COURSE BSc (BIOTECHNOLOGY) III YEAR

PAPER CODE: BBT-301

PAPER TITLE: RECOMBINANT DNA TECHNOLOGY

Production of recombinant pharmaceuticals

By:

DR. ANNIKA SINGH

DEPARTMENT OF BIOTECHNOLOGY

INSTITUTE OF BIOSCIENCE AND BIOTECHNOLOGY

- Group I: protein therapeutics with enzymatic or regulatory activity
- Group II : protein therapeutics with special targeting activity
- Group III : protein vaccines
- Group IV : protein diagnostics

1982	Human insulin, created using recombinant DNA technology
1986	Interferon alfa and muromonab-CD3 approved
1993	CBER's Office of Therapeutics Research and Review (OTRR) formed
1997	First whole chimeric antibody, rituximab, and first humanized antibody, daclizumab, approved
2002	$30 billion share of biotechnological drugs of $400 billion in yearly worldwide pharmaceutical sales
2006	An inhaled form of insulin (Exubera) approved

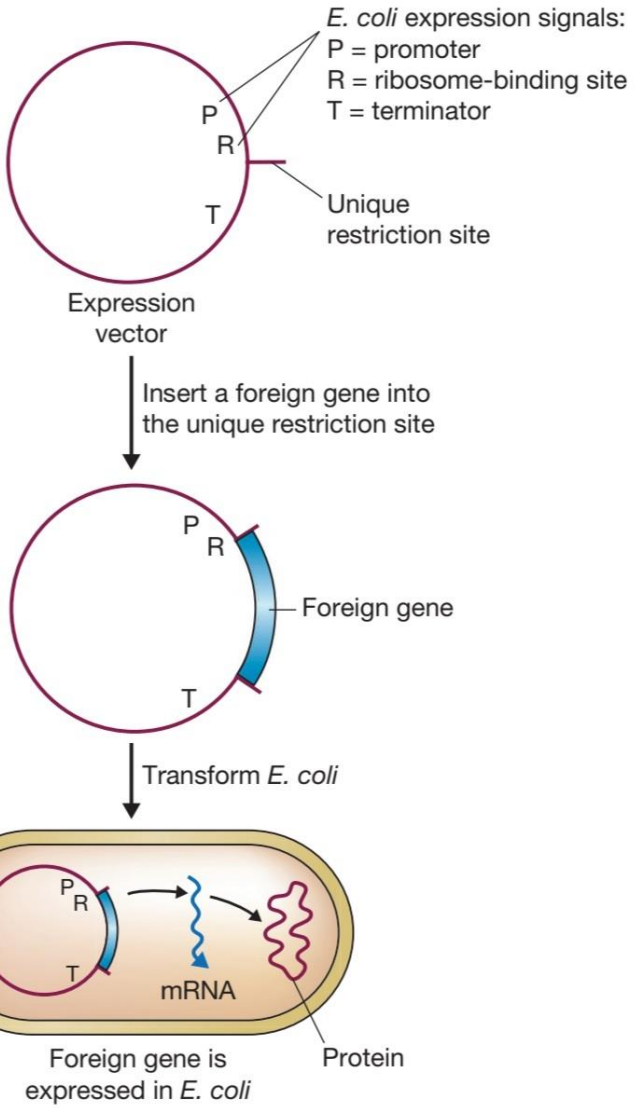
Production of recombinant pharmaceuticals

PROTEIN	USED IN THE TREATMENT OF
α_1-Antitrypsin	Emphysema
Deoxyribonuclease	Cystic fibrosis
Epidermal growth factor	Ulcers
Erythropoietin	Anaemia
Factor IX	Christmas disease
Factor VIII	Haemophilia
Fibroblast growth factor	Ulcers
Follicle-stimulating hormone	Infertility treatment
Granulocyte colony-stimulating factor	Cancers
Insulin	Diabetes
Insulin-like growth factor 1	Growth disorders
Interferon-β	Cancers, AIDS
Interferon-γ	Cancers, rheumatoid arthritis
Interferon-α	Leukaemia and other cancers
Interleukins	Cancers, immune disorders
Lung surfactant protein	Respiratory distress
Relaxin	Used to aid childbirth
Serum albumin	Used as a plasma supplement
Somatostatin	Growth disorders
Somatotrophin	Growth disorders
Superoxide dismutase	Free radical damage in kidney transplants
Tissue plasminogen activator	Heart attack
Tumour necrosis factor	Cancers

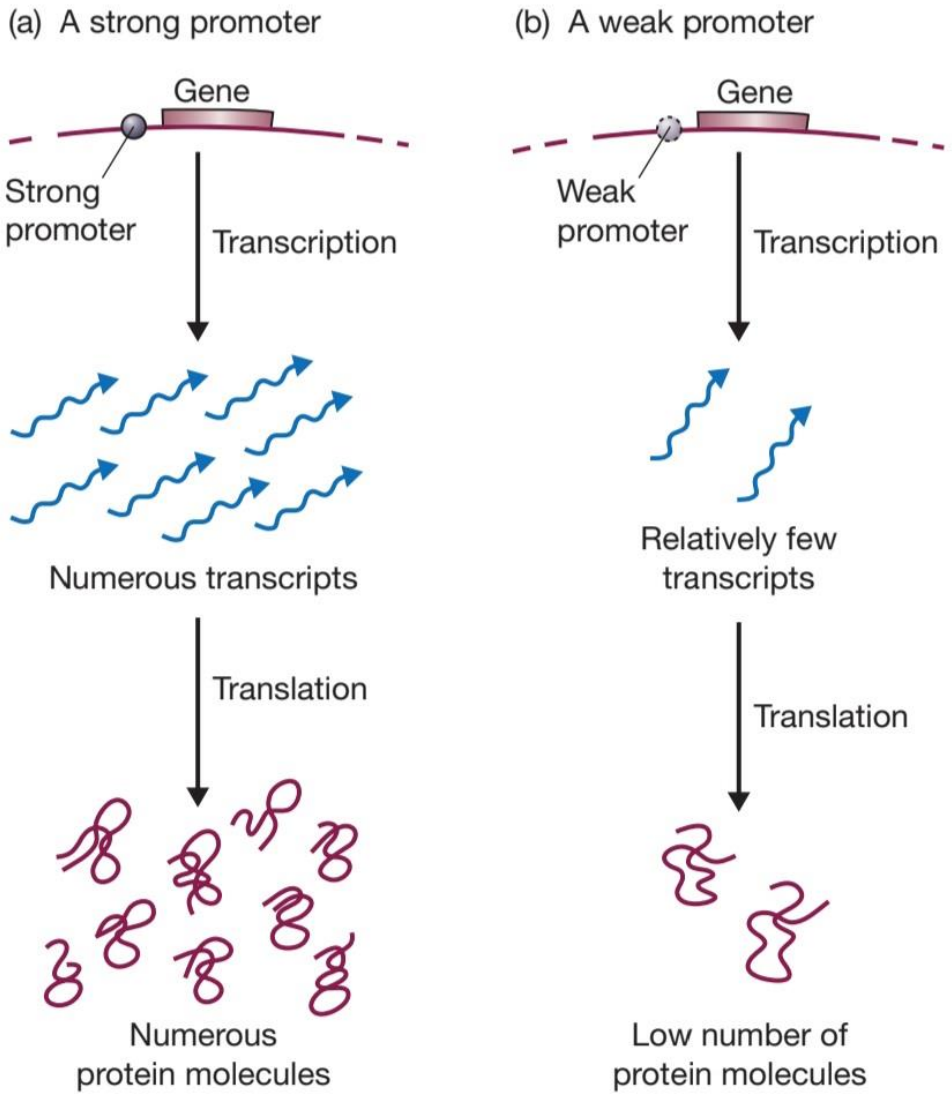
Production of recombinant pharmaceuticals

