation and rheumatic disease. *Biochim. Biophys. Acta.*, **1455**(2-3):219-229.

Bayna, E.M., Shaper, J.H., Shur, B.D., 1988. Temprally specific involvement of cell surface β-1,4-galactosyltransferase during mouse embryo morula compaction. *Cell*, **53**:145-157.

Begovac, P.C., Shur, B.D., 1990. Cell surface galactosyltransferase mediates the initiation of neurite out-growth from PC 12 cells on laminin. *J. Cell Biol.*, **110**(2): 461-470.

Eckstein, D.J., Shur, B.D., 1992. Cell surface beta-1,4-galactosyltransferase is associated with the detergent-insoluble cytoskeleton on migrating mesenchymal cells. *Exp. Cell Res.*, **201**(1):83-90.

Evans, S.C., Youakim, A., Shur, B.D., 1995. Biological consequences of targeting β-1,4-galactosyltransferase to two different subcellular compartments. *Bioessays*, **17**(3):261-268.

Fan, Y., Yu, L., Tu, Q., Gong, R., Jiang, Y., Zhang, Q., Dai, F., Chen, C., Zhao, S., 2002. Molecular cloning, genomic organization, and mapping of β4GalT-VIb, a brain abundant member of β4-galactosyltransferase gene family, to human chromosome 18q12.1. *DNA Seq.* **13**(1):1-8.

Gillespie, P.G., Hudspeth, A.J., 1991. High-purity isolation of bullfrog hair bundles and subcellular and topological localization of constituent proteins. *J. Cell Biol.*, **112**(4):625-640.

Hathaway, H.J., Shur, B.D., 1992. Cell surface beta-1,4-galactosyltransferase functions during neural crest cell migration and neurulation *in vivo*. *J. Cell Biol.*, **117**(2): 369-382.

Hathaway, H.J., Shur, B.D., 1996. Mammary gland morphogenesis is inhibited in transgenic mice that overexpress cell surface beta-1,4-galactosyltranferase. *Development*, **122**(9):2859-2872.

Holm, L., Sander, C., 1983. Protein structure comparison by alignment of distance matrices. *J. Mol. Biol.*, **233**: 123-138.

Huang, Q., Shur, B.D., Begovac, P.C., 1995. Overexpressing cell surface beta-1,4-galactosyltransferase in PC12 cells increases neurite outgrowth on laminin. *J. Cell Sci.*, **108** (Pt 2):839-847.

Lopez, L.C., Youakim, A., Evans, S.C., Shur, B.D., 1991. Evidence for a molecular distinction between Golgi and cell surface forms of β-1,4-galactosytransferase. *J. Bio. Chem.*, **266**(24):15984-15991.

Macek, M.B., Lopez, L.C., Shur, B.D., 1991. Aggregation of beta-1,4-galactosyltransferase on mouse sperm induces the acrosome reaction. *Dev. Biol.*, **147**(2):440-444.

Maillet, C.M., Shur, B.D., 1993. Uvomorulin, LAMP-1, and laminin are substrates for cell surface beta-1,4-galactosyltransferase on F9 embryonal carcinoma cells: comparisons between wild-type and mutant 5.51 att-cells. *Exp. Cell Res.*, **208**(1):282-295.

Maillet, C.M., Shur, B.D., 1994. Perturbing cell surface beta-(1,4)-galactosyltransferase on F9 embryonal carcinoma cells arrests cell growth and induces laminin synthesis. *Cell Sci.*, **107**(Pt 6):1713-1724.

Malissard, M., Zeng, S., Berger, E.G., 2000. Expression of functional soluble forms of human β-1,4-galactosyltransferase I, α-2,6-sialyltransferase, and α -1,3-fucosyltransferase VI in the methylotrophic yeast Pichia pastoris. *Biochem. Biophys. Res. Commun.*, **267**(1):169-173.

Malissard, M., Berger, E.G., 2001. Improving solubility of catalytic domain of human beta-1,4-galactosyltransferase 1 through rationally designed amino acid replacements. *Eur. J. Biochem.*, **268**(15):4352-4358.

Masibay, A.S., Balaji, P.V., Boeggeman, E.E., Qasba, P.K., 1993. Mutational analysis of the golgi retention signal of bovine β-1,4-galactosyltransferase. *J. Bio. Chem.*, **268**(13):9908-9916.

Masri, K.A., Appert, H.E., Fukuda, M.N., 1988. Identification of the full-length coding sequence of human galactosylstransferase(β-N-acetylglucosminide:β-1,4-galactosyltransferase). *Biochem. Biophys. Res. Commun.*, **157**(2):657-663.

