Journal of Cellular Biochemistry 42:117–122 (1990)

Co-Transformation of Lec1 CHO Cells With N-Acetylgucosaminyltransferase 1 Activity and a Selectable Marker

James Ripka, Michael Pierce, and Nevis Fregien

Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, Florida 33101

In animal cells, the enzyme α(1,3)-mannoside-β(1,2)-N-acetylglucosaminyltransferase I (GlcNAc-TI, EC.2.4.1.101) catalyzes the addition of N-acetylglucosamine to the ASN-linked Man GlcNAc oligosaccharide. The Chinese hamster ovary (CHO) mutant cell line Lec1 is deficient in this enzyme activity and, therefore, accumulates mannose-terminating cell surface ASN-linked oligosaccharides. Consequently, Lec1 cells are sensitive to the cytotoxic effects of the mannose-binding lectin Concanavalin A (Con A). Lec1 cells were co-transformed with human DNA from A431 cells and eukaryotic expression plasmids containing the bacterial *neo* gene by calcium phosphate/DNA-mediated transformation. Co-transformants were selected for resistance to Con A and G-418. DNA from a primary co-transformant was purified and used to transform Lec1 cells, resulting in secondary co-transformants. Both primary and secondary co-transformants exhibited in vitro GlcNAc-TI-specific enzyme activity. DNA gel blot analysis indicated that secondary co-transformants contained both human and *neo* sequences.

Key words: glycosyltransferase, gene transfer, DNA-mediated transformation, GlcNAc-TI, Man GlcNAc

Glycosyltransferases are a series of enzymes in the pathway of oligosaccharide biosynthesis. The enzyme α(1,3)-mannoside-β(1,2)-N-acetylglucosaminyltransferase I (GlcNAc-TI) is required for the synthesis of complex-type carbohydrates from mannose-terminating, ASN-linked oligosaccharides. The Lec1 Chinese hamster ovary (CHO) cell line is deficient in GlcNAc-TI enzyme activity [1], and, therefore, expresses ASN-linked Man GlcNAc oligosaccharides on its cell surface glycoproteins. We have previously reported the restoration of GlcNAc-TI enzyme activity to Lec1 cells by DNA-mediated gene transfer and selection for Con A resistance [2]. DNA from secondary transformants did not hybridize to the human repetitive *alu* element. Hence, there was no unique sequence available to recover the transferred human gene from the recipient Lec1 hamster background DNA. Accordingly, an alternative marker DNA was re-

Received May 18, 1989; accepted September 5, 1989.

© 1990 Wiley-Liss, Inc.

quired in order to recover the transforming DNA from the Lec1 cells by molecular cloning. DNA-mediated co-transformation of genes has resulted in the relatively close proximity of target and marker genes [3]. Lec1 cells were co-transformed, therefore, with genomic DNA from A431 cells and expression plasmids containing the *neo* gene, which confers resistance to the antibiotic G-418 in eukaryotic cells, as a marker DNA. Co-transformant colonies expressed both in vitro GlcNAc-TI enzyme activity and resistance to G-418. DNA from secondary co-transformants exhibited a single band hybridizing to a *neo* probe in a southern blot, which should allow the rapid isolation of the human GlcNAc-TI gene by molecular cloning.

MATERIALS AND METHODS

Cell Lines and DNA

Lec1 CHO and parental CHO cells were obtained from the American Type Culture Collection (Rockville, Maryland). A431 cells were a gift of Dr. Margory Nicolson. Cells were cultured in monolayers in alpha plus medium containing 10% fetal calf serum and antibiotics (GiBCO, Grand Island, NY) in a humidified atmosphere with 5% CO_2 at 37°C. The plasmids pβAct-neo7 [4], pBR-neo [4], and pBLUR 8 [5] have been described.

DNA-Mediated Co-Transformation and Selection of Co-Transformants

DNAs were prepared and cells were transformed as previously described [2]. Primary co-transformants were obtained from human A431 genomic DNA (20 μg) and plasmid DNA (5 μg), which contained the bacterial *neo* gene, mixed and co-precipitated with calcium phosphate. Secondary co-transformants were obtained from the genomic DNAs of the primary co-transformants. Co-transformants were selected in the lectin Concanavalin A (Con A) (10 μg/ml) and G-418 (400 μg/ml) when appropriate. Co-transformants were tested for lectin-resistance phenotype against Con A and wheat germ agglutinin (WGA) as described elsewhere [2].

GLcNAc-TI Enzyme Activity Assay

Cell sonicates were incubated with UDP-[^{3}H]GlcNAc and a synthetic trisaccharide acceptor specific for GlcNAc-TI activity [6]. The radiolabeled product was bound to a reverse-phase C-18 Sep-Pak column and eluted with methanol.