rDNA Product	Trade name	Application / Uses
Insulin	Humulin	Diabetes
Growth hormone	Protropin/Humatrope	Pituitary dwarfism
Factor vIII	Kogenate/Recombinate	Hemophilia
Interferon	Intron A	Hairy cell leukemia
Hepatitis B vaccine	Recombinax HB/ Engerix	Hepatitis B
Tissue plasminogen activator	Activase	Myocardial infarction
Dnase	Pulmozyme	Cystic fibrosis
Erythropoietin	Epogen/rocrit	Severe anemia with kidney damage

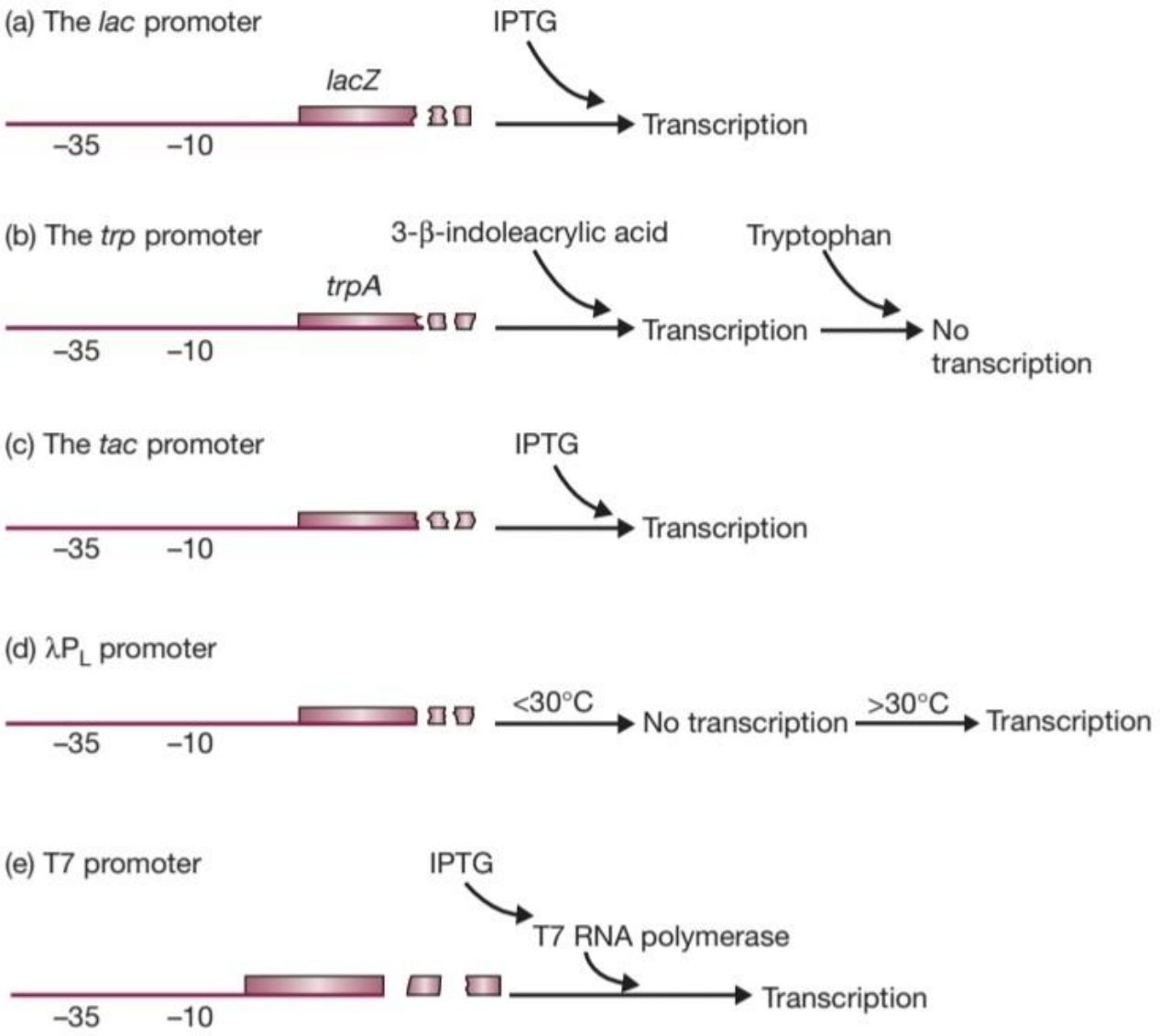
Production of recombinant pharmaceuticals



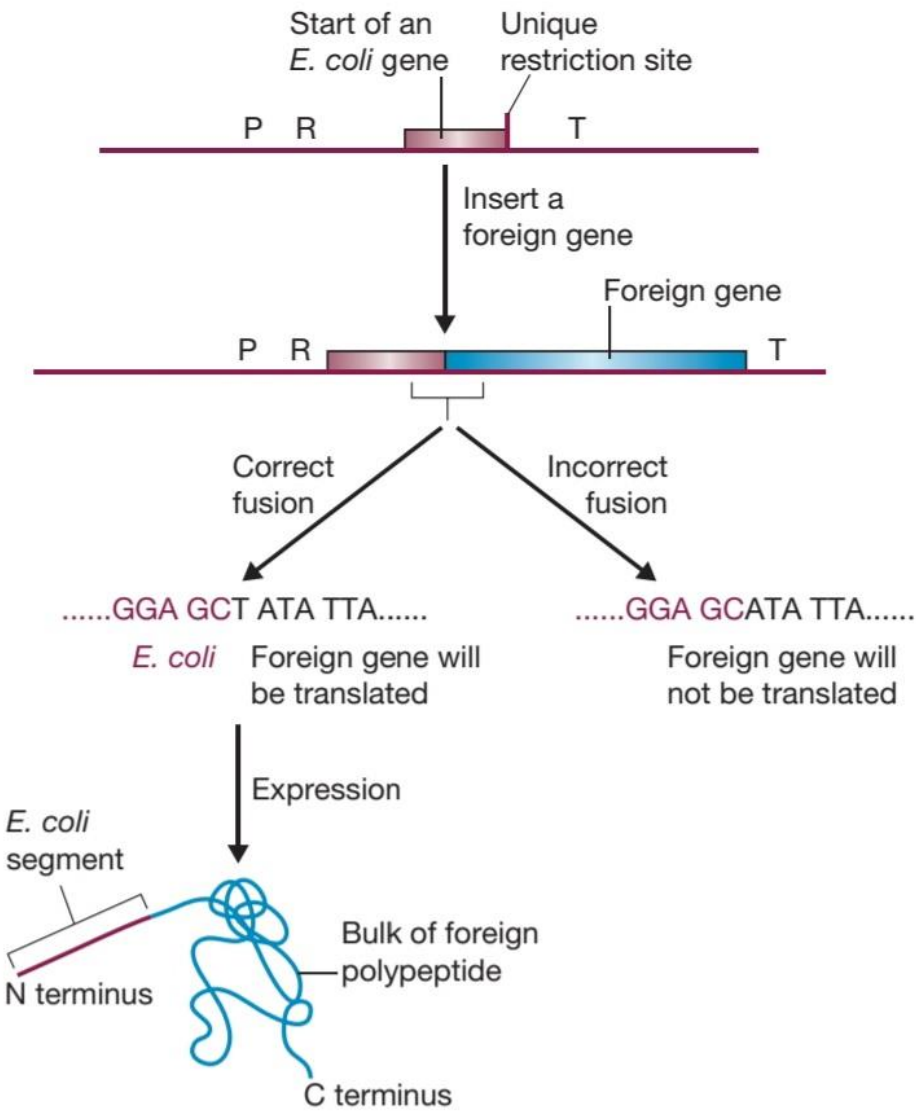
Production of recombinant pharmaceuticals



