Module 2 overview

lecture

1. Introduction to the module
2. Rational protein design
3. Fluorescence and sensors
4. Protein expression

lab

1. Start-up protein eng.
2. Site-directed mutagenesis
3. DNA amplification
4. Prepare expression system

SPRING BREAK

lecture

5. Review & gene analysis
6. Purification and protein analysis
7. Binding & affinity measurements
8. High throughput engineering

lab

5. Induce protein
6. Purify protein
7. Characterize expression
8. Assess protein function

Lecture 4: Protein expression & purification

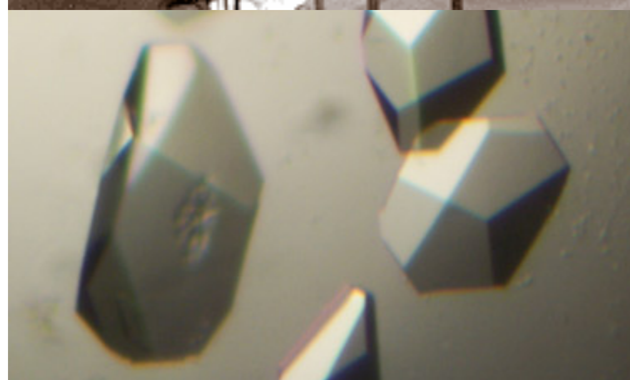
I. Why express & purify proteins?
   A. Scientific applications
   B. Applications in industry, *etc.*

II. Protein expression systems
   A. Alternatives to protein expression
   B. Prokaryotic and eukaryotic systems

Laboratory uses of purified proteins

Biochemistry analysis

Structural biology



Research biochemicals

www.mcgill.ca, images.apple.com,
www.varianinc.com, www.neb.com

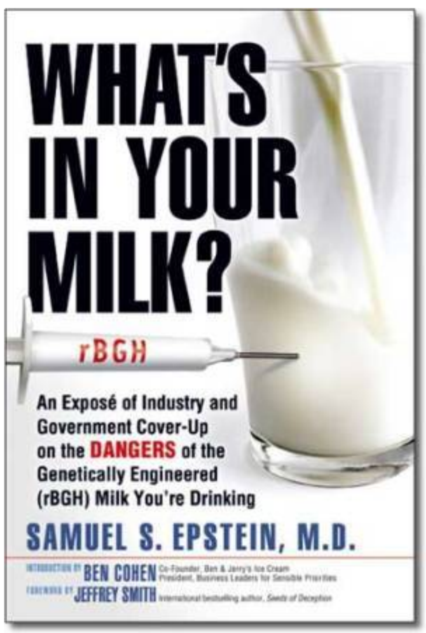
Protein therapeutics

Table 1 Top ten recombinant therapeutic proteins and their global sales between 2001 and 2003

	Product (generic)/ marketing company	2001 ($million)	2002 ($million)	2003 ($million)	Growth (decline) 2002–2003 (%)
	Procrit (epoetin alfa)/ Johnson & Johnson	3,430	4,269	3,986	(6.6)
	Epogen (epoetin alfa)/ Amgen	2,108	2,261	2,435	7.7
	Neupogen (filgrastim)/ Amgen	1,346	1,380	1,268	(8.1)
PEGylated	Neulasta (pegfilgrastim)/ Amgen	0	464	1,255	170.5
	Novolin (insulin systemic)/ Novo Nordisk	2,244	2,255	2,235	(0.9)
	Avonex (interferon beta-1a)/ Biogen IDEC	971	1,034	1,170	13.2
PEGylated	PEG-Intron A franchise (pegylated interferon alpha)/ Schering Plough	1,447	2,736	1,851	(32.3)
TNF ligand binding domain + Fc antibody domain	Enbrel (etanercept)/ Amgen	856	521	1,300	149.5
epo engineered to have additional glycoslyation sites	Aranesp (darbepoetin alfa)/ Amgen	42	416	1,544	271.2
	NeoRecormon (epoetin-beta)/ Roche	479	766	1,318	72.1
	Top ten product sales	*12,923*	*16,102*	*18,362*	*14.0*
	Others	*8,547*	*10,833*	*13,703*	*26.5*
	Total market value	*21,470*	*26,935*	*32,065*	*19.0*

Source: Datamonitor and company-reported information.

Pavlou & Reichert (2004) *Nat. Biotechnol.*



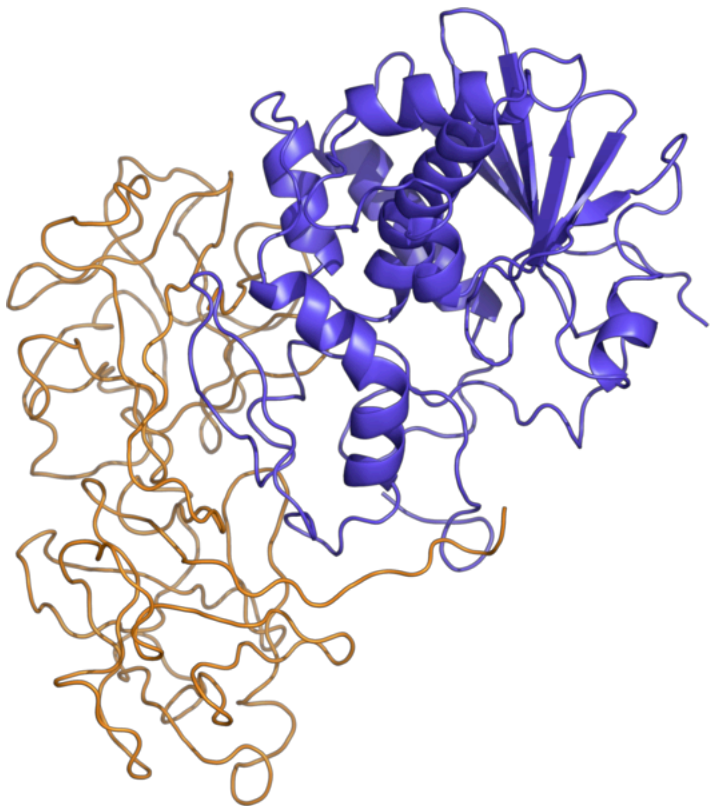
clockwise from top left: s.sears.com, www.beertech.co.uk, www.treatment-skin.com, www.valleynaturals.com, servekrishna.net

