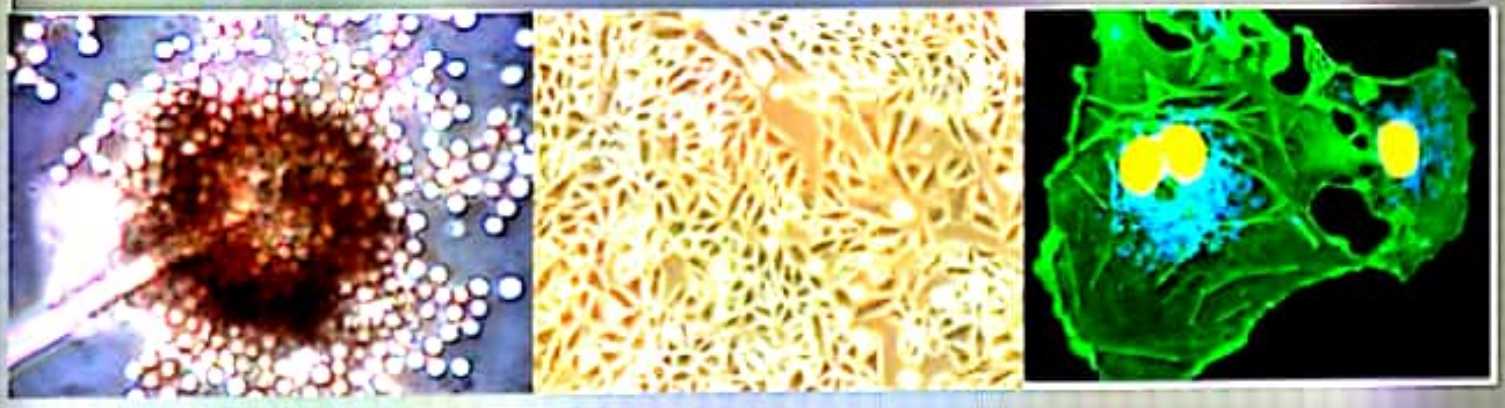
EXPRESSION OF RECOMBINANT PROTEINS IN MAMMALIAN CELL LINES