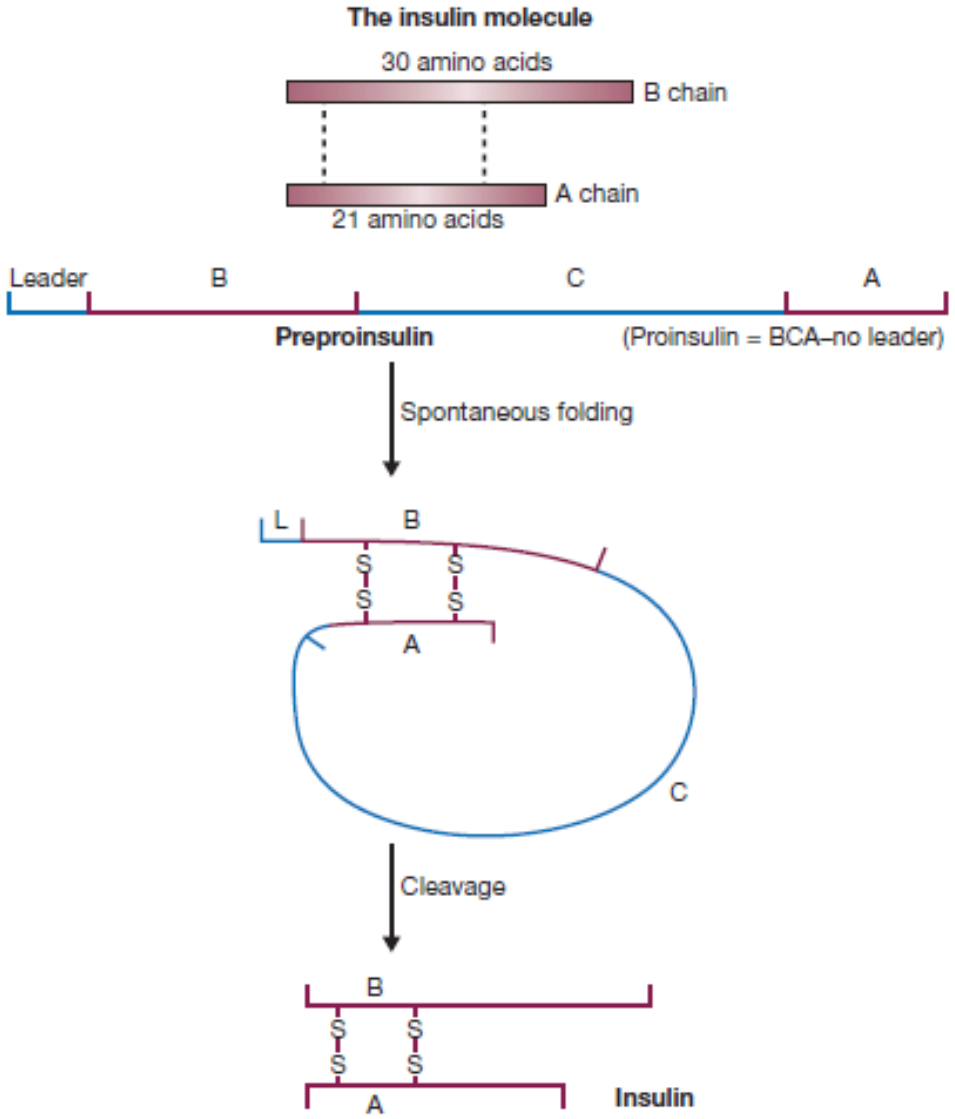
Production of recombinant pharmaceuticals



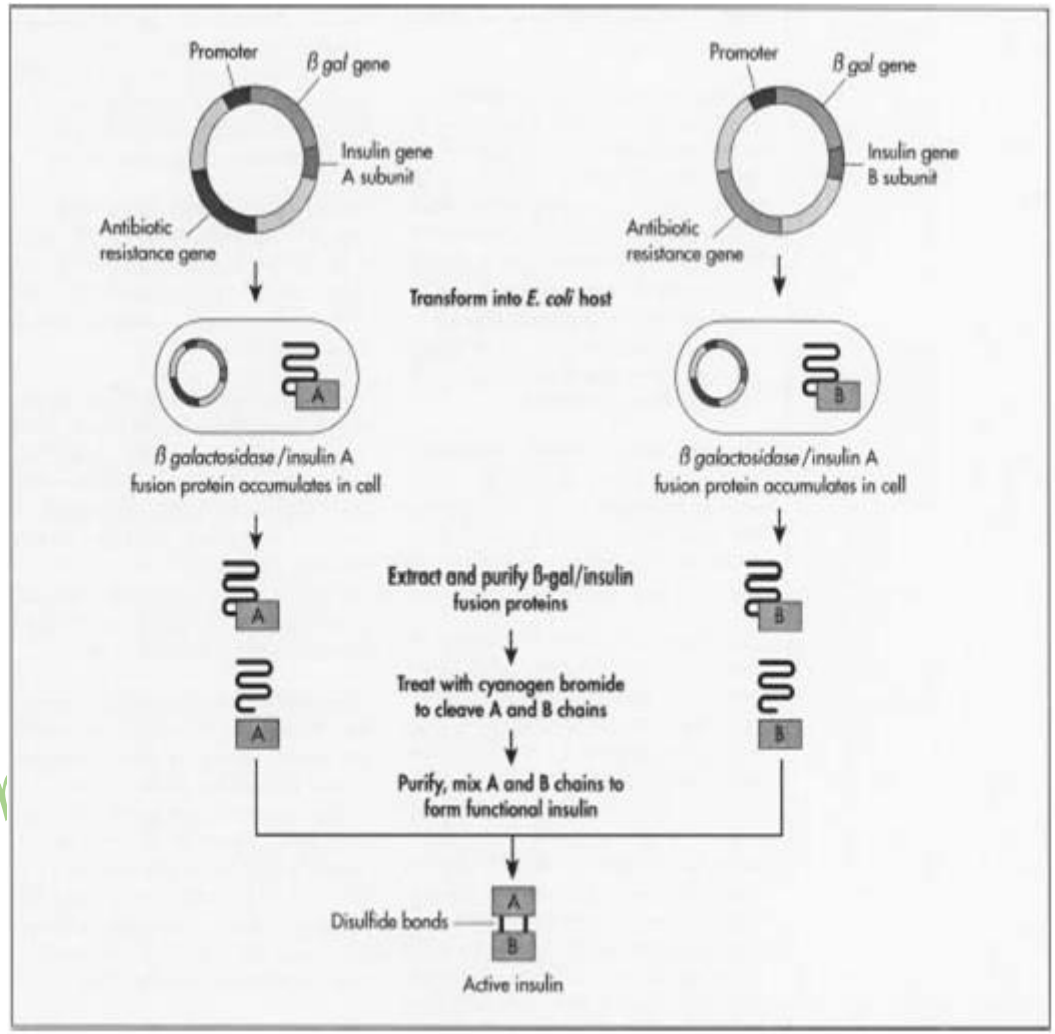
Production of recombinant pharmaceuticals



