

Lecture 6 - Production of Recombinant Proteins II: Eukaryotic Hosts (Yeasts and Mammalian)

BENG168

Instructor: Adam M. Feist, Assistant Professor, Shu Chien - Gene Lay Department of Bioengineering

In-Class Announcements & Follow Up

- Update to previous lecture slides
- In-class announcements

Biomanufacturing Seminar - UCSD Student Group Led

17 July

Dates: January 8 to March 13 every Wednesday via zoom



Time: 9:30 to 10:30 am



Location: Zoom link as follows:

[https://ucsd.zoom.us/j/97526231316__;!!Mih3wA!Fez537p1t7reo2QVvH4Bx8b-hqCXNjmpBNKOFcQcpGsErxZerZ26BUbhtaOO7QIH39RTO6HtAbIet1th\\$](https://ucsd.zoom.us/j/97526231316__;!!Mih3wA!Fez537p1t7reo2QVvH4Bx8b-hqCXNjmpBNKOFcQcpGsErxZerZ26BUbhtaOO7QIH39RTO6HtAbIet1th$)



Who Should Attend: Students in biology, chemical engineering, **bioengineering**, biochemistry, biotechnology, and related fields.



Why Attend: Gain exposure to real-world biotechnology applications, expand your professional network, and explore career opportunities in bioprocessing and biomanufacturing.

4 -1/28/2026 Biologics Drug Development
Gayle Derfus @ Oceanside Gilead Sciences Executive Director, Drug Substance

5 -2/4/2026 Role of Manufacturing Science and Technology: MAbs
Scott Rosenthal @ Oceanside Genentech Executive Director MSAT

6 -Date to be announced Role of Manufacturing Science and Technology: Biosimilars
Latit Saxena @ South Korea Samsung Biologics Senior Director MSAT

7 -2/11/2026 Bioengineer Perspective on Delivering Monoclonal Antibodies
Eric Fallon @ San Diego Genentech/Vir Biotech/Neurocrine

Process Development/MSAT /CMC

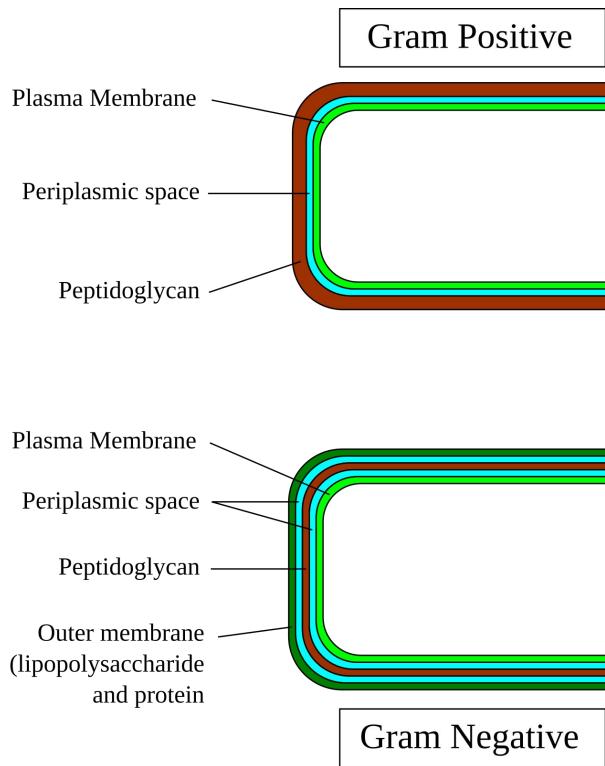
8 -2/18/2026 Manufacturing viral vectors for gene and cell therapy
Daniel Gibbs @ San Diego Cirsium Biosciences CEO

9 -2/25/2026 Bioengineer Organs
Emily Beck @ Minneapolis Miromatrix Director Upstream R&D

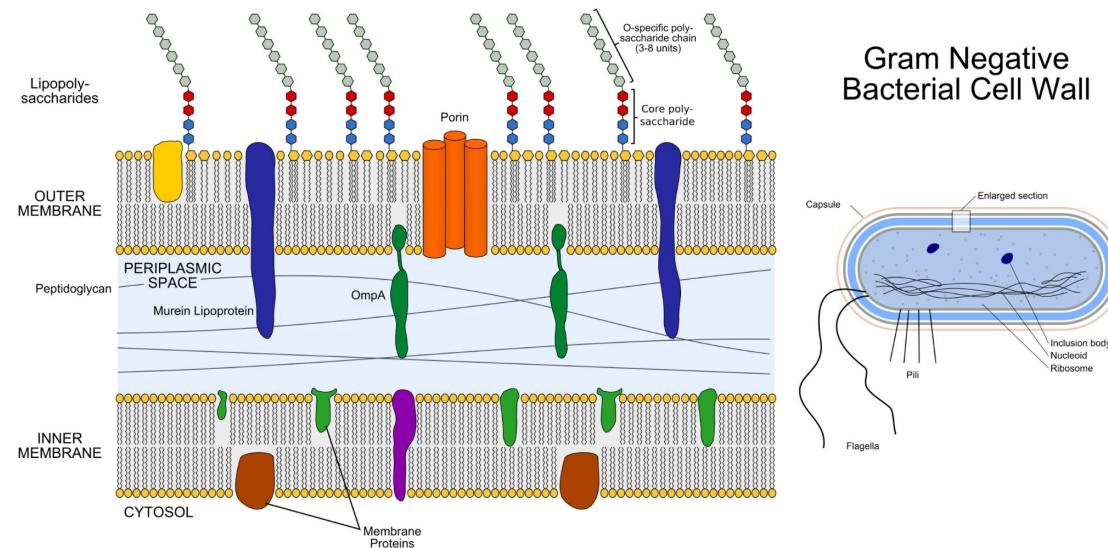
10 -3/4/2026 Automated Experimentation and Evolutionary Engineering of Microbes for Industrial Biotechnology Adam Feist @ UCSD UCSD Bioengineering Assistant Professor

11 -3/11/2026 Industrial Chemicals Bioprocessing: Scaling Technologies
Seth Levine @ San Diego Genomatica Engineering Fellow

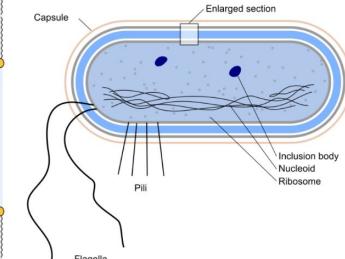
Prokaryotes have 2 major membrane types - reminder



The Gram stain originated in 1884 with Danish physician and bacteriologist [Hans Christian Gram](#).



Gram Negative
Bacterial Cell Wall



Why do cells lose their plasmids?

Biological Mechanisms of Heterologous Plasmid Loss

- **Metabolic Load:** Vector replication and protein synthesis divert **energy (ATP/GTP)** and **resources (nucleotides/amino acids)** from the host.
- **Segregational Instability:** Unequal distribution of plasmids to daughter cells during division creates a growing population of plasmid-less cells. Plasmids and chromosomes replicate at different rates and under different regulatory controls.
- **Toxicity:** Recombinant proteins or enzymes may be lethal to host bacteria - the products interfere with cellular functions.
- **The Result is a Growth Differential:** Cells lacking the plasmid maintain higher growth rates and eventually outcompete producers, dominating the culture.

Module 1: Eukaryotic Protein Processing & *S. cerevisiae* Systems (Source Pages: Chapter 3: 119–131)

- **Eukaryotic post-translational modifications are essential.**
- ***S. cerevisiae* serves as model host.**
- **Optimize secretion and protein folding capacity.**

Why Eukaryotic Host Systems?

- **Authentic Products:** Eukaryotic hosts are required for authentic human therapeutics that must be identical to natural versions in all properties.
- **Protein Folding:** These systems facilitate proper protein folding and the formation of essential disulfide bonds within the endoplasmic reticulum.
- **PTM Capabilities:** These hosts enable post-translational modifications like glycosylation, a process we identified as a major *Lecture 5* limitation for bacteria.

