N-Acetyl-β-Glucosaminidase Accounts for Differences in Glycosylation of Influenza Virus Hemagglutinin Expressed in Insect Cells from a Baculovirus Vector

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The hemagglutinin of fowl plague virus has been expressed in Spodoptera frugiperda (S9) cells and in Estigmene acrea cells by using a baculovirus vector. Structural analysis revealed that the endo-H-resistant N-glycans of HA from S9 cells were predominantly trimannosyl core oligosaccharides, whereas in E. acrea cells most of these cores were elongated by at least one terminal N-acetylglucosamine residue. To understand the difference in carbohydrate structures, enzymes involved in N-glycan processing have been analyzed. The results revealed that the different glycosylation patterns observed are due to an N-acetyl-β-glucosaminidase activity that was found in S9 cells but not in E. acrea cells. This enzyme specifically used the GlcNAcMan,GlcNAc2 oligosaccharide as a substrate. When N-acetyl-β-glucosaminidase or α-mannosidase II was inhibited by specific inhibitors, the amount of terminal N-acetylglucosamine in hemagglutinin from S9 cells was significantly enhanced. These results demonstrate that N-glycosylation in both cell lines follows the classical pathway up to the stage of GlcNAcMan,GlcNAc2 oligosaccharide side chains. Whereas these structures are the end product in E. acrea cells, they are degraded in S9 cells to Man,GlcNAc2 cores by N-acetyl-β-glucosaminidase.

The frequent use of lepidopteran cell lines in the baculovirus expression system (for reviews, see references 22 and 25) has strongly stimulated the interest in the N-glycosylation capacities of insect cells. For most of the glycoproteins expressed so far in insect cells, the oligosaccharide side chains have been reported to differ markedly from their vertebrate counterparts in that they carry only high-mannose-type and truncated trimannosyl N-glycans, frequently with a fucose residue attached to the innermost Asn-linked GlcNAc (for a review, see reference 23). These truncated oligosaccharide structures are not present on glycoproteins from vertebrate cells and are not intermediates in the standard oligosaccharide-processing cascade (15). In vertebrate cells N-glycosylation commences with the en bloc transfer of a dolichol-linked precursor oligosaccharide (Glc3Man,GlcNAc2) to the nascent polypeptide chain in the endoplasmic reticulum. Since this precursor was also found in insect cells (12, 26), it is considered a ubiquitous intermediate of glycoprotein N-glycan biosynthesis in eukaryotes. After transfer, vertebrate cell oligosaccharides are trimmed sequentially by endoplasmic reticulum glucosidases I and II, endoplasmic reticulum α-mannosidase, and Golgi mannosidase I, yielding a Man,GlcNAc2 unit (for a review, see reference 32), which is then elongated by N-acetyl-β-glucosaminyltransferase I (GNT-I) by transfer of the first outer N-acetylglycosamine (GlcNAc) residue (10). The addition of this GlcNAc residue is an absolute requirement for further processing of the glycans by α-mannosidase II, which subsequently removes both terminal mannose residues (11; for a review, see reference 28). The resulting GlcNAcMan,GlcNAc2 oligosaccharides are further elongated, depending on the repertoire of glycosyltransferases. The direct formation of the trimannosyl glycans abundantly found in insect cells is incompatible with this series of reactions, since α-mannosidase II is strictly dependent on the prior action of GNT-I. The same restrictions concerning substrate specificity apply for those vertebrate cell fucosyltransferases which are in charge of transferring fucose residues to the Asn-linked GlcNAc (27). It was therefore suggested that insect cells may harbor unusual mannosidase and fucosyltransferase activities as components of an alternative pathway of glycoprotein biosynthesis. Recent reports, however, have described considerable levels of GNT-I activity (1, 31, 33) and the presence of typical α-mannosidase II (2) and fucosyltransferase (1) activities in different insect cell lines. These data indicate that the N-glycosylation machinery of insect cells comprises the trimming enzymes and some of the elongating glycosyltransferases of the classical pathway.