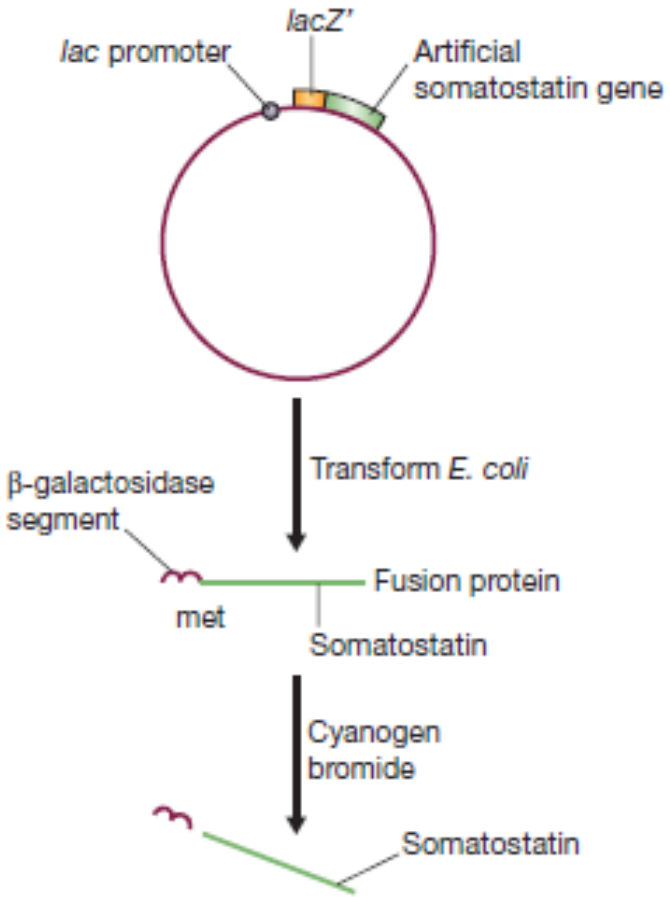
Production of recombinant pharmaceuticals

