

[11] Lectin-Resistant CHO Glycosylation Mutants

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Abstract

Chinese hamster ovary (CHO) mutant cells with a wide variety of alterations in the glycosylation of proteins and lipids have been isolated by selection for resistance to the cytotoxicity of plant lectins. These CHO mutants have been used to characterize glycosylation pathways, to identify genes that code for glycosylation activities, to elucidate functional roles of glycans that mediate biological processes, and for glycosylation engineering. In this chapter, we briefly describe the available panel of lectin-resistant CHO mutants and summarize their glycan alterations and the biochemical and genetic bases of mutation.

Introduction

CHO cells have been very successfully used as somatic cell genetic tools. A significant proportion of the CHO genome is functionally hemizygous (Siminovitch, 1976), which in combination with a high frequency of segregation-like events (Worton, 1978) makes it easy to obtain loss-of-function (recessive) mutant cells. A large number of CHO mutants affected in a wide range of cellular processes have been selected from CHO cell populations (Gottesman, 1985; Hanada and Nishijima, 2000; Nishimoto, 1997). This chapter describes what is known about CHO mutants that were mainly selected for resistance to toxic plant lectins (Stanley, 1983, 1984). Their resistance primarily arises from reduced cell surface binding of the lectin used in selection due to an alteration in the glycans to which that lectin binds. Most of these mutants are affected in *N*- and *O*-glycan synthesis pathways. Some are also affected in the synthesis of glycolipids, glycosaminoglycans (GAGs) or glycosylphosphatidylinositol (GPI) membrane anchors. Chapters 12 and 13 in this volume discuss mutants affected in the GPI and GAG pathways, respectively. Some CHO glycosylation mutants have a defect in one of the components of the conserved oligomeric complex (COG) associated with the Golgi compartment (Oka and Krieger, 2005). However, the affects on glycosylation due to COG defects are indirect and these mutants are not described here.

Glycosylation in CHO Cells

CHO cells have a range of complex and oligomannosyl *N*-glycans with few hybrid structures (Lee *et al.*, 2001). Poly lactosamine chains containing up to six units (Kawar *et al.*, 2005) and polysialic acid (Hong and Stanley, 2003; Muhlenhoff *et al.*, 1996) have been observed as minor species. *O*-glycans of CHO cells include mucins containing up to four sugars but not the core 2 structure (Bierhuizen and Fukuda, 1992; Sasaki *et al.*, 1987), and *O*-fucosylated (Moloney *et al.*, 2000), *O*-glucosylated (Moloney *et al.*, 2000), or *O*-mannosylated (Patnaik and Stanley, 2005) structures. The major glycolipid of CHO cells is GM₃, α 2,3-sialylated lactosylceramide (Stanley, 1980; Warnock *et al.*, 1993). CHO cells also contain heparan sulfate and chondroitin sulfate GAGs (Esko *et al.*, 1985). CHO cells do not express α 1,2-, α 1,3-, or α 1,4-fucosyltransferases (Howard *et al.*, 1987), ST6Gal α 2,6-sialyltransferases that transfer sialic acid (SA) to galactose (Gal) (Sasaki *et al.*, 1987), or GlcNAc-TIII that transfers the bisecting *N*-acetylglucosamine (GlcNAc) (Campbell and Stanley, 1984). CHO cells express the six known β 1,4-galactosyltransferases; however, *B4galt6* is not expressed in the Pro⁻⁵ parent CHO line (Lee *et al.*, 2001). CHO cells lack sulfotransferases required to generate sulfated glycolipids or sulfated *N*- or *O*-glycans (Brockhausen *et al.*, 2001).

Isolation and Characterization of Lectin-Resistant Mutants

CHO parent populations Pro⁻⁵ CHO, Gat⁻² CHO, or CHO-K1 were subjected to selection with and without prior mutagenesis using agents such as 5-azacytidine (5-azaC) and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG). The selective agents were used in single-step or multistep negative selection strategies employing cytotoxic plant lectins (Stanley, 1984) or bacterial toxins in the case of CHO2.38 (Ashida *et al.*, 2006). Most mutants were isolated in a single step for resistance to a single lectin, but others harboring mutations in up to four genes were isolated following selection with multiple lectins in combination or sequentially (Stanley, 1989). The change in lectin sensitivity of a mutant to a panel of lectins reflects the nature of the underlying alteration in glycosylation. Thus, a particular glycosylation gene mutation generates a characteristic lectin-resistance (Lec^R) phenotype. Table I presents the Lec^R phenotype for many mutant lines. Such phenotypic profiles allow rapid classification of new isolates (Stanley, 1989), have provided initial clues to the nature of the biochemical activity affected in the different mutants (e.g., Campbell and Stanley, 1984), have aided in the functional cloning of glycosylation genes (e.g., Kumar *et al.*, 1990), and are of value in designing selections for the isolation and characterization of new glycosylation mutants.

TABLE I
LECTIN-RESISTANCE PHENOTYPE OF CHO GLYCOSYLATION MUTANTS

Cell line	L-PHA ($\mu\text{g/ml}$)	WGA ($\mu\text{g/ml}$)	ConA ($\mu\text{g/ml}$)	Ricin (ng/ml)	LCA ($\mu\text{g/ml}$)	PSA ($\mu\text{g/ml}$)	E-PHA ($\mu\text{g/ml}$)	MOD (pg/ml)	Abrin (ng/ml)
CHO	5	2	18	5	18	50	35	2.5	2.5
Lec1	>1000R	30R	6S	100R	>200R	9R	>10R	4R	300R
Lec1A	>300R	9R	5S	10R	35R	5R	>10R	2R	3R
Lec2	S	11R	—	100S	2S	2S	—	5S	>10S
Lec2B	S	25R	S	3S	S	?	?	?	?
Lec3	S	5R	—	10S	2S	2S	—	25S	?
Lec4	>1000R	R	S	S	S	2S	R	—	?
Lec5	7R	R	R	3R	3R	S	2R	<2R	<6R
Lec8	10R	100R	S	R	10R	2S	>10R	2R	R
Lec9	R	R	—	10R	R	—	—	<5R	15R
LEC10	2S	S	—	20R	—	—	10S	—	20R
LEC11	4R	8R	—	25S	3R	—	R	2R	5S
LEC12	3R	50R	—	4S	2R	—	?	4R	S
Lec13	—	—	—	—	27R	48R	?	—	S
Lec13A	—	—	—	—	3R	9R	?	S	—
LEC14	R	—	—	—	2R	10R	?	—	—
Lec15	—	—	—	4R	—	?	2S	—	4R
LEC16	R	S	S	3R	R	?	—	—	3R
LEC17	—	—	S	35R	—	—	—	—	4R
LEC18	—	—	—	S	16R	39R	?	—	—
Lec19	3S	2S	S	10R	S	?	2S	2R	10R
Lec20	2R	S	S	2R	S	?	7R	3R	2R
Lec21	—	—	—	2R	?	?	—	300R	—
Lec22	S	—	—	5R	—	?	—	—	4R

