What is claimed is:

1. A method of producing an altered N-glycosylation form of a target protein or polypeptide, the method comprising:

providing a cell genetically engineered to comprise at least one modified N-glycosylation activity relative to a non-genetically engineered cell of the same species, the cell being a *Yarrowia lipolytica* or an *Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast, the at least one modified N-glycosylation activity comprising;

expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1; and

introducing into the cell a nucleic acid encoding a target protein or polypeptide, wherein the cell produces the target protein or polypeptide in an altered N-glycosylation form relative to a non-genetically engineered cell of the same species.

2. The method of claim 1, further comprising isolating the altered N-glycosylation form of the target protein.

3. The method of claim 1 or 2, wherein the target protein is an exogenous protein.

4. The method of any of claims 1-3, wherein the target protein is an endogenous protein.

5. The method of any of claims 1-4, wherein the target protein is a mammalian protein.

6. The method of claim 5, wherein the target protein is a human protein.

7. The method of any of claims 1-6, wherein the target protein is a pathogen protein, a lysosomal protein, a growth factor, a cytokine, a chemokine, an antibody or antigen-binding fragment thereof, or a fusion protein.

8. The method of claim 7, wherein the fusion protein is a fusion of a pathogen protein, a lysosomal protein, a growth factor, a cytokine, or a chemokine with an antibody or an antigen-binding fragment thereof.

9. The method of any of claims 1-8, wherein the target protein is a protein associated with a lysosomal storage disorder (LSD).

10. The method of any of claims 1-9, wherein the target protein is glucocerebrosidase or galactocerebrosidase.

11. The method of any of claims 1-9, wherein the target protein is selected from the group consisting of alpha-L-iduronidase, beta-D-galactosidase, beta-glucosidase, beta-hexosaminidase, beta-D-mannosidase, alpha-L-fucosidase, arylsulfatase B, arylsulfatase A, alpha-N-acteylgalactosaminidase, aspartylglucosaminidase, iduronate-2-sulfatase, alpha-glucosaminide-N-acetyltransferase, beta-D-glucoronidase, hyaluronidase, alpha-L-mannosidase, alpha- neuraminidase, phosphotransferase, acid lipase, acid ceramidase, sphinogmyelinase, thioesterase, cathepsin K, and lipoprotein lipase.

12. The method of any of claims 1-11, wherein the altered N-glycosylation form of the target protein or polypeptide protein comprises one or more N-glycan structures.

13. The method of claim 12, wherein the altered N-glycosylation form of the target protein is homogenous.

14. The method of either of claims 12 or 13, wherein the altered N-glycosylation form of the target protein is substantially homogenous.

15. The method of claim 14, wherein the fraction of altered target molecules that contain the altered glycosylation is at least about 20%.

16. The method of claim 14, wherein the fraction of altered target molecules that contain the altered glycosylation is at least about 30%.

17. The method of claim 14, wherein the fraction of altered target molecules that contain the altered glycosylation is at least about 40%.

18. The method of any of claims 1-17, wherein the cell is genetically engineered to comprise at least one further N-glycosylation activity.

19. The method of claim 18, wherein N-glycosylation activity is OCH1 activity.

20. The method of either of claims 18 or 19, wherein the at least one further modification comprises deletion of a gene encoding a protein having the N-glycosylation activity.

21. The method of any of claims 18-20, wherein the at least one further modification comprises expression of a mutant form of a protein having the N-glycosylation activity.

22. The method of any of claims 18-21, wherein the at least one further modification comprises introduction or expression of an RNA molecule that interferes with the functional expression of a protein having the N-glycosylation activity.

23. The method of claim 18, wherein the at least one further modification comprises expression of a protein having N-glycosylation activity.

24. The method of claim 23, wherein the expressed protein is a protein encoded by an exogenous nucleic acid in the cell.

25. The method of either of claims 23 or 24, wherein the protein having N-glycosylation activity is an exogenous protein.

26. The method of any of claims 23-25, wherein the protein having N-glycosylation activity is a mammalian protein.

27. The method of claim 26, wherein the protein having N-glycosylation activity is a human protein.

28. The method of either claim 23 or 24, wherein the protein having N-glycosylation activity is a lower eukaryotic protein.