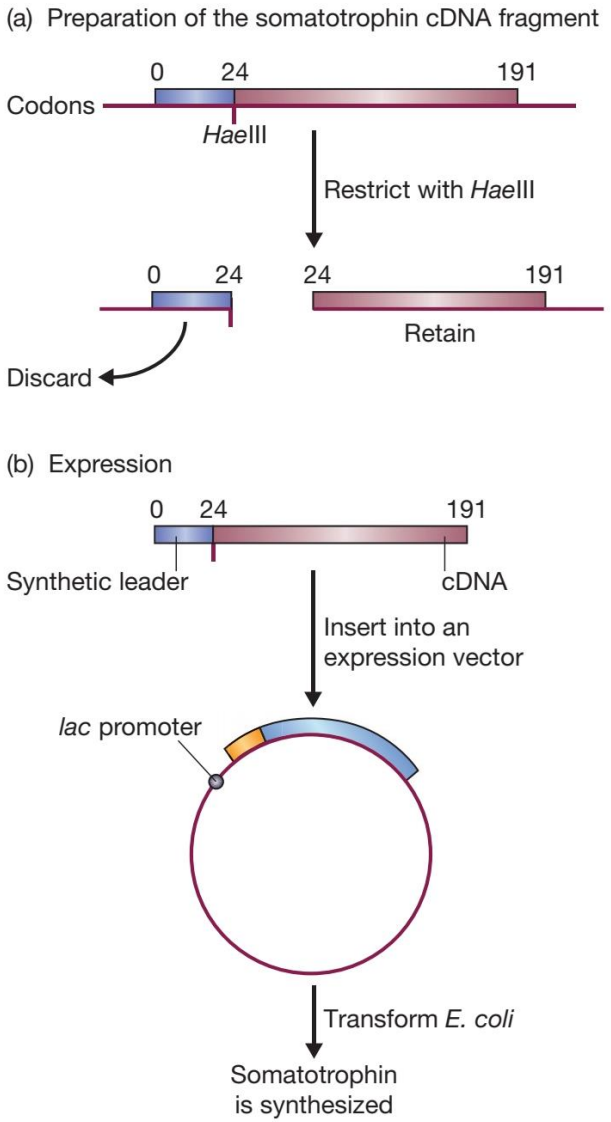


Production of recombinant pharmaceuticals



Production of recombinant pharmaceuticals

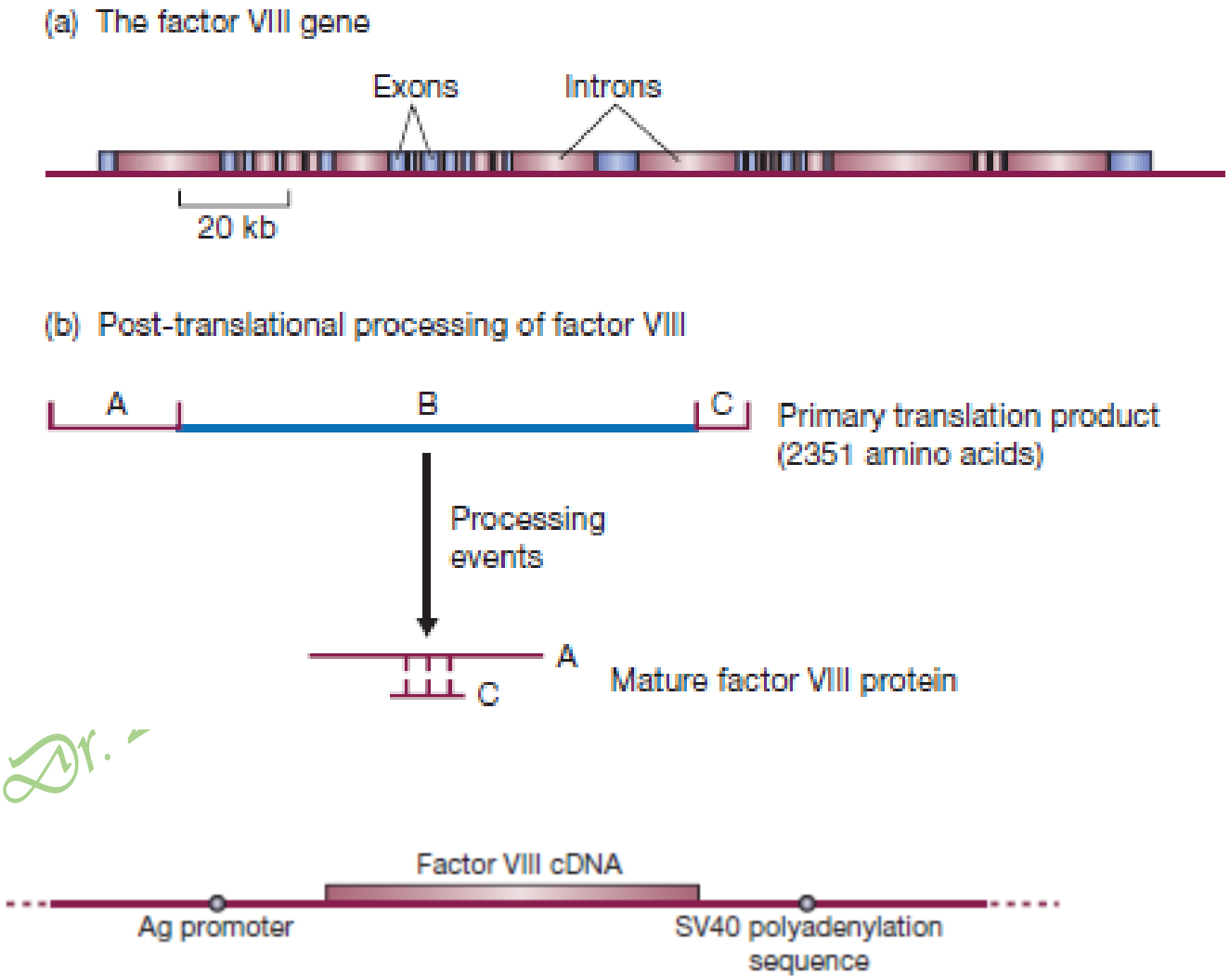




Production of recombinant pharmaceuticals

- The factor VIII gene is very large, over 186 kb in length, and is split into 26 exons and 25 introns.
- The mRNA codes for a large polypeptide (2351 amino acids), which undergoes a complex series of post-translational processing events, eventually resulting in a dimeric protein consisting of a large subunit, derived from the upstream region of the initial polypeptide, and a small subunit from the downstream segment.
- The two subunits contain a total of 17 disulphide bonds and a number of glycosylated sites.
- As might be anticipated for such a large and complex protein, it has not been possible to synthesize an active version in *E. coli*.
- Initial attempts to obtain recombinant factor VIII therefore involved mammalian cells.
- In the first experiments entire cDNA was cloned in hamster cells, but the yields of protein were extremely low.
- This was probably because the post-translational events, although carried out correctly in hamster cells, did not convert all of the initial product into an active form, thus limiting the overall yield

Production of recombinant pharmaceuticals



Production of recombinant pharmaceuticals

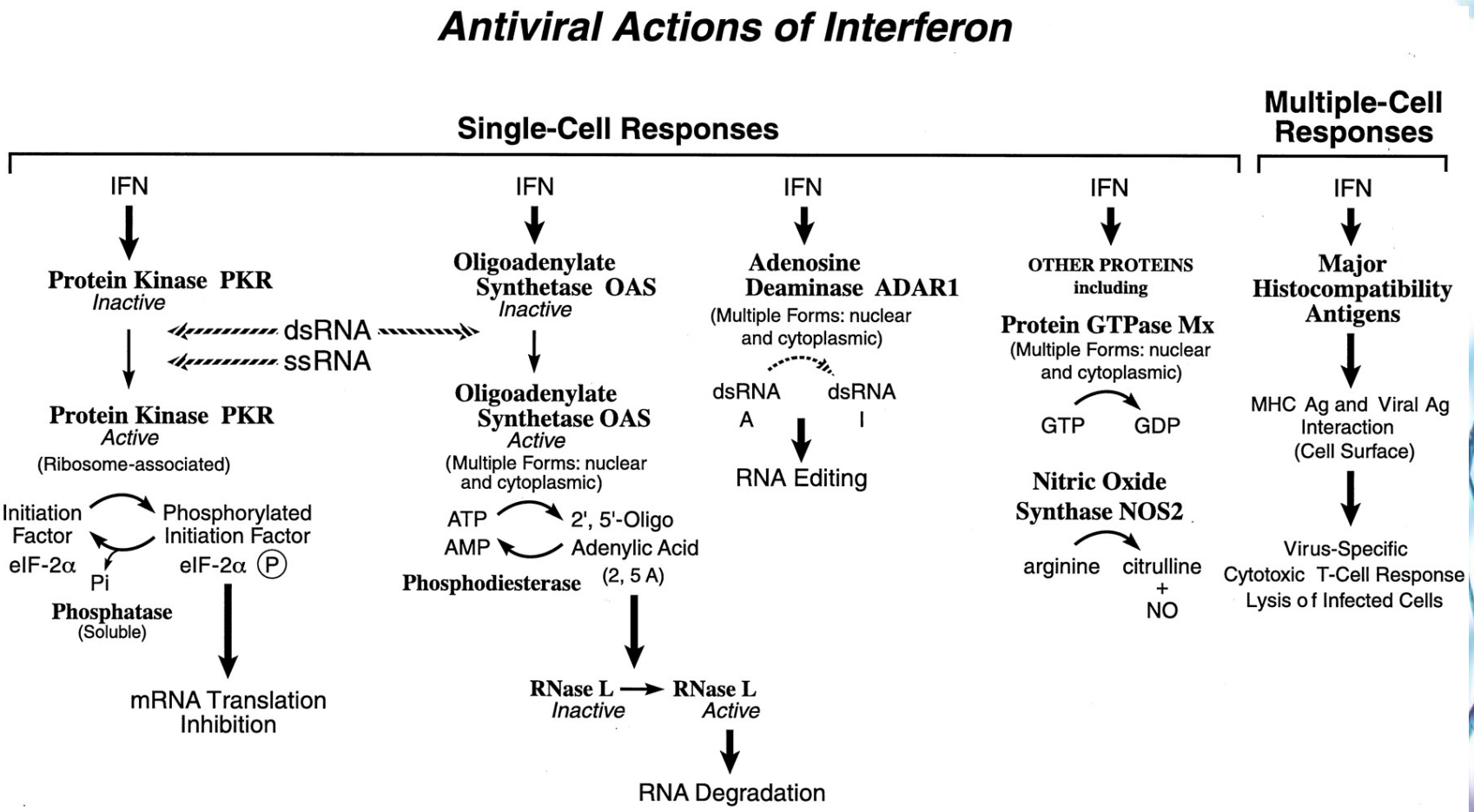
Recombinant Interferon-gamma

Year	Description	References
1954	Hypothesised the presence of a "viral inhibitory factor" in tissues infected by virus	Nagano Y and Kojima Y (1954)
1957	Coined the term "Interferon"	Isaacs A and Lindenmann J (1957)
1961	Evidence that human leucocytes produce IFNs	Gresser I (1961)
1978	Purification of leukocyte IFN	Rubinstein (1978)
1980-81	The IFN genes were cloned	Taniguchi T *et al.* (1980) Pestka S (1981)
1980	Recombinant DNA technology produce large amounts of purified IFNs	Nagata S *et al.* (1980)
1986	US Food and Drug Administration (FDA) approved IFN-α2b (Intron A)	Spiegel RJ (1986)
2001	FDA approved pegylated IFN-α2b (PEG-Intron)	Pham P and Pharm D (2001)
2002	FDA approved pegylated IFN-α2a (Pegasys)	Iafolla M (2002)

