

26912

REVIEW

The production of recombinant glycoproteins with special reference to simple eukaryotes including *Dictyostelium discoideum*

Eva Jung and Keith L. Williams¹

MUCAB (Macquarie University Center for Analytical Biotechnology), Macquarie University, Sydney, NSW, Australia, 2109

Because many recombinantly produced proteins require post-translational modification to be properly folded and active, there is a new emphasis on eukaryotic expression systems. *Dictyostelium discoideum* is a well studied eukaryotic model organism for the investigation of key questions in molecular and cell biology. More recently *D. discoideum* was successfully used as a system for recombinant glycoprotein production. The vegetative amoebae are easy and inexpensive to grow either in axenic culture or on Gram-negative bacteria. Reliable and uncomplicated transformation systems are also well established. This organism harbours the machinery to perform post-translational modifications such as phosphorylation, acylation, formation of glycosylphosphatidylinositol anchors and more importantly N- and O-linked glycosylation. This review provides an overview of glycosylation in different expression systems and focuses on glycosylation in *D. discoideum*.

Production of glycoproteins for biotechnological use

Sustained scientific and medical effort continues to be expended on the development, production and clinical application of recombinant glycoproteins for the therapeutic manipulation of animals and humans. As a result the biotechnology industry has become increasingly interested in the biological and therapeutic implications of post-translational modifications of proteins, and in particular glycosylation because it is the most common form of protein modification. Glycobiology has been described recently as 'the sleeping giant of biotechnology' [1] and the importance of carbohydrate residues on recombinant glycoproteins destined for human therapy is now well recognized [2]. Altered glycosylation of proteins is involved in several diseases [3] and knowledge about the changes might lead to a better understanding of the medical problems. Although a number of important therapeutic

glycoproteins do not require glycosylation for activity *in vivo* (e.g. erythropoietin, interleukin 2, tissue plasminogen activator, γ -interferon, granulocyte colony-stimulating factor and granulocyte/macrophage colony-stimulating factor), in general glycosylation of these proteins is responsible for a decrease in antigenicity or an increase in solubility, biological half-life, stability or protease resistance. Therefore glycosylation is necessary for these to be effective products [4].

The preferred approach for recombinant glycoprotein production will be the characterization of the authentic post-translational modifications to the protein of interest, and the determination of whether post-translational modifications are implicated in the functions mentioned above. For the production of the potential therapeutic glycoprotein, the most appropriate expression host for large-scale glycoprotein production will involve balancing levels of production, cost, reproducibility of glycosylation and stability *in vivo*. A recent review on glycosylation in different expression hosts deals with this problem, but stresses mainly N-glycosylation with reference only to one class of O-glycoforms [5]. Our review complements that of Jenkins et al. [5] by focusing on O-glycosylation in different expression systems, emphasizing glycosylation in the cellular slime mould *Dictyostelium discoideum*.

The textbook view of glycosylation

Oligosaccharides attached to Asn residues of secreted or membrane-bound proteins are described as N-linked (Figure 1A), with N-linked glycoforms falling into three main categories: high-mannose, hybrid and complex. All of these are derived from a precursor oligosaccharide consisting of GlcNAc₂Man₉Glc₃. These three types share the same core structure (GlcNAc₂Man₃) with high-mannose forms having only glucose and/or additional mannose sugars owing to the absence of trimming of the precursor

¹ To whom correspondence should be addressed.

² Abbreviations used: CHO, Chinese hamster ovary; GST, glutathione S-transferase; PsA, prespore-specific antigen; rPsA, recombinant PsA.

on the addition of mannose to the trimmed structure. The hybrid and complex forms are trimmed and then reconstructed with the addition of GlcNAc, Gal, sialic acid and sometimes fucose in animal systems. Whereas N-glycosylation in higher eukaryotes is well characterized [6], less is known about this post-translational protein modification in various simple expression hosts [5].

O-glycosylation (Figure 1B) in different eukaryotic systems is more challenging because O-glycosylation (glycans attached to serine and threonine residues, and to a smaller extent hydroxyproline and hydroxylysine residues) is less well understood and much more complex, with at least seven different reducing terminal linkages known [7,8]. O-glycans are built up in steps with sugars added one at a time. The most common form of O-glycosylation in mammals is the addition of GalNAc linked to serine or threonine residues, and the further addition of

Gal resulting in a common core structure (Gal β 1-3GalNAc α 1-Ser/Thr). This is often further decorated with the addition of up to three sialic acid residues [4].