29. The method of claim 28, wherein the lower eukaryote is a fungus, a trypanosome, or a protozoan.

30. The method of claim 28, wherein the lower eukaryote is selected from the group consisting of *Typanosoma brucei*, *Trichoderma harzianum*, and an *Aspergillus*.

31. The method of claim 1, wherein at least about 30% of the mannosyl residues of a glycoprotein are phosphorylated.

32. The method of any of claims 1-31, further comprising additional processing of the glycoprotein.

33. The method of claim 47, wherein the additional processing occurs *in vitro*.

34. The method of claim 47, wherein the additional processing occurs *in vivo*.

35. The method of any of claims 32-34, wherein the additional processing comprises addition of a heterologous moiety to the modified glycoprotein.

36. The method of claim 35, wherein the heterologous moiety is a polymer or a carrier.

37. The method of any of claims 32-36, wherein the additional processing comprises enzymatic or chemical treatment of the altered N-glycosylation form of the target protein.

38. The method of claim 32, wherein the additional processing comprises treatment of the altered N-glycosylation form of the target protein with a mannosidase, a mannanase, a phosphodiesterase, a glucosidase, or a glycosyltransferase.

39. The method of claim 37, wherein the additional processing comprises treatment of the altered N-glycosylation form of the target protein with hydrofluoric acid.

40. The method of any of claims 32-39, wherein the additional processing comprises phosphorylation of the altered N-glycosylation form of the target protein.

41. An isolated protein having altered N-glycosylation, wherein the protein is produced by the method of any of claims 1-40, and wherein at least 80% of the N-glycans of the protein are phosphorylated.

42. An isolated cell genetically engineered to comprise at least one modified N-glycosylation activity relative to a non-genetically engineered cell, the cell being a *Yarrowia lipolytica, or Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast, the at least one modified N-glycosylation activity comprising expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1.

43. The isolated cell of claim 42, wherein the cell comprises at least one further modification of N-glycosylation activity.

44. The isolated cell of claim 43, wherein N-glycosylation activity is OCH1 activity.

45. The isolated cell of either of claims 43 or 44, wherein the further modification comprises deletion of a gene encoding a protein having the N-glycosylation activity.

46. The isolated cell of either of claims 43 or 44, wherein the modification comprises expression of a mutant form of a protein having the N-glycosylation activity.

47. The isolated cell of either of claims 43 or 44, wherein the modification comprises introduction or expression of an RNA molecule that interferes with the functional expression of a protein having the N-glycosylation activity.

48. The isolated cell of claim 43, wherein the modification comprises expression of a protein having N-glycosylation activity.

49. The isolated cell of claim 48, wherein the protein having N-glycosylation activity is a human protein.

50. Use of a protein of claim 41 in the manufacture of a medicament for treating a disorder in a subject, the disorder is treatable by administration of a protein having altered N-glycosylation, and wherein the subject is one having, or suspected of having, a disease treatable by administration of a protein having altered N-glycosylation.

51. The use of claim 50, wherein the disorder is a metabolic disorder.

52. The use of claim 51, wherein the metabolic disorder is a lysosomal storage disorder (LSD).

53. The use of claim 52, wherein the lysosomal storage disorder is Gaucher disease, Tay-Sachs disease, Pompe disease, Niemann-Pick disease, or Fabry disease.

54. The use of any of claims 50-53, wherein the protein is one associated with a LSD.

55. The use of claim 54 wherein the protein is glucocerebrosidase or alpha-galactosidase.

56. The use of claim 54, wherein the protein is selected from the group consisting of alpha-L-iduronidase, beta-D-galactosidase, beta-glucosidase, beta-hexosaminidase, beta-D-mannosidase, alpha-L-fucosidase, arylsulfatase B, arylsulfatase A, alpha-N-acteylgalactosaminidase, aspartylglucosaminidase, iduronate-2-sulfatase, alpha-glucosaminide-N-acetyltransferase, beta-D-glucoronidase, hyaluronidase, alpha-L-mannosidase, alpha-neurominidase, phosphotransferase, acid lipase, acid ceramidase, sphinogmyelinase, thioesterase, cathepsin K, and lipoprotein lipase.

