Manipulating genes to improve protein expression

BIOL 426/626
Approaches to Molecular Biology

Class 12: Manipulating genes to improve protein expression

- Learning Goal
  - To understand the various methods for expressing novel gene products or to control the expression of normal proteins including methods to mutate existing genes or construct genes *de novo*.
- Learning Objectives
  - Explain the use of specific expression systems used in bacteria, yeast and higher eukaryotic cells
  - Compare methods for site-directed mutagenesis and explain which method would be best suited for a variety of situations
  - Explain the process of gene assembly and analyze some of the reasons for applying this technique rather than using in vitro mutagenesis
- Reading assignment:
  - Dale From Genes to Genomes: Chapter 7

What is the purpose of engineering genes?

- For biotechnology…

Livestock feed

$150 billion industry

Vaccines & antibodies

Pharmaceuticals

Genetic engineering companies…

Abbott A Promise for Life
Bristol-Myers Squibb
Genentech
Johnson & Johnson
OPTIMER PHARMACEUTICALS, INC.
ALLERGAN
Celgene
genzyme
MedImmune
Otsuka
AMGEN
Centocor Ortho Biotech Inc.
GILEAD
MERCK
P&G
astellas
Daiichi-Sankyo
gsk GlaxoSmithKline
Takeda MILLENNIUM THE TAKEDA ONCOLOGY COMPANY
Roche
Bayer
DAINIPPON SUMITOMO PHARMA
ImClone Systems Incorporated
NOVARTIS
SANOFI
Bayer HealthCare
Eisai
INGENIX.
SEPRACOR
Boehringer Ingelheim
ENDO PHARMACEUTICALS
Janssen.
novo nordisk
UPSHER-SMITH

campbellalliance.com

What purposes can you imagine for gene engineering?

Aims of gene engineering?

- **Improve protein production**
- Increase enzyme specific activity
- Modify cofactor requirement
- Simplify protein purification
- Stabilize protein to degradation
- Alter protein cellular localization
- Modify enzyme substrate specificity
- Analyze the structural basis for protein function

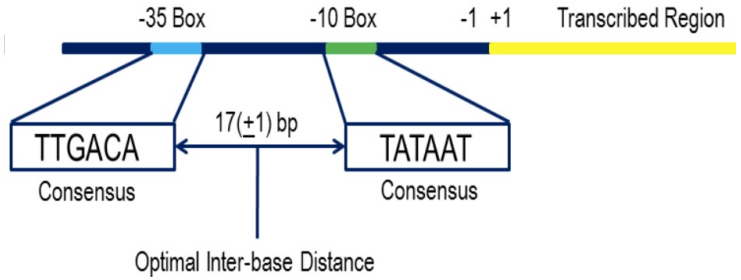
How might protein production be increased?

- Discuss…

Promoter strength?

- The simplest method to increase protein production
- *Increase* the rate of transcript initiation

Typical bacterial promoter



oxfordgenetics.com

Promoter strength?

- The simplest method to increase protein production
- *Increase* the rate of transcript initiation

Simple eukaryotic promoter

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Promoter strength?

- The simplest method to increase protein production
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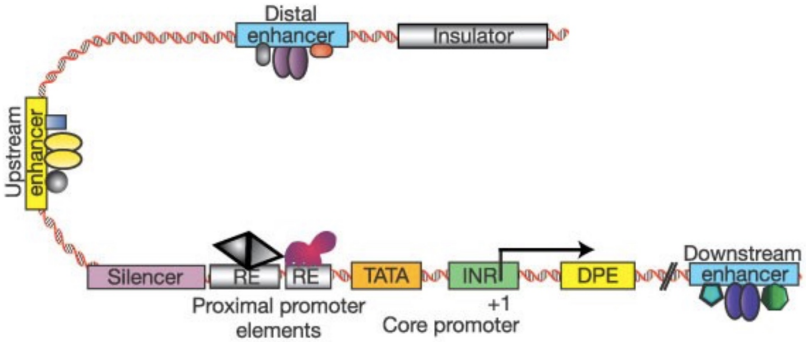
Complex eukaryotic promoter