Changes in O-glycosylation are observed in several diseases and much work has been put into understanding mucins that are heavily O-glycosylated (GalNAc form) secreted or cell surface proteins. Altered mucin glycosylation is common in cancer [9,10].

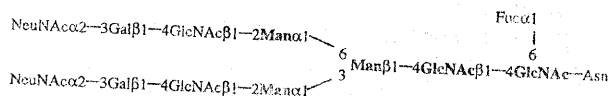
O-glycosylated proteins are also found in the cytoplasm of the cell with a single GlcNAc linked to isolated or clustered serine or threonine residues [11,12].

Cell culture expression systems

The benchmark system for the expression of 'authentic' recombinant glycoproteins is mammalian, Chinese hamster ovary (CHO), cell lines (Table 1). CHO cells are well characterized and have contributed much to the understanding of N-glycosylation [13-15]. There have been many glycosylation mutants isolated for studies on carbohydrate function and they have been characterized biochemically. The disadvantages of using CHO cells for recombinant glycoprotein production include expensive culture conditions and variability of glycosylation. This can arise as a result of: spontaneous mutations during transformation and serial cultivation steps to enhance production levels [13,16], the cell culture environment, e.g. levels of glucose, ammonia ions and hormonal content [17]; and the presence of secreted sialidases, which can modify the product after secretion [18].

To give a flavour of how the CHO system performs consider the expression of Glycophorin A. Glycophorin A is the major sialoglycoprotein of human erythrocytes, containing one N-glycan and 16 O-glycans [19,20]. The O-glycans of this protein consist mainly of short oligosaccharides with one, two or three sialic acid residues linked

A) N-linked glycan



B) O-linked glycan

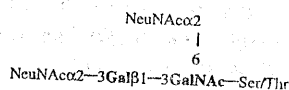


Figure 1

One example of a complex N-linked oligosaccharide (A) and a common O-linked glycan (B) in mammals [4]. (A) The N-glycan is linked to the amide group of an Asn residue and consists of a GlcNAc $_2$ Man $_3$ core structure (indicated in bold) further modified with Gal, GlcNAc, Fuc and NeuNAc residues. (B) The O-glycan is attached to the hydroxy group of a Ser or Thr residue. The reducing terminal linkage is GalNAc, which is further modified with Gal and NeuNAc residues. The core structure is indicated in bold.

Table 1 Glycosylation in different expression hosts

N-glycosylation in different expression hosts is covered only superficially and for a more comprehensive review see [5]. 'High Man' indicates the ability of the cell to add the oligosaccharide precursor (Glc $_2$ Man $_9$ GlcNAc $_2$) to the amide group of an Asn residue within the sequon Asn-Xaa-Ser/Thr (Xaa not Pro). Processing of the precursor oligosaccharide means the ability to trim mannose residues; complex structures involve the addition of galactose and sialic acid. Under O-glycosylation, only the reducing terminal linkages to Ser or Thr residues within a polypeptide chain are indicated here. Single GlcNAc residues are normally found in the cytoplasm of the cell; *D. discoideum* uses this linkage on secreted proteins. Symbols: +, oligo/monosaccharide positively identified, --, not yet found.

	N-glycosylation			O-glycosylation				
	High Man	Processing	Complex	GalNAc	GlcNAc	Man	Fuc	-P GlcNAc ^a
CHO cells	+	+	+	+	+	---	+	---
<i>S. frugiperda</i>	+	+	---	+	+	---	+	---
<i>S. cerevisiae</i>	+	---	---	---	---	+	---	---
<i>D. discoideum</i>	+	+	---	---	+	---	+	+

^a Only in CHO cells; see text.

^b Whether or not insect cells can form complex N-glycans is disputed [26].

^c In *S. cerevisiae* very large mannose structures (50-150 mannose residues) can be constructed.

to a common core sugar Gal β 1-3GalNAc α 1-Ser/Thr, with the disialylated structure being the most abundant. The expression of recombinant glycophorin A in CHO cells was authentic with respect to Ser/Thr glycosylation sites occupied. However, no trisialylated derivatives and mainly monosialylated oligosaccharides were found. Furthermore recombinant glycophorin A was shown to express a small amount of the Tn antigen (GalNAc α 1-Ser/Thr), owing to the failure of the Gal β 1-3 transferase to modify all O-glycans [19].