http://www.youtube.com/watch?v=bHWuj5WPvtU&feature=related

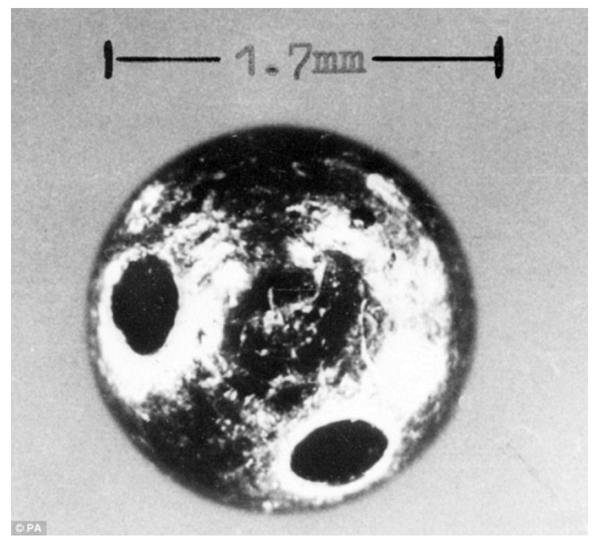
Ricin

A chain: 267 aa (blue); inhibits protein synthesis by degrading ribosomes

B chain: 262 aa (brown); adheres to cell surface to promote internalization



www.wikipedia.com



http://www.dailymail.co.uk/news/article-2143522

ricin extracted from castor beans and loaded into bullet

APHIS 301-851-3300

National Select Agent Registry

CDC 404-718-2000

HHS SELECT AGENTS AND TOXINS

★ Abrin
★ Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
★ Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus[1]
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus[1]
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
★ Ricin
Rickettsia prowazekii

OVERLAP SELECT AGENTS AND TOXINS

Bacillus anthracis *
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus[1]

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Avian influenza virus[1]
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum[1]
Mycoplasma mycoides[1]
Newcastle disease virus[1,2]
Peste des petits ruminants virus
Rinderpest virus*

and more...

Protein-based bioterrorism

"Aerosols were dispersed at multiple sites in downtown Tokyo, Japan, and at US military installations in Japan on at least 3 occasions between 1990 and 1995 by the Japanese cult Aum Shinrikyo. These attacks failed, apparently because of faulty microbiological technique, deficient aerosol-generating equipment, or internal sabotage."

"The US biological weapons program first produced botulinum toxin during World War II."

"After the 1991 Persian Gulf War, Iraq admitted to the United Nations inspection team to having produced 19,000 L of concentrated botulinum toxin, of which approximately 10,000 L were loaded into military weapons. These 19,000 L of concentrated toxin are not fully accounted for and constitute approximately 3 times the amount needed to kill the entire current human population by inhalation."

Arnon *et al.* (2001) *JAMA*

www.rickross.com

www.pbs.org

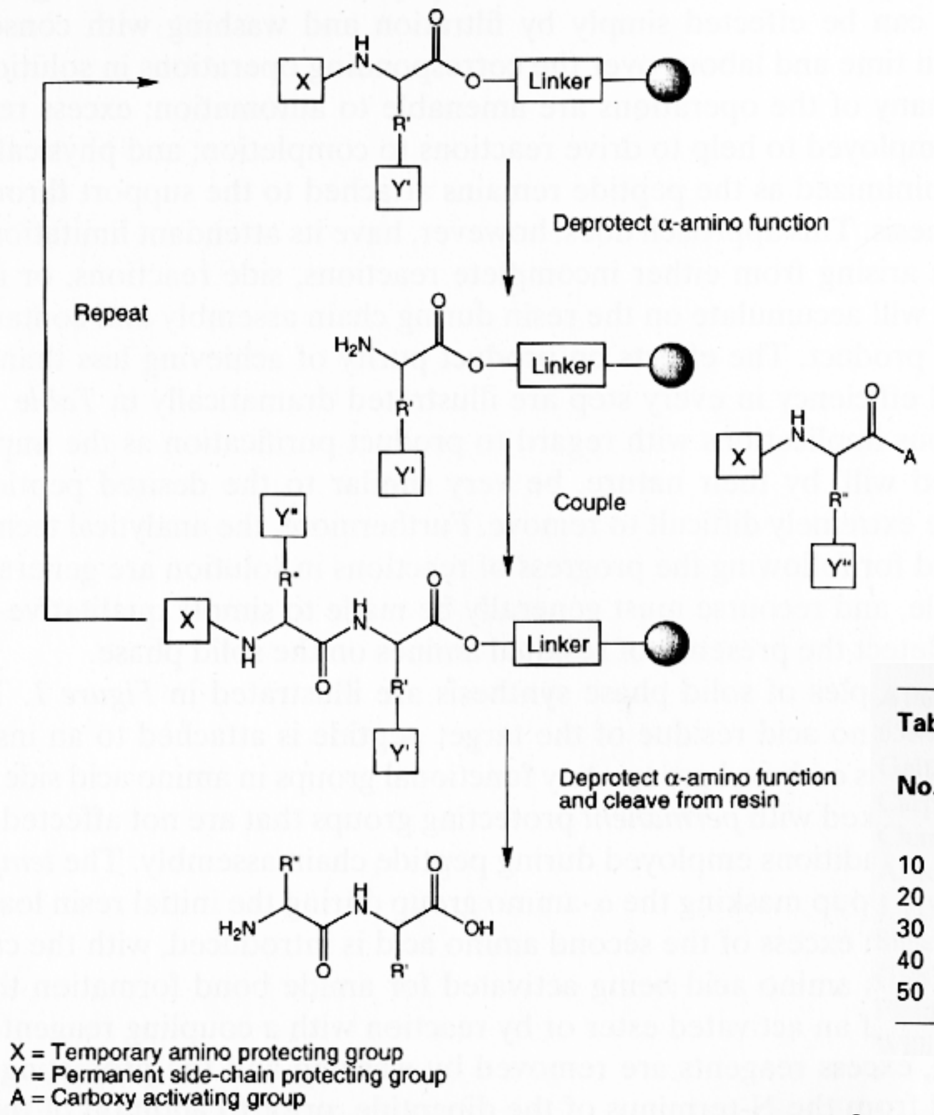
How can proteins be produced?