oxfordgenetics.com

Increase promoter basal activity

- Most studies focus on simple promoters
  - Many of the most active viral promoters are quite simple
  - Complexity allows sensitive regulation of activity (positive & negative) but not necessarily maximal activity
- Alter promoter sequence elements
  - Increase similarity to consensus
  - Alter spacing
  - Substitute consensus sequences (alternative promoters)
- Substitute entire promoters
  - Use promoters recognized by viral RNA polymerase
    - More frequent initiation
    - Faster elongation rates

Increase activated promoter activity

- Introduce binding sites for transcriptional activation proteins
  - Viral activators
    - E.g. Herpes simplex virus VP16
    - Very strong transcription activation activity
    - VP16 cannot bind DNA—normally binds to promoter-bound accessory protein
    - Normally, VP16 is fused to a site-specific DNA binding protein
- Use activators requiring a small molecule cofactor
  - E.g., yeast (*Saccharomyces cerevisiae*) Gal4 protein
    - Requires presence of galactose sugar
    - Does recognize DNA
- To localize activators to the promoter its binding site must be introduced upstream of the promoter

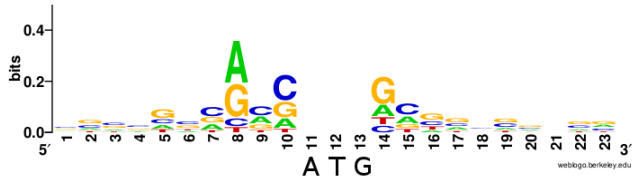
Altering protein synthesis?

- Initiation sequences

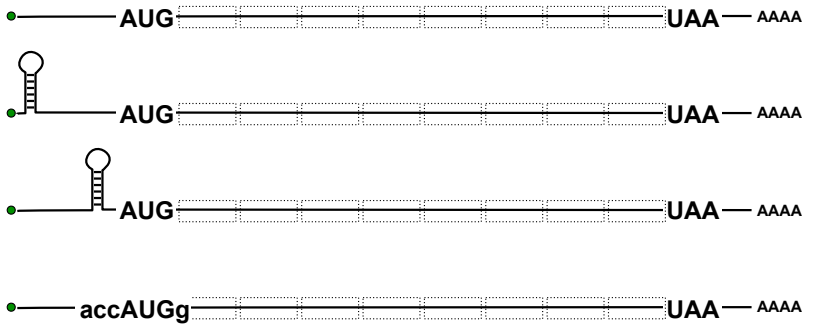
Bacterial ribosome binding site

http://www.brainkart.com

Eukaryotic “Kozak” consensus site



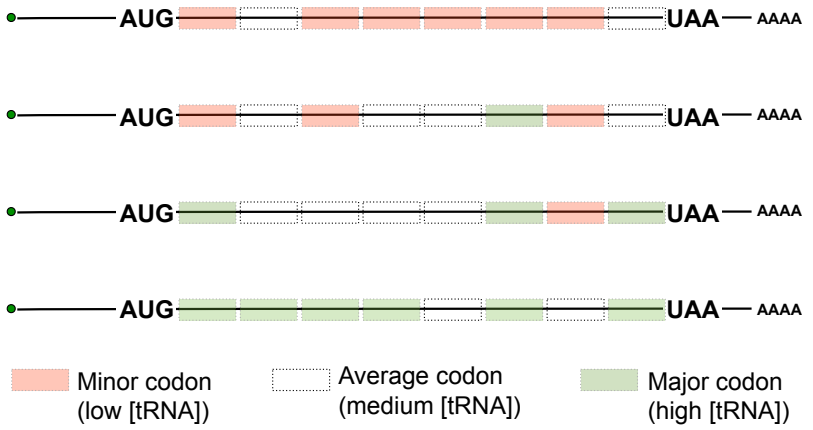
Post-transcriptional effects on protein expression



Codon usage?