Tissue culture systems such as CHO cells can never represent a 'complete' mammalian organism, where the same molecule in different tissues is glycosylated in different ways [21]. Therefore further strategies in terms of glycosylation engineering will need to be adopted [15,22]. This involves the use of glycosylation mutants, transformants with new glycosyltransferases, or modifications of the purified glycoprotein *in vitro* with defined glycosyltransferases.

A less expensive, but not as well studied, cell culture expression system capable of high-level expression involves insect *Spodoptera frugiperda* (SF9 or SF21) cells infected with baculovirus (Table 1). Investigations on N-glycosylation in this system so far have led to the general belief that SF9 cells can trim high-mannose side chains to three to nine mannose residues and that there is a fucosyltransferase that adds fucose to the asparagine-linked GlcNAc residue [23–25]. However, whether or not this system can process the trimmed side chains to complex structures containing galactose and/or sialic acid is disputed [26].

The well-known mammalian O-glycoform Gal β 1-3GalNAc α 1-Ser/Thr has been reported on secreted recombinant monocyte chemoattractant protein 1 in SF9 cells [27]. Studies on the expression of the human cytomegalovirus tegument basic phosphoprotein showed the potential of the insect system to express cytoplasmic glycoproteins modified with a single O-linked GlcNAc [28].

Expression in simple eukaryotes

The expression of recombinant proteins in simple eukaryotes has several advantages, as the costs for cultivation are decreased and these systems represent stable organisms that are easily maintained and are often genetically manipulable.

The expression of glycoproteins in the yeast *Saccharomyces cerevisiae* leads to heavily mannosylated oligosaccharides (Table 1). Most N-linked oligosaccharide chains in *S. cerevisiae* are an extreme high-mannose type with typically 50–150 mannose residues forming the outer chains [29]. N-linked oligosaccharide structures on secreted proteins from *S. cerevisiae* have been shown to be

exceedingly antigenic when introduced into mammals, which makes the production of glycoproteins from *S. cerevisiae* for use as human therapeutics unattractive. To avoid hypermannosylation, glycosylation mutants, e.g. *mn-9*, have been created [30].

O-glycosylation of secreted proteins in *S. cerevisiae* also differs exceedingly to mammals as it is catalysed in the endoplasmic reticulum by a dolichyl-phosphate-D-mannose-protein O-D-mannosyltransferase that requires a dolichyl phosphate mannose as an intermediate [31]. The reducing terminal monosaccharide becomes extended with up to four additional mannose residues [32], a type of O-glycosylation found in fungi but not in other eukaryotes. There is also evidence that *S. cerevisiae* uses different peptide recognition sites for O-glycosylation from those used for mammalian O-glycosylation [33].

Glycosylation in the methylotrophic yeast *Pichia pastoris* differs from *S. cerevisiae* with respect to the processing of N-linked oligosaccharides. Although high-mannose N-glycans are formed, these are limited to less than 30 mannose residues [34,35]. Whether or not this leads to decreased immunogenicity in mammals needs to be determined.

Little is known about glycosylation in filamentous fungal expression systems (e.g. *Aspergillus*, *Neurospora* and *Trichoderma* species) for secreted recombinant proteins. Although (as in yeast) O-linked glycans on secreted proteins are composed of mannose residues, N-linked oligosaccharides are described to be more diverse than in yeast [36].

D. discoideum

Apart from yeast and fungi, *D. discoideum* is another inexpensive eukaryotic expression system whose genetics, cell biology and development have been extensively studied. Recently *D. discoideum* has been used to express several recombinant (glyco)proteins (Table 2) [37,38]. The levels of proteins expressed so far are in the range of mg/l for secreted proteins, although very little attention has been paid to optimizing expression. In our studies on glutathione S-transferase (GST) fusion proteins we purified 0.5 mg/l secreted glycoprotein in a one-step purification (E. Jung, A.A. Gooley, N.H. Packer, M.B. Slade, K.L. Williams and W. Dittrich, unpublished work).

