CLAIMS

1. A method of making a producer cell line that is capable of producing a recombinant polypeptide product (i) in increased quantity or (ii) having improved quality, the method comprising:

a) contacting a population of cells with a first inhibitor of a protein degradation pathway;

b) evaluating the effect of the first inhibitor on the quantity or the quality of an endogenously or exogenously expressed polypeptide produced by a first cell present in the population of cells by comparison with a reference value;

c) selecting and isolating the first cell or progeny of the first cell wherein the first cell or progeny of the first cell produces the endogenously or exogenously expressed polypeptide (i) in increased quantity or (ii) having improved quality compared to the reference value; and

d) establishing a producer cell line from the first cell or progeny of the first cell;

thereby making the producer cell line that is capable of producing the recombinant polypeptide product (i) in increased quantity or (ii) having improved quality.

2. The method of claim 1, wherein the first inhibitor is a proteasome inhibitor, a ubiquitin pathway inhibitor, or an ERAD inhibitor.

3. The method of claim 1, wherein the polypeptide produced by the first cell is a recombinant protein encoded by a first exogenous nucleic acid.

4. The method of claim 1, wherein

the quality of the polypeptide produced by the first cell is improved, and the quality is selected from the proportion of the group consisting of:

the polypeptide with respect to unwanted isoforms, product fragments, or truncated forms;

properly folded product;

functional or enzymatically active product;

aggregated product; and

fragmented or unwanted isoforms.

5. The method of claim 1, wherein:

(a) the first inhibitor is a proteasome inhibitor, wherein the proteasome inhibitor inhibits or reduces the activity of one or more of the 20S core subunit, the 19S regulatory subunit, the 11S regulatory particle, or a chaperone protein that assists in proteasome assembly;

(b) the first inhibitor is an ERAD inhibitor, wherein the ERAD inhibitor inhibits or reduces the activity of one or more of calnexin/calreticulin, UDP-glucose-glycoprotein glucosyltransferase, ER degradation enhancing α-mannosidase-like protein (EDEM), ER mannosidase I, Sec61, CDC48p (VCP/p97),

Hrd1, Doa10, Ubc6, Ubc1, Cue1, or Ubc7; or

(c) the first inhibitor is a ubiquitin pathway inhibitor, wherein the ubiquitin pathway inhibitor inhibits or reduces the activity of one or more of: E1 ubiquitin activating enzyme, E2 ubiquitin conjugating enzyme, or E3 ubiquitin ligase.

6. The method of claim 5, wherein the first inhibitor is a proteasome inhibitor selected from: MG132, epoxomicin, bortezomib, ixazomib, carfilzomib, disulfiram, CEP-18770, ONX 0912, salinosporamide, LLnV, CEP1612, lactacystin, PS-341, and eponomicin, or wherein the ERAD inhibitor is eeyarestatin I.

7. The method of claim 1, wherein the population of cells is contacted with a concentration of the first inhibitor that is sufficient to reduce culture viability, by about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%, as compared to the culture viability before the culture is contacted with the first inhibitor or as compared to a culture that is not contacted with the first inhibitor.

8. The method of claim 1, wherein:

the first inhibitor is MG-132 and the concentration of MG-132 is less than 0.5 μM; or

the first inhibitor is epoxomicin and the concentration of epoxomicin is less than 0.05 μM

epoxomicin.

9. The method of claim 1, wherein the population of cells is contacted by or cultured in the presence of the first inhibitor for 24, 48, 72, 96, or more hours.

10. The method of claim 1, wherein the population of cells is contacted by the first inhibitor 24, 48, 72, 96, or 168 hours prior to or after a MSX selection step, or simultaneously with a MSX selection step.

11. The method of claim 1, wherein the first cell is selected from the group consisting of Hela, HEK293, H9, HepG2, MCF7, Jurkat, NIH3T3, PC12, PER.C6, BHK, VERO, SP2/0, NS0, YB2/0, EB66, C127, L cell, COS, QC1-3, CHOK1, CHOK1SV, Potelligent CHOK1SV, CHO GS knockout, CHOK1SV GS-KO, CHOS, CHO DG44, CHO DXB11, CHOZN, and a CHO cell.

12. The method of claim 1, wherein the polypeptide produced by the first cell is evaluated for a parameter related to a physical or functional property selected from the group consisting of primary sequence, glycosylation, primary, secondary, tertiary, or quaternary structure, activity, degree of glycosylation, degree of aggregation, proportion of the recombinant polypeptide having a preselected monomeric, dimeric, or trimeric structure, and the proportion of the recombinant polypeptide having a preselected non-denatured or non-aggregated structure.

13. The method of claim 1, further comprising introducing a first exogenous nucleic acid that encodes the recombinant polypeptide product into the population of cells, prior to step a).