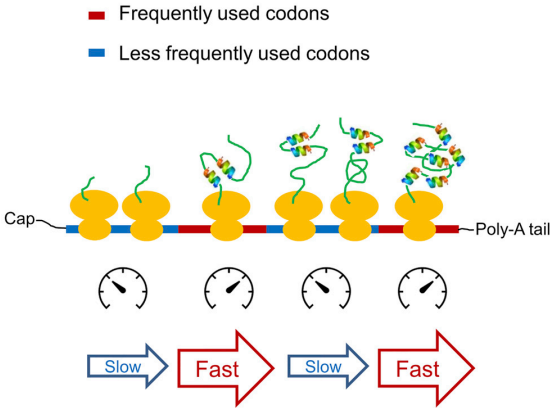
- Protein expression can be affected by choice of codons

Post-transcriptional effects on protein expression



Effects of altering codon usage: misfolding

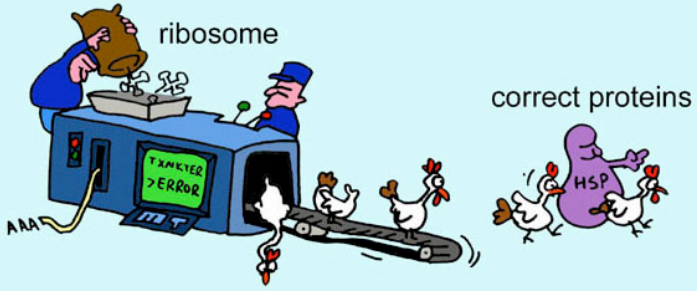
- Poorly translated codons can promote pausing to allow nascent protein folding



Effects of altering codon usage: misreading

- Use of poorly recognized codons can result in misreading

Protein synthesis uses most cellular energy

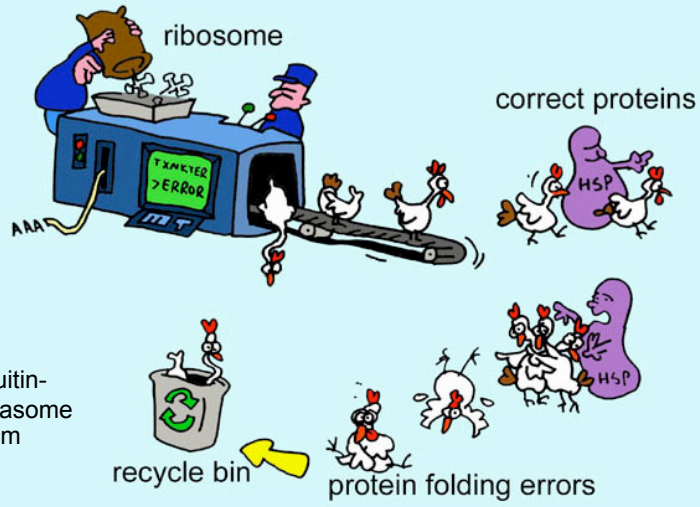


Uses ~1/2 of cellular energy

$t_{1/2}$ ≈ many hours

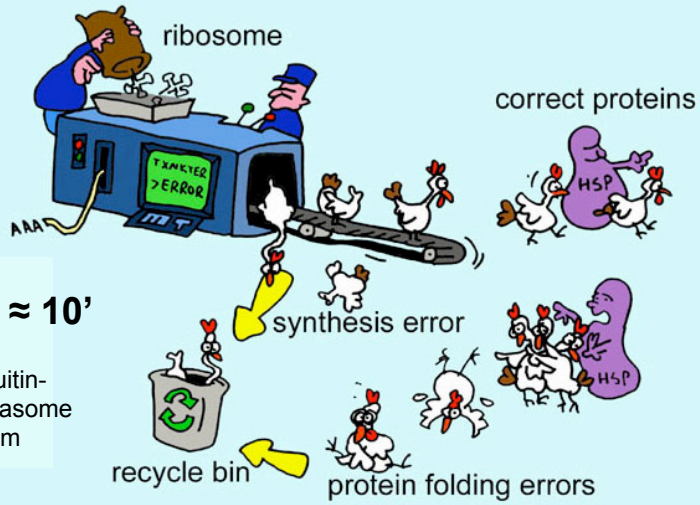
Yewdell et al. *Nature Rev Immunol* 3:952 2003