1. Purify from natural source
   advantages: no chemistry or DNA manipulation required, proteins likely to fold properly, assemble with native cofactors, *etc.*
   disadvantages: usually only practical for high abundance proteins, source-specific purification method required

2. Synthesize *de novo*
   advantages: no DNA manipulation required, synthesis methods well established, proteins produced are relatively pure
   disadvantages: relatively expensive, becomes extremely difficult for polypeptides > 50 amino acids

3. Express and purify from a dedicated expression system
   advantages: cheap and frequently high-yield, versatile expression systems already established
   disadvantages: cloning required, troubleshooting often needed to obtain high expression and proper folding

Solid phase peptide synthesis is a reliable technique for generating short polypeptides



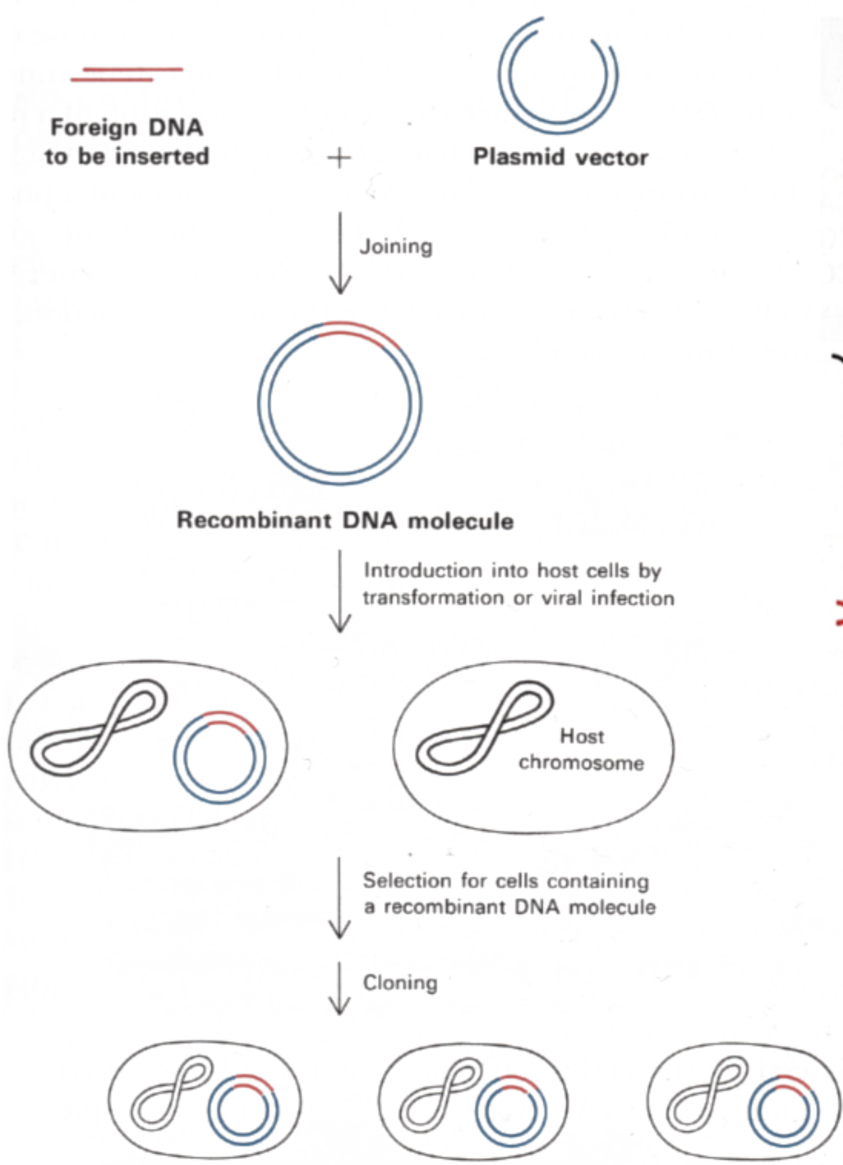
www.pitt.edu

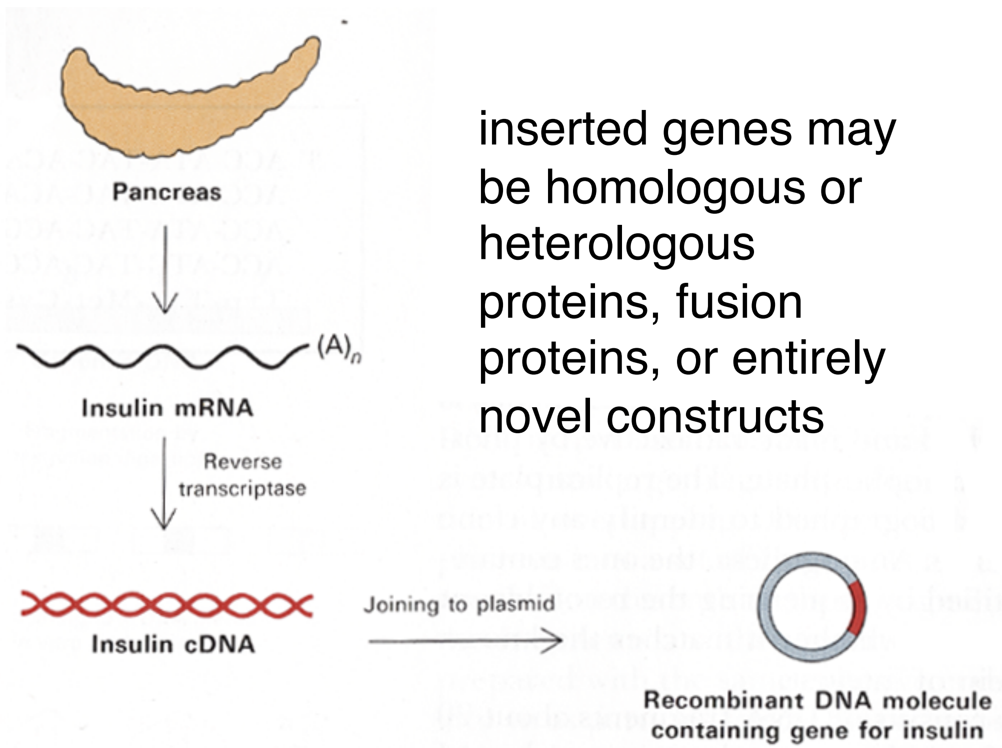
Table 1. Effects of accumulated errors on final product yields

No. of reactions		**Yield of each reaction (%)**			
		100	**99**	**95**	**90**
10	Overall yields	100	90	60	35
20		100	81	36	12
30		100	74	21	4
40		100	67	13	1
50		100	61	8	< 1