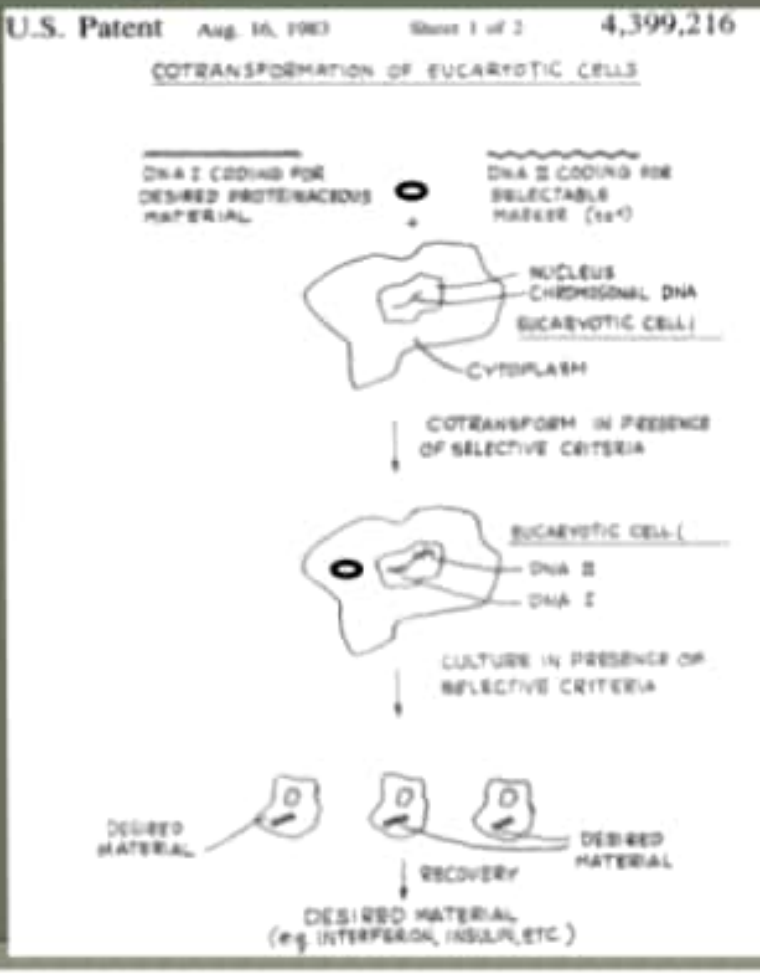
Mammalian Cell Expression Systems

capacity to properly fold and assemble proteins and add humanlike posttranslational Modifications

commercially available therapeutic proteins are produced in mammalian cells.

Outline of Recombinant DNA Technology

1. isolation of the DNA fragments (e.g. A human gene) to be cloned
2. Insertion of the isolated gene into a suitable vector(e.g. a plasmid) to create a recombinant DNA
3. Introduction of the recombinant vector into suitable organism/cell– host
4. Multiplication & selection of clones containing the recombinant molecules
5. Expression of gene to produce the required product (Recombinant proteins)



Established mammalian cell lines

CHO: an epithelial cell line derived from the ovaries of Chinese hamsters (Cricetulus griseus)

DG44: a CHO cell line (Marker-DHFR)
DUK-B11: a CHO cell line (Marker-GS)

NS0: a myeloma cell line derived from B lymphocytes of mice (Mus musculus)

HEK293: an epithelial cell line derived from human embryonic kidney cells transformed with adenovirus DNA

Established mammalian cell lines

BHK: a cell line derived from the kidney cells of baby Syrian golden hamsters

COS: fibroblast cell lines derived from the kidney cells (SV40 transformed) of African green monkeys (Cercopithecus aethiops

PER.C6: a trademarked cell line (derived from a human retinal cell) developed and owned by Crucell Holland BV

TRANSIENT GENE EXPRESSION

cell types used for transient gene expression

- COS ,
- human embryonic kidney(HEK)-293 cells

convenient method for the rapid production of small quantities of protein for initial characterization

rapid testing of vector functionality as well as optimization of different combinations of promoters and other elements in expression vectors

STABLE GENE EXPRESSION

cell types used for stable gene expression
CHO DG44,
CHO-K1
PER.C6
NS0
Sp2/0
BHK

stable integration of plasmid into the host chromosome

STABLE GENE EXPRESSION

Used for long-term (stable) gene expression and when high yields of heterologous proteins are required.

About 140 recombinant proteins are currently approved for therapeutic use, most of them are produced in CHO cells.

Vector design

The first vector was based on a simian virus that can replicate in several mammalian species.

its use is restricted to small inserts because only a limited amount of DNA can be packaged into the viral capsid.

Other vectors that can accommodate larger amounts of cloned DNA are :

Adenovirus: which can be maintained as a multicopy plasmid in some mammalian cells

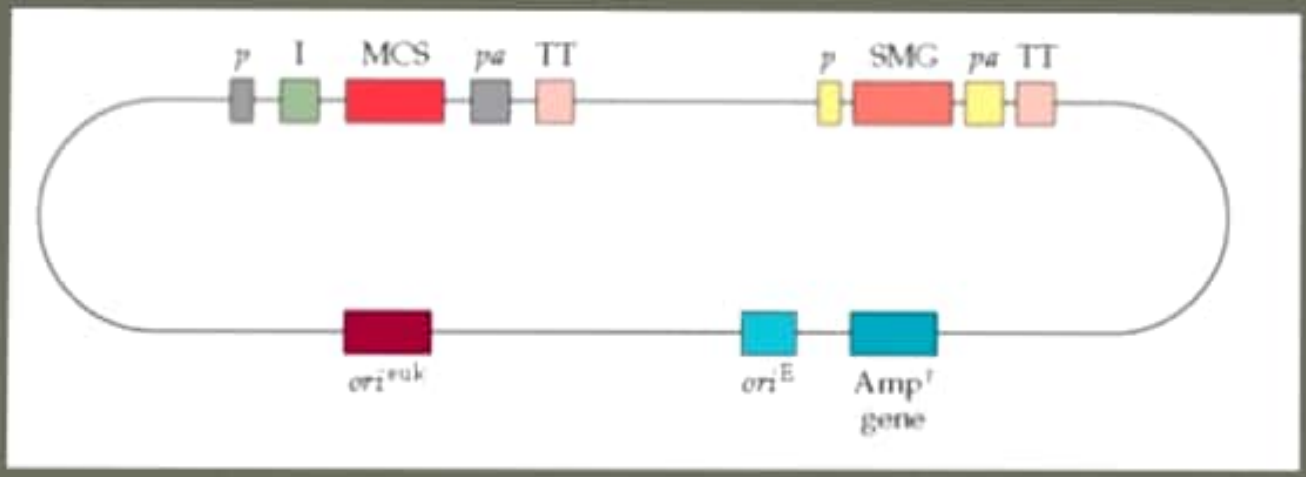
Adeno-associated virus: which can integrate into specific sites in the host chromosome