Misfolded proteins are destroyed



Yewdell et al. *Nature Rev Immunol* 3:952 2003

Proteins synthesized in error are also destroyed

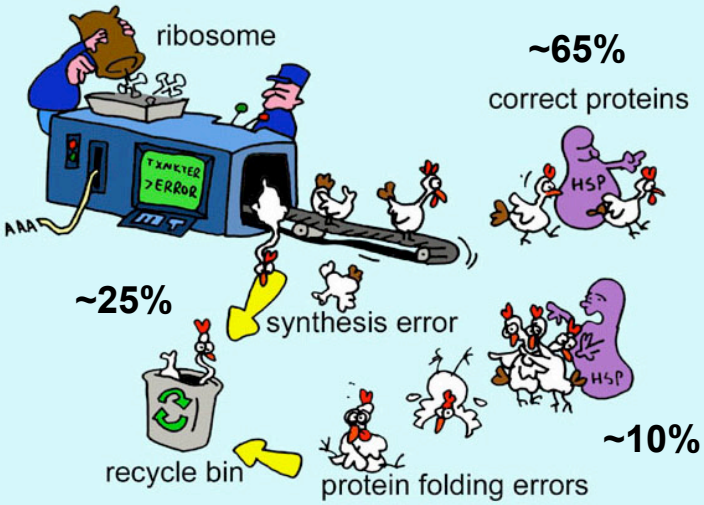


$t_{1/2} \approx 10'$

Ubiquitin-proteasome system

Yewdell et al. *Nature Rev Immunol* 3:952 2003

Proteins synthesized in error are also destroyed



Yewdell et al. *Nature Rev Immunol* 3:952 2003

Poisson distribution of errors

Fraction correct proteins = $P_0 \approx 0.75$

$P_0 = e^{-m}$

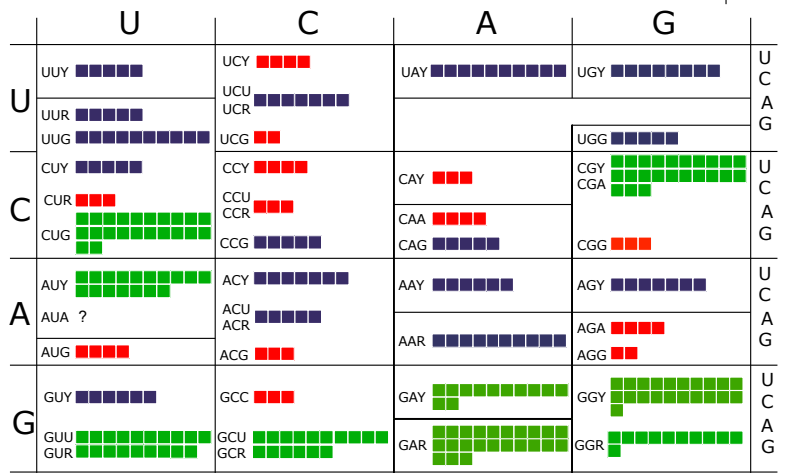
m = 0.29 errors/gene

Avg. gene = 460 codons

Avg. error frequency **≈ 6 x 10^{-4}/codon**

Yewdell et al. *Nature Rev Immunol* 3:952 2003

Distribution of tRNA abundance in E. coli



13 tRNAs | 16 tRNAs | 9 tRNAs

Data on tRNA abundance from Dong et al. 1996

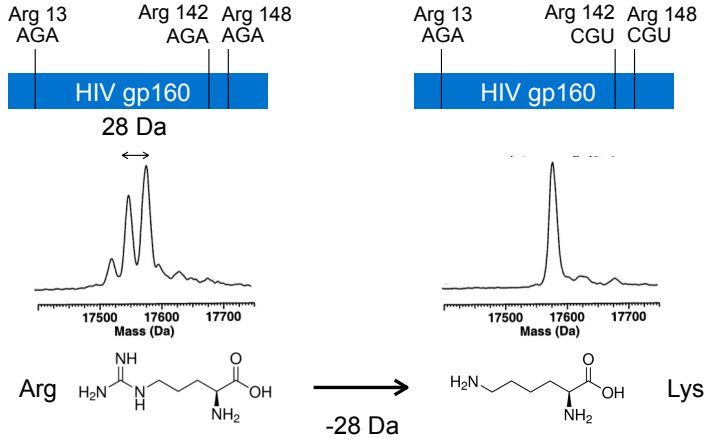
S. cerevisiae has a different tRNA distribution

	U	C	A	G	
U	UUY	UCY	UAY	UGY	U C A G
	UUA	UCA			
	UUG	UCG		UGG	
C	CUY	CCY	CAY	CGY CGA	U C A G
	CUN	CCR	CAA		