(continued)

TABLE I (continued)

Cell line	L-PHA ($\mu\text{g/ml}$)	WGA ($\mu\text{g/ml}$)	ConA ($\mu\text{g/ml}$)	Ricin (ng/ml)	LCA ($\mu\text{g/ml}$)	PSA ($\mu\text{g/ml}$)	E-PHA ($\mu\text{g/ml}$)	MOD (pg/ml)	Abrin (ng/ml)
Lec23	>58R	10R	5S	4R	—	?	8R	R	8R
Lec24	2R	2R	R	12R	4R	?	2R	R	12R
Lec25	R	—	—	4R	—	?	R	—	3R
Lec26	—	—	—	10R	S	?	—	—	16R
Lec27	—	—	—	12R	—	?	—	R	4R
Lec28	R	S	S	4R	S	?	S	R	12R
LEC29	R	R	R	10S	R	?	?	?	?
LEC30	10R	50R	R	S	4R	?	?	?	?
LEC31	?	?	?	?	?	R	3-7R	4R	?
Lec32	?	17R	?	500R	?	?	?	?	?

—, same as wild-type CHO; ?, unknown; L-PHA, leuko-phytohemagglutinin from *Phaseolus vulgaris*; WGA, wheat germ agglutinin; ConA, concanavalin A; Ricin, *Ricinus communis* lectin II; LCA, *Lens culinaris* lectin; PSA, *Pisum sativum* lectin; E-PHA, erythro-phytohemagglutinin from *Phaseolus vulgaris*; MOD, modeccin.

Note: Fold resistance (R) or sensitivity (S) compared to wild-type parental CHO D_{10} value (lectin concentration at 10% relative plating efficiency); S or R given alone are less than twofold different from wild-type CHO.

Most of the mutants have been named numerically with the prefix Lec or LEC (Table I). This nomenclature was first defined in 1983 by Stanley. Lec mutants are so called because they have lost an activity (loss of function), whereas LEC mutants have acquired an activity not detected in parent cells and are gain of function. All Lec mutants are recessive in that their defect is complemented in somatic cell hybrids formed with parent cells. Some loss-of-function mutants with distinct but related Lec^R phenotypes belong to the same complementation group. Thus, complementation analysis identifies mutants that, in spite of having apparently different phenotypes, are mutated in the same gene. For example, the Lec1 and Lec1A mutants belong to the same complementation group (number 1) and they are both mutated in the *Mgat1* gene (Chaney and Stanley, 1986; Chen and Stanley, 2003; Chen *et al.*, 2001b). Mutants with multiple glycosylation defects belong to multiple complementation groups (e.g., Lec3.2.8.1). All LEC mutants are dominant in that somatic cell hybrids formed with parent CHO exhibit the mutant phenotype. However, one dominant mutant was found to arise from a loss-of-function mutation in a negative regulatory factor (Zhang *et al.*, 1999).

The mutants in Table I have been characterized extensively at the structural, biochemical, and/or genetic level. Figure 1 summarizes the sites of many of the glycosylation defects in terms of the sugar added in gain-of-function mutants (+) or the sugar that is not transferred (–) in the case of loss-of-function mutants to *N*-glycan, *O*-fucose, *O*-mannose, and mucin *O*-glycan structures. Tables II and III summarize what is known about the glycan structural changes in each mutant, the biochemical basis of these changes, and the molecular basis of mutation where known. The precise genetic change leading to the activation of a glycosylation gene in the gain-of-function mutants is largely unknown. On the other hand, the mutation in the glycosylation gene affected in many of the loss-of-function mutants has been identified (Table IV). Whereas revertants generally do not arise at high frequency, some null mutant phenotypes are “leaky,” perhaps because of redundancy at a biochemical level. Some mutants, however, revert at a frequency of 10^{−4} (Chen *et al.*, 2005). Interestingly, all the different genes sequenced from clones and independent CHO mutants show remarkably little variation in sequence, thus allowing point mutations to be easily identified in glycosylation mutants. However, this is likely to be because these isolates have not been in continuous culture for more than about 2–4 mo.

Availability and Culture of the Mutants

The Pro^{−5} and CHO-K1 parental CHO cells and the Lec1, Lec2, and Lec8 mutants are available from the American Type Culture Collection (ATCC;

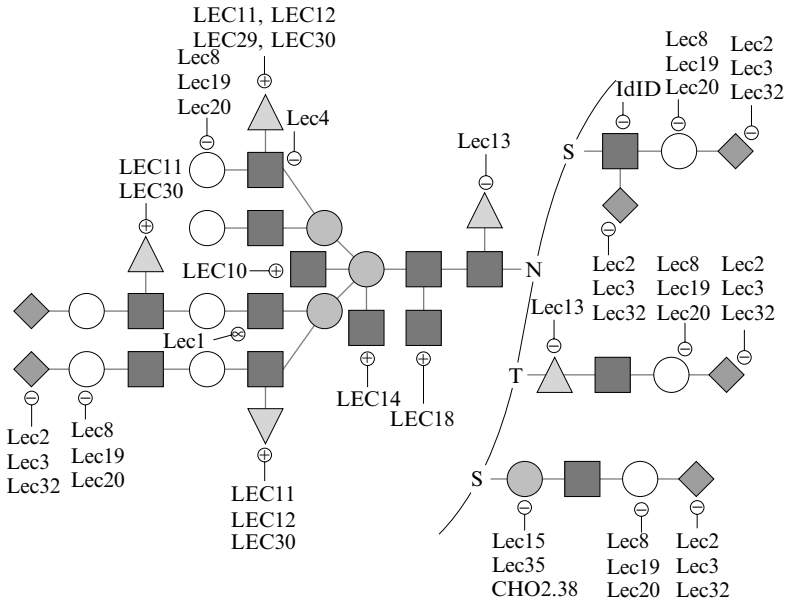


FIG. 1. This diagram illustrates the altered *N*-glycans, mucin *O*-glycans, *O*-fucose, and *O*-mannose glycans in many of the CHO glycosylation mutants described here. The major glycolipid in CHO cells is GM₃, which is affected in mutants defective for transfer or synthesis of CMP-SA or UDP-Gal. Proteoglycans are affected in mutants defective for the synthesis or transfer of UDP-Gal. A loss or reduction of a sugar residue at a particular position is indicated with the minus (–) sign, whereas the gain of a sugar residue is indicated with a plus (+) sign. Asparagine, threonine, or serine residues bearing the different glycans are indicated by their amino acid code. Sugar symbols: Gray triangle, fucose; gray circle, mannose; white circle, galactose; black square, *N*-acetylglucosamine; white square, *N*-acetylgalactosamine; gray trapezoid, sialic acid.