DNA Gel Blot Analysis

DNA samples (10 μg) were digested with EcoR1, electrophoresed in 0.8% agarose, and transferred to nitrocellulose [7]. The filters were hybridized to gel isolated *neo* [4] and *alu* [5] inserts labeled to a specific activity of 5–8 $\times 10^8$ cpm/μg probe [8]. Blots were washed with 0.2×standard saline citrate (SSC) at 55°C for the *neo* probe and 1×SSC at 50°C for the *alu* probe. Blots were exposed to X-ray film for 2 days.

RESULTS

DNA-Mediated Co-Transformation of Lec1 CHO Cells

Lec1 CHO cells were cotransformed with human DNA from A431 cells and expression plasmid DNA containing the *neo* gene by co-precipitation with calcium

TABLE I. DNA-Mediated Co-Transformation of Lec1 CHO Cells*

Transformation	Donor DNA	Selective conditions		Total colonies/ dishes	Colonies with parental CHO phenotype/ No. tested	Transformation frequency
		Con A	G-418			
1	A431 + pβACT-neo7	+	+	2/3	1/2	3.3×10^{-7}
2	A431 + pSV2-neo	+	+	7/6	1/7	1.7×10^{-7}
3	Primary transformant	+	+	2/4.5	1/2	2.2×10^{-7}
4	Primary transformant	−	+	22/1.5	6/6	4.0×10^{-6}
5	Primary transformant	+	−	2/3	1/2	3.3×10^{-7}

*Lec1 cells were co-transformed with the indicated DNA, and co-transformant colonies were detected after 10–14 days of growth in selective conditions. Colonies were picked and tested for the CHO lectin-resistance phenotype and in vitro GlcNAc-T1 activity. The co-transformants obtained in each transformation were as follows: 1, colony 3.1, clone 3.1.3, clone 3.1.5; 2, colony 53.1; 3, colony 27.4.1; 4, colony 22.1.6; 5, colony 22.2.1.

phosphate. Primary co-transformants were co-selected with Con A and G-418 at a frequency of approximately 2–3 $\times 10^{-7}$ (Table I). Transformation of Lec1 cells with only genomic A431 DNA and selection in Con A results in a transformation frequency of approximately 10^{-6} [2]. Primary co-transformant colonies 3.1 and 53.1 (Table I; transformations 1 and 2, respectively) were able to proliferate in tissue culture in the presence of both Con A and G-418. These co-transformants exhibited the parental CHO lectin-resistance phenotype of resistance to Con A and sensitivity to WGA (data not shown). This result demonstrates that the co-transformants were expressing complex cell-surface carbohydrates. To eliminate extraneous human and *neo* sequences, DNA from co-transformant colony 3.1 was purified and used to transform Lec1 cells. Two secondary co-transformants were selected (Table I, transformations 3 and 4) and termed 22.2.1 and 22.1.6, respectively.

To have a homogeneous population of co-transformant cells, colony 3.1 was cloned by limiting dilution, resulting in two clones 3.1.3 and 3.1.5. DNA from clone 3.1.5 was

TABLE II. GlcNAc-T1 Enzyme Activity of CO-Transformant Colonies

Cell line	GlcNAc-T1 activity (nmol/mg × h)	Relative activity (%)
A431	19.47 ± 9.7	100.0
Parental CHO	9.98 ± 4.2	51.3
Lec1 CHO	<0.06 ± 0.07	<0.3
Primary co-transformants		
Colony 3.1	24.42 ± 7.6	125.4
Clone 3.1.3	24.75 ± 7.7	127.1
Clone 3.1.5	45.85 ± 13.6	235.5
Colony 53.1	41.80 ± 4.8	214.7
Secondary co-transformants		
Colony 22.1.6	10.11 ± 1.3	51.9
Colony 22.2.1	10.43 ± 0.03	53.6
Colony 27.4.1	21.45 ± 5.1	110.2