N-Glycans of Estigmene acrea cell-derived HA contain terminal N-acetylglucosamine. The present study was undertaken to elucidate the biosynthetic route leading to the truncated trimannosyl side chains observed in Spodoptera frugiperda (S9) cells (18) and to find an insect cell system that allows further elongation steps. The E. acrea cell line (9) proved to be a promising candidate to fulfill this demand. Western blot (immunoblot) analysis revealed that hemagglutinin (HA) of influenza virus strain A/FPV/Rostock/34 (H7/N1) expressed from a recombinant baculovirus (19) in these cells had a significantly higher molecular mass, particularly in the HA1 subunit, than did HA from S9 cells (Fig. 1A). After removal of all N-linked oligosaccharide side chains by treatment with peptide-N′-N-acetyl-β-glucosaminyl)asparagine amidase F (N-glycosidase F), this difference was no longer detectable (data not shown), indicating that the higher molecular mass of HA from E. acrea cells was due to an increased oligosaccharide moiety. The amount of terminal GlcNAc residues present on the oligosaccharide side chains of HA was determined by an in vitro assay in which these residues were radiolabeled by the transfer of tritiated galactose catalyzed by galactosyltransferase (33). Carbohydrates of HA expressed in E. acrea cells were about 20 times more accessible to in vitro galactosylation than S9 cell-
FIG. 1. Characterization of N-linked oligosaccharide side chains of HA from *E. acerea* cells. (A) Comparison of HA produced in SF9 (lanes 1) and *E. acerea* (lanes 2) cells. HA expressed from a recombinant baculovirus was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subsequent Western blotting. The blot was analyzed with polyclonal anti-fowl plague virus serum (left panel). (Right panel) Identification of terminal GlcNAc residues on oligosaccharide side chains of HA by in vitro galactosylation (33). Cell lysates containing equal amounts of HA protein were incubated with UDP-[6-3H]galactose and galactosyltransferase. HA was immunoprecipitated with anti-fowl plague virus antiserum and analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis. Protein bands were quantitated densitometrically. Uncleaved HA and its cleavage products HA1 and HA2 are indicated. (B) Fractionation and characterization of endo-H-resistant HA N-glycans from *E. acerea* cells. Oligosaccharides released with N-glycosidase F were treated with a-fucosidase and fractionated by HPAEC (chromatogram a) as reported previously (33). Eluate fractions were immediately neutralized with acetic acid and monitored for radioactivity. Peaks (I to IV) were pooled and characterized by gel filtration on a Biogel P-4 matrix (33) (chromatograms b to e). Glycans of peak III were analyzed before (b) and after (c) treatment with N-acetyl-β-hexosaminidases from beef kidney and jack beans; glycans of peak IV were analyzed before (d) and after (e) treatment with N-acetyl-β-glucosaminidase from *D. pneumoniae*. Arabic numbers indicate the elution positions of isomaltosyl oligosaccharides with the given number of glycosyl residues.
derived HA glycans (Fig. 1A). The structures of the N-glycans of E. acrea cell-derived HA were studied by a sequence of well-established chromatographic steps (7, 8, 33). To this end, HA was metabolically labeled with [2-3H]mannose during expression in E. acrea cells and subsequently purified by immunofinity chromatography according to a procedure reported previously (33). Following treatment with trypsin, the resulting glycopeptides were digested with endo-β-N-acetylglucosaminidase H (endo H), and the oligosaccharides released (50% of total radioactivity) were separated from residual glycopeptides by preparative Biogel P-4 chromatography (33). Endo-H-resistant glycans were liberated by N-glycosidase F and isolated by reversed-phase high-performance liquid chromatography. The remaining peptides were devoid of any radioactivity, indicating that the oligosaccharide side chains had been completely removed from the tryptic fragments. High-pH anion-exchange chromatography (HPAEC) (20) of the products formed by total hydrolysis of the endo-H-resistant glycan fraction revealed that about 30 and roughly 70% of the radioactive label resided in fucosyl and mannosyl residues, respectively (data not shown), indicating that radiolabeled mannose was partly appropriated to comprise the liberated fucose residues (data not shown). Peak II appeared at 2.9 glucose units in HPAEC and at about 7 glucose units in Biogel P-4 chromatography, which is typical for the trimannosyl core structure (data not shown). Peak IV emerged at 3.7 glucose units in HPAEC and at about 9 glucose units in Biogel P-4 chromatography. After treatment with N-acetyl-β-glucosaminidase from D. pneumoniae, this peak exhibited the same elution profile as peak II. In view of the specificity of this enzyme (34), peak IV was considered a trimannosyl core structure carrying one terminal (β-1,2)-linked GlcNAc, which could be assigned to the (α-1,3)-bound mannose residue by comparison with the HPAEC elution profiles of corresponding reference compounds. Peak III eluted at 3.1 glucose units in HPAEC and at about 11 glucose units in Biogel P-4 chromatography. Following treatment with a mixture of N-acetyl-β-hexosaminidases from beef kidney and jack beans, the elution position of the glycan shifted to that observed for the trimannosyl core structure. This shift was consistent with the removal of two GlcNAc residues. Since the latter enzymes do not discriminate between different linkage positions, further structural assignments of these monosaccharide constituents were not possible. Nevertheless, these data show that a small fraction (about 16%) of endo-H-resistant N-glycans from E. acrea cells comprised structures with two GlcNAc residues, indicating the presence in these cells of a GlcNAc transferase acting in addition to GNT-I. Endo-H-glycans were investigated by gel filtration on a Biogel P-4 column (data not shown). After treatment with α-mannosidase from jack beans, two oligosaccharide peaks were obtained, one major peak eluting at about 3 glucose units, representing the Man α 1-4GlcNAc disaccharide unit, and a minor peak at about 6 glucose units. Simultaneous treatment with N-acetyl-β-glucosaminidase from D. pneumoniae led to the disappearance of this minor peak. These results showed that the endo-H-sensitive glycan fraction largely consisted of oligomannosidic structures (93%) and a minor fraction (7%) of hybrid type structures carrying terminal GlcNAc.