Chan & White (2000) *Fmoc Solid Phase Peptide Synthesis*

E. coli are the most common host for recombinant gene expression



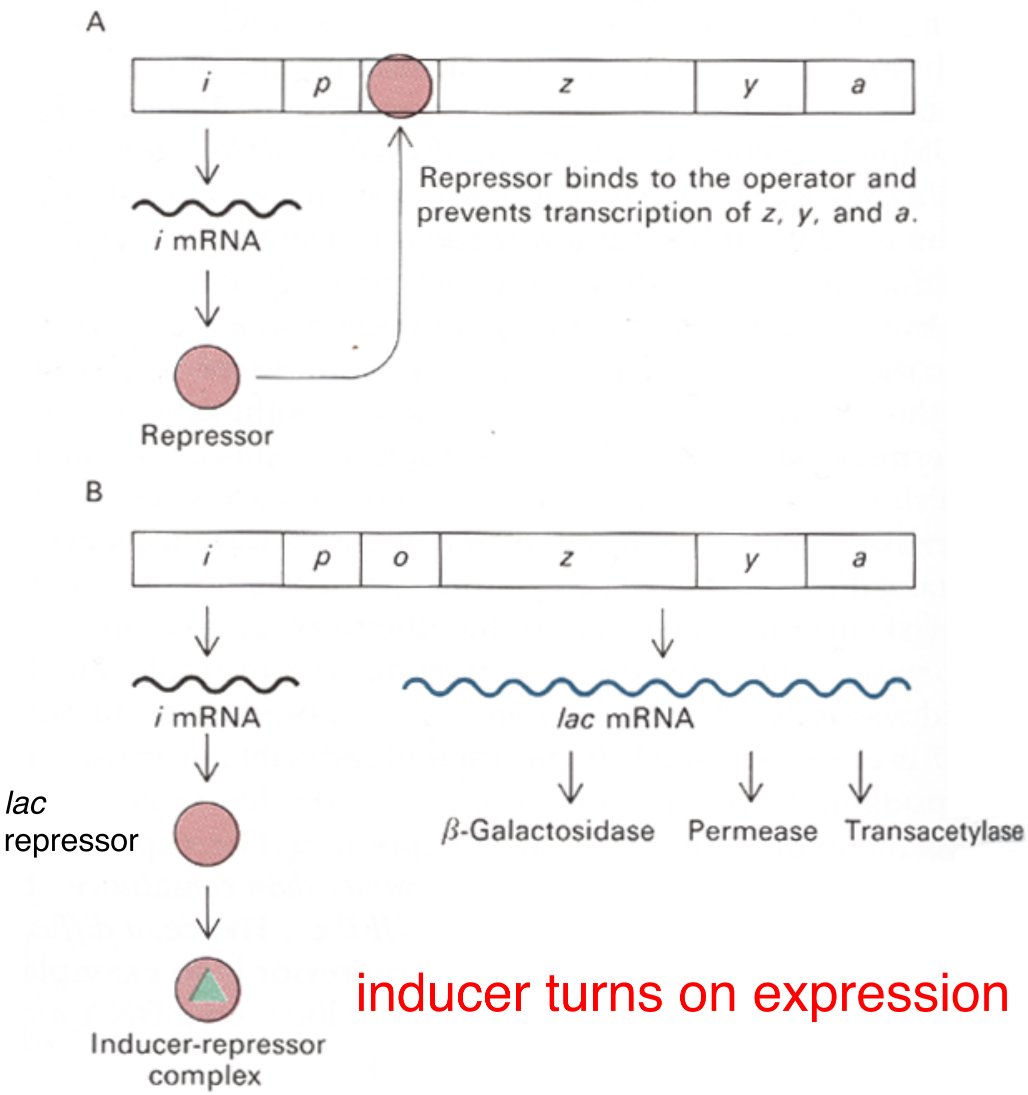


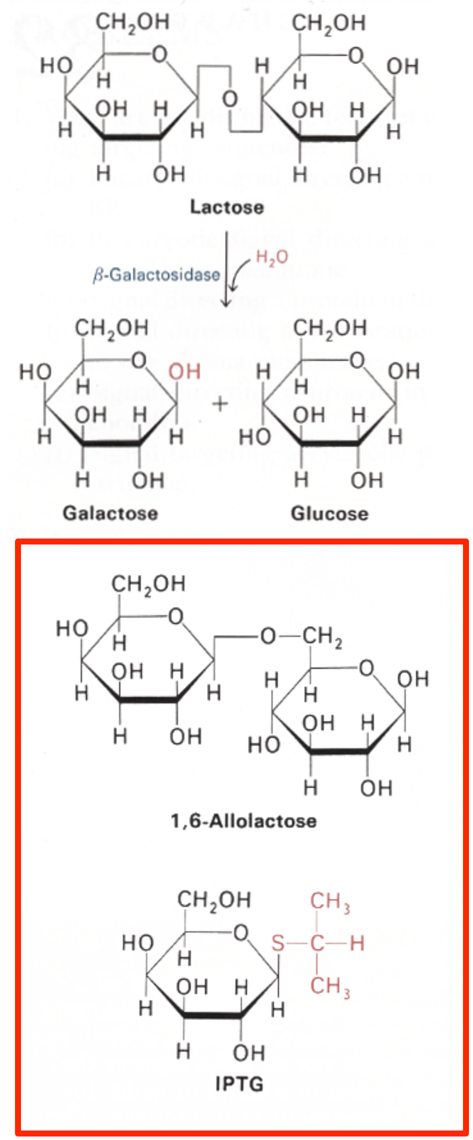
inserted genes may be homologous or heterologous proteins, fusion proteins, or entirely novel constructs