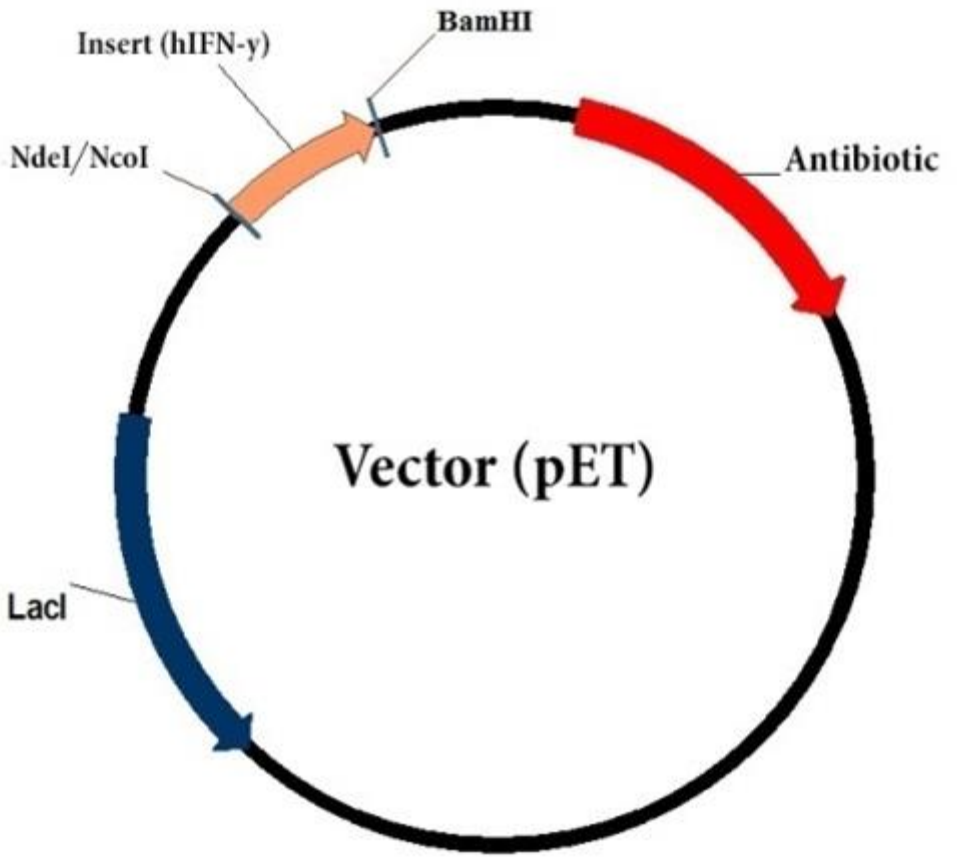
Production of recombinant pharmaceuticals

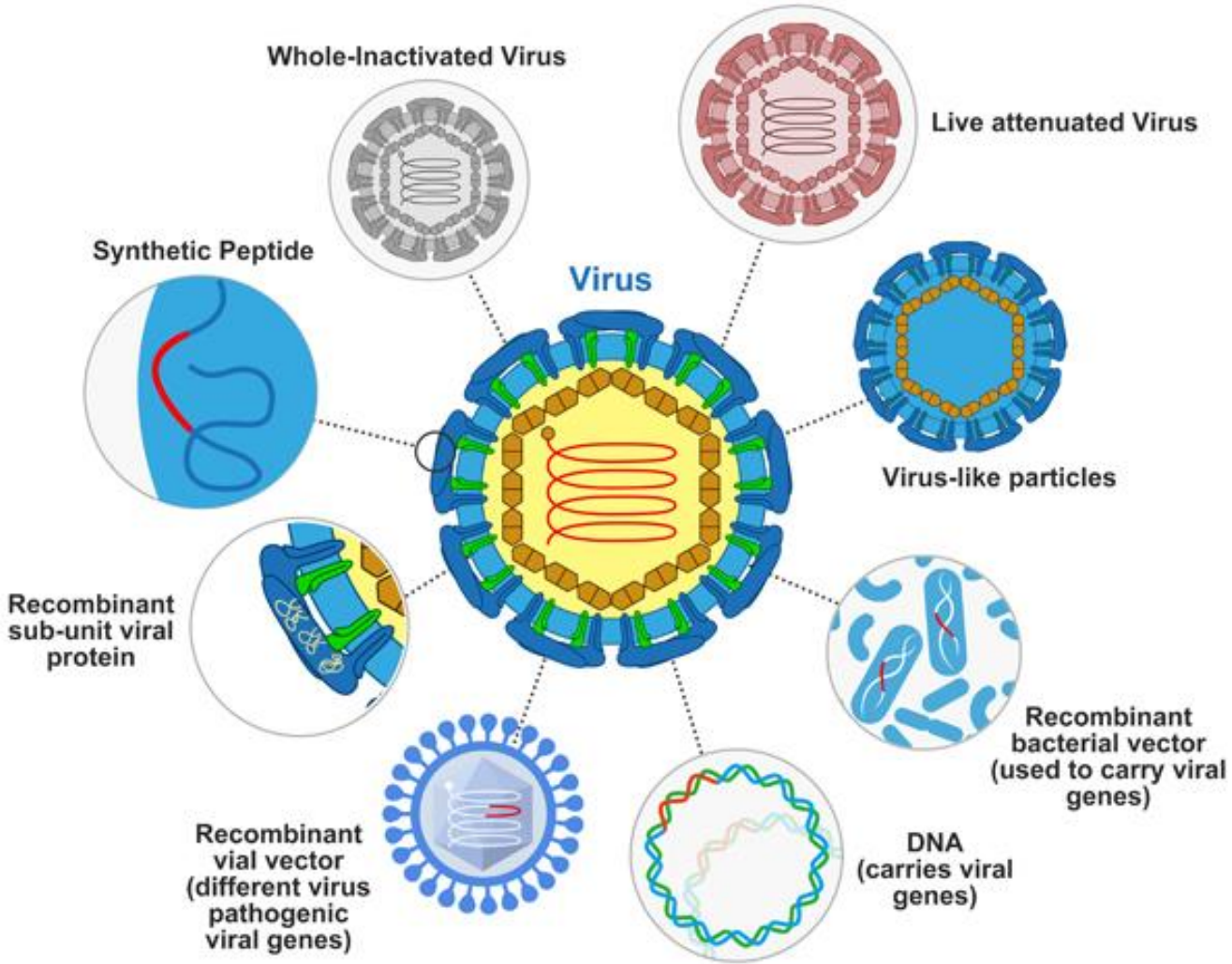
- **Recombinant Interferon-gamma**
- The expression of the interferon is induced by a unique set of stimulus and is produced by T-lymphocytes and NK cells.
- The *hifn-γ* gene encodes a 143 amino-acid residue long polypeptide and contains two sites of glycosylation: Asn^{25} and Asn^{97}.
- Interferon-gamma has a structure of a glycosylated homodimer.
- Interferon-gamma is pleiotropic cytokine, playing crucial role in the innate and acquired immunity.
- The IFN-γ cytokine, the only member of the type II interferon family, is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by Th1 CD4 and CD8 cytotoxic T lymphocyte (CTL) effector T cells upon the development of antigen-specific immunity
- It influences on antiviral and antibacterial protection of an organism, regulation of a cellular cycle (apoptosis) and participates in the inflammatory process.
- The first expression of recombinant interferon-gamma in *E.coli* cells was carried out in 1982. rIFN-γ is accumulated in *E.coli* cells in the inclusion bodies.