Considerable progress has been made in understanding glycosylation in *D. discoideum*, which is quite different from that in yeast and fungi (Table 1). As in animals, there are changes in glycosylation depending on the developmental stage of the life cycle and in different cell types [39–41]. Various glycosylation mutants have been isolated, defining at least five genes. These include defects in the *modA* locus

Table 2 Heterologous recombinant proteins successfully produced in *D. discoideum*

Protein	Mode of expression	Reference
<i>S. japonicum</i> GST	s/r ^a	[66]
Rotavirus VP7	s/r/m ^b	[67]
Human IgE receptor	s	[68]
Human muscarinic receptor	m	[69]
Malaria circumsporozoite antigen	m ^d	[70]
Human antithrombin III	s	[71]

^aTwo different constructs: secreted form ([66], and E. Jung, A.A. Gooley, N.H. Packer, M.B. Slade, K.L. Williams and W. Dittrich, unpublished work) and GST expressed intracellularly (E. Jung, unpublished work)

^bLocation of VP7 is dependent on Ca^{2+} concentration when a secretion signal is used.

^cI. Wilson, M.B. Slade and K.L. Williams, unpublished work.

^dGlycosylphosphatidylinositol-anchor signal used.

(type 1: N-glycosylation; failure to trim the N-glycan precursor), *modB* locus (type 2: O-glycosylation; defect leading to the absence of GlcNAc on secreted proteins) and the *modC*, *modD* and *modE* loci (all with defects in fucosylation on O-glycans) ([42], and references therein).

N-linked glycosylation

N-glycosylation is mostly high-mannose in early development, changing to trimmed glycoforms in later development [40]. The assembly of proteoglycans is also different in late development [41].

In early development, processing involves removal of glucose residues leading to several classes of high-mannose structures [40]. About half of these structures are neutral and endoglycosidase H-sensitive [43]. In late growth and early development there is an increase in fucosylated endoglycosidase H-resistant species [44], of which many are also sulphated and phosphorylated [40,45]. Another class does not contain fucose residues but is modified with sulphate and phosphate groups [40,45,46]. Some of these high-mannose structures also contain a peripheral GlcNAc residue [43,47].

Glycosylation during late development is characterized by the removal of glucose residues, the trimming of up to four mannose residues, the incorporation of fucose and less sulphation and phosphorylation [40]. Prespore cells are described as being 2–3-fold more active than prestalk cells in the assembly of N-linked glycans, and 20-fold more active in their fucosylation [41]. There is no evidence for incorporation of galactose, galactosamine or sialic acid at any stage [48], although there is one publication that contradicts this [49]. Despite the limited processing of N-glycans leading to some unusual structures, *D. discoideum* N-linked glycoforms are not highly immunogenic in mammals, as there have been no antibodies raised against

N-glycoforms found on any of the *D. discoideum* multicellular glycoproteins [42]. Almost all studies on N-linked glycans have been performed on pooled sugars; in future, studies on N-glycans from individual molecules will be needed.

O-linked glycosylation

Although it is clear that a number of reducing terminal linkages occur in animal O-glycoforms, in general there is almost no literature on O-glycans, other than oligosaccharides with reducing terminal GalNAc on recombinant proteins. One exception is epidermal growth factor (EGF) domains within the blood coagulation factor XII and tissue plasminogen activator, where O-fucose has been reported [50]. We have begun a systematic study of O-glycosylation in *D. discoideum* and have found several reducing terminal linkages.

O-linked GlcNAc

Studies on the recombinant prespore-specific antigen (rPsA), expressed during vegetative growth, showed the addition of single GlcNAc residues within a threonine-rich domain (PTVT)_{3–5} [51]. The same domain in authentic PsA isolated from prespore cells (late development) is further modified with fucose residues and phosphate [52]. The exact linkage between GlcNAc and fucose remains to be determined, but the GlcNAc is the reducing terminal linkage. This indicates that the elaboration of the O-Ser/Thr monosaccharides is dependent on the developmental stage.

To characterize the acceptor sites for this type of glycosylation (addition of GlcNAc to Thr) we expressed and purified chemical amounts of GST fusion proteins containing only one repeat (PTVTPT) fused to the GST C-terminus (E. Jung, A.A. Gooley, N.H. Packer, M.B. Slade, K.L. Williams and W. Dittrich, unpublished work). This peptide motif was sufficient as a signal for O-glycosylation because it was glycosylated in the same way as rPsA, although it was fused to a heterologous protein. Replacement of threonine by serine residues in this peptide (PSVSPS) also yielded a glycosylated peptide modified with single GlcNAc residues, but not all of the serine residues were glycosylated. The different glycosylation pattern led to a suggestion of two different UDP-GlcNAc:polypeptide glucosaminyltransferases responsible for threonine and serine residues, respectively. The O-linked glycosylation of both peptides and (r)PsA presented the *modB*-dependent carbohydrate-specific epitope identified by the monoclonal antibody MUD50. Because there has so far been no report of GalNAc linked to serine or threonine residues in *D. discoideum*, it seems that in this organism GlcNAc might

substitute for GalNAc in secreted and membrane-anchored proteins. Single GlcNAc residues are also found on the major cell-surface glycoprotein of *Trypanosoma cruzi* Y-strain, although 80% of the GlcNAc residues are further modified by galactose [53].