Once a foreign gene has been introduced, how does protein expression take place?

Stryer (1988) *Biochemistry, 3rd ed.*

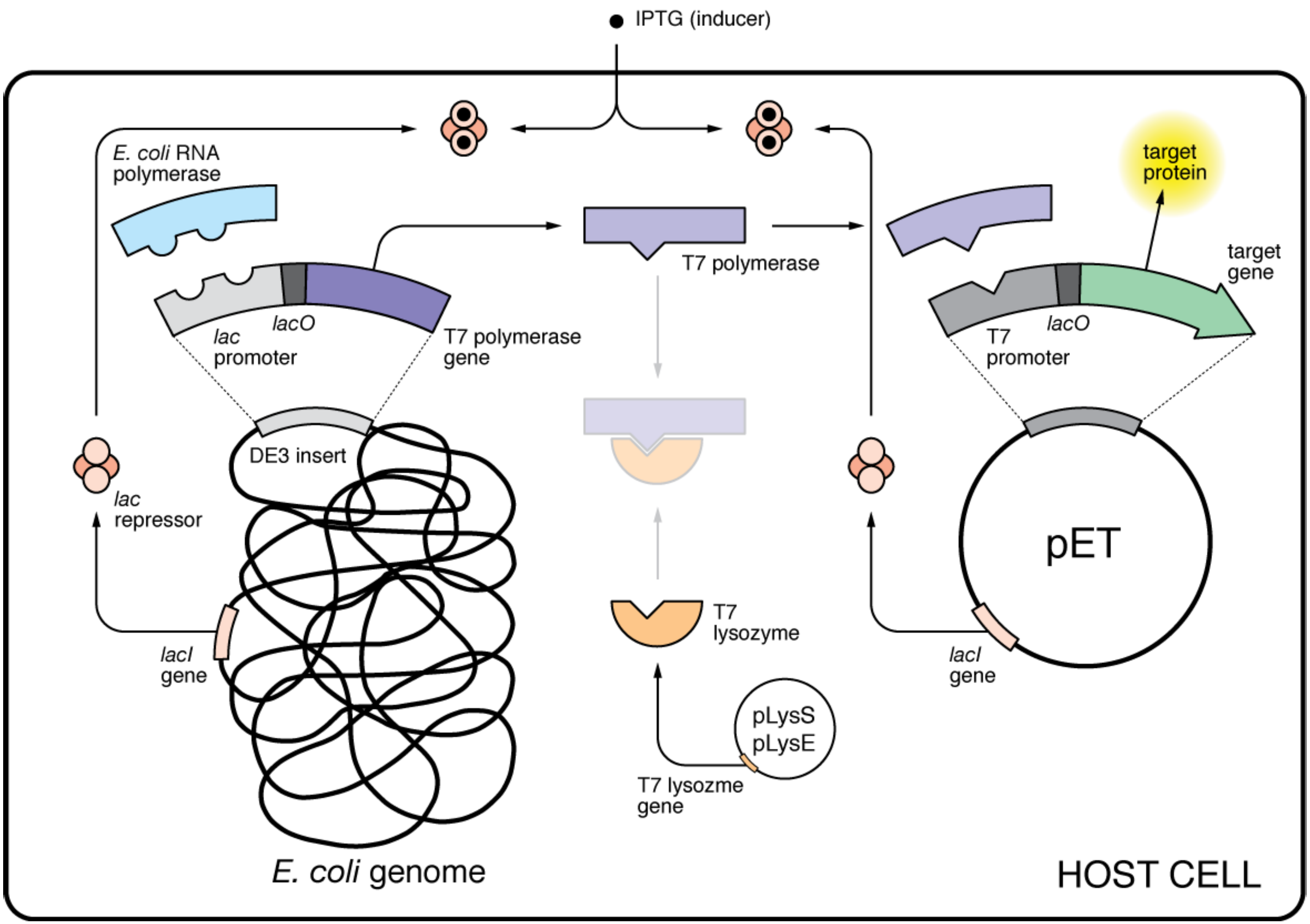
The *lac* operon is the basis for the most common bacterial protein expression systems; allows bacterial growth to be dissociated from protein expression