Table 3.1 Production of recombinant human proteins in various biological hosts

Parameter	Bacteria	Yeast	Mammalian cell culture	Transgenic plants
Glycosylation	None	Incorrect	Correct	Generally correct; small differences
Multimeric proteins assembled	Limited	Limited	Limited	Yes
Production costs	Low-medium	Medium	High	Very low
Protein folding accuracy	Low	Medium	High	High
Protein yield	High	Medium-high	Low-medium	Medium
Scale-up capacity	High	High	Low	Very high
Scale-up costs	High	High	High	Low
Time required	Low	Low-medium	Medium-high	High
Skilled workers required	Medium	Medium	High	Low
Acceptable to regulators	Yes	Yes	Yes	Not yet

Post-translational Modifications (PTMs)

- **Proteolytic Cleavage:** Many proteins are synthesized as inactive precursors that require specific removal of leader or internal peptides.
- **Functional Activation:** Cleavage is essential to transform molecules like preproinsulin into active, mature insulin for clinical use.
- **Complexity:** Eukaryotes possess the specialized enzymes and organelles, like the Golgi apparatus, to perform these precise cuts.

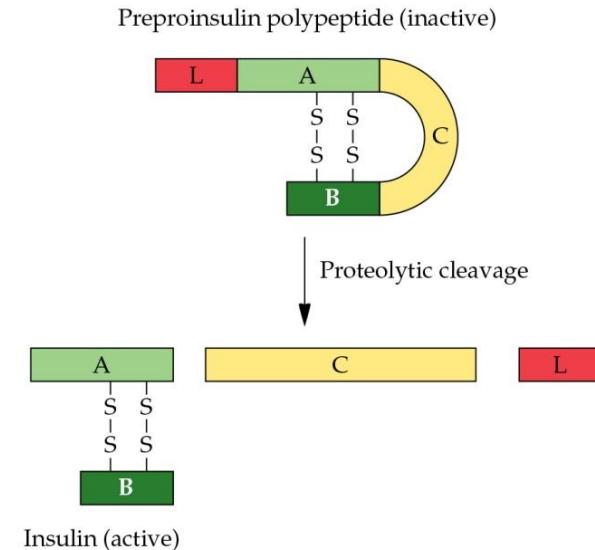


Figure 3.24 Cleavage of inactive preproinsulin to yield active mature insulin. Proteases remove the leader peptide (L) and an internal peptide (C), yielding a protein that consists of peptides A and B linked by disulfide bonds.

Post-translational Modifications (PTMs)

- **O-linked Glycosylation:** This process entails adding specific sugar groups to the hydroxyl group of **Serine (S)** or **Threonine (T)** residues.
- **N-linked Glycosylation:** Sugars are attached to the amide group of **Asparagine (N)**, which is critical for protein stability and biological activity.
- **Biological Function:** PTMs are not just decorative; they determine how long a drug lasts in the bloodstream and how it binds receptors.

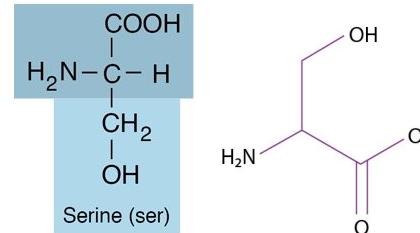
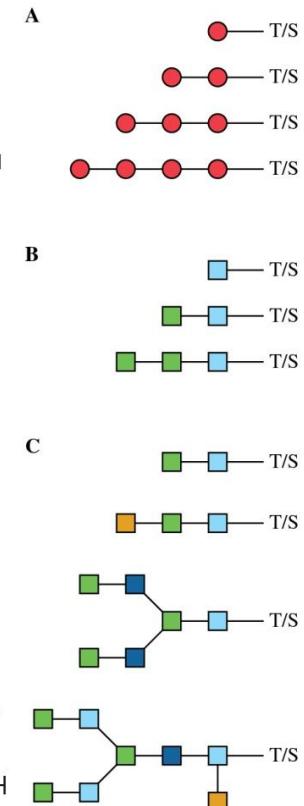
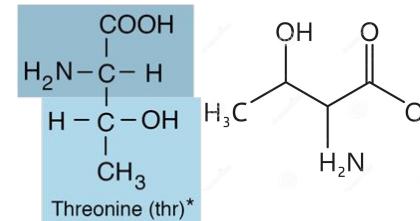
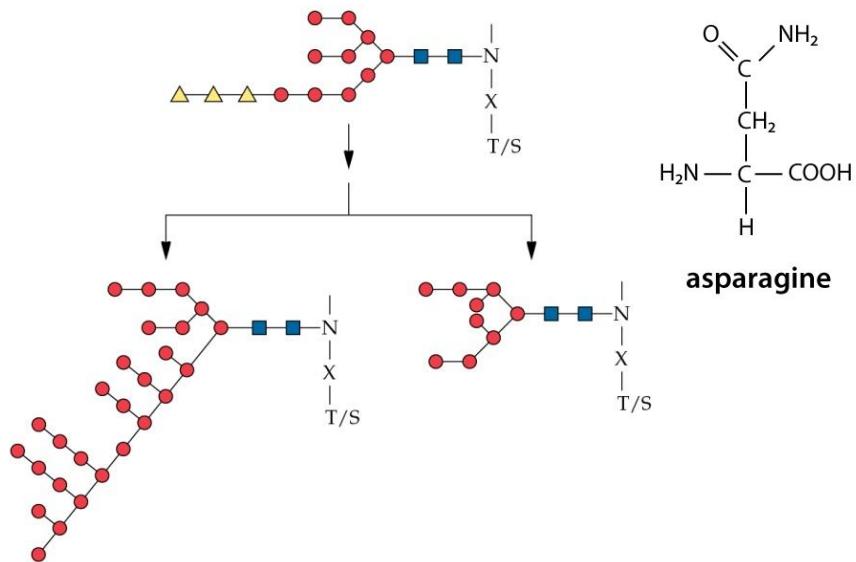


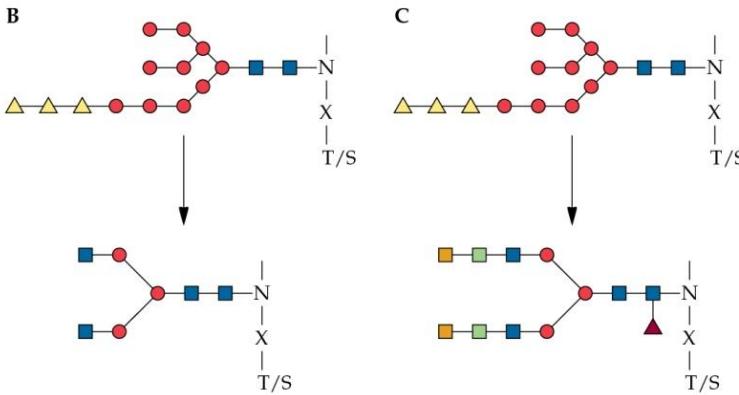
Figure 3.25 Examples of some O-linked oligosaccharides in yeasts (**A**), insects (**B**), and mammals (**C**). O-linked oligosaccharides have a number of arrangements with different combinations of sugars. Some of the more prevalent forms are shown here. S, serine; T, threonine; red circles, mannose; light blue squares, N-acetylgalactosamine; green squares, galactose; orange squares, sialic acid; dark blue squares, N-acetylglucosamine.



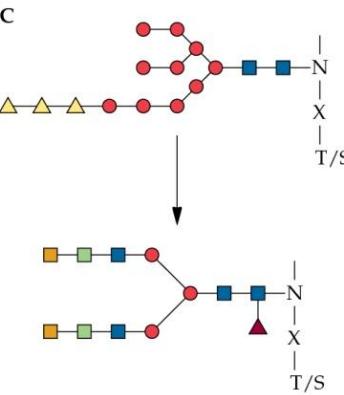
A



B



C



- **N-linked Glycosylation:** Sugars are attached to the amide group of **Asparagine (N)**, which is critical for protein stability and biological activity.

- **Therapeutic Importance:** Approximately 50% of all human proteins are glycosylated, including antibodies, blood factors, and hormones.