O-linked fucose and phosphoglycosylation

A well characterized protein expressed in late development is the spore coat protein SP96, which is modified with fucose and GlcNAc residues [41]. Recently we have determined two reducing terminal linkages on SP96. These are O-Fuc and O-P-GlcNAc respectively (M. Mreyen, N.H. Packer, A. Champion, A.A. Gooley, and K.L. Williams, unpublished work). Direct O-linkage of fucose to Ser/Thr is known to occur in the epidermal growth factor domains of several human blood proteins [54,55] and has been described in CHO cells [56].

The other type of modification, called phosphoglycosylation, was first identified in proteinases of *D. discoideum*, present in vegetative cells [57–60], and the enzyme responsible was recently identified [61]. Phosphoglycosylation has yet to be described in higher eukaryotes, although phosphoserine-linked glycans (decorated Man-P-Ser) are found on a secreted protein from *Leishmania mexicana* [62,63], and phosphothreonine-linked glycans (decorated Xyl-P-Thr) are found on cell-surface glycoproteins of *T. cruzi* (P.A. Haynes, M.A.J. Ferguson and G.A.M. Cross, unpublished work).

Cytoplasmic glycosylation

The linkage of single GlcNAc residues to threonine and serine is found on cytoplasmic glycoproteins in mammals [11,12]. We are currently investigating whether *D. discoideum* has a cytoplasmic UDP-GlcNAc:polypeptide glucosaminyltransferase in addition to the one(s) for secreted and cell-surface proteins. So far only one cytosolic glycoprotein from *D. discoideum* has been identified (FP21) and it is modified with fucose, galactose and xylose [64,65]. Although the reducing terminal linkages have not yet been determined, this example indicates the presence of various glycosyltransferases within the cytosol of *D. discoideum*.

Conclusions

Only now are we beginning to understand the capabilities of different expression hosts with respect to N-glycosylation, whereas O-glycosylation remains a mystery. We predict that in the next 5 years research in the fields of glycobiology and recombinant protein expression will provide a library of expression systems, where one can choose a system for the desired modifications. With respect to O-glycosylation, *D. discoideum* is an organism

where the possibilities of engineering various O-glycoforms are excellent.

Acknowledgments

E.J. thanks the DAAD for support from the HSPII/AUFE program; K.L.W. thanks the Australian Research Council and the National Health and Medical Research Council for support for his work on expression of recombinant proteins in *D. discoideum*.

References

- 1 Wrotnowski, C. (1996) Genetic Engineering News (May 15), 7, 29
- 2 Cumming, D.A. (1991) Glycobiology 1, 115–130
- 3 Varki, A. (1993) Glycobiology 3, 97–130
- 4 Goochee, C.F., Gramer, M.J., Andersen, D.C., Bahr, J.B. and Rasmussen, J.R. (1991) Bio/Technology 9, 1347–1355
- 5 Jenkins, N., Parekh, R.B. and James, D.C. (1996) Nature Biotechnol. 14, 975–981
- 6 Kornfeld, R. and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–646
- 7 Gooley, A.A. and Williams, K.L. (1994) Glycobiology 4, 413–417
- 8 Hansen, J.E., Lund, O., Engelbrecht, J., Bohr, H., Nielson, J.O., Hansen, J.S. and Brunak, S. (1995) Biochem. J. 308, 801–813
- 9 Carraway, K.L. and Hull, S.R. (1991) Glycobiology 1, 131–138
- 10 Verma, M. and Davidson, E.A. (1994) Glycoconjugate J. 11, 172–179
- 11 Haltiwanger, R.S., Kelly, W.G., Roquemore, E.P., Blomberg, M.A., Dong, D.L.-Y., Kreppel, L., Chou, T.-Y. and Hart, G.W. (1992) Biochem. Soc. Trans. 20, 264–269
- 12 Ku, N.-O. and Omary, M.B. (1995) J. Biol. Chem. 270, 11820–11827
- 13 Stanley, P. (1984) Annu. Rev. Genet. 18, 525–552
- 14 Stanley, P. (1987) Trends Genet. 3, 77–81
- 15 Stanley, P. and Ioffe, E. (1995) FASEB J. 9, 1436–1444
- 16 Stanley, P. (1989) Mol. Cell. Biol. 9, 377–383
- 17 Goochee, C.F. and Monica, T. (1990) Bio/Technology 8, 421–427
- 18 Gramer, M.J., Goochee, C.F., Chock, V.Y., Brousseau, D.T. and Sliwkowski, M.B. (1995) Bio/Technology 13, 692–698
- 19 Pahlsson, P., Blackall, D.P., Ugorski, M., Czerwinski, M. and Spitalnik, S.L. (1994) Glycoconjugate J. 11, 43–50
- 20 Pisano, A., Redmond, J.W., Williams, K.L. and Gooley, A.A. (1993) Glycobiology 3, 429–435
- 21 Parekh, R.B., Tse, A.G.D., Dwek, R.A., Williams, A.F. and Rademacher, T.W. (1987) EMBO J. 6, 1233–1244