Production of recombinant pharmaceuticals



Production of recombinant pharmaceuticals




Whole-Inactivated Virus
Live attenuated Virus
Synthetic Peptide
Virus
Virus-like particles
Recombinant
sub-unit viral
protein
Recombinant
bacterial vector
(used to carry viral
genes)
Recombinant
vial vector
(different virus
pathogenic
viral genes)
DNA
(carries viral
genes)

Recombinant vaccine

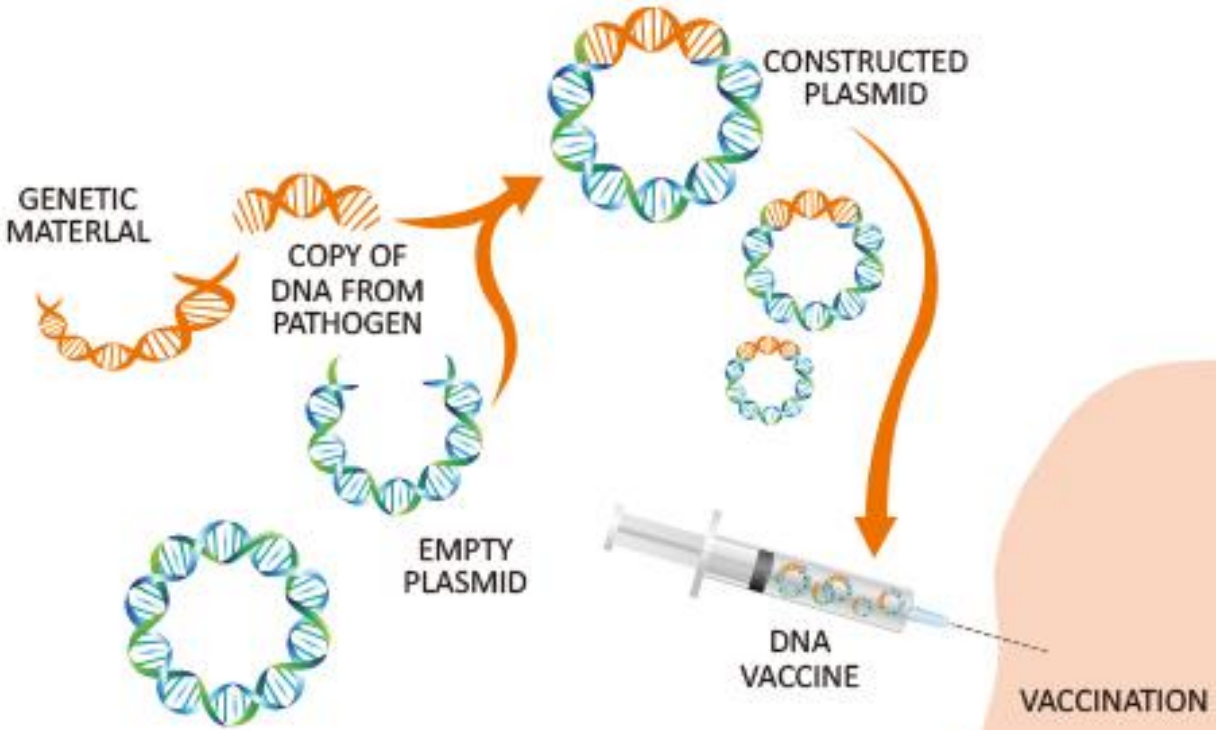
A recombinant vaccine is a vaccine produced through recombinant DNA technology. This involves inserting the DNA encoding an antigen (such as a bacterial surface protein) that stimulates an immune response into bacterial or mammalian cells, expressing the antigen in these cells and then purifying it from them.

Recombinant vaccines can be classified into two major categories.

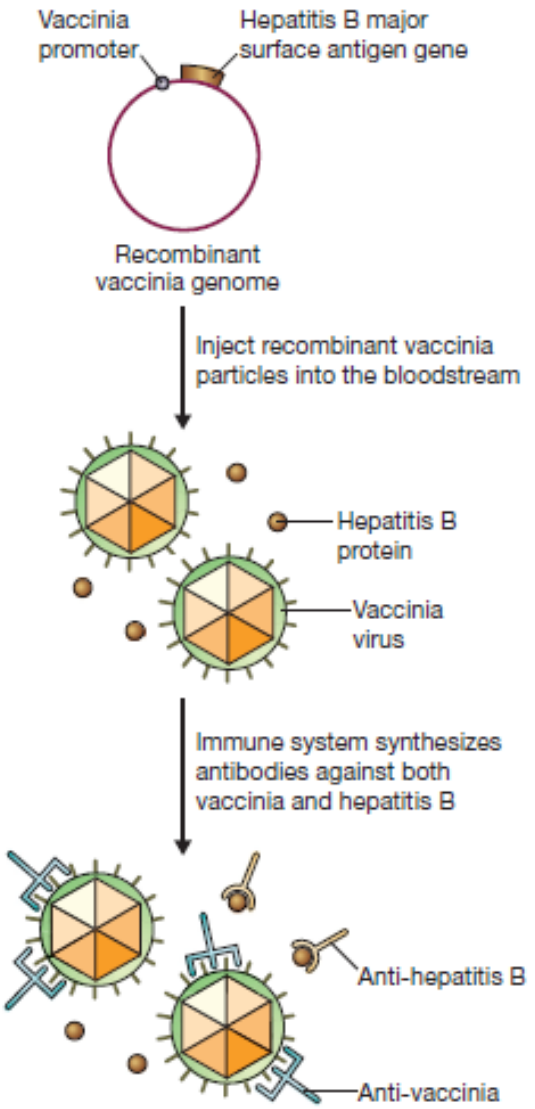
- DNA vaccines
- Recombinant (protein subunit) vaccines

DNA vaccines

These vaccines usually consist of synthetic DNA containing the gene that encodes the disease-agent protein. Usually, the plasmid DNA used as vaccine is propagated in bacteria such as *E. coli* and they are isolated and purified for injection. This "naked" DNA is usually injected intramuscularly or intradermally. The principle behind a DNA vaccine is that the antigen can be expressed directly by host cells in a way that simulates viral infection and invokes an immune response from the host.