Figure 3.26 Examples of some N-linked oligosaccharides in yeasts (A), insects (B), and mammals (C). All N-linked glycosylations in eukaryotes start with the same initial group, which is subsequently trimmed and then elaborated in diverse ways within and among species. Some yeast sites have 15 or fewer mannose units (core series), and others have more (outer-chain family). In *S. cerevisiae*, the oligosaccharides frequently have 50 or more mannose units. An asparagine (N) next to any amino acid (X) followed by either threonine (T) or serine (S) can be targeted for glycosylation. Red circles, mannose; dark blue squares, *N*-acetylglucosamine; yellow triangles, glucose; green squares, galactose; orange squares, sialic acid; maroon triangle, fucose.

Eukaryotic Expression Vector Features

- **Regulatory Signals:** Vectors must include a eukaryotic promoter, polyadenylation signals, and transcription termination sequences to ensure efficient mRNA processing.
- **Selection Mechanisms:** A selectable marker gene specific for eukaryotic host cells is required to identify successfully transformed or transfected cells.
- **Shuttle Functionality:** These vectors act as "shuttles," *Lecture 1 cloning tools*, containing *E. coli* origins and markers for easy replication and manipulation in bacteria.

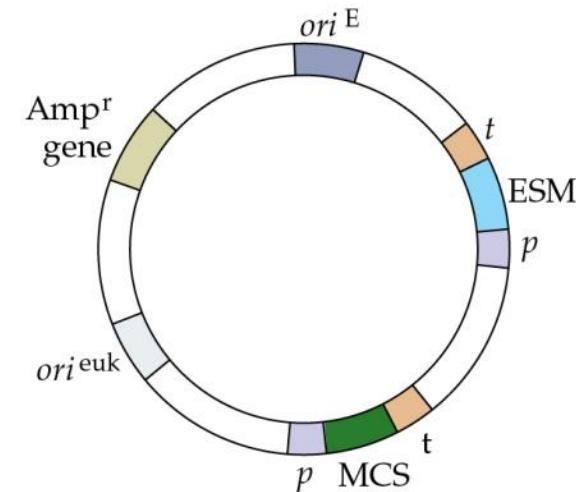
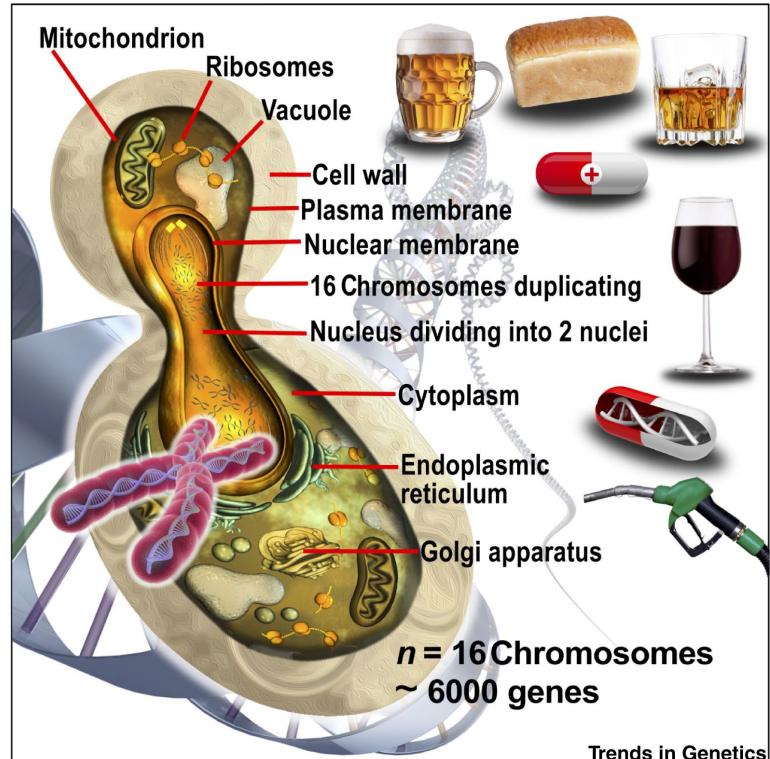


Figure 3.27 Generalized eukaryotic expression vector. The major features of a eukaryotic expression vector are a eukaryotic transcription unit with a promoter (*p*), a multiple cloning site (MCS) in which to insert a target gene, and a DNA segment with termination and polyadenylation signals (*t*). A eukaryotic origin of replication (*ori^{euk}*) enables replication of the vector in a eukaryotic host, and a eukaryotic selectable marker (ESM) gene enables selection of eukaryotic cells carrying the vector. An origin of replication that functions in *E. coli* (*ori^E*) and an *E. coli* selectable marker (Amp^r) gene are useful during the cloning of the heterologous gene.

Saccharomyces cerevisiae as a Model Host - Brewer's Yeast

- **Safety Status:** *S. cerevisiae* is "Generally Recognized as Safe" (GRAS) by the FDA, making it ideal for human drug production.
 - "Best studied" eukaryote.
 - Used for millennia in fermentation.
- **Characterized Genetics:** As the first eukaryote sequenced (1996), its biology is well understood, facilitating precise genetic engineering.
- **Secretion Focus:** Sc naturally secretes very few endogenous proteins, which significantly simplifies the downstream purification.
- **Supply Dominance:** A biotechnological workhorse, currently producing over 50% of the world's supply of recombinant insulin.



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Table 3.11 Recombinant proteins produced by *S. cerevisiae* expression systems^a

Vaccines
Hepatitis B virus surface antigen
Malaria circumsporozoite protein
HIV-1 envelope protein
Diagnostics
Hepatitis C virus protein
HIV-1 antigens
Human therapeutic agents
Epidermal growth factor
Insulin
Insulin-like growth factor
Platelet-derived growth factor
Proinsulin
Fibroblast growth factor
Granulocyte-macrophage colony-stimulating factor
α1-Antitrypsin
Blood coagulation factor XIIIa
Hirudin
Human growth factor
Human serum albumin

^aHIV-1, human immunodeficiency virus type 1.

Yeast Vectors: YEp, YIp, and YAC

- **Yeast episomal plasmid (YEp):** Based on high-copy 2- μm plasmids, these circular vectors replicate independently and are the most common tool for protein production.
- **Integrating Vectors (YIp):** These lack an origin of replication and must integrate into the host genome for stable maintenance.
- **Yeast Artificial Chromosomes (YAC):** As shown in Figure 3.29, YACs are linear vectors mimicking natural chromosomes, designed for carrying massive DNA segments (~100 kb).
- **Stability and Capacity:** While YEps prioritize high protein yield, and YIps are good for chromosomal integration, YACs provide unmatched stability for analyzing **large transcription units** and **creating genomic libraries**.

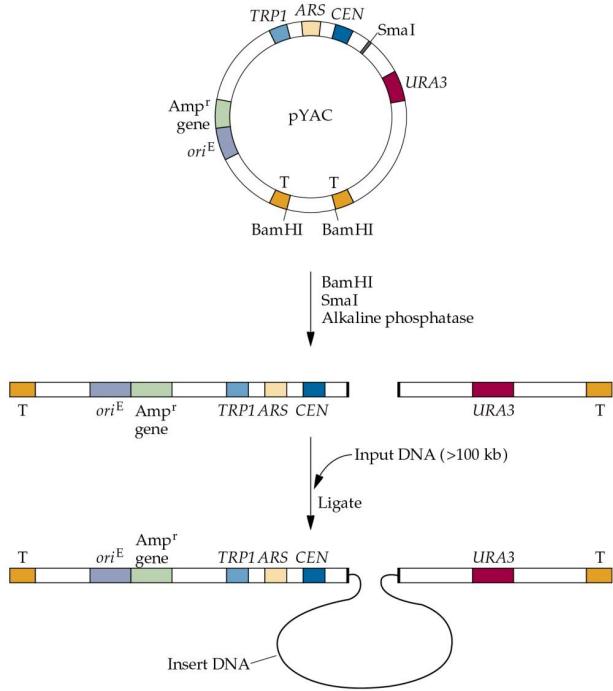


Figure 3.29 YAC cloning system. The YAC plasmid (pYAC) has an *E. coli* selectable marker (*Amp^r* gene), an origin of replication that functions in *E. coli* (*ori^E*), and yeast DNA sequences, including *URA3*, *CEN*, *TRP1*, and *ARS*. *CEN* provides centromere function, *ARS* is a yeast autonomous replicating sequence that is equivalent to a yeast origin of replication, *URA3* is a functional gene of the uracil biosynthesis pathway, and *TRP1* is a functional gene of the tryptophan biosynthesis pathway. The *T* regions are yeast chromosome telomeric sequences. The *SmaI* site is the cloning insertion site. pYAC is first treated with *SmaI*, *BamHI*, and alkaline phosphatase and then ligated with size-fractionated (100-kb) input DNA. The final construct carries cloned DNA and can be stably maintained in double-mutant *ura3* and *trp1* cells.