Manassas, VA). Other mutants are available from investigator laboratories. The MI8–5, Lec9, Lec15, and Lec35 mutants are temperature sensitive and should be grown at 34°. These four mutants and the IdID mutant, as well as cells derived from CHO-K1, should be grown in monolayer, whereas the other mutants can be cultured in suspension or monolayer. For certain purposes, dialyzed serum should be used when culturing Lec13 cells as fucose in serum can be used by the cells to generate GDP-fucose, partially bypassing the defect in Lec13 (Chen *et al.*, 2001a). Similarly, the IdID mutation can be bypassed in the presence of undialyzed serum (Kingsley *et al.*, 1986). Lec3 cells are known to salvage SA from serum proteins and lipids for conversion to CMP-SA (Hong and Stanley, 2003).

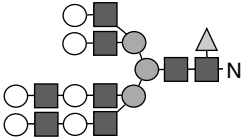
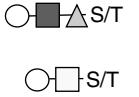
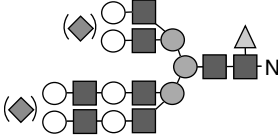
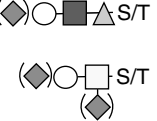
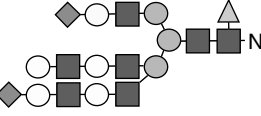
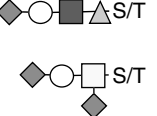
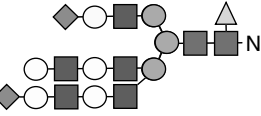
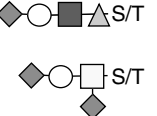
TABLE II
GLYCOSYLATION DEFECTS IN LECTIN-RESISTANT CHO MUTANTS

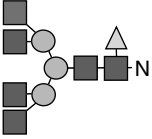
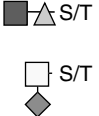
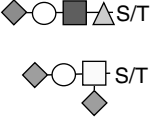
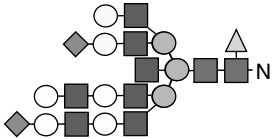
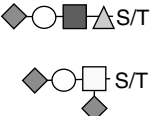
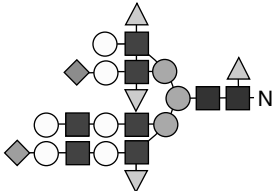
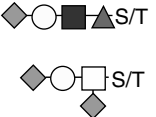
CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans ^a	Predicted <i>O</i> -glycans ^a	Most recent reference
Gat ⁻ 2 (parent)	—	—			Lee et al., 2001
Pro ⁻ 5 (parent)	↓ Gal on <i>N</i> -glycans	No expression of <i>B4galt6</i>			Lee et al., 2001
Lec1	↓ GlcNAc-TI	Insertion/deletion in <i>Mgat1</i> ORF			Chen and Stanley, 2003
Lec1A	K _m mutant of GlcNAc-TI	Point mutation in <i>Mgat1</i> ORF	Oligomannosyl 		Chen et al., 2001b

↓ Complex and hybrid
↑ Oligomannosyl

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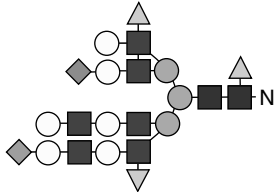
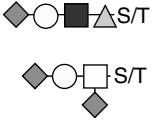
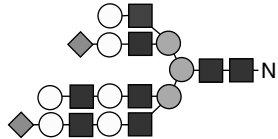
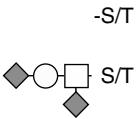
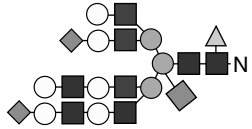
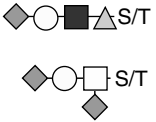
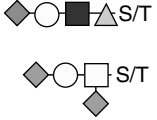
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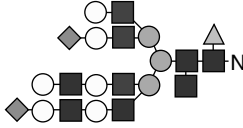
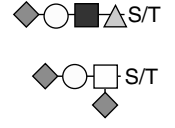
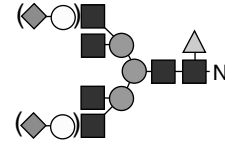
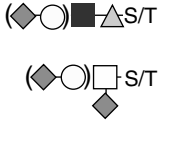
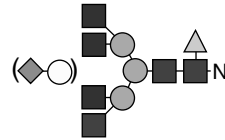
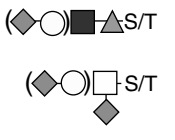
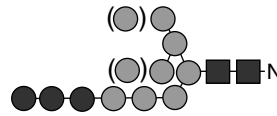
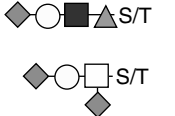
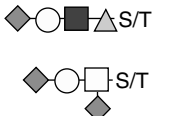
CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans ^a	Predicted <i>O</i> -glycans ^a	Most recent reference
Lec2	↓ CMP-sialic acid Golgi transporter	Mutation in <i>Slc35a1</i> ORF			Eckhardt <i>et al.</i>, 1998
Lec3	↓ UDP-GlcNAc 2-epimerase	Mutation in <i>Gne</i> ORF (epimerase domain)			Hong and Stanley, 2003
Lec4	↓ GlcNAc-TV	Deletion in <i>Mgat5</i> ORF			Weinstein <i>et al.</i>, 1996
Lec4A	Mislocalized GlcNAc-TV	Point mutation in <i>Mgat5</i> ORF			Weinstein <i>et al.</i>, 1996

Lec8	↓ UDP-Gal Golgi transporter	Mutation in <i>Slc35a2</i> ORF			Oelmann et al., 2001
Lec9	? ↓ polyprenol reductase synthesis	?	Decreased occupancy of <i>N</i> -glycosylation sites		Rosenwald and Krag, 1990
LEC10 LEC10A LEC10B	↑ GlcNAc-TIII	Activation of <i>Mgat3</i>			Stanley et al., 2005
LEC11 LEC11A LEC11B	↑ Fuc-TVIB ↑ Fuc-TVIA ↑ Fuc-TVIB	Activation of <i>Fut6</i> (<i>Fut6A</i> or <i>Fut6B</i>)			Zhang et al., 1999

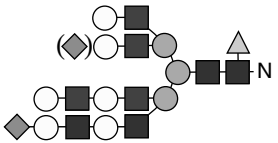



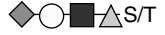


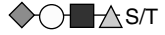


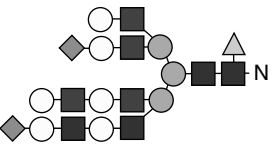


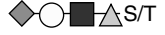


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TABLE II (continued)

CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans ^a	Predicted <i>O</i> -glycans ^a	Most recent reference
LEC12	↑ Fuc-TIX	Activation of <i>Fut9</i>			Patnaik et al., 2000
Lec13	↓ GDP-Man-4,6-dehydratase	↓ Expression of <i>Gm5s</i>			Ohyama et al., 1998; Sullivan et al., 1998
LEC14	↑ GlcNAc-TVII	?			Raju and Stanley, 1998
Lec15	↓ Dol-P-Man synthase	Allelic loss and <i>Dpm2</i> inactivation	Decreased occupancy of <i>N</i> -glycosylation sites		Maeda et al., 1998

LEC18	↑ GlcNAc-TVIII	?			Raju and Stanley, 1998
Lec19	↓ β-1,4-galactosyl-transferases	↓ Expression of six <i>B4galt6</i> genes			Lee et al., 2003
Lec20	↓ β4GalT-1	Inactivation or ↓ expression of <i>B4galt1</i>			Lee et al., 2001
Lec23	↓ α-glucosidase I	Point mutation in <i>Gcs1</i> ORF			Hong et al., 2004
Lec24	Slightly truncated lipid-linked oligosaccharides	?	Decreased occupancy of <i>N</i> -glycosylation sites		S. S. Krag, unpublished observations

(continued)

Lec32	↓ CMP-sialic acid synthetase	↓ expression of <i>Cmas</i>		  	Potvin <i>et al.</i> , 1995
Lec35	Accumulation of Man ₅ GlcNAc ₂ -P-P-dolichol	Inactivation of <i>Mpdul</i>	Decreased occupancy of <i>N</i> -glycosylation sites	  	Anand <i>et al.</i> , 2001
CHO2.38	↓ Dol-P-Man synthase	Mutation in <i>Dpm3</i>	Decreased occupancy of <i>N</i> -glycosylation sites	  	Ashida <i>et al.</i> , 2006
IdlD	↓ UDP-Gal/Glc-4-epimerase	?		 	Kingsley <i>et al.</i> , 1986
MI8-5	↓ glucosylated Man ₀ GlcNAc ₂ -P-P-dolichol	?	Decreased occupancy of <i>N</i> -glycosylation sites	  	Quellhorst <i>et al.</i> , 1999

^aComplex *N*-glycans of CHO parent cells may have polylectosamines (Lee *et al.*, 2001); other *N*-glycans include oligomannosyl and hybrid types; *O*-mannose glycans on α -dystroglycan will also be affected, as will the synthesis of GM₃ in mutants that do not transfer Gal or SA. Sugar symbols: gray triangle, fucose; gray circle, mannose; white circle, galactose; black square, *N*-acetylglucosamine; white square, *N*-acetylgalactosamine; gray trapezoid, sialic acid; black circle, glucose.

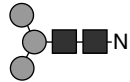
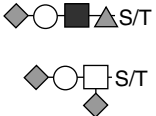
TABLE III
CHO MUTANTS WITH MULTIPLE GLYCOSYLATION DEFECTS

CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans ^a	Predicted <i>O</i> -glycans ^a	Most recent reference
Lec3.2	↓ UDP-GlcNAc 2-epimerase, ↓ CMP-sialic acid Golgi transporter	Mutations in <i>Gne</i> and <i>Slc35a1</i> ^b		○■△S/T ○□S/T	Hong and Stanley, 2003
Lec3.2.1	↓ UDP-GlcNAc 2-epimerase, ↓ CMP-sialic acid Golgi transporter, ↓ GlcNAc-TI	Mutations in <i>Gne</i> , <i>Mgat1</i> and <i>Slc35a1</i> ^b		○■△S/T ○□S/T	Hong and Stanley, 2003
Lec3.2.8	↓ CMP-sialic acid Golgi transporter, ↓ UDP-Gal Golgi transporter	Mutations in <i>Gne</i> , <i>Slc35a2</i> , <i>Slc35a1</i> ^b		■△S/T □S/T	Hong and Stanley, 2003
Lec3.2.8.1	↓ UDP-GlcNAc 2-epimerase,	Mutations in <i>GNE</i> , <i>Mgat1</i> , <i>Slc35a2</i> , <i>Slc35a1</i> ^b		■△S/T □S/T	Hong and Stanley, 2003

Lec4.8	<p>↓ CMP-sialic acid Golgi transporter, ↓ UDP-Gal Golgi transporter, ↓ GlcNAc-TI</p> <p>↓ GlcNAc-TV, ↓ UDP-Gal Golgi transporter</p>	<p>Mutations in <i>Mgat5</i> and <i>Slc35a2</i></p>		<p>■△S/T</p> <p>□S/T</p> <p>◆</p>	<p>Oelmann et al., 2001</p>
Lec4A.8	<p>Mislocalized GlcNAc-TV, ↓ UDP-Gal Golgi transporter</p>	<p>Mutations in <i>Mgat5</i> and <i>Slc35a2</i></p>		<p>■△S/T</p> <p>□S/T</p> <p>◆</p>	<p>Oelmann et al., 2001</p>
LEC10.8	<p>↑ GlcNAc-TIII, ↓ UDP-Gal Golgi transporter</p>	<p>Mutations in <i>Mgat3</i> and <i>Slc35a2</i></p>		<p>■△S/T</p> <p>□S/T</p> <p>◆</p>	<p>Stanley et al., 1991</p>
ldID.Lec1	<p>↓ UDP-Glc-4 epimerase, ↓ GlcNAc-TI</p>	<p>Mutation in <i>Mgat1</i> and ?</p>		<p>■△S/T</p> <p>-S/T</p>	<p>Chen and Stanley, 2003</p>

(continued)

TABLE III (continued)

CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans ^a	Predicted <i>O</i> -glycans ^a	Most recent reference
Lec35.Lec1	↓ GlcNAc-TI and no synthesis of Man ₆ GlcNAc ₂ -P-P-dolichol	Inactivation of <i>Mpdul</i> and mutation in <i>Mgat1</i>			Chen and Stanley, 2003

^a Complex *N*-glycans of CHO parent cells may have polylactosamines (Lee *et al.*, 2001); other *N*-glycans include oligomannosyl and hybrid types; *O*-mannose glycans on α -dystroglycan will also be affected, as will the synthesis of GM₃ in mutants that do not transfer Gal or SA.

^b Based on genetic complementation analysis (Stanley, 1989).

Sugar symbols: gray triangle, fucose; gray circle, mannose; white circle, galactose; black square, *N*-acetylglucosamine; white square, *N*-acetylgalactosamine; gray trapezoid, sialic acid; black circle, glucose.