57. The use of any of claims 50-56, further comprising determining whether the subject has a disease treatable by administration of a protein having altered N-glycosylation.

58. The use of any of claims 50-57, wherein the subject is a human.

59. A method of producing an altered N-glycosylation form of a target protein, the method comprising:

contacting a target protein with a cell lysate prepared from a cell genetically engineered to comprise at least two modifications of N-glycosylation activity relative to a non-genetically engineered cell of the same species, the cell being a *Yarrowia lipolytica* or an *Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast, the at least two modifications of N-glycosylation activity comprising: a deficiency in OCH1 activity; and

expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1, and

wherein the contacting of the target protein with the cell lysate results in an altered N-glycosylation form of the target protein.

60. A method of producing an altered N-glycosylation form of a target protein, the method comprising contacting a target protein with one or more proteins having N-glycosylation activity, wherein the one or more proteins having N-glycosylation activity are obtained from a cell genetically engineered to comprise at least two modifications of N-glycosylation activity relative to a non-genetically engineered cell of the same species, the cell being a *Yarrowia lipolytica* or an *Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast, the at least two modifications of N-glycosylation activity comprising:

a deficiency in OCH1 activity; and

expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1, and

wherein contacting the target molecule with the one or more proteins having N-glycosylation activity results in an altered N-glycosylation form of the target protein.

61. A substantially pure culture of *Yarrowia lipolytica* or *Arxula adeninivorans* cells, a substantial number of which are genetically engineered to comprise at least two modifications of N-glycosylation activity comprising:

a deficiency in OCH1 activity; and expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1.

62. The substantially pure culture of cells of claim 61, wherein the culture of cells contains one or more subpopulations of cells, each subpopulation comprising a different modified glycosylation activity.

What is claimed is:

1. A method of producing an altered N-glycosylation form of a target protein or polypeptide, the method comprising:

providing a cell genetically engineered to comprise at least one modified N-glycosylation activity relative to a non-genetically engineered cell of the same species, the cell being a *Yarrowia lipolytica* or an *Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast, the at least one modified N-glycosylation activity comprising;

expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1; and

introducing into the cell a nucleic acid encoding a target protein or polypeptide, wherein the cell produces the target protein or polypeptide in an altered N-glycosylation form relative to a non-genetically engineered cell of the same species.

2. The method of claim 1, further comprising isolating the altered N-glycosylation form of the target protein.

3. The method of claim 1 or 2, wherein the target protein is an exogenous protein.

4. The method of any of claims 1-3, wherein the target protein is an endogenous protein.

5. The method of any of claims 1-4, wherein the target protein is a mammalian protein.

6. The method of claim 5, wherein the target protein is a human protein.

7. The method of any of claims 1-6, wherein the target protein is a pathogen protein, a lysosomal protein, a growth factor, a cytokine, a chemokine, an antibody or antigen-binding fragment thereof, or a fusion protein.

8. The method of claim 7, wherein the fusion protein is a fusion of a pathogen protein, a lysosomal protein, a growth factor, a cytokine, or a chemokine with an antibody or an antigen-binding fragment thereof.

9. The method of any of claims 1-8, wherein the target protein is a protein associated with a lysosomal storage disorder (LSD).

10. The method of any of claims 1-9, wherein the target protein is glucocerebrosidase or galactocerebrosidase.

11. The method of any of claims 1-9, wherein the target protein is selected from the group consisting of alpha-L-iduronidase, beta-D-galactosidase, beta-glucosidase, beta-hexosaminidase, beta-D-mannosidase, alpha-L-fucosidase, arylsulfatase B, arylsulfatase A, alpha-N-acteylgalactosaminidase, aspartylglucosaminidase, iduronate-2-sulfatase, alpha-glucosaminide-N-acetyltransferase, beta-D-glucoronidase, hyaluronidase, alpha-L-mannosidase, alpha- neuraminidase, phosphotransferase, acid lipase, acid ceramidase, sphinogmyelinase, thioesterase, cathepsin K, and lipoprotein lipase.