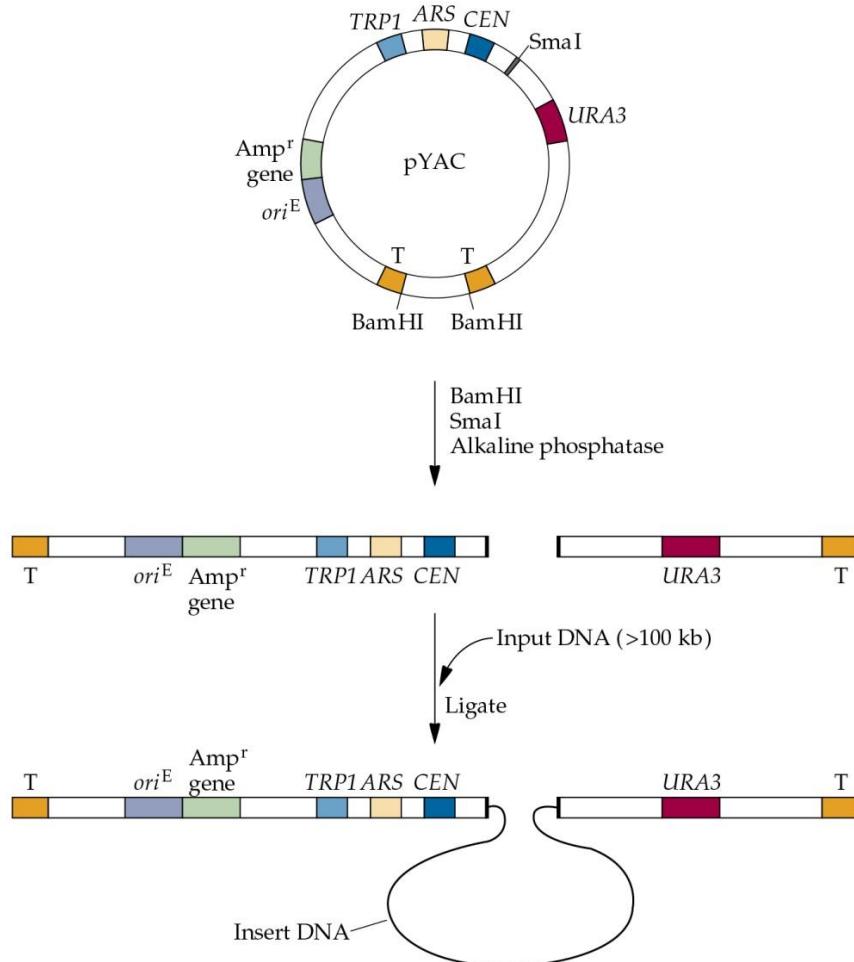


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Directing Protein Secretion

- **Signal Peptides:** Recombinant proteins are fused to a signal sequence, typically from the yeast mating factor α gene.
- **Processing Pathway:** The signal directs the protein to the endoplasmic reticulum and Golgi for export and purification.
- **Purification Ease:** Secreted proteins are much easier to purify from the medium than from a complex cell lysate.

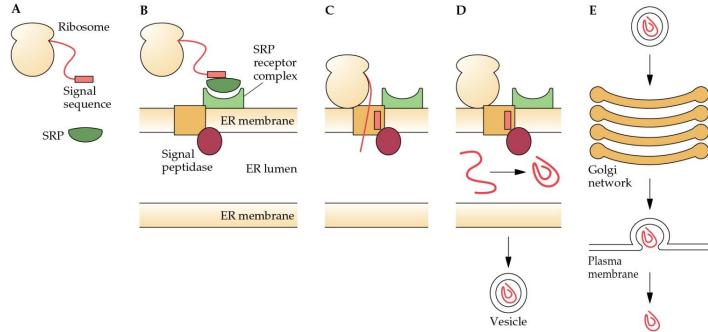


Figure 3.30 Protein secretion pathway in eukaryotes. **(A)** A signal recognition particle (SRP) binds to the signal sequence of a secretory protein. **(B)** The SRP attaches to an SRP receptor on the endoplasmic reticulum (ER) membrane. **(C)** The secretory protein is translocated into the lumen of the ER, and a signal peptidase removes the signal sequence. **(D)** The secretory protein is folded, partially modified, and packaged in a transport vesicle intended for the Golgi network. **(E)** The ER-released vesicle carrying the secretory protein enters the Golgi network at the *cis* face and passes through the Golgi stack, where it is further modified. After it is sorted, a plasma membrane-specific vesicle is formed at the *trans* face of the Golgi network. The secretory transport vesicle fuses with the plasma membrane and releases the secretory protein to the extracellular environment.

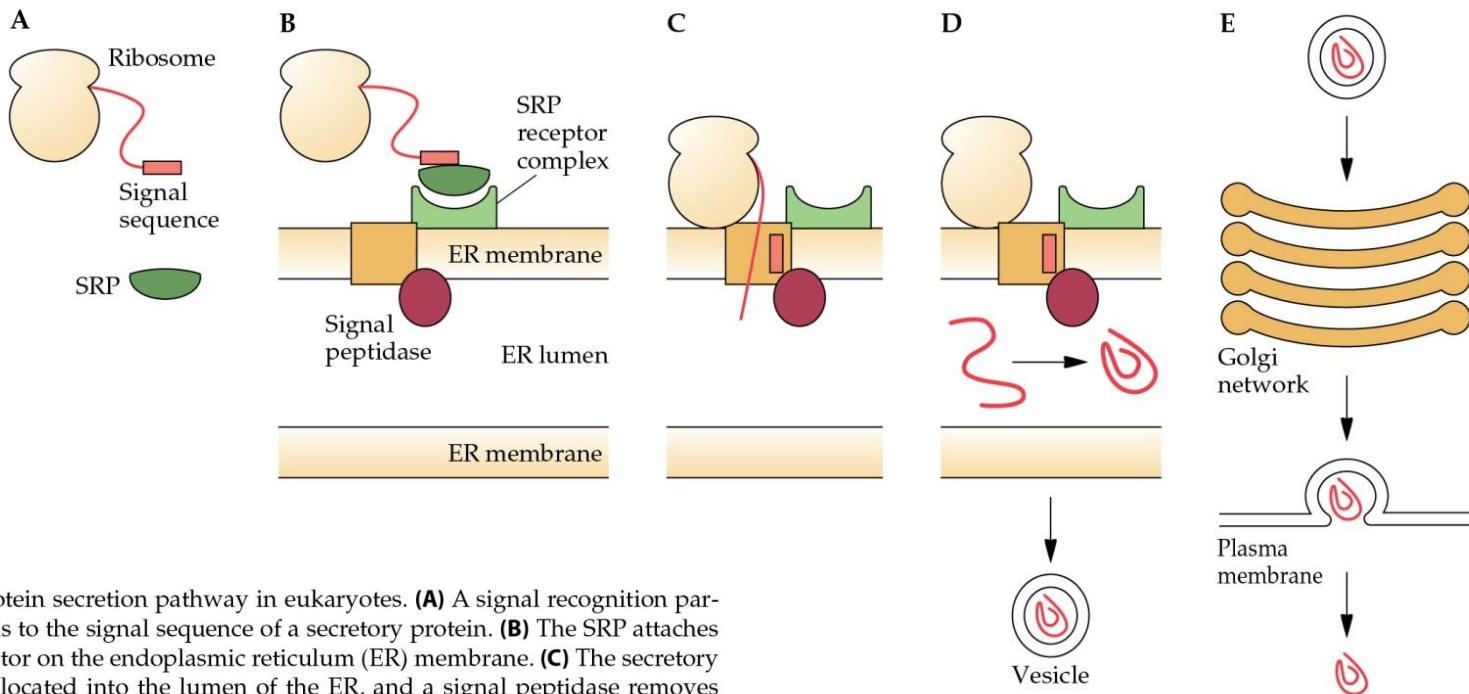


Figure 3.30 Protein secretion pathway in eukaryotes. **(A)** A signal recognition particle (SRP) binds to the signal sequence of a secretory protein. **(B)** The SRP attaches to an SRP receptor on the endoplasmic reticulum (ER) membrane. **(C)** The secretory protein is translocated into the lumen of the ER, and a signal peptidase removes the signal sequence. **(D)** The secretory protein is folded, partially modified, and packaged in a transport vesicle intended for the Golgi network. **(E)** The ER-released vesicle carrying the secretory protein enters the Golgi network at the *cis* face and passes through the Golgi stack, where it is further modified. After it is sorted, a plasma membrane-specific vesicle is formed at the *trans* face of the Golgi network. The secretory transport vesicle fuses with the plasma membrane and releases the secretory protein to the extracellular environment.