TABLE IV
GENE MUTATIONS IN LOSS-OF-FUNCTION CHO GLYCOSYLATION MUTANTS

Mutant (clone number)	Parental CHO	Gene	DNA sequence alteration ^a	Protein sequence alteration	References
Lec1A (2A and 2C)	Pro ⁻⁵ CHO	<i>Mgat1</i>	634G>A	212D>N	Chen et al., 2001b
Lec1A (3E)	Gat ⁻² CHO	<i>Mgat1</i>	907C>A	303R>W	Chen et al., 2001b
Lec1A (5J)	Pro ⁻⁵ CHO	<i>Mgat1</i>	907C>A	303R>W	Chen et al., 2001b
Lec1 (1C)	Pro ⁻⁵ CHO	<i>Mgat1</i>	330–362 33 bp insertion	Frameshift and premature stop at codon 114	Chen and Stanley, 2003
Lec1 (1N)	Gat ⁻² CHO	<i>Mgat1</i>	784C>T	262R>stop	Chen and Stanley, 2003
Lec1 (3C)	Pro ⁻⁵ CHO	<i>Mgat1</i>	702–705 4 bp insertion	Frameshift and premature stop at codon 245	Chen and Stanley, 2003
Lec2 (4C)	Pro ⁻⁵ CHO	<i>Slc35a1</i>	575–751 deletion	192G-251F>V	Eckhardt et al., 1998
Lec2 (1E3)	CHO-K1	<i>Slc35a1</i>	195–574 duplication	Frameshift at codon 193	Eckhardt et al., 1998
Lec2 (6B2)	CHO-K1	<i>Slc35a1</i>	752–886 deletion	251F-296T>S	Eckhardt et al., 1998
Lec2 (8G8)	CHO-K1	<i>Slc35a1</i>	194–195 deletion	Frameshift at codon 67	Eckhardt et al., 1998
Lec2 (9D3)	CHO-K1	<i>Slc35a1</i>	566G>A	189G>E	Eckhardt et al., 1998
Lec3 (4B)	Pro ⁻⁵ CHO	<i>Gne</i>	103G>T	35E>stop	Hong and Stanley, 2003
Lec3 (6F)	Gat ⁻² CHO	<i>Gne</i>	394G>A	135G>E	Hong and Stanley, 2003

(continued)

TABLE IV (continued)

Mutant (clone number)	Parental CHO	Gene	DNA sequence alteration ^a	Protein sequence alteration	References
Lec4	Pro ⁻ 5 CHO	<i>Mgat5</i>	403–1224 822 bp insertion	Frameshift at codon 135 and premature stop at codon 156	Weinstein <i>et al.</i> , 1996
Lec4A	Pro ⁻ 5 CHO	<i>Mgat5</i>	563T>G	188L>R	Weinstein <i>et al.</i> , 1996
Lec8	Pro ⁻ 5 CHO	<i>Slc35a2</i>	275–374 deletion	92E>stop	Oelman <i>et al.</i> , 2001
Lec8 (5H)	Pro ⁻ 5 CHO	<i>Slc35a2</i>	636–638 deletion	213S deletion	Oelman <i>et al.</i> , 2001
Lec8 (1C)	Gat ⁻ 2 CHO	<i>Slc35a2</i>	844G>A	281G>D	Oelman <i>et al.</i> , 2001
Lec15 (B4-2-1)	Pro ⁻ 5 CHO	<i>Dpm1</i>	29G>A	10G>E	Pu <i>et al.</i> , 2003
Lec20 (6A)	Gat ⁻ 2 CHO	<i>B4galt1</i>	311 bp deletion of exons 4 and 5 in coding region	Truncated protein of 214 aa	Lee <i>et al.</i> , 2001
Lec23 (11C)	Pro ⁻ 5 CHO	<i>GcsI</i>	1320C>T	440S>F	Hong <i>et al.</i> , 2004
Lec35.1	CHO-K1	<i>Mpdu1</i>	Gross gene disruption		Anand <i>et al.</i> , 2001
Lec3.2.1	Pro ⁻ 5 CHO	<i>Mgat1</i>	~400 bp deletion in coding region	Not known	Chen and Stanley, 2003
Lec3.2.8.1	Pro ⁻ 5 CHO	<i>Mgat1</i>	1154G insertion	Frameshift at codon 385 and premature stop at codon 392	Chen and Stanley, 2003
Lec9.1	Pro ⁻ 5 CHO	<i>Mgat1</i>	310C insertion	Frameshift at codon 104 and premature stop at codon 116	Chen and Stanley, 2003
LEC10.8	Pro ⁻ 5 CHO	<i>Slc35a2</i>	364T>C	122Y>H	Oelman <i>et al.</i> , 2001
CHO2.38	CHO-K1 ^b	<i>Dpm3</i>	108–115 8 bp deletion	Frameshift at codon 36	Ashida <i>et al.</i> , 2006

^a A of the ATG start codon is nucleotide number 1.

^b Transfected with multiple copies of various genes.

TABLE V
VARIOUS APPLICATIONS OF SOME OF THE CHO GLYCOSYLATION MUTANTS

Mutant	Biochemical alteration	Observation	References
Lec1	No GlcNAcT-I	An adhesin of <i>Candida glabrata</i> specifically recognizes asialo-lactosyl <i>N</i> -glycans on human epithelial cells	Cormack <i>et al.</i> , 1999
Lec2	↓ CMP-sialic acid Golgi transporter	Identification of a novel type of CDG II _f resulting from mutation in CMP-sialic acid transporter	Martinez-Duncker <i>et al.</i> , 2005
Lec4	Loss of GlcNAc-TV	<i>Gly-2</i> of <i>C. elegans</i> encodes GlcNAc-TV	Warren <i>et al.</i> , 2002
Lec8	↓ UDP-Gal Golgi transporter	Molecular cloning of <i>Arabidopsis</i> UDP-Gal transporter genes	Bakker <i>et al.</i> , 2005
LEC10	Expression of GlcNAcT-III	Bisecting GlcNAc of complex <i>N</i> -glycans of human IgG1 is not important for antibody-dependent cellular cytotoxicity	Shinkawa <i>et al.</i> , 2003
LEC11	Expression of FucT-VI	Neuronal protection in stroke by CR1 possessing sLe ^x moieties	Huang <i>et al.</i> , 1999
LEC12	Expression of FucT-IX	VIM-2 glycan may be a ligand for E-selectin	Patnaik <i>et al.</i> , 2004
Lec13	↓ GDP-Man-4,6-dehydratase	Fucose facilitates Jagged1-induced Notch signaling	Chen <i>et al.</i> , 2001a
Lec15	↓ Dol-P-Man synthase	Protein <i>C</i> -mannosylation uses dolichol-phosphate mannose as a precursor	Doucey <i>et al.</i> , 1998
Lec20	↓ β4GalT-I	Fringe modulation of Jagged1 induced Notch signaling requires the action of β4GalT-I	Chen <i>et al.</i> , 2001a
Lec23	↓ α-glucosidase I	ERp57 interacts with both calnexin and calreticulin in the absence of their glycoprotein substrates	Oliver <i>et al.</i> , 1999
Lec3.2.8.1	↓ UDP-GlcNAc 2-epimerase, ↓ CMP-sialic acid Golgi translocase, ↓ UDP-Gal Golgi translocase, no GlcNAc-TI	Sialylation has no effect on the overall structure of the stalk-like region of CD8	Merry <i>et al.</i> , 2003
Lec35	No synthesis of Man ₆ GlcNAc ₂ -P-P-dolichol	Requirement for <i>O</i> -mannosylation of α-dystroglycan for binding LCMV virus	Imperiali <i>et al.</i> , 2005

