

## [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 GM<sub>3</sub>,  $\alpha$ 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  $\alpha$ 1,2-,  $\alpha$ 1,3-, or  $\alpha$ 1,4-fucosyltransferases (Howard *et al.*, 1987), ST6Gal  $\alpha$ 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  $\beta$ 1,4-galactosyltransferases; however, *B4galt6* is not expressed in the Pro<sup>-5</sup> 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<sup>-5</sup> CHO, Gat<sup>-2</sup> 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<sup>R</sup>) phenotype. Table I presents the Lec<sup>R</sup> 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 ( $\text{ng/ml}$ )	LCA ( $\mu\text{g/ml}$ )	PSA ( $\mu\text{g/ml}$ )	E-PHA ( $\mu\text{g/ml}$ )	MOD ( $\text{pg/ml}$ )	Abrin ( $\text{ng/ml}$ )
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-*Phaseolus vulgaris* hemagglutinin; WGA, wheat germ agglutinin; ConA, concanavalin A; Ricin, *Ricinus communis* lectin II; LCA, *Lens culinaris* lectin; PSA, *Pisum sativum* lectin; E-PHA, erythro-*Phaseolus vulgaris* hemagglutinin; 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<sup>R</sup> 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<sup>−4</sup> (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<sup>−5</sup> and CHO-K1 parental CHO cells and the Lec1, Lec2, and Lec8 mutants are available from the American Type Culture Collection (ATCC;

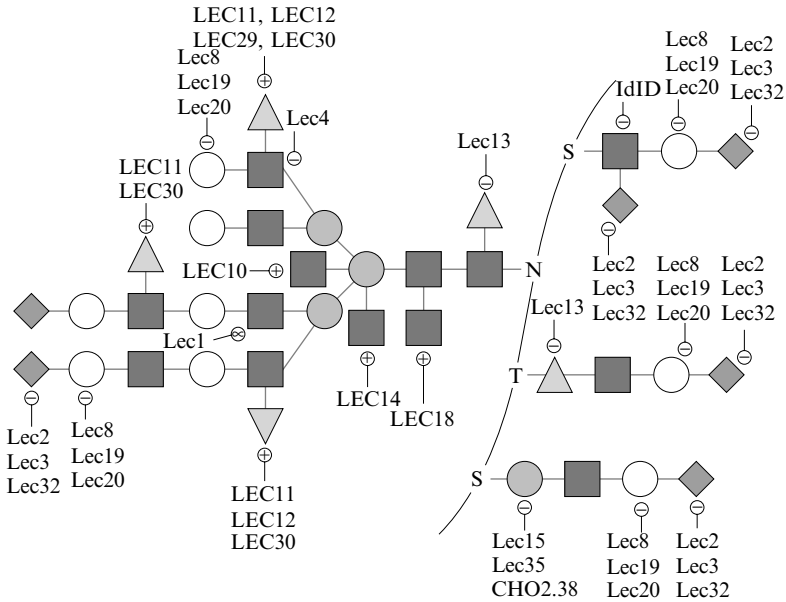
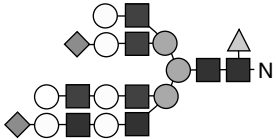
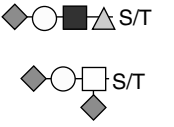
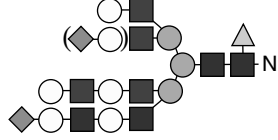
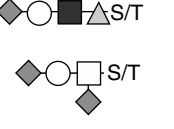
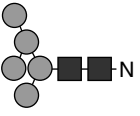
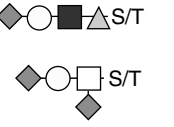
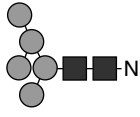
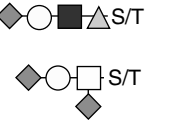


FIG. 1. This diagram illustrates the altered *N*-glycans, mucin *O*-glycans, *O*-fucose, and *O*-mannose glycans in many of the CHO glycosylation mutants described here. The major glycolipid in CHO cells is GM<sub>3</sub>, which is affected in mutants defective for transfer or synthesis of CMP-SA or 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

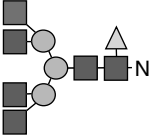
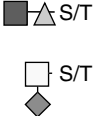
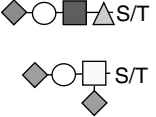
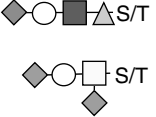
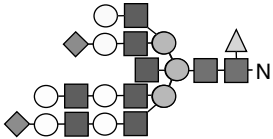
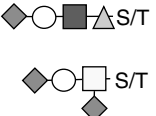
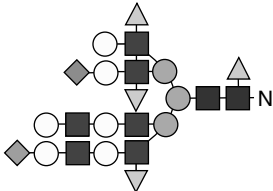
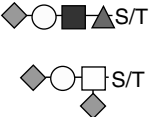
CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans <sup>a</sup>	Predicted <i>O</i> -glycans <sup>a</sup>	Most recent reference
Gat <sup>-</sup> 2 (parent)	—	—			<a href="#">Lee et al., 2001</a>
Pro <sup>-</sup> 5 (parent)	↓ Gal on <i>N</i> -glycans	No expression of <i>B4galt6</i>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