Optimizing Yeast Protein Folding

- **Molecular Chaperones:** Overexpressing chaperones like BiP helps prevent the formation of insoluble protein aggregates, similar to the inclusion bodies discussed in *Lecture 5*.
- **Enzymatic Aid:** Overproducing Protein Disulfide Isomerase (PDI) significantly increases the yield of active proteins that require complex internal covalent bonds.
- **Upregulating UPR:** Engineering the "Unfolded-Protein Response" via the Hac1 transcription factor increases the cell's overall capacity to fold and process overexpressed proteins.

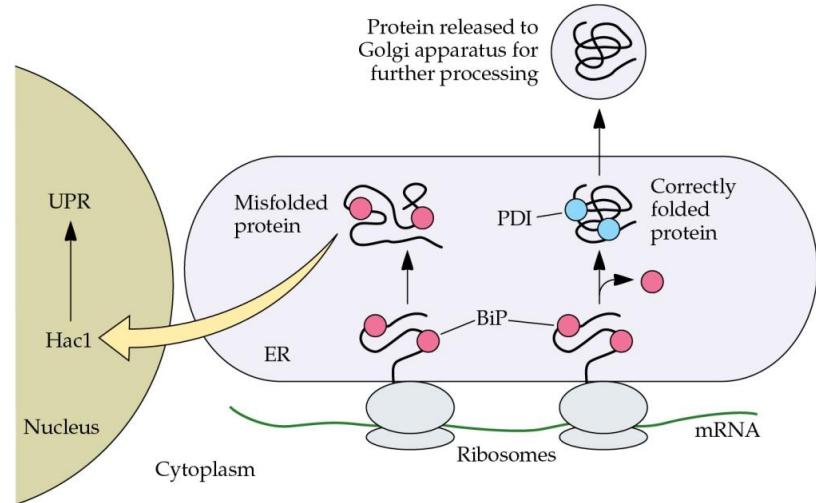


Figure 3.31 Summary of protein folding in the endoplasmic reticulum of yeast cells. During synthesis on ribosomes associated with the endoplasmic reticulum (ER), nascent proteins are bound by the chaperones BiP and calnexin, which aid in the correct folding of the protein. Protein disulfide isomerases (PDI) catalyze the formation of disulfide bonds between cysteine amino acids that are nearby in the folded protein. Quality control systems ensure that only correctly folded proteins are released from the ER. Proteins released from the ER are transported to the Golgi apparatus for further processing. Prolonged binding of BiP to misfolded proteins leads to activation of the *S. cerevisiae* transcription factor Hac1, which controls the expression of several proteins that mediate the unfolded-protein response (UPR). Data from Gasser et al., *Microb. Cell Fact.* 7:11–29, 2008.

Video - Concepts in the module or a demonstration

- Post-Translational Modifications (7min01s)

In class: watch 1min47s

More videos

- Protein Expression Vectors (9min59s)
 - applications, expression hosts, etc

Module 2: Advanced Fungal Systems & Mammalian Cells

(Source Pages: Chapter 3: 132–137; 145–155)

- Engineering 'humanized' yeast glycosylation.**
- Advantages of mammalian cell culture.**
- Enhancing high-level stable expression.**

Advantages of *Pichia pastoris*

- **Metabolic Flexibility:** *P. pastoris* is a methylotrophic yeast capable of utilizing methanol as its sole carbon and energy source.
- **High-Density Scaling:** Unlike *S. cerevisiae*, it does not produce toxic ethanol at high cell densities, allowing for massive biomass accumulation.
- **Superior Secretion:** This system is capable of secreting extremely high levels of recombinant proteins directly into the culture medium.



The yeast formerly known as ***Pichia pastoris*** has been reclassified, with the common industrial strains now officially named ***Komagataella phaffii***.

Why the Change: Genetic analysis revealed that the strains used in labs (like GS115, X-33) belonged to a different species than the original *Pichia pastoris*.

The Hyperglycosylation Problem

- **Excessive Mannose:** Native yeast often add 50–150 mannose units to N-linked oligosaccharide side chains, which can interfere with protein function.
- **Immunogenicity Risks:** The specific α -1,3 linkages produced by yeast can be highly antigenic or allergenic to human patients.
- **Clearance Rates:** Non-human sugar patterns often cause the human body to clear the drug from the blood too quickly.

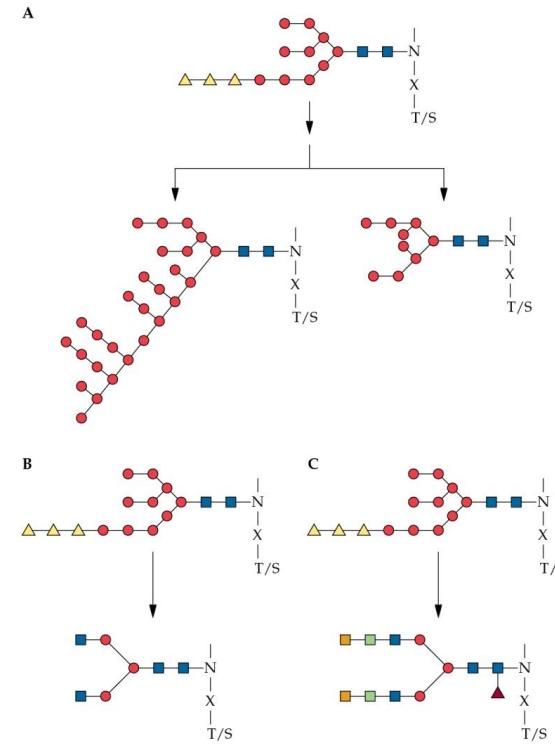


Figure 3.26 Examples of some N-linked oligosaccharides in yeasts (A), insects (B), and mammals (C). All N-linked glycosylations in eukaryotes start with the same initial group, which is subsequently trimmed and then elaborated in diverse ways within and among species. Some yeast sites have 15 or fewer mannose units (core series), and others have more (outer-chain family). In *S. cerevisiae*, the oligosaccharides frequently have 50 or more mannose units. An asparagine (N) next to any amino acid (X) followed by either threonine (T) or serine (S) can be targeted for glycosylation. Red circles, mannose; dark blue squares, N-acetylglucosamine; yellow triangles, glucose; green squares, galactose; orange squares, sialic acid; maroon triangle, fucose.

"Humanizing" *Pichia pastoris*

- **Hyperglycosylation Issue:** Native *S. cerevisiae* often adds too much **mannose**, which can be antigenic in human patients.
- **Pathway Engineering:** Eliminating yeast-specific enzymes and adding mammalian glycosyltransferases ensures human-like sugar patterns.
- **Clinical Success:** Used to produce properly sialylated monoclonal antibodies like Herceptin for cancer treatment.