Applications of the Mutants

The CHO mutants have been used to address a large variety of functional questions in glycobiology over the years. Examples of some of these applications are listed in [Table V](#). They include glycosylation engineering of experimental and therapeutic glycoproteins, as well as cloning and characterization of glycosylation genes. Panels of mutants have been used to characterize glycans involved in biological processes such as the binding of an amebic lectin ([Ravdin *et al.*, 1989](#)), recognition of sialylated Le^X by selectins ([Polley *et al.*, 1991](#)), binding of different galectins to the mixture of cell surface glycans ([Patnaik *et al.*, 2005](#)), and the functional glycosylation of α -dystroglycan ([Patnaik and Stanley, 2005](#)).

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References

- Anand, M., Rush, J. S., Ray, S., Doucey, M. A., Weik, J., Ware, F. E., Hofsteenge, J., Waechter, C. J., and Lehrman, M. A. (2001). Requirement of the Lec35 gene for all known classes of monosaccharide-P-dolichol-dependent glycosyltransferase reactions in mammals. *Mol. Biol. Cell* **12**, 487–501.
- Ashida, H., Maeda, Y., and Kinoshita, T. (2006). DPM1, the catalytic subunit of dolichol-phosphate mannose synthase, is tethered to and stabilized on the endoplasmic reticulum membrane by Dpm3. *J. Biol. Chem.* **281**, 896–904.
- Bakker, H., Routier, F., Oelmann, S., Jordi, W., Lommen, A., Gerardy-Schahn, R., and Bosch, D. (2005). Molecular cloning of two Arabidopsis UDP-galactose transporters by complementation of a deficient Chinese hamster ovary cell line. *Glycobiology* **15**, 193–201.
- Bierhuizen, M. F., and Fukuda, M. (1992). Expression cloning of a cDNA encoding UDP-GlcNAc:Gal beta 1-3-GalNAc-R (GlcNAc to GalNAc) beta 1-6GlcNAc transferase by gene transfer into CHO cells expressing polyoma large tumor antigen. *Proc. Natl. Acad. Sci. USA* **89**, 9326–9330.
- Brockhausen, I., Vavasseur, F., and Yang, X. (2001). Biosynthesis of mucin type O-glycans: Lack of correlation between glycosyltransferase and sulfotransferase activities and CFTR expression. *Glycoconj. J.* **18**, 685–697.
- Campbell, C., and Stanley, P. (1984). A dominant mutation to ricin resistance in Chinese hamster ovary cells induces UDP-GlcNAc:glycopeptide beta-4-N-acetylglucosaminyltransferase III activity. *J. Biol. Chem.* **259**, 13370–13378.
- Chaney, W., and Stanley, P. (1986). Lec1A Chinese hamster ovary cell mutants appear to arise from a structural alteration in N-acetylglucosaminyltransferase I. *J. Biol. Chem.* **261**, 10551–10557.
- Chen, J., Moloney, D. J., and Stanley, P. (2001a). Fringe modulation of Jagged1-induced Notch signaling requires the action of beta 4galactosyltransferase-1. *Proc. Natl. Acad. Sci. USA* **98**, 13716–13721.

- Chen, W., and Stanley, P. (2003). Five Lec1 CHO cell mutants have distinct Mgat1 gene mutations that encode truncated N-acetylglucosaminyltransferase I. *Glycobiology* **13**, 43–50.
- Chen, W., Tang, J., and Stanley, P. (2005). Suppressors of alpha(1,3)fucosylation identified by expression cloning in the LEC11B gain-of-function CHO mutant. *Glycobiology* **15**, 259–269.
- Chen, W., Unligil, U. M., Rini, J. M., and Stanley, P. (2001b). Independent Lec1A CHO glycosylation mutants arise from point mutations in N-acetylglucosaminyltransferase I that reduce affinity for both substrates. Molecular consequences based on the crystal structure of GlcNAc-TI(.). *Biochemistry* **40**, 8765–8772.
- Cormack, B. P., Ghorri, N., and Falkow, S. (1999). An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. *Science* **285**, 578–582.
- Doucey, M. A., Hess, D., Cacan, R., and Hofsteenge, J. (1998). Protein C-mannosylation is enzyme-catalysed and uses dolichyl-phosphate-mannose as a precursor. *Mol. Biol. Cell* **9**, 291–300.
- Eckhardt, M., Gotza, B., and Gerardy-Schahn, R. (1998). Mutants of the CMP-sialic acid transporter causing the Lec2 phenotype. *J. Biol. Chem.* **273**, 20189–20195.
- Esko, J. D., Stewart, T. E., and Taylor, W. H. (1985). Animal cell mutants defective in glycosaminoglycan biosynthesis. *Proc. Natl. Acad. Sci. USA* **82**, 3197–3201.
- Gottesman, M. M. (1985). “Molecular Cell Genetics.” Wiley, New York.
- Hanada, K., and Nishijima, M. (2000). Selection of mammalian cell mutants in sphingolipid biosynthesis. *Methods Enzymol.* **312**, 304–317.
- Hong, Y., and Stanley, P. (2003). Lec3 CHO mutants lack UDP-GlcNAc 2-epimerase activity due to mutations in the epimerase domain of the Gne gene. *J. Biol. Chem.* **278**, 53045–53054.
- Hong, Y., Sundaram, S., Shin, D. J., and Stanley, P. (2004). The Lec23 Chinese hamster ovary mutant is a sensitive host for detecting mutations in alpha-glucosidase I that give rise to congenital disorder of glycosylation IIb (CDG IIb). *J. Biol. Chem.* **279**, 49894–49901.
- Howard, D. R., Fukuda, M., Fukuda, M. N., and Stanley, P. (1987). The GDP-fucose: N-acetylglucosaminide 3-alpha-L-fucosyltransferases of LEC11 and LEC12 Chinese hamster ovary mutants exhibit novel specificities for glycolipid substrates. *J. Biol. Chem.* **262**, 16830–16837.
- Huang, J., Kim, L. J., Mealey, R., Marsh, H. C., Jr., Zhang, Y., Tenner, A. J., Connolly, E. S., Jr., and Pinsky, D. J. (1999). Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. *Science* **285**, 595–599.
- Imperiali, M., Thoma, C., Pavoni, E., Brancaccio, A., Callewaert, N., and Oxenius, A. (2005). O Mannosylation of alpha-dystroglycan is essential for lymphocytic choriomeningitis virus receptor function. *J. Virol.* **79**, 14297–14308.
- Kawar, Z. S., Haslam, S. M., Morris, H. R., Dell, A., and Cummings, R. D. (2005). Novel poly-GalNAcbeta1-4GlcNAc (LacdiNAc) and fucosylated poly-LacdiNAc N-glycans from mammalian cells expressing beta1,4-N-acetylgalactosaminyltransferase and alpha1,3-fucosyltransferase. *J. Biol. Chem.* **280**, 12810–12819.
- Kingsley, D. M., Kozarsky, K. F., Hobbie, L., and Krieger, M. (1986). Reversible defects in O-linked glycosylation and LDL receptor expression in a UDP-Gal/UDP-GalNAc 4-epimerase deficient mutant. *Cell* **44**, 749–759.
- Kumar, R., Yang, J., Larsen, R. D., and Stanley, P. (1990). Cloning and expression of N-acetylglucosaminyltransferase I, the medial Golgi transferase that initiates complex N-linked carbohydrate formation. *Proc. Natl. Acad. Sci. USA* **87**, 9948–9952.
- Lee, J., Park, S. H., Sundaram, S., Raju, T. S., Shaper, N. L., and Stanley, P. (2003). A mutation causing a reduced level of expression of six beta4-galactosyltransferase genes is the basis of the Lec19 CHO glycosylation mutant. *Biochemistry* **42**, 12349–12357.