Table 1 presents a comparative overview of the oligosaccha-

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**TABLE 1. Structures proposed for N-glycans of HA expressed in insect cells**

<table>
<thead>
<tr>
<th>Glycan</th>
<th>Peak&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Oligosaccharide structure&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Relative abundance (%) in:</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>E. acrea</td>
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<tr>
<td>Endo-H resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Manα6&lt;sup&gt;4′&lt;/sup&gt;</td>
<td>Manαβ4GlcNAcβ4GlcNAc</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Manα3</td>
<td>Manαβ4GlcNAcβ4GlcNAc</td>
<td>&gt;90</td>
</tr>
<tr>
<td>IV</td>
<td>GlcNAcβ2Manα3&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Manαβ4GlcNAcβ4GlcNAc</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Manα6&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Manαβ4GlcNAcβ4GlcNAc</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Endo-H sensitive</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>III</td>
<td>GlcNAcβ&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Manαβ4GlcNAcβ4GlcNAc</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Manα3</td>
<td>Manαβ4GlcNAcβ4GlcNAc</td>
<td>93</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>100</td>
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<tr>
<td></td>
<td>B</td>
<td>Manαβ4GlcNAcβ4GlcNAc</td>
<td>7</td>
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</table>

<sup>a</sup> See Fig. 1.

<sup>b</sup> Fucosylation has not been taken into consideration. The numbering of sugar residues is indicated by boldface type.

<sup>c</sup> Results given here for the N-glycans from Sf9 cells are taken from references 18 and 33.
Man5GlcNAc2 side chains of ovalbumin (33). Ovalbumin was then precipitated and aliquots were checked for radioactivity, which was taken as a measure for GNT-I activity present in the tested lysates. The GNT-I activities of various amounts of cell lysate were assayed in vitro by monitoring the transfer of tritiated GlcNAc from UDP-[6-3H]GlcNAc to Man5GlcNAc2 side chains of ovalbumin (33). Ovalbumin was then precipitated with trichloroacetic acid and washed, and the pellet was redissolved in NaOH. Aliquots were checked for radioactivity, which was taken as a measure for GNT-I activity present in the tested lysates.