12. The method of any of claims 1-11, wherein the altered N-glycosylation form of the target protein or polypeptide protein comprises one or more N-glycan structures.

13. The method of claim 12, wherein the altered N-glycosylation form of the target protein is homogenous.

14. The method of either of claims 12 or 13, wherein the altered N-glycosylation form of the target protein is substantially homogenous.

15. The method of claim 14, wherein the fraction of altered target molecules that contain the altered glycosylation is at least about 20%.

16. The method of claim 14, wherein the fraction of altered target molecules that contain the altered glycosylation is at least about 30%.

17. The method of claim 14, wherein the fraction of altered target molecules that contain the altered glycosylation is at least about 40%.

18. The method of any of claims 1-17, wherein the cell is genetically engineered to comprise at least one further N-glycosylation activity.

19. The method of claim 18, wherein N-glycosylation activity is OCH1 activity.

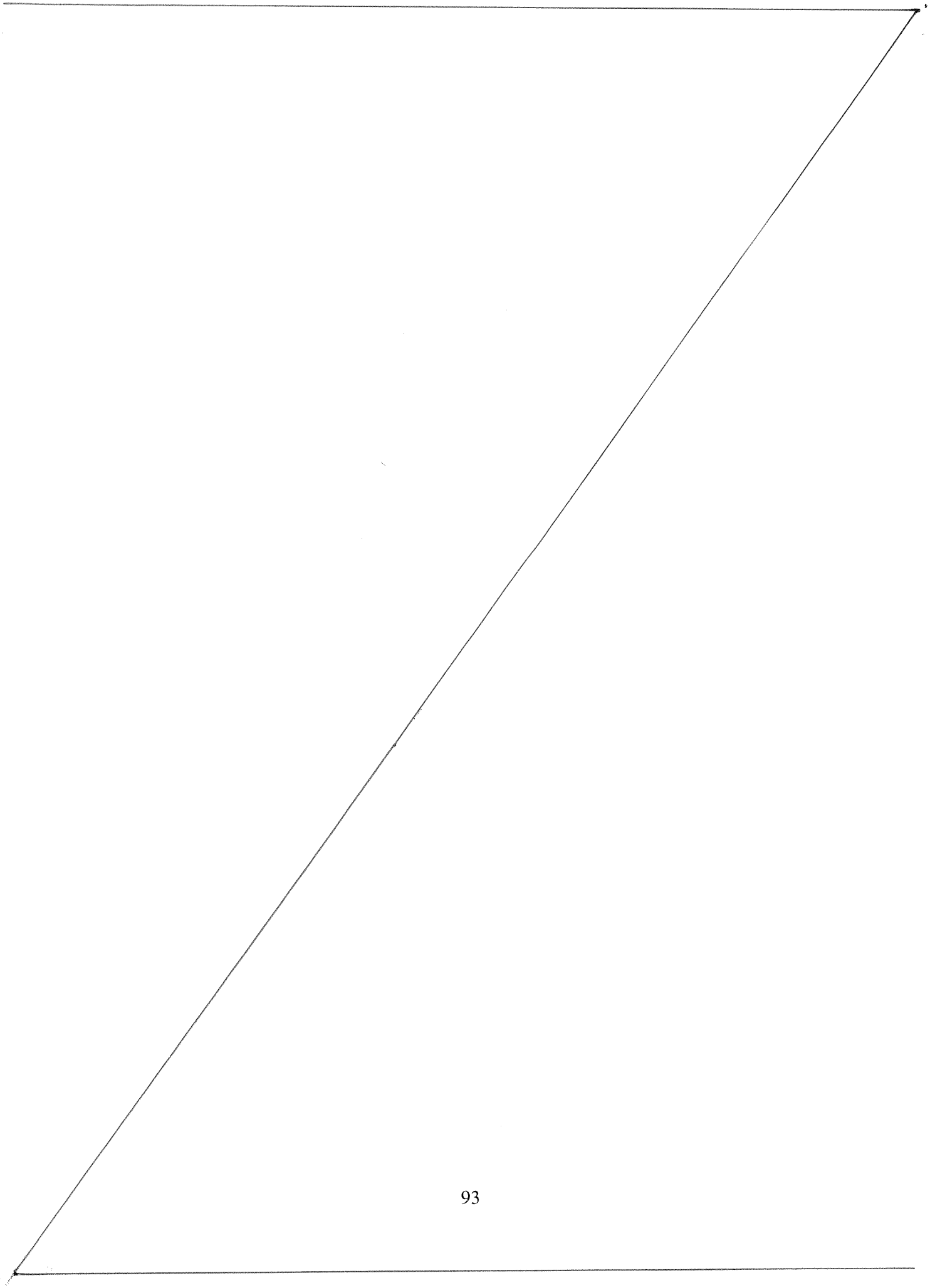
20. The method of either of claims 18 or 19, wherein the at least one further modification comprises deletion of a gene encoding a protein having the N-glycosylation activity.