- Lee, J., Sundaram, S., Shaper, N. L., Raju, T. S., and Stanley, P. (2001). Chinese hamster ovary (CHO) cells may express six β 4-galactosyltransferases (β 4GalTs). Consequences of the loss of functional β 4GalT-1, β 4GalT-6, or both in CHO glycosylation mutants. *J. Biol. Chem.* **276**, 13924–13934.
- Maeda, Y., Tomita, S., Watanabe, R., Ohishi, K., and Kinoshita, T. (1998). DPM2 regulates biosynthesis of dolichol phosphate-mannose in mammalian cells: Correct subcellular localization and stabilization of DPM1, and binding of dolichol phosphate. *EMBO J.* **17**, 4920–4929.
- Martinez-Duncker, I., Dupre, T., Piller, V., Piller, F., Candelier, J. J., Trichet, C., Tchernia, G., Oriol, R., and Mollicone, R. (2005). Genetic complementation reveals a novel human congenital disorder of glycosylation of type II, due to inactivation of the Golgi CMP-sialic acid transporter. *Blood* **105**, 2671–2676.
- Merry, A. H., Gilbert, R. J., Shore, D. A., Royle, L., Miroshnychenko, O., Vuong, M., Wormald, M. R., Harvey, D. J., Dwek, R. A., Classon, B. J., Rudd, P. M., and Davis, S. J. (2003). O-glycan sialylation and the structure of the stalk-like region of the T cell co-receptor CD8. *J. Biol. Chem.* **278**, 27119–27128.
- Moloney, D. J., Shair, L. H., Lu, F. M., Xia, J., Locke, R., Matta, K. L., and Haltiwanger, R. S. (2000). Mammalian Notch1 is modified with two unusual forms of O-linked glycosylation found on epidermal growth factor-like modules. *J. Biol. Chem.* **275**, 9604–9611.
- Muhlenhoff, M., Eckhardt, M., Bethe, A., Frosch, M., and Gerardy-Schahn, R. (1996). Autocatalytic polysialylation of polysialyltransferase-1. *EMBO J.* **15**, 6943–6950.
- Nishimoto, T. (1997). Isolation and characterization of temperature-sensitive mammalian cell cycle mutants. *Methods Enzymol.* **283**, 292–309.
- Oelmann, S., Stanley, P., and Gerardy-Schahn, R. (2001). Point mutations identified in Lec8 Chinese hamster ovary glycosylation mutants that inactivate both the UDP-galactose and CMP-sialic acid transporters. *J. Biol. Chem.* **276**, 26291–26300.
- Ohyama, C., Smith, P. L., Angata, K., Fukuda, M. N., Lowe, J. B., and Fukuda, M. (1998). Molecular cloning and expression of GDP-D-mannose-4,6-dehydratase, a key enzyme for fucose metabolism defective in Lec13 cells. *J. Biol. Chem.* **273**, 14582–14587.
- Oka, T., and Krieger, M. (2005). Multi-component protein complexes and Golgi membrane trafficking. *J. Biochem. (Tokyo)* **137**, 109–114.
- Oliver, J. D., Roderick, H. L., Llewellyn, D. H., and High, S. (1999). ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin. *Mol. Biol. Cell* **10**, 2573–2582.
- Patnaik, S. K., and Stanley, P. (2005). Mouse large can modify complex N- and mucin O-glycans on alpha-dystroglycan to induce laminin binding. *J. Biol. Chem.* **280**, 20851–20859.
- Patnaik, S. K., Potvin, B., and Stanley, P. (2004). LEC12 and LEC29 gain-of-function Chinese hamster ovary mutants reveal mechanisms for regulating VIM-2 antigen synthesis and E-selectin binding. *J. Biol. Chem.* **279**, 49716–49726.
- Patnaik, S. K., Potvin, B., Carlsson, S., Sturm, D., Leffler, H., and Stanley, P. (2005). Complex N-glycans are the Major Ligands for Galectin-1, Galectin-3 and Galectin-8 on Chinese Hamster Ovary Cells. *Glycobiology* (in press).
- Patnaik, S. K., Zhang, A., Shi, S., and Stanley, P. (2000). alpha(1,3)fucosyltransferases expressed by the gain-of-function Chinese hamster ovary glycosylation mutants LEC12, LEC29, and LEC30. *Arch. Biochem. Biophys.* **375**, 322–332.
- Polley, M. J., Phillips, M. L., Wayner, E., Nudelman, E., Singhal, A. K., Hakomori, S., and Paulson, J. C. (1991). CD62 and endothelial cell-leukocyte adhesion molecule 1 (ELAM-1)