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TABLE II (continued)

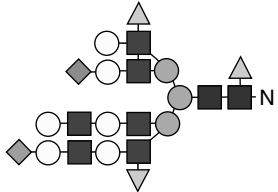
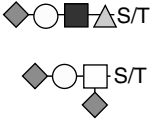
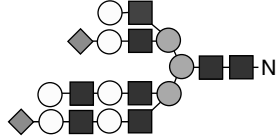
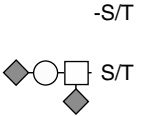
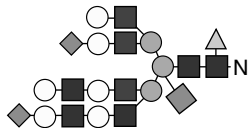
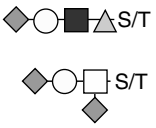
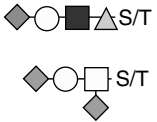
CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans <sup>a</sup>	Predicted <i>O</i> -glycans <sup>a</sup>	Most recent reference
Lec2	↓ CMP-sialic acid Golgi transporter	Mutation in <i>Slc35a1</i> ORF			<a href="#">Eckhardt <i>et al.</i>, 1998</a>
Lec3	↓ UDP-GlcNAc 2-epimerase	Mutation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

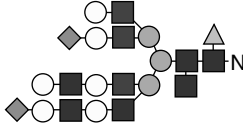
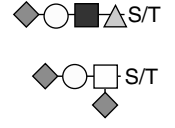
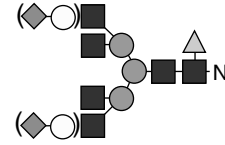
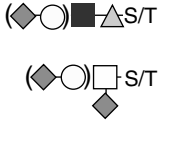
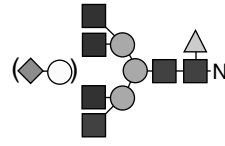
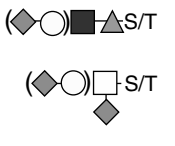
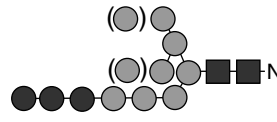
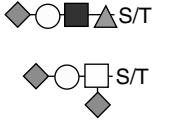
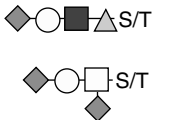


Lec8	↓ UDP-Gal Golgi transporter	Mutation in <i>Slc35a2</i> ORF			<a href="#">Oelmann et al., 2001</a>	
Lec9	? ↓ polyprenol reductase synthesis	?	Decreased occupancy of <i>N</i> -glycosylation sites			<a href="#">Rosenwald and Krag, 1990</a>
LEC10 LEC10A LEC10B	↑ GlcNAc-TIII	Activation of <i>Mgat3</i>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

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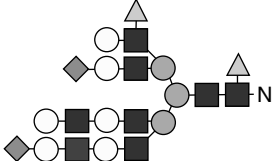
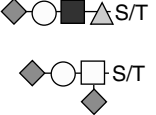
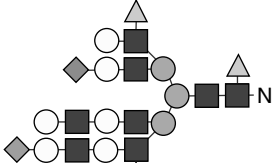
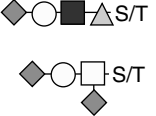
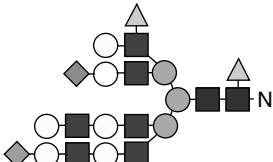
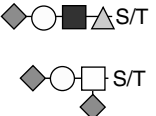
TABLE II (continued)

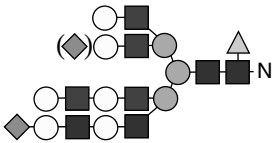

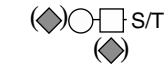

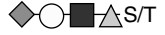
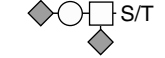

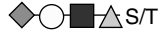
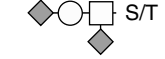

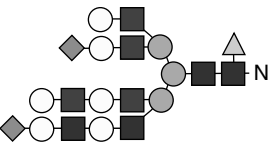


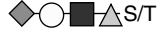
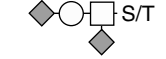

CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans <sup>a</sup>	Predicted <i>O</i> -glycans <sup>a</sup>	Most recent reference
LEC12	↑ Fuc-TIX	Activation of <i>Fut9</i>			<a href="#">Patnaik et al., 2000</a>
Lec13	↓ GDP-Man-4,6-dehydratase	↓ Expression of <i>Gm5</i>			<a href="#">Ohyama et al., 1998;</a> <a href="#">Sullivan et al., 1998</a>
LEC14	↑ GlcNAc-TVII	?			<a href="#">Raju and Stanley, 1998</a>
Lec15	↓ Dol-P-Man synthase	Allelic loss and <i>Dpm2</i> inactivation	Decreased occupancy of <i>N</i> -glycosylation sites		<a href="#">Maeda et al., 1998</a>

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)