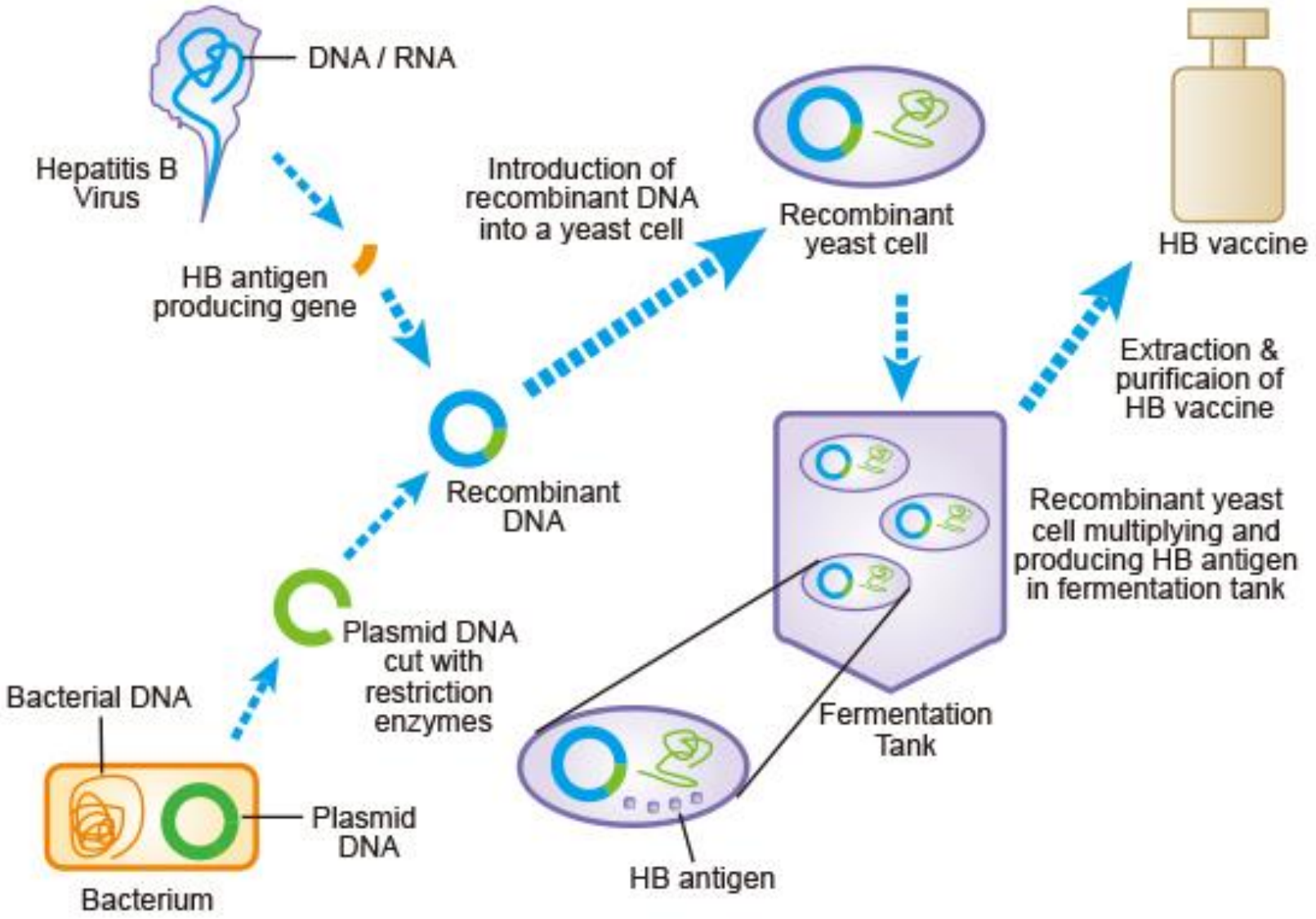

CONSTRUCTED
PLASMID
GENETIC
MATERLAL
COPY OF
DNA FROM
PATHOGEN
EMPTY
PLASMID
DNA
VACCINE
VACCINATION

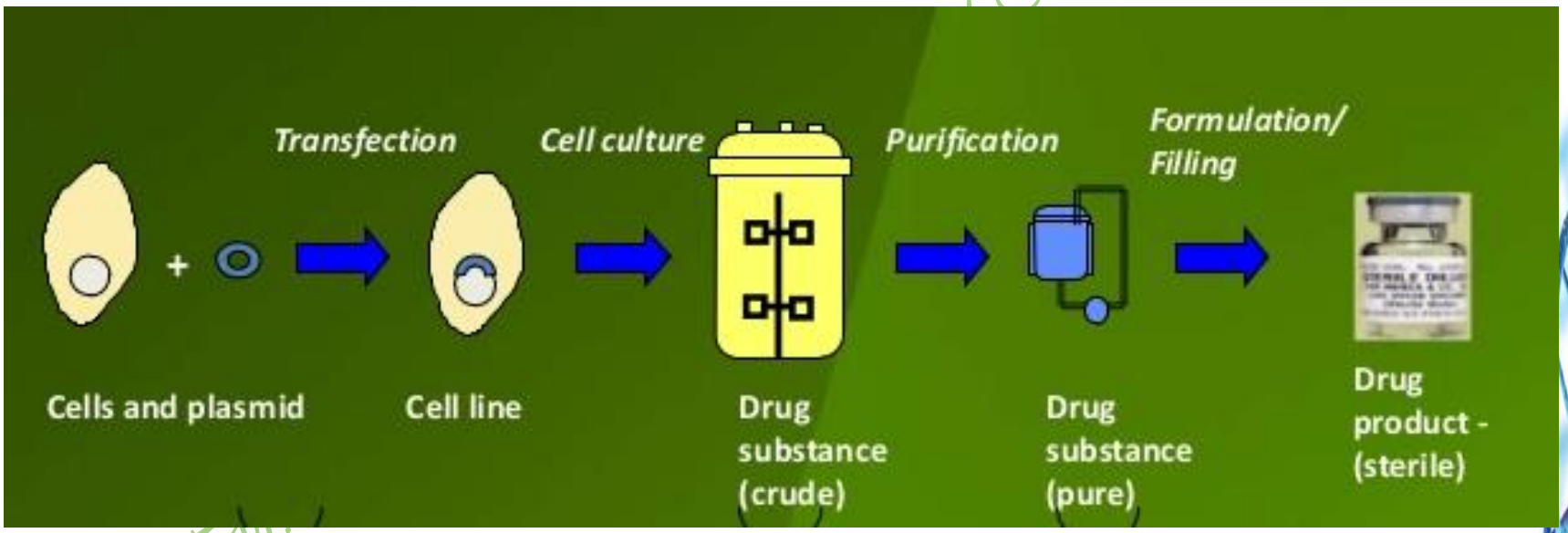
Production of recombinant pharmaceuticals



Recombinant subunit protein vaccine

- These are subunit vaccines containing only a fraction of the pathogenic organism.
- Often time these are synthetic peptides that represent the protein component that induces an immune response.
- But they can also consist of protein subunits (antigens) expressed in a heterologous expression system (*E. coli*, yeast, insect etc.) using recombinant protein expression technologies.
- Most of the vaccines under investigation today are based on such purified recombinant proteins or subunits of antigens.


DNA / RNA
Hepatitis B Virus
HB antigen producing gene
Introduction of recombinant DNA into a yeast cell
Recombinant yeast cell
HB vaccine
Extraction & purificaion of HB vaccine
Recombinant DNA
Recombinant yeast cell multiplying and producing HB antigen in fermentation tank
Plasmid DNA cut with restriction enzymes
Bacterial DNA
Fermentation Tank
Plasmid DNA
HB antigen
Bacterium


Transfection
Cell culture
Purification
Formulation/
Filling
+
Cells and plasmid
Cell line
Drug
substance
(crude)
Drug
substance
(pure)
Drug
product -
(sterile)