21. The method of any of claims 18-20, wherein the at least one further modification comprises expression of a mutant form of a protein having the N-glycosylation activity.

22. The method of any of claims 18-21, wherein the at least one further modification comprises introduction or expression of an RNA molecule that interferes with the functional expression of a protein having the N-glycosylation activity.

23. The method of claim 18, wherein the at least one further modification comprises expression of a protein having N-glycosylation activity.

24. The method of claim 23, wherein the expressed protein is a protein encoded by an exogenous nucleic acid in the cell.



25. The method of either of claims 23 or 24, wherein the protein having N-glycosylation activity is an exogenous protein.

26. The method of any of claims 23-25, wherein the protein having N-glycosylation activity is a mammalian protein.

27. The method of claim 26, wherein the protein having N-glycosylation activity is a human protein.

28. The method of either claim 23 or 24, wherein the protein having N-glycosylation activity is a lower eukaryotic protein.

29. The method of claim 28, wherein the lower eukaryote is a fungus, a trypanosome, or a protozoan.

30. The method of claim 28, wherein the lower eukaryote is selected from the group consisting of *Typanosoma brucei*, *Trichoderma harzianum*, and an *Aspergillus*.

31. The method of claim 1, wherein at least about 30% of the mannosyl residues of a glycoprotein are phosphorylated.

32. The method of any of claims 1-31, further comprising additional processing of the glycoprotein.

33. The method of claim 47, wherein the additional processing occurs *in vitro*.

34. The method of claim 47, wherein the additional processing occurs *in vivo*.

35. The method of any of claims 32-34, wherein the additional processing comprises addition of a heterologous moiety to the modified glycoprotein.

36. The method of claim 35, wherein the heterologous moiety is a polymer or a carrier.

37. The method of any of claims 32-36, wherein the additional processing comprises enzymatic or chemical treatment of the altered N-glycosylation form of the target protein.

38. The method of claim 32, wherein the additional processing comprises treatment of the altered N-glycosylation form of the target protein with a mannosidase, a mannanase, a phosphodiesterase, a glucosidase, or a glycosyltransferase.

39. The method of claim 37, wherein the additional processing comprises treatment of the altered N-glycosylation form of the target protein with hydrofluoric acid.

40. The method of any of claims 32-39, wherein the additional processing comprises phosphorylation of the altered N-glycosylation form of the target protein.

41. An isolated protein having altered N-glycosylation, wherein the protein is produced by the method of any of claims 1-40.

42. An isolated cell genetically engineered to comprise at least one modified N-glycosylation activity relative to a non-genetically engineered cell, the cell being a *Yarrowia lipolytica, or Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast of the same species, the at least one modified N-glycosylation activity comprising expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1.

43. The isolated cell of claim 42, wherein the cell comprises at least one further modification of N-glycosylation activity.

44. The isolated cell of claim 43, wherein N-glycosylation activity is OCH1 activity.

45. The isolated cell of either of claims 43 or 44, wherein the further modification comprises deletion of a gene encoding a protein having the N-glycosylation activity.