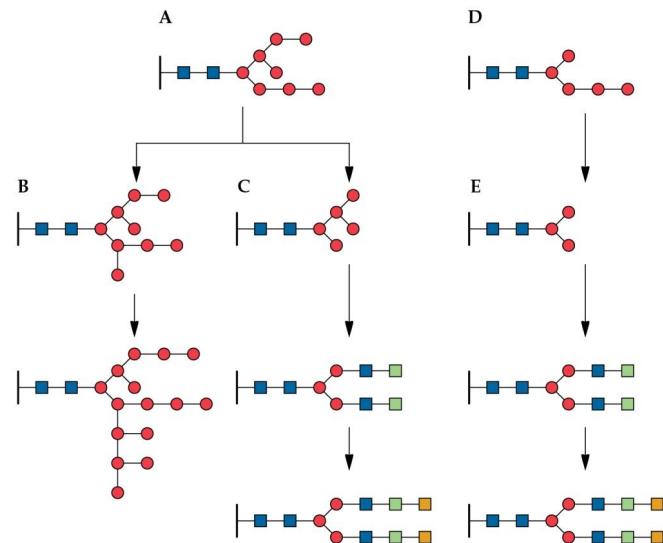


Figure 3.33 Differential processing of glycoproteins in *P. pastoris*, humans, and "humanized" *P. pastoris*. Initial additions of sugar residues to glycoproteins in the endoplasmic reticulum are similar in human and *P. pastoris* cells (**A**). However, further N-glycosylation in the Golgi apparatus differs significantly between the two cell types. N-glycans are hypermannosylated in *P. pastoris* (**B**), while in humans, mannose residues are trimmed and specific sugars are added, leading to termination of the oligosaccharide in sialic acid (**C**). *P. pastoris* cells have been engineered to produce enzymes that process glycoproteins in a manner similar to that of human cells. In "humanized" *P. pastoris*, a recombinant glycoprotein produced in the endoplasmic reticulum (**D**) is transported to the Golgi apparatus, where it is further processed to yield a properly sialylated glycoprotein (**E**). Blue squares, *N*-acetylglucosamine; red circles, mannose; green squares, galactose; orange squares, sialic acid. Data from Hamilton and Gerngross, *Curr. Opin. Biotechnol.* 18:387–392, 2007.

The AOX1 Promoter System

- **Methanol Induction:** The Alcohol Oxidase 1 (AOX1) promoter is tightly regulated and only becomes active in the presence of methanol.
- **Transcriptional Strength:** Upon induction, the AOX1 protein can represent as much as 30% of the total cellular protein in the host. So, our target gene / protein can also be expressed at a high level.
- **Phased Growth:** This system allows manufacturers to separate the biomass growth phase from the protein induction phase for maximum yield.

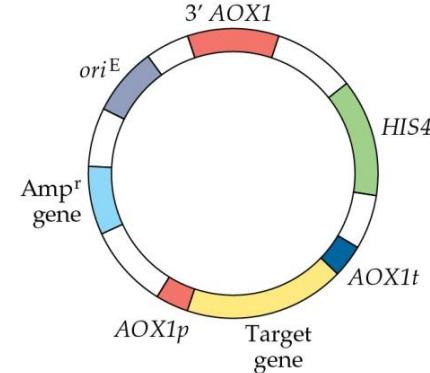


Figure 3.34 *P. pastoris* integrating expression vector. The target gene is cloned between the promoter (*AOX1p*) and termination-polyadenylation sequence (*AOX1t*) of the *P. pastoris* alcohol oxidase 1 gene. The *HIS4* gene encodes a functional histidinol dehydrogenase of the histidine biosynthesis pathway. The ampicillin resistance (*Amp^r*) gene and an origin of replication (*ori^E*) function in *E. coli*. The segment marked 3' *AOX1* is a piece of DNA from the 3' end of the alcohol oxidase 1 gene of *P. pastoris*. A double recombination event between the *AOX1p* and 3' *AOX1* regions of the vector and the homologous segments of chromosome DNA results in the integration of the DNA carrying the target gene and the *HIS4* gene into a *P. pastoris* chromosome (Fig. 3.35B).

Wide-Range Fungal Vectors

- **Modular Construction:** These vector systems use interchangeable modules for promoters, origins, and selection markers to suit different fungal hosts.
- **Species Flexibility:** Researchers can rapidly construct customized vectors for *S. cerevisiae*, *P. pastoris*, or *H. polymorpha* using modular kits.
- **Host Screening:** This allows for "trial and error" screening to determine which specific fungus produces the highest active protein levels.

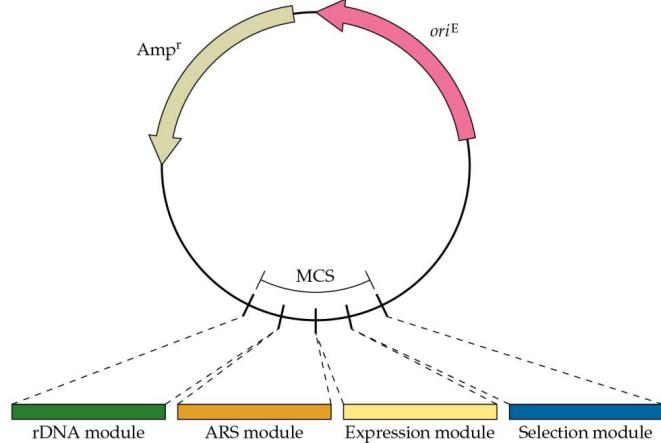


Figure 3.36 A wide-range yeast vector system for expression of heterologous genes in several different yeast hosts. The basic vector contains a multiple cloning site (MCS) for insertion of selected modules containing appropriate sequences for chromosomal integration (rDNA module), replication (ARS module), selection (selection module), and expression (expression module) of a target gene in a variety of yeast host cells (Table 3.13 shows examples of interchangeable modules). Sequences for maintenance (*ori^E*) and selection (*Amp^r*) of the vector in *E. coli* are also included.

Filamentous Fungi Bioreactors - Complex Morphology

- **Hyphal Growth:** Multicellular fungi grow as branching hyphae, providing a massive surface area for large-scale protein secretion.
- **Industrial Workhorses:** Species like *Aspergillus niger* are used extensively in the food and paper industries to produce commercial enzymes.
- **Secretion Masters:** These fungi can be engineered to add mammalian-like sugars, offering a potential alternative to "humanized" yeast.

Table 3.14 Some recombinant proteins produced by filamentous fungal expression systems

Recombinant protein	Host cell	Main application
α -Amylase	<i>Aspergillus niger</i> , <i>Aspergillus oryzae</i>	Starch processing, food industry
Aspartyl protease	<i>Aspergillus nidulans</i> , <i>A. oryzae</i>	Food industry
Cellulase	<i>Trichoderma reesei</i>	Textile, pulp and paper industries
Chymosin	<i>A. niger</i>	Food industry
Immunoglobulin G	<i>A. niger</i>	Pharmaceutical industry
Insulin	<i>A. niger</i>	Pharmaceutical industry
Interleukin-6	<i>A. niger</i>	Pharmaceutical industry
Laccase	<i>A. niger</i> , <i>T. reesei</i>	Textile, pulp and paper industries
Manganese peroxidase	<i>A. niger</i>	Chemical industry
Lactoferrin	<i>A. oryzae</i>	Pharmaceutical industry
Lipase, thermophilic	<i>A. oryzae</i>	Detergent
Lysozyme	<i>A. niger</i>	Pharmaceutical industry
Phytase	<i>T. reesei</i>	Food industry
Xylanase	<i>A. niger</i> , <i>T. reesei</i>	Textile, pulp and paper, food industries

Why Choose Mammalian Hosts?

- **Product Quality:** Between 2014 and 2018, 84% of approved recombinant human therapeutics were produced in mammalian cells.
- **Complex PTMs:** Essential for antibodies requiring accurate post-translational modifications that yeast and bacteria cannot perfectly replicate.
- **CHO Cells:** Chinese Hamster Ovary cells are the most common because they produce human-like glycans and grow well in high-density suspension.

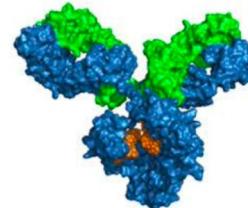
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Protein folding accuracy	Low	Medium	High	High
Protein yield	High	Medium-high	Low-medium	Medium
Scale-up capacity	High	High	Low	Very high
Scale-up costs	High	High	High	Low
Time required	Low	Low-medium	Medium-high	High
Skilled workers required	Medium	Medium	High	Low
Acceptable to regulators	Yes	Yes	Yes	Not yet

Why Choose Mammalian Hosts?