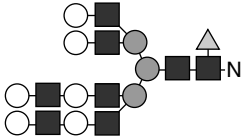
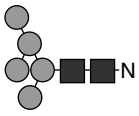
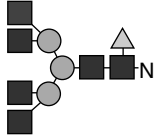
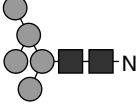
TABLE II (continued)

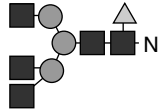
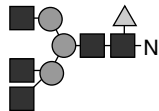
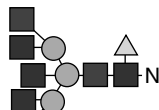
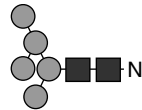
CHO line	Biochemical change	Genetic change	Predicted <i>N</i> -glycans <sup>a</sup>	Predicted <i>O</i> -glycans <sup>a</sup>	Most recent reference
LEC29	↑ Fuc-TIX	Activation of <i>Fut9</i>			<a href="#">Patnaik <i>et al.</i>, 2000</a>
LEC30	↑ Fuc-TIV and Fuc-TIX	Activation of <i>Fut4</i> and <i>Fut9</i>			<a href="#">Patnaik <i>et al.</i>, 2000</a>
LEC31	↑ $\alpha$ -1,3-fucosyltransferase	Activation of <i>Fut9</i>			S. K. Patnaik and P. Stanley, unpublished observations

Lec32	↓ CMP-sialic acid synthetase	↓ expression of <i>Cmas</i>		  	Potvin <i>et al.</i> , 1995
Lec35	Accumulation of Man <sub>5</sub> GlcNAc <sub>2</sub> -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 <sub>0</sub> GlcNAc <sub>2</sub> -P-P-dolichol	?	Decreased occupancy of <i>N</i> -glycosylation sites	  	Quellhorst <i>et al.</i> , 1999

<sup>a</sup> Complex *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  $\alpha$ -dystroglycan will also be affected, as will the synthesis of GM<sub>3</sub> 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.

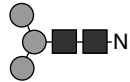
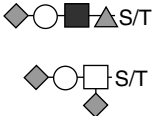
TABLE III  
CHO MUTANTS WITH MULTIPLE GLYCOSYLATION DEFECTS

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

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><a href="#">Oelmann et al., 2001</a></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><a href="#">Oelmann et al., 2001</a></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><a href="#">Stanley et al., 1991</a></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><a href="#">Chen and Stanley, 2003</a></p>

(continued)

TABLE III (continued)

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

<sup>a</sup> 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  $\alpha$ -dystroglycan will also be affected, as will the synthesis of GM<sub>3</sub> in mutants that do not transfer Gal or SA.

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

(continued)

TABLE IV (continued)

Mutant (clone number)	Parental CHO	Gene	DNA sequence alteration <sup>a</sup>	Protein sequence alteration	References
Lec4	Pro <sup>-</sup> 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 <sup>-</sup> 5 CHO	<i>Mgat5</i>	563T>G	188L>R	Weinstein <i>et al.</i> , 1996
Lec8	Pro <sup>-</sup> 5 CHO	<i>Slc35a2</i>	275–374 deletion	92E>stop	Oelman <i>et al.</i> , 2001
Lec8 (5H)	Pro <sup>-</sup> 5 CHO	<i>Slc35a2</i>	636–638 deletion	213S deletion	Oelman <i>et al.</i> , 2001
Lec8 (1C)	Gat <sup>-</sup> 2 CHO	<i>Slc35a2</i>	844G>A	281G>D	Oelman <i>et al.</i> , 2001
Lec15 (B4-2-1)	Pro <sup>-</sup> 5 CHO	<i>Dpm1</i>	29G>A	10G>E	Pu <i>et al.</i> , 2003
Lec20 (6A)	Gat <sup>-</sup> 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 <sup>-</sup> 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 <sup>-</sup> 5 CHO	<i>Mgat1</i>	~400 bp deletion in coding region	Not known	Chen and Stanley, 2003
Lec3.2.8.1	Pro <sup>-</sup> 5 CHO	<i>Mgat1</i>	1154G insertion	Frameshift at codon 385 and premature stop at codon 392	Chen and Stanley, 2003
Lec9.1	Pro <sup>-</sup> 5 CHO	<i>Mgat1</i>	310C insertion	Frameshift at codon 104 and premature stop at codon 116	Chen and Stanley, 2003
LEC10.8	Pro <sup>-</sup> 5 CHO	<i>Slc35a2</i>	364T>C	122Y>H	Oelman <i>et al.</i> , 2001
CHO2.38	CHO-K1 <sup>b</sup>	<i>Dpm3</i>	108–115 8 bp deletion	Frameshift at codon 36	Ashida <i>et al.</i> , 2006

<sup>a</sup> A of the ATG start codon is nucleotide number 1.

<sup>b</sup> 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 <sub>f</sub> 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 <sup>x</sup> 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 <sub>6</sub> GlcNAc <sub>2</sub> -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<sup>X</sup> 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  $\alpha$ -dystroglycan ([Patnaik and Stanley, 2005](#)).

### Acknowledgments

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## [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