46. The isolated cell of either of claims 43 or 44, wherein the modification comprises expression of a mutant form of a protein having the N-glycosylation activity.

47. The isolated cell of either of claims 43 or 44, wherein the modification comprises introduction or expression of an RNA molecule that interferes with the functional expression of a protein having the N-glycosylation activity.

48. The isolated cell of claim 43, wherein the modification comprises expression of a protein having N-glycosylation activity.

49. The isolated cell of claim 48, wherein the protein having N-glycosylation activity is a human protein.

50. Use of a protein of claim 41 in the manufacture of a medicament for treating a disorder in a subject, the disorder is treatable by administration of a protein having altered N-glycosylation, and wherein the subject is one having, or suspected of having, a disease treatable by administration of a protein having altered N-glycosylation.

51. The use of claim 50, wherein the disorder is a metabolic disorder.

52. The use of claim 51, wherein the metabolic disorder is a lysosomal storage disorder (LSD).

53. The use of claim 52, wherein the lysosomal storage disorder is Gaucher disease, Tay-Sachs disease, Pompe disease, Niemann-Pick disease, or Fabry disease.

54. The use of any of claims 50-53, wherein the protein is one associated with a LSD.

55. The use of claim 54 wherein the protein is glucocerebrosidase or alpha-galactosidase.

56. The use of claim 54, wherein the protein is selected from the group consisting of alpha-L-iduronidase, beta-D-galactosidase, beta-glucosidase, beta-hexosaminidase, beta-D-mannosidase, alpha-L-fucosidase, arylsulfatase B, arylsulfatase A, alpha-N-acteylgalactosaminidase, aspartylglucosaminidase, iduronate-2-sulfatase, alpha-glucosaminide-N-acetyltransferase, beta-D-glucoronidase, hyaluronidase, alpha-L-mannosidase, alpha-

neurominidase, phosphotransferase, acid lipase, acid ceramidase, sphinogmyelinase, thioesterase, cathepsin K, and lipoprotein lipase.

57. The use of any of claims 50-56, further comprising determining whether the subject has a disease treatable by administration of a protein having altered N-glycosylation.

58. The use of any of claims 50-57, wherein the subject is a human.

59. A method of producing an altered N-glycosylation form of a target protein, the method comprising:

contacting a target protein with a cell lysate prepared from a cell genetically engineered to comprise at least two modifications of N-glycosylation activity relative to a non-genetically engineered cell of the same species, the cell being a *Yarrowia lipolytica* or an *Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast, the at least two modifications of N-glycosylation activity comprising: a deficiency in OCH1 activity; and

expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1, and

wherein the contacting of the target protein with the cell lysate results in an altered N-glycosylation form of the target protein.

60. A method of producing an altered N-glycosylation form of a target protein, the method comprising contacting a target protein with one or more proteins having N-glycosylation activity, wherein the one or more proteins having N-glycosylation activity are obtained from a cell genetically engineered to comprise at least two modifications of N-glycosylation activity relative to a non-genetically engineered cell of the same species, the cell being a *Yarrowia lipolytica* or an *Arxula adeninivorans* cell or a cell from a related species of dimorphic yeast, the at least two modifications of N-glycosylation activity comprising:

a deficiency in OCH1 activity; and

expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1, and

wherein contacting the target molecule with the one or more proteins having N-glycosylation activity results in an altered N-glycosylation form of the target protein.

61. A substantially pure culture of *Yarrowia lipolytica* or *Arxula adeninivorans* cells, a substantial number of which are genetically engineered to comprise at least two modifications of N-glycosylation activity comprising:

a deficiency in OCH1 activity; and expression of a phosphorylation-promoting protein or biologically active variant thereof that promotes mannosyl phosphorylation of the target protein or polypeptide, wherein the phosphorylation-promoting protein or biologically active variant thereof is MNN4 or PNO1.

62. The substantially pure culture of cells of claim 61, wherein the culture of cells contains one or more subpopulations of cells, each subpopulation comprising a different modified glycosylation activity.