- 22 Stanley, P. (1992) *Glycobiology* **2**, 99-107
- 23 Jarvis, D.L. and Summers, M.D. (1992) in *Recombinant DNA Vaccines: Rationale and Strategies* (Isaacson, R.E., ed.), pp. 265-291, Dekker, New York
- 24 O'Reilly, D.R., Miller, L.K. and Luckow, V.A. (1992) *Baculovirus Expression Vectors: A Laboratory Manual*, pp. 1-347, Freeman, New York
- 25 Jarvis, D.L. (1993) in *Insect Cell Culture Engineering* (Goosen, M.F.A., Daugulis, A. and Faulkner, P., eds.), pp. 193-217, Dekker, New York
- 26 Jarvis, D.L. and Finn, E.E. (1995) *Virology* **212**, 500-511
- 27 Ishii, K., Yamagami, S., Tanaka, H., Motoki, M., Suwa, Y. and Endo, N. (1995) *Biochem. Biophys. Res. Commun.* **206**, 955-961
- 28 Greis, K.D., Gibson, W. and Hart, G.W. (1994) *J. Virol.* **68**, 8339-8349
- 29 Kukuruzinska, M.A., Bergh, M.L.E. and Jackson, B.J. (1987) *Annu. Rev. Biochem.* **56**, 915-944
- 30 Ballou, C.E. (1990) *Methods Enzymol.* **185**, 440-470
- 31 Strahl-Bolsinger, S. and Tanner, W. (1991) *Eur. J. Biochem.* **196**, 185-190
- 32 Herscovics, A.O. and Orlean, P. (1993) *FASEB J.* **7**, 540-550
- 33 Kalsner, I., Schneider, F.J., Geyer, R., Ahorn, H. and Maurefogy, I. (1992) *Glycoconjugate J.* **9**, 209-216
- 34 Grinna, L.S. and Tschopp, J.F. (1989) *Yeast* **5**, 107-115
- 35 Cregg, J.M., Vedvick, T.S. and Raschke, W.C. (1993) *Bio/Technology* **11**, 905-910
- 36 Peberdy, J.F. (1994) *Trends Biotechnol.* **12**, 50-57
- 37 Williams, K.L., Emslie, K.R. and Slade, M.B. (1995) *Curr. Opin. Biotechnol.* **6**, 538-542
- 38 Emslie, K.R., Slade, M.B. and Williams, K.L. (1995) *Trends Microbiol.* **3**, 1-5
- 39 Freeze, H. (1992) in *Developmental Glycobiology of Dictyostelium discoideum* (Fukuda, M., ed.), pp. 285-317, CRC Press, Boca Raton
- 40 Ivatt, R.J., Das, O.P., Henderson, E.J. and Robbins, P.W. (1984) *Cell* **38**, 561-567
- 41 Riley, G.R., West, C.M. and Henderson, E.J. (1993) *Glycobiology* **3**, 165-177
- 42 Champion, A., Griffiths, K., Gooley, A.A., Gonzalez, B.Y., Gritzali, M., West, C.M. and Williams, K.L. (1995) *Microbiology* **141**, 785-797
- 43 Hobbs, L.J., Das, O.P. and Henderson, E.J. (1990) *Dev. Genet.* **11**, 473-483
- 44 Tschursin, E., Riley, G.R. and Henderson, E.J. (1989) *Differentiation* **40**, 1-9
- 45 Das, O.P. and Henderson, E.J. (1986) *Anal. Biochem.* **158**, 390-398
- 46 Freeze, H.H. (1986) *Mol. Cell. Biochem.* **72**, 45-65
- 47 Couso, R., van Halbeek, H., Reinhold, V. and Kornfeld, S. (1987) *J. Biol. Chem.* **262**, 4521-4527