1	Keytruda	\$29.5 B	
2	Ozempic	\$17.5 B	
3	Dupixent	\$14.1 B	
4	Biktarvy	\$13.4 B	
5	Eliquis	\$13.3 B	
6	Skyrizi	\$11.7 B	
7	Darzalex	\$11.7 B	
8	Mounjaro	\$11.5 B	
9	Stelara	\$10.4 B	
10	Trikafta	\$10.2 B	

Top Selling Drugs in 2024



5/10 of top drugs are glycoproteins, produced in CHO cells

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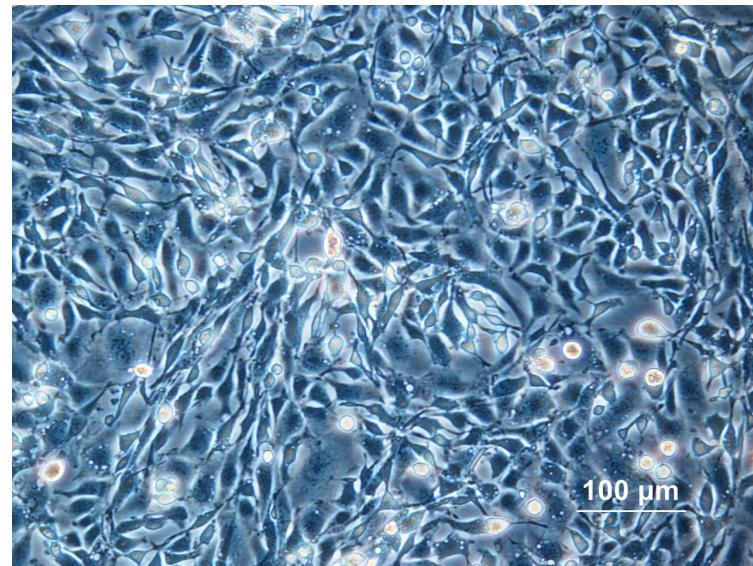
Emerging demands for:

- ADCs, multi-specific mAbs
- Antiviral mAbs
- Virus for gene therapy
- Antivenom
- Cytokines for cell therapies

* USD

Culturing Mammalian Cells vs. Microbes

- **Slower Growth Rates:** Mammalian cells divide much slower than microbes; whereas bacteria double in minutes, animal cells take many hours or days.
- **Complex Nutrient Requirements:** Cultures require expensive, specialized media often containing growth factors, unlike the simple, inexpensive media used for microbes.
- **Physical Fragility:** Lacking a rigid cell wall, mammalian cells are shear-sensitive and achieve lower cell densities in bioreactors compared to bacteria.



<https://www.genengnews.com/topics/bioprocessing/fresh-hotspots-idd-for-more-productive-cho-cell-lines/>

Mammalian Vector Components

- **Viral Elements:** Use strong constitutive promoters and enhancers from human viruses like SV40 or Cytomegalovirus (CMV).
- **mRNA Stability:** Include polyadenylation signals at the 3' end to ensure efficient processing and long-term mRNA stability.
- **Shuttle Capability:** Retain a bacterial origin of replication and selectable markers for easy cloning and manipulation in *E. coli*.

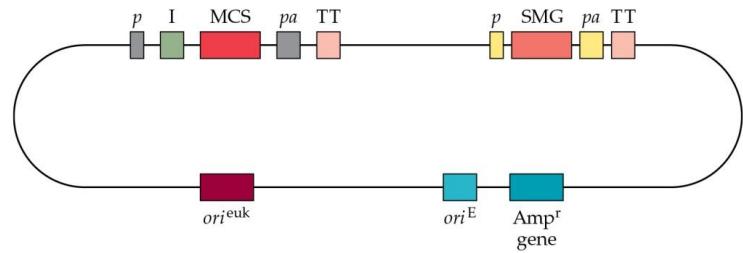


Figure 3.43 Generalized mammalian expression vector. Target genes inserted into the multiple cloning site (MCS) and selectable marker gene (SMG) are under the control of eukaryotic promoter (*p*), polyadenylation (*pa*), and transcription termination (TT) sequences. An intron (I) enhances the production of recombinant protein. Propagation of the vector in *E. coli* and mammalian cells depends on the origins of replication *ori*^E and *ori*^{euk}, respectively. The ampicillin resistance (Amp^r) gene is used for selecting transformed *E. coli*.

Translational Control Sequences

- **Start Site Recognition:** The Kozak sequence (GCCGCC(A/G)CCAUGG) is essential for the ribosome to correctly identify the translation start codon.
- **Secretion Guidance:** N-terminal signal sequences direct the newly synthesized protein into the secretory pathway for extracellular release.
- **Efficiency Elements:** Utilize 5' and 3' untranslated regions (UTRs) to enhance the overall translation rate and protect mRNA from degradation.



Figure 3.45 Translation control elements. A target gene can be fitted with various sequences that enhance translation and facilitate both secretion and purification, such as a Kozak sequence (K), signal sequence (S), protein affinity tag (T), proteolytic cleavage site (P), and stop codon (SC). The 5' and 3' UTRs increase the efficiency of translation and contribute to mRNA stability.

Multimeric Protein Assembly

- **Stoichiometry Control:** Use bicistronic vectors containing viral-derived **Internal Ribosomal Entry Sites (IRES)** to express two subunits from one mRNA.
- **Simultaneous Expression:** This allows for the balanced production of both light and heavy chains required for complex antibodies.

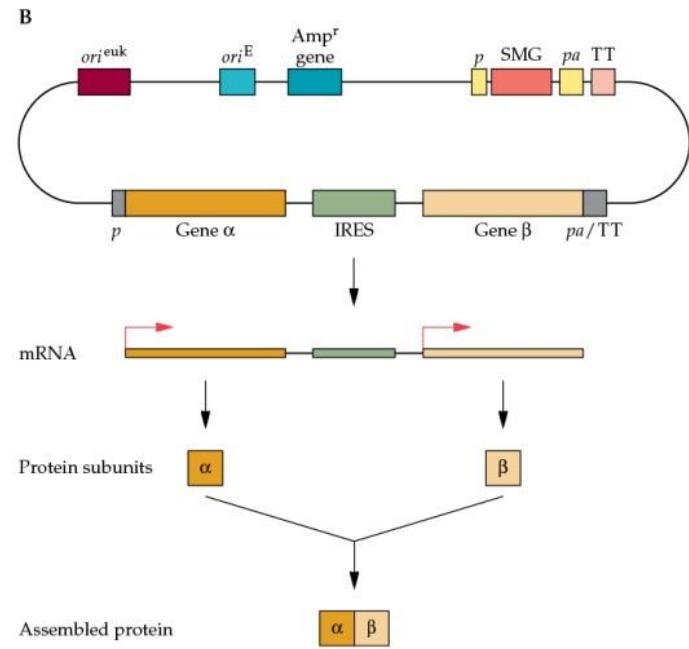


Figure 3.46 Expression vectors for production of heterodimeric or -tetrameric proteins in mammalian cells. **(A)** Two-gene expression vector. The target genes (gene α and gene β) encode subunits of a protein dimer ($\alpha\beta$). The target genes are inserted into a vector and are under the control of different eukaryotic promoter (p), polyadenylation (pa), and transcription termination (TT) sequences. Each sub-unit is translated from a separate mRNA, and a functional protein dimer ($\alpha\beta$) is assembled. **(B)** Bicistronic expression vector. Each target gene (gene α and gene β) is inserted into a vector on either side of a sequence for an IRES. The

two genes and the IRES sequence form a transcription unit under the control of a single eukaryotic promoter. Translation of the mRNA occurs from the 5' end and internally (right-angled arrows). Both subunits (α and β) are synthesized and assembled into a functional protein dimer ($\alpha\beta$). Both expression vectors carry origins of replication for *E. coli* (ori^E) and mammalian cells (ori^{euk}), a selectable marker (Amp^r) for selecting transformed *E. coli*, and a selectable marker gene (SMG) that is under the control of eukaryotic promoter (p), polyadenylation (pa), and transcription termination (TT) sequences.