McDonald, O.B., Chen, W.J., Ellis, B., Hoffman, C., Overton, L., Rink, M., Smith, A., Marshall, C.J., Wood, E.R., 1999. A scintillation proximity assay for the Raf/MEK/ERK kinase cascade: high-throughput scrceening and identification of selective enzyme inhibitors. *Anal. Biochem.,* **268**(2):318-329.

Mengle, G.L., Mccoy, H.M.F., Tiemeier, D.C., 1991. Genomic structure and expression of human beta-1,4-galactosyltransferase. *Biochem. Biophy. Res. Commun.*, **176**:1269-1276.

Nomura, T., Takizawa, M., Aoki, J., Arai, H., Inoue, K., Wakisaka, E., Yoshizuka, N., Imokawa, G., Dohmae, N., Takio, K., Hattori, M., Matsuo, N., 1988. Purification, cDNA cloning, and expression of the UDP-Gal: glucosylceramide β-1,4-galactosyltransferase from rat brain. *J. Biol. Chem.*, **273**(22):13570-13577.

Park, J.E., Lee, K.Y., Do, S.I., Lee, S.S., 2002. Expression and characterization of β-1,4-galactosyltransferase from *Neisseria meningitisdis* and *Neisseria gonorrhoeae*. *J. Biochem. Mol. Biol.*, **35**(3):330-336.

Posern, G., Zheng, J., Knudsen, B.S., Kardinal, C., Muller, K.B., Voss, J., Shishido, T., Cowburn, D., Cheng, G., Wang, B., Kruh, G.D., Burrell, S.K., Jacobson, C.A., Lenz. D.M., Zamborelli, T.J., Adermann, K., Hanafusa, H., Feller, S.M., 1998. Development of highly selective SH3 binding peptides for Crk and CRKL which disrupt Crk-complexes with DOCK180, SoS and C3G. *Oncogene*, **16**(15):1903-1912.

Sambrook, J., Fritsh, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, 2nd Edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Shi, X., Amindari, S., Paruchuru, K., Skalla, D., Burkin, H., Shur, B.D., Miller, D.J., 2001. Cell surface beta-1,4-galactosyltransferase-I activates G protein-dependent exocytotic signaling. *Development*, **128**(5):645-654.

Smith, D.B., Johnson, K.S., 1988. Single-step purifycation of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene*, **67**(1):31-40.

Tomiya, N., Howe, D., Aumiller, J.J., Pathak, M., Park, J., Palter, K.B., Jarvis, D.L., Betenbaugh, M.J., Lee, Y.C., 2003. Complex-type biantennary N-glycans of recombinant human transferrin from *Trichoplusia ni* insect cells expressing mammalian {beta}-1,4-galactosyltransferase and {beta}-1,2-*N*-acetylgluco-saminyltransferase II. *Glycobiology*, **13**(1):23-34.

Uehara, K., 1998. Structural analysis of milk and testis β-1,4-galactosyltransferase gene products. *Cell structure and function*, **23**:43-48.

Vadaie, N., Hulinsky, R.S., Jarvis, D.L., 2002. Identification and characterization of a *Drosophila melanogaster* ortholog of human β-1,4-galactosyltransferase VII. *Glycobiology*, **12**(10):589-597.

Vrielink, A., Rüger, W., Driessen, H.P., Freemont, P.S., 1994. Crystal structure of the DNA modifying enzyme β-glucosyltransferase in the presence or in the absence of the substrate uridine diphosphoglucose. *EMBO J.*, **13**(15):3413-3422.

Wassler, M.J., Shur, B.D., 2000. Clustering of cell surface beta-1,4-galactosyltransferase I induces transient tyrosine phosphorylation of focal adhesion kinase and loss of stress fibers. *J. Cell Sci.*, **113**(Pt 2):237-245.

Wassler, M.J., Foote, C.J., Gelman, I.H., Shur, B.D., 2001. Functional interaction between the SSeCKS scaffolding protein and the cytoplasmic domain of beta-1,4-galactosyltransferase. *J. Cell Sci.*, **114**:2291-2300.

Yang, R.Y., Hsu, D.K., Liu, F.T., 1996. Expression of galectin-3 modulates T-cell growth and apoptosis. *Proc. Natl. Acad. Sci. U.S.A*, **93**(13):6737-6742.

Youakim A., Hathaway H.J., Miller, D.J., Gong, X., Shur, B.D., 1994. Overexpressing sperm surface β-1,4-galactosyltransferase in transgenic mice affects multiple aspects of sperm-egg interactions. *J. Cell Biol.*, **126**(6):1573-1583.

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