14. The method of claim 13, further comprising introducing a second exogenous nucleic acid encoding a selection marker into the population of cells , wherein the second exogenous nucleic acid is introduced prior to step a), and wherein the second exogenous nucleic acid is introduced simultaneously with the introduction of the first exogenous nucleic acid.

15. The method of claim 14, wherein the second exogenous nucleic acid encodes glutamine synthetase.

16. The method of claim 1, further comprising determining if the first cell comprises one or more exogenous nucleic acids integrated into a nucleic acid of the first cell, wherein determining if the first cell comprises one or more exogenous nucleic acids integrated into a nucleic acid of the first cell consists of a selection step selected from the group consisting of MSX selection, MTX selection, antibiotic selection, yeast growth selection, and selection based on color change or surface expression of a marker.

17. The method of claim 16, further comprising an additional selection step, wherein the selection step is selected from the group consisting of FACS, magnetic separation, colony picking, microfluidic cell sorting, and microfluidic cell destruction.

18. The method of claim 1, further comprising introducing to the population of cells an agent that assists in protein folding comprising a nucleic acid encoding a chaperone protein or component of the protein folding pathway.

19. The method of claim 1 , further comprising after a) and before b): culturing the first cell, or progeny of the first cell, in the absence of the first inhibitor for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more passages or for at least 12, 24, 48, or 72 hours.

20. The method of claim 1, further comprising contacting the population of cells with a second inhibitor of a protein degradation pathway.

21. The method of claim 20, wherein the population of cells is contacted with the first and second inhibitors of protein degradation concurrently or sequentially.

22. The method of claim 20, wherein the first inhibitor is selected from the group consisting of:

(i) MG132, epoxomicin, and eeyarestatin I and second inhibitor of protein degradation is selected from MG132, epoxomicin, and eeyarestatin I;

(ii) a proteasome inhibitor and the second inhibitor is a proteasome inhibitor;

(iii) a proteasome inhibitor and the second inhibitor is an ERAD inhibitor or an ubiquitin pathway inhibitor;

(iv) an ERAD inhibitor and the second inhibitor is an ERAD inhibitor;

(v) an ERAD inhibitor and the second inhibitor is an ubiquitin pathway inhibitor or a proteasome inhibitor;

(vi) the first inhibitor is an ubiquitin pathway inhibitor and the second inhibitor is an ubiquitin pathway inhibitor; and

(vii) the first inhibitor is an ubiquitin pathway inhibitor and the second inhibitor is an ERAD inhibitor or a proteasome inhibitor.

23. The method of claim 1, wherein the population of cells is a eukaryotic cell.

24. The method of claim 1, wherein the population of cells is selected from the group consisting of mouse, rat, Chinese hamster, Syrian hamster, monkey, ape, human, dog, camel, horse, ferret, cat, an insect, a plant, duck, parrot, fish, and yeast.

25. The method of claim 1, wherein the quantity or the quality of the endogenously or exogenously expressed polypeptide produced in step (b) is evaluated by comparison with the quantity or quality of the polypeptide produced by a cell that has not been contacted by the inhibitor of protein degradation.

26. A method of making a producer cell line that produces a recombinant polypeptide (i) in increased quantity or (ii) having improved quality, the method comprising:

a) contacting a population of cells which comprise an exogenous nucleic acid encoding the recombinant polypeptide and which express the recombinant polypeptide with a first inhibitor of a protein degradation pathway;

b) evaluating the effect of the first inhibitor on the quantity or the quality of the recombinant polypeptide produced by a first cell present in the population of cells by comparison with a reference value;

c) selecting and isolating the first cell or progeny of the first cell wherein the first cell or progeny of the first cell produces the recombinant polypeptide (i) in increased quantity or (ii) having improved quality compared to the reference value; and

d) establishing a producer cell line from the first cell or progeny of the first cell;

thereby making the producer cell line that produces the recombinant polypeptide (i) in increased quantity or (ii) having improved quality.

27. The method of claim 26, wherein the quantity or the quality of the polypeptide produced in step (b) is evaluated by comparison with the quantity or quality of the polypeptide produced by a cell that has not been contacted by the inhibitor of protein degradation.

28. The method of claim 1, wherein the first cell is isolated after step (a) and before step (b).

29. The method of claim 1, wherein the producer cell line secretes the recombinant polypeptide product.

30. The method of claim 1, wherein the producer cell line produces the recombinant polypeptide product in the absence of the first inhibitor of a protein degradation pathway.

31. The method of claim 1, further comprising introducing an exogenous nucleic acid that expresses the recombinant protein into the producer cell line.

32. The method of claim 16, wherein the selection step is antibiotic selection and the antibiotic selection comprises selection for resistance to an antibiotic selected from the group consisting of hygromycin, neomycin (G418), zeocin, puromycin, or blasticidin.