Vector design

http://vectordb.atcg.com

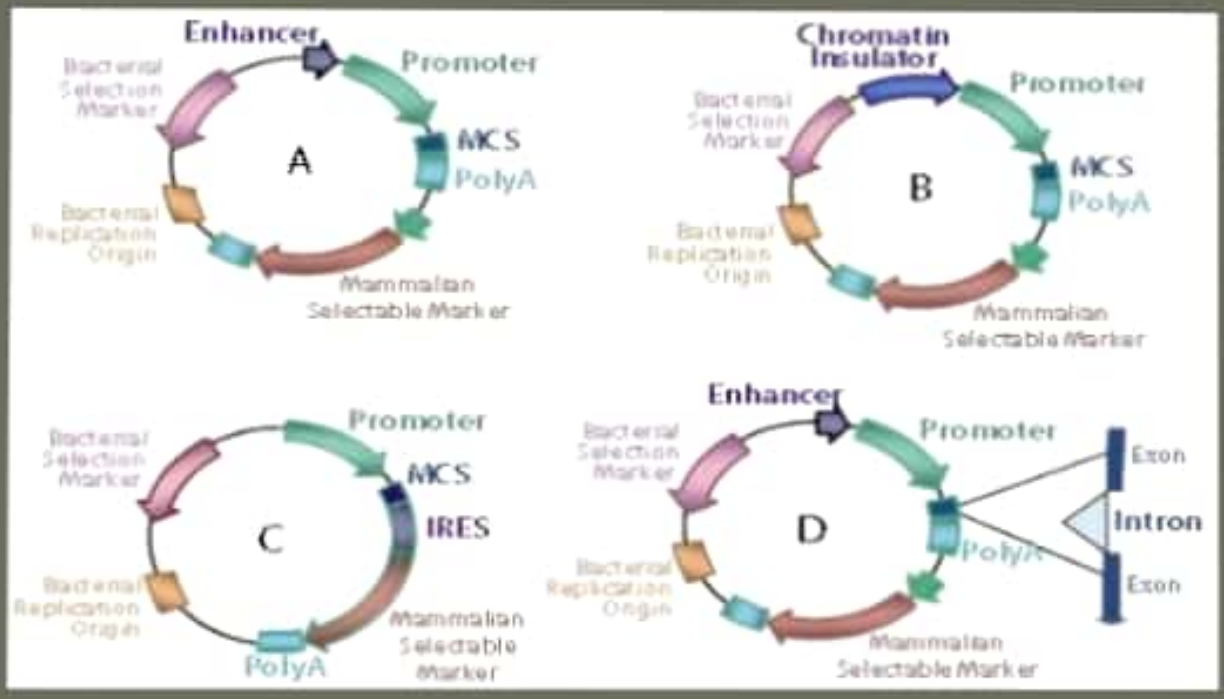
VectorDB contains information on more than 2600 vectors, including phage, plasmid, phasmid, cosmid, viral, and YAC vectors. The database has a search engine and contains annotation and sequence information for many of the vectors. In addition, vectors which are also in GenBank have direct links to that database.