			CAG	CGG	
A	AUY	ACY	AAY	AGY	U C A G
	AUA	ACA	AAA	AGA	
	AUG	ACG	AAG	AGG	
G		GCY	GAY	GGY	U C A G
	GUA	GCR	GAA	GGA	
	GUG		GAG	GGG	

12 tRNAs 18 genes | 15 tRNAs 87 genes | 14 tRNAs 174 genes

Consequence of high-level protein expression

- Overexpressing an HIV-1 viral protein in *E. coli* (over 50% total cell protein



Calderone et al., JMB (1996) 262:407

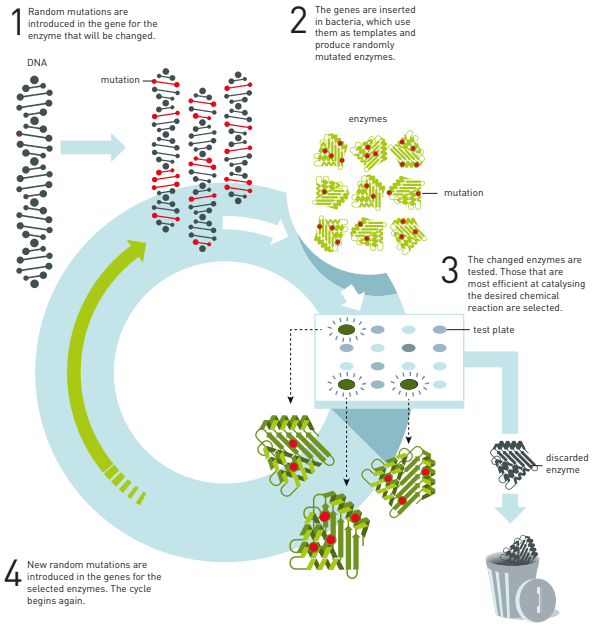
2018 Nobel Prize in Chemistry

Ill. Niklas Elmehed. © Nobel Media
Frances H. Arnold

Ill. Niklas Elmehed. © Nobel Media
George P. Smith

Ill. Niklas Elmehed. © Nobel Media
Sir Gregory P. Winter

The Nobel Prize in Chemistry 2018 was divided, one half awarded to Frances H. Arnold "for the directed evolution of enzymes", the other half jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies."



Reading for next time:

- Blount et al. (2012) Rational diversification of a promoter providing fine-tuned expression and orthogonal regulation for synthetic biology. PLoS One 7:e33279.