Firstly, HA was expressed in cells cultivated in the presence of 10 μM swainsonine, a specific inhibitor of α-mannosidase II (30). Terminal GlcNAc residues on resulting HA N-glycans were quantitated by in vitro galactosylation. For HA from Sf9 cells, the presence of swainsonine in the culture medium caused a significant increase in the amount of terminal GlcNAc residues. Furthermore, the inhibition of the removal of mannose residues by swainsonine treatment led to increased molecular masses of the HA bands (Fig. 3A). These results indicate that GlcNAcMan5GlcNAc2 is made under these conditions, which would not be the case if the precursor Man5GlcNAc2 were depleted by the presumptive atypical mannosidase. The data are therefore not compatible with the presence of such an enzyme in Sf9 cells. In E. aerea cells, the number of terminal GlcNAc residues is slightly reduced after swainsonine treatment. This observation indicates that the remaining mannose residues (mannoses A and B, cf. Table 1) probably interfere with the addition of the second terminal GlcNAc residue.

We then analyzed the effects of the N-acetyl-β-glucosaminidase inhibitor 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (21). Expression of HA in Sf9 cells in the presence of the N-acetyl-β-glucosaminidase inhibitor resulted in a marked increase of N-glycans with terminal GlcNAc residues as shown by in vitro galactosylation. This effect was not observed in E. aerea cells (Fig. 3B). These results, together with those obtained after swainsonine treatment, clearly demonstrate that the formation of truncated oligosaccharides in Sf9 cells is the result of an N-acetyl-β-glucosaminidase that is absent in E. aerea cells.

N-acetyl-β-glucosaminidase from Sf9 cells specifically cleaves GlcNAcMan5GlcNAc2 oligosaccharide. To further characterize the differences in glycoprotein processing in the two cell lines, the enzymes involved were analyzed with radiolabeled oligosaccharides of the structures [3H]GlcNAcMan5GlcNAc2 to

**FIG. 2.** Comparison of GNT-I levels of Sf9 and E. aerea (EA) cells. The GNT-I activities of various amounts of cell lysate were assayed in vitro by monitoring the transfer of tritiated GlcNAc from UDP-[6-3H]GlcNAc to Man5GlcNAc2 side chains of ovalbumin (33). Ovalbumin was then precipitated with trichloroacetic acid and washed, and the pellet was redissolved in NaOH. Aliquots were checked for radioactivity, which was taken as a measure for GNT-I activity present in the tested lysates.

**FIG. 3.** Effect of glycosidase inhibitors on HA N-glycan biosynthesis in Sf9 and E. aerea (EA) cells. (A) HA was expressed in the two cell lines in the absence (−) and presence (+) of 10 μM swainsonine (Swa) in the culture medium. (B) HA was expressed in both cell lines in the absence (0) or presence of increasing concentrations (10, 50, and 100 μM) of the N-acetyl-β-glucosaminidase inhibitor (GI). In both cases, cells were lysed 48 h after infection with the recombinant baculovirus used for HA expression (33). Lysates containing identical amounts of HA were then subjected to in vitro galactosylation by incubation with UDP-[6-3H]galactose and galactosyltransferase (33). Subsequently, HA was immunoprecipitated with polyclonal anti-fowl plague virus serum and subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis. Protein bands were visualized by fluorography.
[3H]GlcNAcMan5GlcNAc2 as the substrates. To generate these compounds, a previously described system was used in which the GNT-I activity of Sf9 cells was increased by transient expression of human GNT-I from a recombinant baculovirus (33). Lysates of these cells were incubated with Man5GlcNAc2 and UDP-[6-3H]GlcNAc. Reaction products were isolated by HPAEC (data not shown). Four radiolabeled glycan peaks were obtained and identified by their elution properties in HPEAC and gel filtration on Biogel P-4. The first peak represented liberated tritiated GlcNAc, whereas peaks 2 to 4 contained oligosaccharide compounds with three to five mannosyl residues, all carrying terminal [3H]GlcNAc in (β-1,2)-linkage to the (α-1,3) arm ([3H]GlcNAcMan3GlcNAc2) to [3H]GlcNAcMan5GlcNAc2). The formation of products with three (or four) mannosyl residues indicates (partial) trimming by a typical α-mannosidase II, which is in agreement with a recent report demonstrating the presence of such an enzyme activity in three different insect cell lines (2).