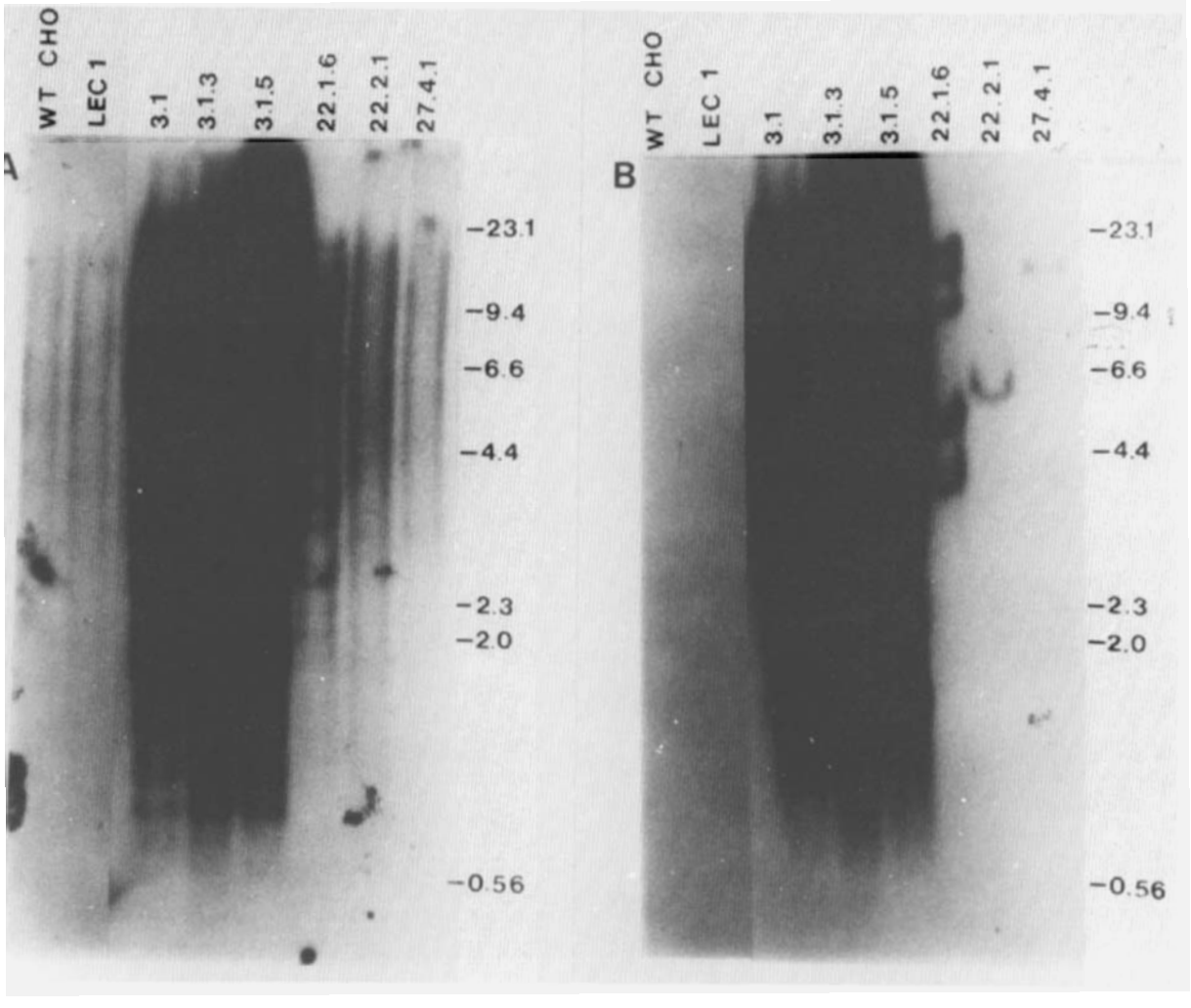
A
WT CHO
LEC 1
3.1
3.1.3
3.1.5
22.1.6
22.2.1
27.4.1
-23.1
-9.4
-6.6
-4.4
-2.3
-2.0
-0.56
B
WT CHO
LEC 1
3.1
3.1.3
3.1.5
22.1.6
22.2.1
27.4.1
-23.1
-9.4
-6.6
-4.4
-2.3
-2.0
-0.56

Figure 1A,B.

purified and used to transform Lec1 cells. A secondary co-transformant was selected (Table I, transformation 5) with Con A, named 27.4.1. All three secondary co-transformants, 22.2.1, 22.1.6, and 27.4.1, proliferated in the presence of both Con A and G-418 and demonstrated the parental CHO lectin-resistance phenotype.

N-Acetylglucosaminyltransferase 1 Activity of Co-Transformants

Lec1 CHO cells exhibited no detectable in vitro GlcNAc-TI-specific activity, whereas A431 cells demonstrated approximately twofold more GlcNAc-TI activity than parental CHO cells (Table II). The two primary co-transformant colonies, 3.1 and 53.1, exhibited up to twofold more GlcNAc-TI activity than A431 cells. Clone 3.1.3 and colony 3.1 exhibited a similar level of GlcNAc-TI activity. However, clone 3.1.5 demonstrated in vitro GlcNAc-TI activity that was twofold higher than colony 3.1, suggesting that the original colony 3.1 had co-transformant cells expressing two distinct levels of in vitro GlcNAc-TI-specific activity. The in vitro GlcNAc-TI activity of the primary co-transformants, which is 270–470% of the parental CHO cell GlcNAc-TI-specific activity, is thus much higher than that seen in previous primary transformants (4–100%) with only genomic A431 cell DNA [2]. Secondary cotransformants exhibited two- to fourfold less in vitro GlcNAc-TI activity than the primary co-transformants (Table II). A decrease in GlcNAc-TI activity was also noted in previous secondary transformants [2].