Stryer (1988) *Biochemistry, 3rd ed.*

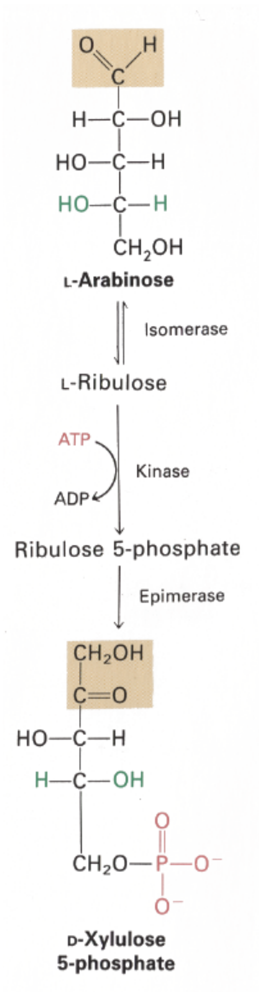
T7 expression system

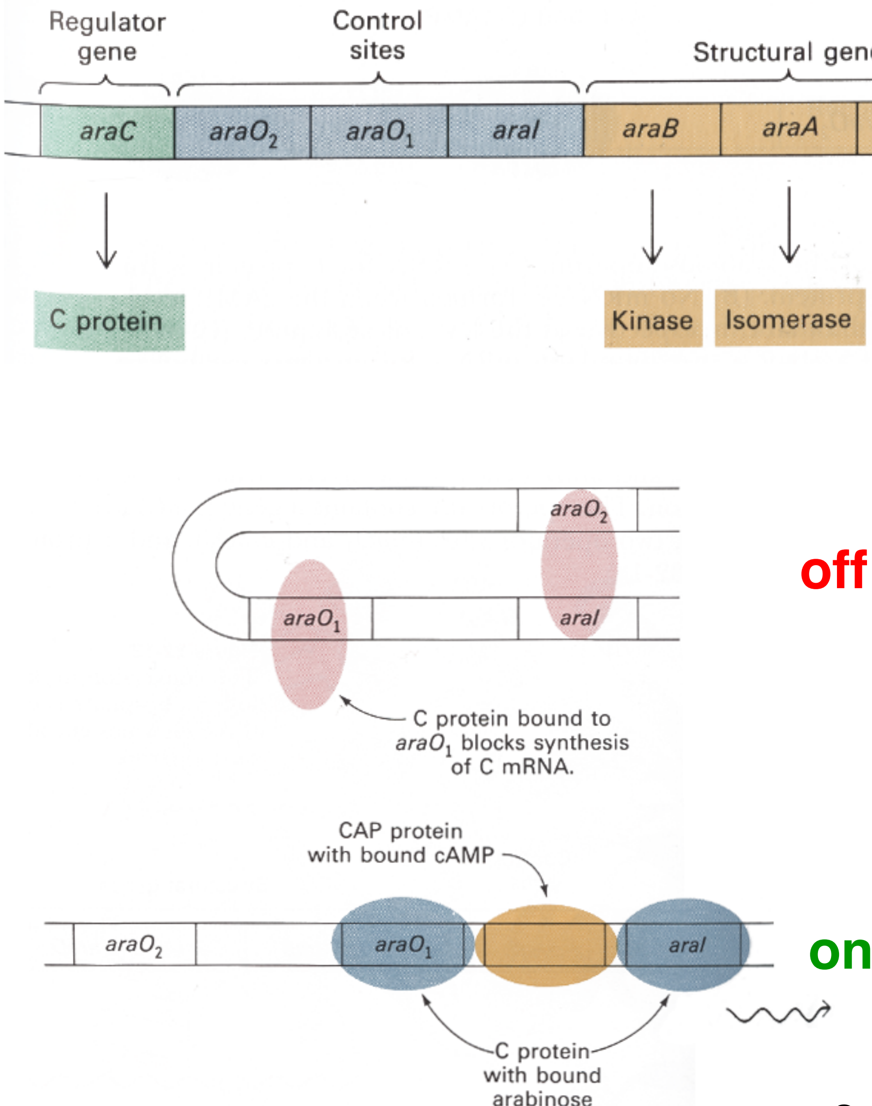


http://farm8.staticflickr.com/7024/6464583867_16c8ccdc9a_z.jpg

http://www.biomm.com/index.php?p=3,2

Other induction systems can also be used for protein expression in *E. coli:* arabinose system is considered to be more tightly controlled than the *lac* operon