Isolated [3H]GlcNAcMan5GlcNAc2 oligosaccharides were incubated with lysates from Sf9 and E. aceroid cells to monitor N-acetyl-β-glucosaminidase activity. To mimic the conditions of the in vivo inhibition experiments (described above), lysates were prepared from cells infected with a recombinant baculovirus to express HA (Fig. 3). Reaction products were characterized by HPAEC. For lysates from Sf9 cells, [3H]GlcNAcMan5GlcNAc2 species were processed by both typical α-mannosidase II and N-acetyl-β-glucosaminidase activities, as indicated by the generation of [3H]GlcNAcMan4GlcNAc2 and [3H]GlcNAcMan5GlcNAc2, and liberated [3H]GlcNAc residues (Fig. 4A). Lysates of E. aceroid cells, on the other hand, exhibited only a very weak N-acetyl-β-glucosaminidase activity, whereas the level of α-mannosidase II activity was even higher than that in Sf9 cell lysates.

To elucidate the precise substrate specificity of the N-acetyl-β-glucosaminidase, the [3H]GlcNAcMan5GlcNAc2 to [3H]GlcNAcMan5GlcNAc2 components were individually subjected to incubation with cell lysates in the presence of swainsonine, thus preventing simultaneous action of α-mannosidase II. Products obtained were again identified by HPAEC. As summarized in Fig. 4B, incubation of [3H]GlcNAcMan5GlcNAc2 and [3H]GlcNAcMan5GlcNAc2 with lysates of Sf9 cells resulted only in minute release of [3H]GlcNAc residues (less than 2 and about 5% of total radioactivity, respectively). Thus, these two oligosaccharides are poor substrates for this enzyme. For [3H]Glc

FIG. 4. Analysis of activity levels and substrate specificity of N-acetyl-β-glucosaminidase activity in Sf9 and E. acera (EA) cells. (A) With identical amounts of total protein, cell lysates from both cell lines, prepared after infection with a recombinant baculovirus, were incubated with the [3H]GlcNAcMan5GlcNAc2 substrate generated as outlined in the text. The resulting products were fractionated by analytical HPAEC as described elsewhere (33). (B) Lysates containing equivalent amounts of total protein from Sf9 and E. aceroid cells, prepared after infection with a recombinant baculovirus to express HA (33), were incubated with [3H]GlcNAcMan5GlcNAc2, (GNM4) (I), [3H]GlcNAcMan4GlcNAc2 (GNM3) (II), and [3H]GlcNAcMan5GlcNAc2, (GNM5) (III), respectively, in the presence of 10 μM swainsonine. Reaction products were again characterized by analytical HPAEC (as described for panel A). The relative abundance of the carbohydrate compounds thereby detected is indicated by the height of the respective columns. GN, [3H]GlcNAc.
NacMan3GlcNAc2 oligosaccharides, however, substantial removal of \(^{[3}H\)GlcNAc residues (about 40\%) was observed. Hence, it can be concluded that the GlcNAc residues liberated in the absence of swainsonine (Fig. 4A) originated from structures already trimmed by \(\alpha\)-mannosidase II. In lysates of \(E.\ acrea\) cells, \(N\)-acetyl-\(\beta\)-glucosaminidase activity was hardly detectable. Even with the \(^{[3}H\)GlcNAcMan3GlcNAc2, substrate, less than 2\% of the total \(^{[3}H\)GlcNAc was released (Fig. 4B). Thus, the results obtained by the in vitro studies closely corresponded to those obtained with intact cells by using HA as a reporter glycoprotein (cf. Fig. 3). The swainsonine effect observed in intact SF9 cells is obviously due to the strict substrate specificity of the \(N\)-acetyl-\(\beta\)-glucosaminidase activity for \(^{[3}H\)GlcNAcMan3GlcNAc2, the formation of which is prevented by swainsonine treatment.