DNA Southern Blot Analysis

Primary and secondary co-transformants were examined for the presence of human DNA sequences by hybridization to the human repetitive *alu* sequence. Primary co-transformants exhibited a large number of bands hybridizing to the *alu* probe (Fig. 1A). Secondary co-transformant 22.1.6 exhibited several bands hybridizing to the human repetitive sequence probe, whereas 22.2.1 and 27.4.1 showed little or no hybridization at this stringency.

The primary co-transformants also showed a large number of bands hybridizing to the *neo* probe (Fig. 1B). Secondary co-transformant 22.1.6 demonstrated a number of discrete bands hybridizing to the *neo* probe, and co-transformants 22.2.1 and 27.4.1 showed a single band hybridizing to *neo*. Secondary co-transformants 22.2.1 and 27.4.1 were also digested with Bam H1 prior to hybridization with *neo*. Each co-transformant exhibited a single band, although the mobilities differed from those seen in Eco R1 digested DNA (data not shown). These results suggest that the *neo* sequence is present at only a single site in the genome of the these two secondary co-transformants.

The DNA gel blot hybridization indicates that the secondary co-transformants have taken up exogenous DNA. In addition, two secondaries showed a single band hybridizing to the marker *neo* DNA sequence.

Fig. 1. DNA blot hybridization. Genomic DNAs were digested, transferred to nitrocellulose, probed with *alu* (**A**), and subsequently reprobed with *neo* (**B**). Lane 1, parental CHO (WT CHO); lane 2, Lec1 CHO (LEC 1); lane 3, primary co-transformant colony 3.1; lanes 4 and 5, primary co-transformant clones 3.1.3 and 3.1.5; lanes 6–8, secondary co-transformant colonies 22.1.6, 22.2.1, and 27.4.1. The positions of size standards in kilobases are shown at the right of each panel.

DISCUSSION

Lec1 CHO cells have been co-transformed with human DNA and a marker sequence, the bacterial *neo* gene. Lec1 CHO cells have not been observed to revert to GlcNAc-TI enzyme activity either spontaneously or by mock transfections [2]. In addition, the in vitro GlcNAc-TI-specific enzyme activity expressed in the primary co-transformants is two- to fourfold higher than that demonstrated by parental CHO cells. These results suggest that the human gene for GlcNAc-TI enzyme activity has been transferred to the recipient Lec1 CHO cells. Using a similar approach, Ernst et al. [4] have transformed and expressed a human fucosyltransferase activity in mouse cells that lack the enzyme activity. The three secondary cotransformants did not exhibit a single discrete band cohybridizing to the human repetitive element *alu*. This may be due to the lack of an *alu* sequence in or near the gene coding for the human GlcNAc-TI gene. It is also possible that a human GlcNAc-TI gene-associated *alu* element may lack homology to the pBlur 8 probe used with the hybridization conditions in these experiments.

Co-transformation of genes has been shown to result in the close proximity of target and marker DNA [3]. Two secondary co-transformants, 22.2.1 and 27.4.1, exhibited a discrete, single band hybridizing to the *neo* probe. These bands presumably confer resistance to the antibiotic G-418 in these cells. Hence, genomic DNA libraries constructed from co-transformants 22.2.1 and 27.4.1 may contain a single *neo* sequence per genome. If one of the two independent co-transformants, 22.2.1 or 27.4.1, has in fact human sequences or the human gene coding for GlcNAc-TI activity near the *neo* sequence, the human GlcNAc-TI gene should be readily isolated from genomic phage or cosmid libraries.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health grants CA 35377 (M.P.) and AR 38872 (N.F.). M.P. is a recipient of an American Cancer Society Research Award.

REFERENCES

1. Narasimhan S, Stanley P, Schachter H: J Biol Chem 252:3926–3933, 1977.
2. Ripka J, Pierce M, Fregien N: Biochem Biophys Res Commun 159:554–560, 1989.
3. Perucho M, Hanahan D, Wigler M: Cell 22:309–317, 1980.
4. Fregien N, Davidson N: Gene 48:1–10, 1986.
5. Jelinek WR, Toomey TP, Leinwand L, Duncan CH, Biro PA, Choudary PV, Weissman SM, Rubin CM, Houck CM, Deininger PL, Schmidt CW: Proc Natl Acad Sci USA 77:1398–1402, 1980.
6. Palcic M, Heerze LD, Pierce M, Hindsgaul O: Glycoconjagate J 5:49–63, 1988.
7. Southern EM: J Mol Biol 98:503–517, 1975.
8. Feinberg AP, Vogelstein B: Anal Bioch 137:266–267, 1984.
9. Ernst LK, Rajan VP, Larsen RD, Ruff MM, Lowe JB: J Biol Chem 264:3436–3447, 1989.