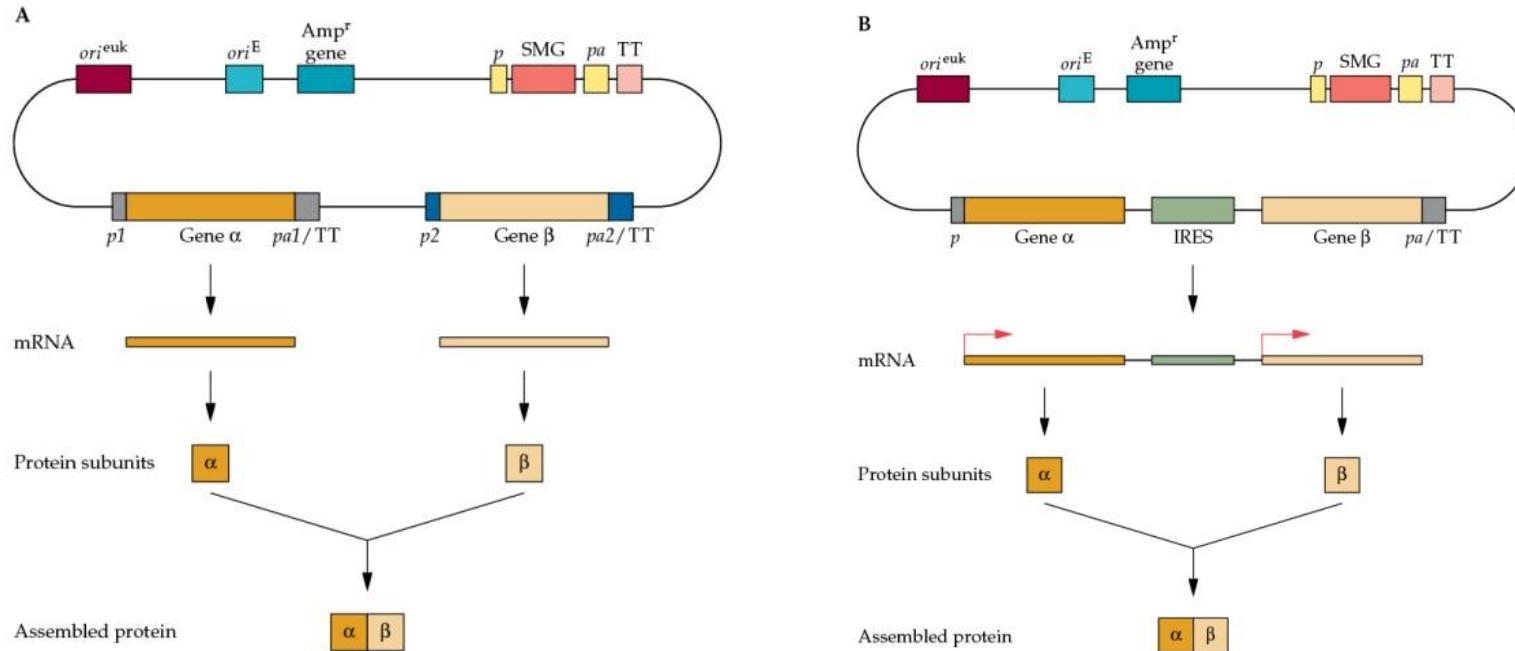


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Increasing Host Cell Viability

- **Apoptosis Inhibition:** Overexpress the MDM2 protein to inhibit p53-mediated cell death caused by high-density bioreactor stress.
- **Stress Defense:** Delaying natural cell death allows the culture to remain productive for a significantly longer duration.
- **Yield Enhancement:** Maintaining high cell viability directly results in higher final concentrations of the target therapeutic protein.

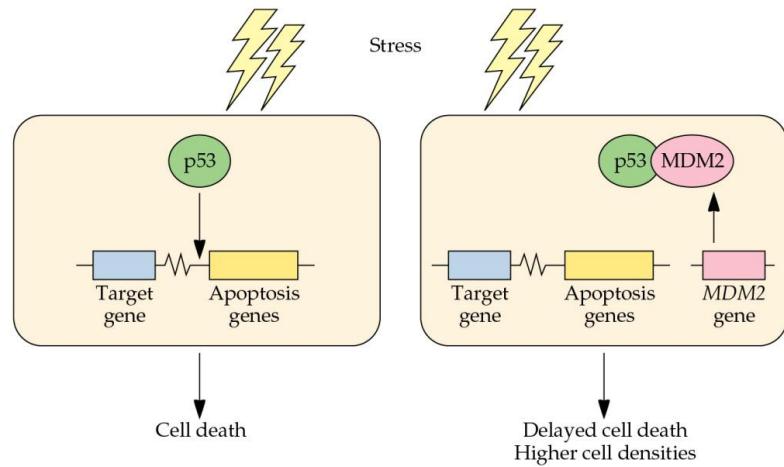


Figure 3.47 Strategy to increase yields of recombinant mammalian cells. Cell death (apoptosis), stimulated by the transcription factor p53, can lead to decreased yields of recombinant mammalian cells grown under stressful conditions in large bioreactors. To prevent cell death, the gene encoding MDM2 is introduced into mammalian cells. The MDM2 protein binds to p53 and prevents it from inducing expression of proteins required for apoptosis. Engineered cells not only showed delayed cell death, but also achieved higher cell densities in bioreactors.

Chromatin and Epigenetic Control Strategies

- **Positional Effects:** The site where a gene integrates into a chromosome can 'silence' its expression if the DNA is too tightly packed
- **Silencing Prevention:** Use Stabilizing and Antirepressor (STAR) elements to prevent the formation of heterochromatin around the transgene site.
- **Epigenetic Relaxation:** Recruit Histone Acetyltransferases (HATs) to chemically relax the DNA structure, making it more accessible to RNA polymerase.

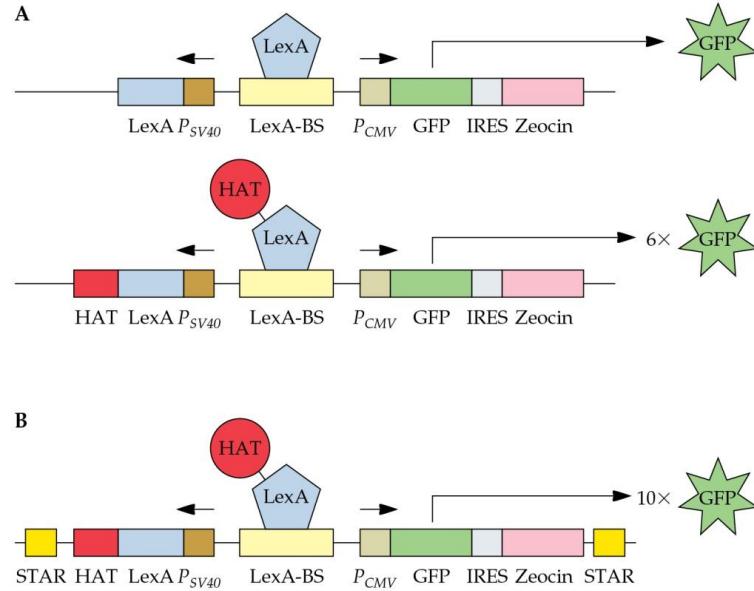


Figure 3.50 Strategies to increase expression of recombinant proteins in mammalian cells by altering chromatin structure. Local "relaxation" of chromosome condensation, which leads to increased transcription of genes in the region, can be achieved by the addition of an acetyl group to DNA-packaging proteins known as histones. Histone acetylation is catalyzed by the enzyme histone acetyltransferase (HAT). **(A)** To increase the expression of a recombinant protein, HAT was directed to the site of target gene (GFP gene) insertion in a mammalian chromosome. HAT was expressed as a fusion protein with the LexA protein that binds to a specific DNA sequence (LexA-BS) inserted upstream of the CMV promoter (P_{CMV}) that directs expression of GFP. Production of the HAT-LexA fusion protein under the control of the SV40 promoter (P_{SV40}) increased expression of GFP 6-fold compared to production of the LexA protein alone. **(B)** Insertion of STAR elements on both sides of the expression cassette further increased GFP expression. The gene encoding resistance to the antibiotic Zeocin was included as a selectable marker and was expressed from an IRES. The arrows above the promoter boxes indicate the direction of transcription.

Video - Concepts in the module or a demonstration

- Epigenetics & Chromatin (8min41s)
in class: 3:05–4:10

More videos

- CHO cells - Chinese hamster ovary cells used in molecular biology and pharmaceutical biotechnology
- Next Generation CHO Transient Expression
- Genentech - here in Oceanside and makes antibodies in a beautiful facility

The End