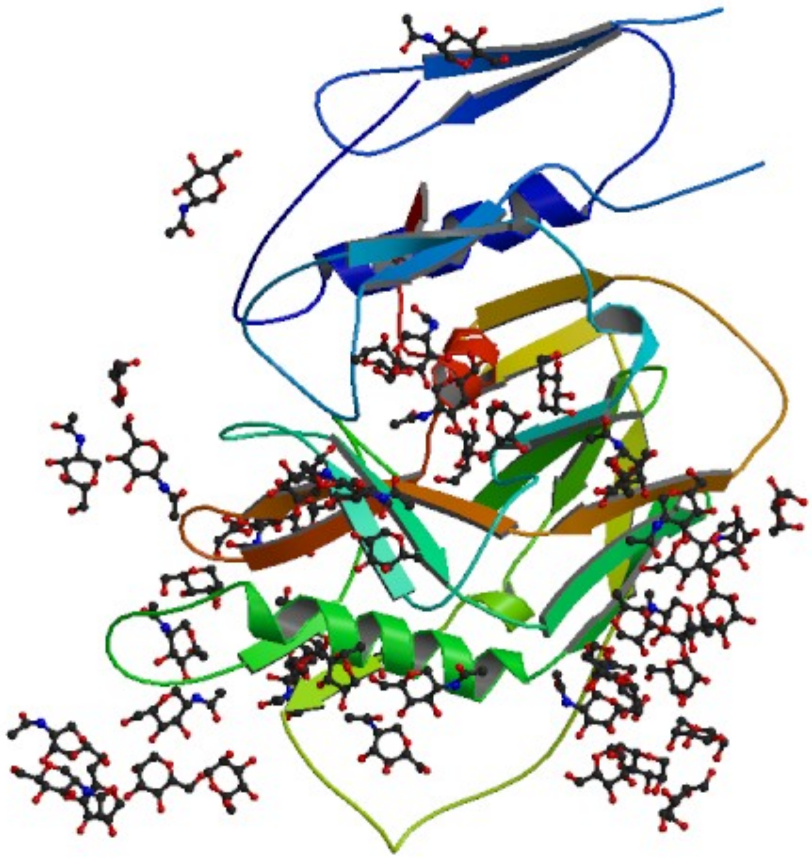


ara system is also compatible with T7-based vectors

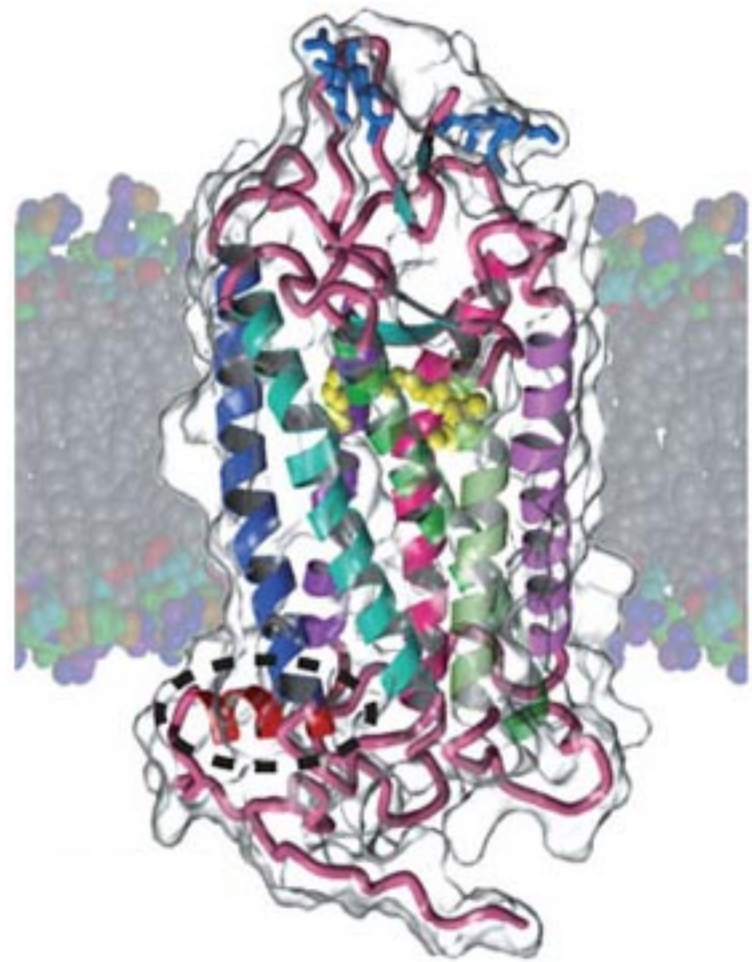
Stryer (1988) *Biochemistry, 3rd ed.*

Differences between prokaryotic *vs.* eukaryotic proteins sometimes require eukaryotic expression systems.