Generalized mammalian expression vector



Multiple cloning site(MCS)
Selectable marker gene (SMG)
Eukaryotic promoter(p)
Polyadenylation (pa)
Termination of transcription (TT) sequences
Intron (I)

Key Features of Mammalian Expression System



A-In a basic expression vector, gene coding sequences are inserted into a multiple cloning site (MCS) under control of a 5′ promoter (with or without an enhancer element) and a 3′ polyadenylation sequence. The selectable marker is under control of a separate set of regulatory elements. Sequences for propagation of the plasmid in bacteria are present on the vector backbone.

B- In vectors containing chromatin insulators (e.g., MARs) or chromatin opening elements (e.g., UCOEs), the element is typically placed upstream — and possibly also downstream — of the promoter.

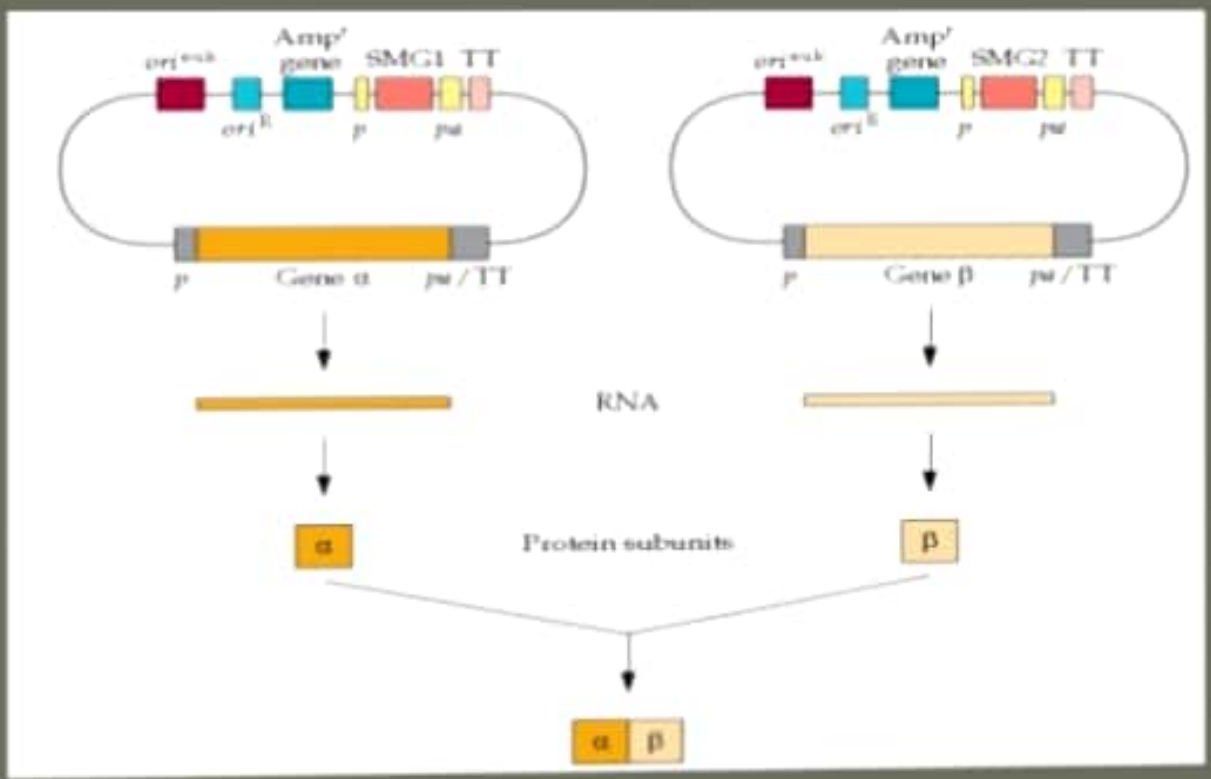
C -Bistronic vectors contain a single cassette for expression of a gene of interest inserted into the MCS and a selectable marker, separated by the IRES and under control of an upstream promoter and 3′ polyA.

D -To facilitate expression, one or more introns are frequently inserted into the coding sequence for a gene of interest.