- 48 Ivatt, R.J., Das, O.P., Henderson, E.J. and Robbins, P.W. (1981) *J. Supramol. Struct. Cell. Biochem.* **17**, 359-368
- 49 Yoshida, M., Fuse, G., Matsui, T. and Ouchi, S. (1992) *Biochem. Biophys. Res. Commun.* **188**, 794-798
- 50 Harris, R.J., Victor, T.L. and Spellman, M.W. (1992) *J. Biol. Chem.* **267**, 5102-5107
- 51 Zachara, N.E., Packer, N.H., Temple, M.D., Slade, M.B., Jardine, D.R., Karuso, P., Moss, C.J., Mabbutt, B.C., Curmi, P.M.G., Williams, K.L. and Gooley, A.A. (1996) *Eur. J. Biochem.* **238**, 511-518
- 52 Haynes, P.A., Gooley, A.A., Ferguson, M.A.J., Redmond, J.W. and Williams, K.L. (1993) *Eur. J. Biochem.* **216**, 729-737
- 53 Previato, J.O., Jones, C., Goncalves, L.P.B., Watt, R., Travassos, L.R. and Mendonca-Previato, L. (1994) *Biochem. J.* **301**, 151-159
- 54 Harris, R.J., Ling, V.L. and Spellman, M.W. (1992) *J. Biol. Chem.* **267**, 5102-5107
- 55 Harris, R.J. and Spellman, M.W. (1993) *Glycobiol.* **3**, 219-224
- 56 Stults, N.L. and Cummings, R.D. (1993) *Glycobiol.* **3**, 589-596
- 57 Gustafson, G.L. and Milner, L.A. (1980) *J. Biol. Chem.* **255**, 7208-7210
- 58 Gustafson, G.L. and Gander, J.E. (1984) *Methods Enzymol.* **107**, 172-183
- 59 Metha, D.P., Ichikawa, M., Salimath, P.V., Etchison, J.R., Haak, R., Manzi, A. and Freeze, H.H. (1996) *J. Biol. Chem.* **271**, 10897-10903
- 60 Souza, G.M., Hirai, J., Mehta, D.P. and Freeze, H.H. (1995) *J. Biol. Chem.* **270**, 28938-28945
- 61 Freeze, H.H. and Ichikawa, M. (1995) *Biochem. Biophys. Res. Commun.* **208**, 384-389
- 62 Ilg, T., Overath, P., Ferguson, M.A.J., Rutherford, T., Campbell, D.G. and McConville, M.J. (1994) *J. Biol. Chem.* **269**, 24073-24081
- 63 Ilg, T., Stierhof, Y.D., Craik, D., Simpson, R., Hardman, E. and Bacic, A. (1996) *J. Biol. Chem.* **271**, 21583-21596
- 64 Gonzalez-Yanes, B., Cicero, J.M., Brown, R.D. and West, C.M. (1992) *J. Biol. Chem.* **267**, 9595-9605
- 65 Kozarov, E., van der Wel, H., Field, M., Gritzali, M., Brown, R.D. and West, C.M. (1995) *J. Biol. Chem.* **270**, 3022-3030
- 66 Dittrich, W., Williams, K.L. and Slade, M.B. (1994) *Bio/Technology* **12**, 614-618
- 67 Emslie, K.R., Miller, J.M., Slade, M.B., Dormitzer, P.R., Greenberg, H.B. and Williams, K.L. (1995) *J. Virol.* **69**, 1747-1754
- 68 Reference deleted.
- 69 Voith, G. and Dingermann, T. (1995) *Pharmazie* **50**, 758-762
- 70 Fasel, N., Begdadi-Pais, C., Bernard, M., Bron, C., Corradin, G. and Reymond, C.D. (1992) *Gene* **111**, 157-163
- 71 Dingermann, T., Troidl, E.-M., Broeker, M. and Nerke, K. (1991) *Appl. Microbiol. Biotechnol.* **35**, 496-503