These two proteins exemplify characteristics that frequently call for eukaryotic expression:

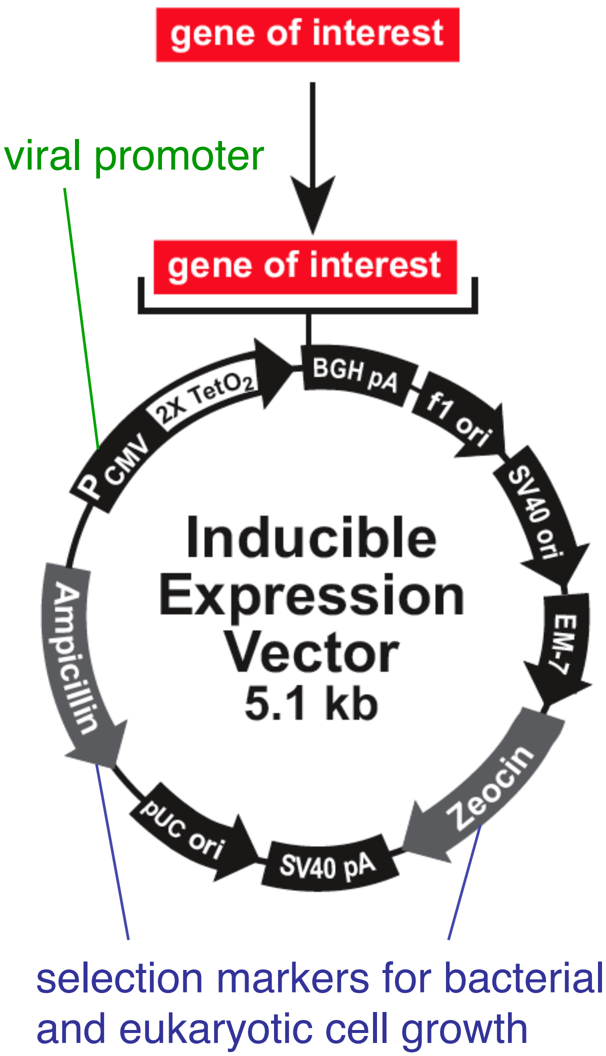


www.rcsb.pdb.org

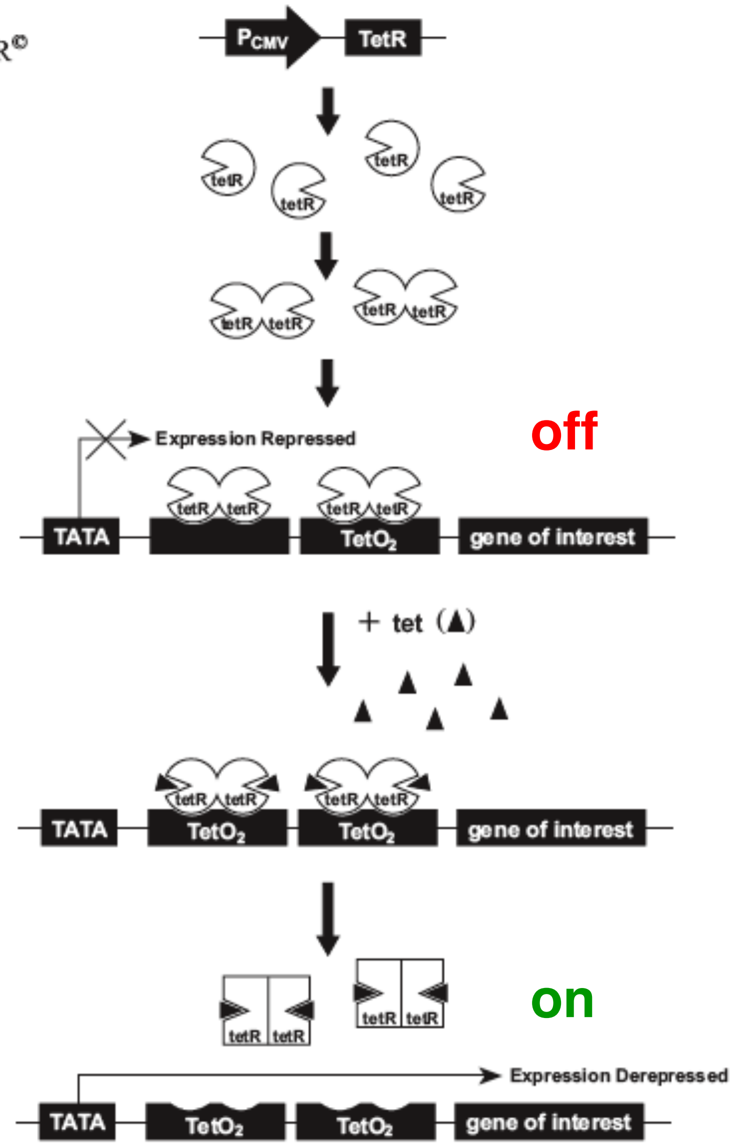


www.rikenresearch.riken.jp

Eukaryotic expression vectors share features with bacterial systems



1. Tet repressor (tetR) protein is expressed from pcDNA6/TR© in cultured cells.
2. TetR homodimers bind to Tet operator 2 ($TetO_2$) sequences in the inducible expression vector, repressing transcription of the gene of interest.
3. Upon addition, tetracycline (tet) binds to tetR homodimers.
4. Binding of tet to tetR homodimers causes a conformational change in tetR, release from the Tet operator sequences, and induction of transcription from the gene of interest.



Invitrogen (2006) *T-REx System*

Prokaryotic *vs.* eukaryotic protein expression

property	*prokaryotic*	*higher eukaryotic*
yield/(L culture)	1-100 mg	widely variable
cost/(L medium)	~$5	~$50
introduction of DNA	transformation of competent cells	viral or nonviral transfection
handling	sterile needles, *etc*.	tissue culture hood
cell incubation	shaking incubator	usu. w/CO_2-control
induction	usually IPTG	none, tetracycline
glycosylation, *etc.*	no	yes
notes	best for small, globular proteins	best for complex, eukaryotic proteins