For example, in yeast, additional mannose subunits are added to Man8GlcNAc2 by mannosyltransferases and mannan polymerases to yield high-mannose type N-glycans. In contrast, mannose sugars are removed from the human Man8GlcNAc2 to yield Man5GlcNAc2, followed by three sequential reactions involving the enzymes N-acetylglucosaminyltransferase I (GnTI), mannosidase II (Mns II), and N-acetylglucosaminyltransferase II (GnTII), to convert Man5GlcNAc2 into GlcNAc2Man3GlcNAc2.

[0006] The differences between the glycosylation process in mammalian and fungal cells pose a challenge to the expression of glycosylated mammalian proteins in fungal cells since glycoproteins with high-mannose type N-glycans are not suitable for therapeutic use in humans (De Pourcq et al., 2010; Wildt and Gerngross, *Nature Reviews Microbiology*, 3: 119-128). Consequently, studies have been conducted to re-engineer the glycosylation pathways in yeast and fungal species to enable them to express recombinant human proteins. The general approach in glycoengineering of yeast or fungal cells has been to disrupt endogenous genes that are involved in formation of high-mannose type N-glycans. These gene disruptions can be combined with over-expression of endogenous mannosidases and/or glycosyltransferases and glycosidases from different species (Chiba et al., 1998, *J Biol Chem* 273: 26298-304; Kainz et al., 2008, *Appl Environ Microbiol* 74: 1076-86; Maras et al., 1997, *Euro J Biochem* 249: 701-07; Maras et al., 1999, *Febs Letters* 452: 365-70; Hamilton et al., 2003, *Science* 301: 1244-6; De Pourcq et al., 2010). However, the production of glycosylated mammalian proteins in non-mammalian cells still requires complicated and time-consuming genetic engineering and can be inefficient at producing a desired glycoprotein.

[0007] Thus, a need remains in the art for a simpler and more efficient system to express complex N-glycans in non-mammalian cells.

SUMMARY

[0008] Described herein are compositions including recombinant proteins having N-acetylglucosaminyltransferase activity. Further described herein are methods of producing complex N-glycans and methods of producing Man3GlcNAc2 glycans.

[0008a] In one aspect, there is provided a recombinant protein having N-acetylglucosaminyltransferase activity, wherein the recombinant protein catalyzes the transfer of N-acetylglucosamine to a terminal Manα3 residue and catalyzes the transfer of N-acetylglucosamine to a terminal Manα6 residue of an acceptor glycan, and

wherein the recombinant protein is a fusion protein comprising an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain.

[0009] O~~Thus one~~ ~~aspect~~embodiment includes recombinant proteins having N-acetylglucosaminyltransferase activity, where the recombinant proteins catalyze the

acetamidase (amdS), fungal glyceraldehyde-3-phosphate dehydrogenase (gpd), yeast alcohol dehydrogenase, yeast alcohol oxidase, yeast lactase, yeast 3-phosphoglycerate kinase, yeast triosephosphate isomerase, bacterial α-amylase, bacterial Spo2, or SSO. Another aspect includes host cells containing the expression vector of any of the preceding embodiments.

[0015] Another ~~aspect~~embodiment includes methods of producing the recombinant protein of any the preceding embodiments, including the steps of introducing an isolated polynucleotide that encodes the recombinant protein into a host cell, and culturing the host cell such that the recombinant protein is expressed. In certain embodiments, the methods further include a step of purifying the recombinant protein from the host cell. In certain embodiments that may be combined with the preceding embodiments, the host cell is a fungal cell. In certain embodiments, the fungal cell is selected from yeast or filamentous fungus.

[0016] Another aspect includes methods of producing a complex N-glycan including the steps of providing a host cell, where the host cell contains a polynucleotide encoding a fusion protein as disclosed above containing an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain, and culturing the host cell such that the fusion protein is expressed, where the fusion protein catalyzes the transfer of N-acetylglucosamine to a terminal Manα3 residue and N-acetylglucosamine to a terminal Manα6 residue of an acceptor glycan to produce a complex N-glycan. In certain embodiments, the complex N-glycan is attached to a molecule selected from an amino acid, a peptide, or a polypeptide. In certain embodiments, the molecule is a heterologous polypeptide. In certain embodiments that may be combined with the preceding embodiments, the acceptor glycan is Man3. In certain embodiments that may be combined with the preceding embodiments, the complex N-glycan is GlcNAcβ2Manα3(GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAc. In certain embodiments that may be combined with the preceding embodiments, the host cell is a eukaryotic cell. In certain embodiments that may be combined with the preceding embodiments, the host cell is a fungal cell. In certain embodiments, the fungal cell is a yeast cell selected from *S. cerevisiae*, *K. lactis*, *P. pastoris*, *H. polymorpha*, *C. albicans*, *Schizosaccharomyces*, or *Yarrowia*. In certain embodiments that may be combined with the preceding embodiments, the fungal cell is a filamentous fungal cell selected from *Trichoderma* sp., *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*,