Most of the \(N\)-acetyl-\(\beta\)-glucosaminidases described previously are lysosomal enzymes taking part in various degradation reactions (for a review, see reference 4). The enzyme identified in this study is clearly distinguishable from its lysosomal counterparts by its pronounced substrate specificity dependent on the prior action of \(\alpha\)-mannosidase II and its definite involvement in HA N-glycan maturation, which points to the localization of this \(N\)-acetyl-\(\beta\)-glucosaminidase within the exocytotic pathway of SF9 cells. However, the \(N\)-acetyl-\(\beta\)-glucosaminidase described here is probably identical to an enzyme that has been observed recently when lysates of three other lepidopteran cell lines were analyzed in vitro with synthetic oligosaccharides as substrates (3). Our study extends this work by showing that this enzyme is involved in glycoprotein synthesis in intact cells and is therefore a constituent of the N-glycosylation pathway. Because of its crucial implications for the nature of insect cell-derived N-glycans, this enzyme can be considered the missing link between the intermediates of the conventional N-glycosylation cascade and the truncated oligosaccharides observed in insect cells. Accordingly, it becomes clear now that N-glycosylation in SF9 cells initially follows the classical route. After trimming of oligomannosidic side chains to Man\(_{2}\)GlcNAc\(_{2}\), a terminal GlcNAc is attached by GNT-I, and the resulting GlcNAcMan\(_{3}\)GlcNAc\(_{2}\) is converted by \(\alpha\)-mannosidase II and partially by fucosyltransferase to GlcNAcMan\(_{2}\)GlcNAc\(_{2}\) and GlcNAcMan\(_{2}\)GlcNAc\(_{2}\{-\text{Fuc}\}.\) Thus, it is obvious that N-acetyl-\(\beta\)-glucosaminidase catalyzes the final step in this course of events, thereby leading to a dead end of N-glycosylation in many insect cells, with the \(E.\ acrea\) cells being the only exception known so far. Not consistent with this concept are studies in which neuraminic acid-containing complex N-glycans have been described in human plasminogen expressed from a baculovirus vector in SF21 cells (5, 6). However, neither we nor others have been able to confirm the presence of full length N-glycans in such cells (16, 17).

In conclusion, with the \(E.\ acrea\) cells we have identified an insect cell line capable to transferring terminal GlcNAc residues to nearly all accessible N-glycans. Moreover, we have demonstrated that the almost complete absence of such glycans on proteins derived from SF9 cells is not due to the lack of GNT-I activity but is caused by \(N\)-acetyl-\(\beta\)-glucosaminidase, which removes terminal GlcNAc residues. This enzyme is responsible for the abundance of trimannosyl core oligosaccharides on proteins expressed in SF9 cells and constitutes a major difference from \(E.\ acrea\) cells, in which such an activity is not detectable. Since expression levels and other posttranslational modifications of HA from \(E.\ acrea\) cells, such as trimerization and cleavage activation, closely resemble those observed with HA from SF9 cells, the \(E.\ acrea\) cell line has proved to be a useful tool for the expression of recombinant glycoproteins by using baculovirus vectors. Moreover, since N-glycosylation does not terminate in a dead end alley as in the case of most other avertebrate cells analyzed to date, \(E.\ acrea\) cells promise to be particularly suitable for engineering studies of the synthesis of fully elongated complex N-glycans in insect systems (33).

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REFERENCES

virus vector. EMBO J. 5:1359–1365.