- recognize the same carbohydrate ligand, sialyl-Lewis x. *Proc. Natl. Acad. Sci. USA* **88**, 6224–6228.
- Potvin, B., Raju, T. S., and Stanley, P. (1995). Lec32 is a new mutation in Chinese hamster ovary cells that essentially abrogates CMP-N-acetylneuraminic acid synthetase activity. *J. Biol. Chem.* **270**, 30415–30421.
- Pu, L., Scoocca, J. R., Walker, B. K., and Krag, S. S. (2003). A single point mutation resulting in an adversely reduced expression of DPM2 in the Lec15.1 cells. *Biochem. Biophys. Res. Commun.* **312**, 555–561.
- Quellhorst, G. J., Jr., O'Rear, J. L., Cacan, R., Verbert, A., and Krag, S. S. (1999). Nonglycosylated oligosaccharides are transferred to protein in MI8–5 Chinese hamster ovary cells. *Glycobiology* **9**, 65–72.
- Raju, T. S., and Stanley, P. (1998). Gain-of-function Chinese hamster ovary mutants LEC18 and LEC14 each express a novel N-acetylglucosaminyltransferase activity. *J. Biol. Chem.* **273**, 14090–14098.
- Ravdin, J. I., Stanley, P., Murphy, C. F., and Petri, W. A., Jr. (1989). Characterization of cell surface carbohydrate receptors for *Entamoeba histolytica* adherence lectin. *Infect. Immun.* **57**, 2179–2186.
- Rosenwald, A. G., and Krag, S. S. (1990). Lec9 CHO glycosylation mutants are defective in the synthesis of dolichol. *J. Lipid Res.* **31**, 523–533.
- Sasaki, H., Bothner, B., Dell, A., and Fukuda, M. (1987). Carbohydrate structure of erythropoietin expressed in Chinese hamster ovary cells by a human erythropoietin cDNA. *J. Biol. Chem.* **262**, 12059–12076.
- Shinkawa, T., Nakamura, K., Yamane, N., Shoji-Hosaka, E., Kanda, Y., Sakurada, M., Uchida, K., Anazawa, H., Satoh, M., Yamasaki, M., Hanai, N., and Shitara, K. (2003). The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *J. Biol. Chem.* **278**, 3466–3473.
- Siminovitch, L. (1976). On the nature of heritable variation in cultured somatic cells. *Cell* **7**, 1–11.
- Stanley, P. (1980). Altered glycolipids of CHO cells resistant to wheat germ agglutinin. In "American Chemical Society Symposium Series, No. 128, Cell Surface Glycolipids" (C. C. Sweeley, ed.), pp. 213–221. American Chemical Society.
- Stanley, P. (1983). Selection of lectin-resistant mutants of animal cells. *Methods Enzymol.* **96**, 157–184.
- Stanley, P. (1984). Glycosylation mutants of animal cells. *Annu. Rev. Genet.* **18**, 525–552.
- Stanley, P. (1989). Chinese hamster ovary cell mutants with multiple glycosylation defects for production of glycoproteins with minimal carbohydrate heterogeneity. *Mol. Cell. Biol.* **9**, 377–383.
- Stanley, P., Sundaram, S., and Sallustio, S. (1991). A subclass of cell surface carbohydrates revealed by a CHO mutant with two glycosylation mutations. *Glycobiology* **1**, 307–314.
- Stanley, P., Sundaram, S., Tang, J., and Shi, S. (2005). Molecular analysis of three gain-of-function CHO mutants that add the bisecting GlcNAc to N-glycans. *Glycobiology* **15**, 43–53.
- Sullivan, F. X., Kumar, R., Kriz, R., Stahl, M., Xu, G. Y., Rouse, J., Chang, X. J., Boodhoo, A., Potvin, B., and Cumming, D. A. (1998). Molecular cloning of human GDP-mannose 4,6-dehydratase and reconstitution of GDP-fucose biosynthesis *in vitro*. *J. Biol. Chem.* **273**, 8193–8202.
- Warnock, D. E., Roberts, C., Lutz, M. S., Blackburn, W. A., Young, W. W., Jr., and Baenziger, J. U. (1993). Determination of plasma membrane lipid mass and composition

- in cultured Chinese hamster ovary cells using high gradient magnetic affinity chromatography. *J. Biol. Chem.* **268**, 10145–10153.
- Warren, C. E., Krizus, A., Roy, P. J., Culotti, J. G., and Dennis, J. W. (2002). The *Caenorhabditis elegans* gene, *gly-2*, can rescue the N-acetylglucosaminyltransferase V mutation of Lec4 cells. *J. Biol. Chem.* **277**, 22829–22838.
- Weinstein, J., Sundaram, S., Wang, X., Delgado, D., Basu, R., and Stanley, P. (1996). A point mutation causes mistargeting of Golgi GlcNAc-TV in the Lec4A Chinese hamster ovary glycosylation mutant. *J. Biol. Chem.* **271**, 27462–27469.
- Worton, R. G. (1978). Karyotypic heterogeneity in CHO cell lines. *Cytogenet. Cell Genet.* **21**, 105–110.
- Zhang, A., Potvin, B., Zaiman, A., Chen, W., Kumar, R., Phillips, L., and Stanley, P. (1999). The gain-of-function Chinese hamster ovary mutant LEC11B expresses one of two Chinese hamster FUT6 genes due to the loss of a negative regulatory factor. *J. Biol. Chem.* **274**, 10439–10450.

[12] CHO Glycosylation Mutants: GPI Anchor

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Abstract

Glycosylphosphatidylinositol (GPI) is used for anchoring many cell surface proteins to the plasma membrane. Biosynthesis of GPI anchor, its attachment to proteins, and modification of GPI-anchored proteins (GPI-APs) en route to the plasma membrane are complex processes (Ferguson, 1999; Kinoshita and Inoue, 2000). GPI-AP-defective mutant cell lines derived from CHO and other cells have been very useful in elucidating GPI biosynthetic pathway and cloning genes involved in these processes. In this chapter, we overview GPI-AP biosynthesis, establishment and characterization of GPI-AP-defective mutant cell lines, expression cloning using those mutant cells, and characteristics of GPI-AP-defective mutant cell lines.

Overview of GPI-AP Biosynthesis

Biosynthesis of GPI

GPI is synthesized by the sequential additions of sugars and other components to phosphatidylinositol (PI) in the endoplasmic reticulum (ER) (Fig. 1) (Ferguson, 1999; Kinoshita and Inoue, 2000). At least eight reaction steps are required for generation of GPI that is competent for attachment to proteins (Fig. 2). The biosynthetic pathway is initiated by the transfer of *N*-acetylglucosamine (GlcNAc) from UDP-GlcNAc to PI on the