Chrysosporium, *Chrysosporium lucknowense*, *Filibasidium*, *Fusarium*, *Gibberella*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Myrothecium*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, or *Tolypocladium*. In certain embodiments that may be combined with the preceding embodiments, the host cell further contains a polynucleotide encoding a UDP-GlcNAc transporter. In certain embodiments that may be combined with the preceding embodiments, the host cell has a reduced level of activity of a dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase compared to the level of activity in a wild-type host cell. In certain embodiments, the host cell has a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type host cell. In certain embodiments, the *alg3* gene is deleted from the host cell. In certain embodiments that may be combined with the preceding embodiments, the host cell has a reduced level of activity of an α-1,6-mannosyltransferase compared to the level of activity in a wild-type host cell. In certain embodiments, the host cell has a reduced level of expression of an *och1* gene compared to the level of expression in a wild-type host cell. In certain embodiments, the *och1* gene is deleted from the host cell. In certain embodiments that may be combined with the preceding embodiments, the host cell further contains a polynucleotide encoding an α-1,2-mannosidase. In certain embodiments that may be combined with the preceding embodiments, the host cell further contains a polynucleotide encoding a β-1,4-galactosyltransferase. In certain embodiments that may be combined with the preceding embodiments, the host cell further contains a polynucleotide encoding a sialyltransferase. In certain embodiments that may be combined with the preceding embodiments, the host cell is a *Trichoderma* cell that has a reduced level of activity of a dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase compared to the level of activity in a wild-type *Trichoderma* cell. In certain embodiments that may be combined with the preceding embodiments, the host cell is a yeast or fungal cell that has a reduced level of activity of a dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase and a reduced level of activity of an alpha-1,6-mannosyltransferase compared to the levels of activity in a wild-type yeast cell and further contains a polynucleotide encoding an α-1,2-mannosidase.

[0017] Another ~~aspect~~embodiment includes methods of producing a complex N-glycan including the steps of providing a *Trichoderma* host cell, where the host cell has a reduced level of

expression of an *alg3* gene compared to the level of expression in a wild-type host cell and contains a first polynucleotide encoding an N-acetylglucosaminyltransferase I catalytic domain and a second polynucleotide encoding an N-acetylglucosaminyltransferase II catalytic domain, and culturing the host cell to produce a complex N-glycan.

[0017a] In another aspect, there is provided a method of producing a complex N-glycan, comprising:

(1) providing a *Trichoderma* host cell, wherein the host cell has a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type host cell and comprises a recombinant protein as defined above; and,

(2) culturing the host cell to produce a complex N-glycan.

[0018] Another ~~aspect~~embodiment includes methods of producing a complex N-glycan including the steps of incubating a fusion protein containing an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain, an acceptor glycan, and an N-acetylglucosamine donor together in a buffer, where the fusion protein catalyzes the transfer of N-acetylglucosamine to a terminal Manα3 residue and N-acetylglucosamine to a terminal Manα6 residue of an acceptor glycan to produce a complex N-glycan. In certain embodiments, the acceptor glycan is attached to a molecule selected from an amino acid, a peptide, or a polypeptide. In certain embodiments, the molecule is a heterologous polypeptide. In certain embodiments, the acceptor glycan is Man3. In certain embodiments that may be combined with the preceding embodiments, the N-acetylglucosamine donor is a UDP-GlcNAc transporter.

[0019] Another ~~aspect~~embodiment includes filamentous fungal cells containing a mutation of *alg3* and Man3GlcNAc2, where the Man3GlcNAc2 includes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% (mol %) of neutral N-glycans secreted by the cells. The neutral N-glycans may be attached to a molecule selected from the group consisting of an amino acid, a peptide, and a polypeptide. In certain embodiments, the mutation of *alg3* is a deletion of *alg3*. In certain embodiments that may be combined with the preceding embodiments, the cell is a *Trichoderma reesei* cell. In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further contains a first polynucleotide encoding an N-

acetylglucosaminyltransferase I catalytic domain and a second polynucleotide encoding an N-acetylglucosaminyltransferase II catalytic domain. In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further contains a polynucleotide encoding a fusion protein containing an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain.

[0020] Another ~~aspect~~embodiment includes methods of producing a Man3GlcNAc2 glycan in a host cell including the steps of providing a host cell with a reduced level of activity of a mannosyltransferase compared to the level of activity in a wild-type host cell and culturing the host cell to produce a Man3GlcNAc2 glycan, where the Man3GlcNAc2 glycan includes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or100% (mol %) of the neutral N-glycans secreted by the host cell. The neutral N-glycans may be attached to a molecule selected from an amino acid, a peptide, and a polypeptide. In certain embodiments, the Man3GlcNAc2 glycan is attached to a heterologous polypeptide. In certain embodiments that may be combined with the preceding embodiments, the mannosyltransferase is a dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase. In certain embodiments that may be combined with the preceding embodiments, the host cell has a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type host cell. In certain embodiments, the *alg3* gene is deleted from the host cell. In certain embodiments that may be combined with the preceding embodiments, the host cell is a *Trichoderma* cell. In certain embodiments that may be combined with the preceding embodiments, the level of activity of alpha-1,6-mannosyltransferase in the host cell is reduced compared to the level of activity in a wild-type host cell. In certain embodiments that may be combined with the preceding embodiments, the host cell contains an endogenous polynucleotide encoding an α-1,2-mannosidase.

[0021] Another aspect includes a filamentous fungal cell having a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type filamentous fungal cell, where the filamentous fungal cell contains a recombinant protein of any of the preceding embodiments. In certain embodiments, the *alg3* gene contains a mutation. Preferably, the recombinant protein has N-acetylglucosaminyltransferase activity, where the recombinant protein catalyzes the transfer of N-acetylglucosamine to a terminal Manα3 residue and catalyzes the transfer of N-acetylglucosamine to a terminal Manα6 residue of an acceptor glycan, and where the recombinant protein is a fusion protein containing an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain. In certain embodiments, the mutation of the *alg3* gene is a deletion of the *alg3* gene. In certain embodiments that may be combined with the preceding embodiments, the fusion protein is encoded by a polynucleotide operably linked to a promoter. In certain embodiments, the promoter is an