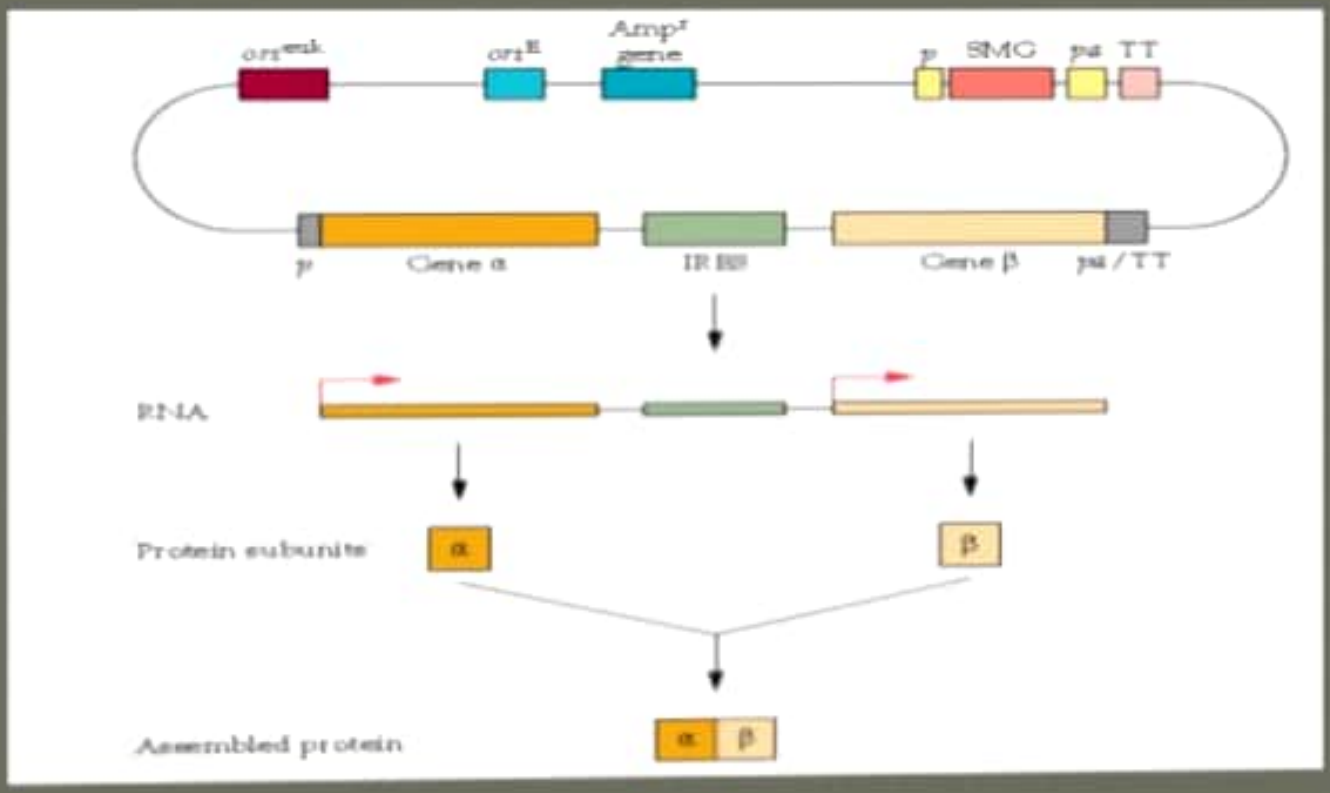
Translation control elements

K: Kozak sequence, e.g.,GCCGCC(A or G)CCAUGG in vertebrates
S: Signal sequence to facilitate secretion
T: Protein sequence (tag) to enhance the purification of the heterologous protein
P: proteolytic cleavage sequence that enables the tag to be removed from the heterologous protein
SC: Stop codon
5' and 3' (UTRs) untranslated regions is important for efficient translation and mRNA stability

Two-vector expression system



Bicistronic expression vector



Selectable Markers for Mammalian Expression Vectors

TABLE 7.7 Selective marker gene systems for mammalian cells

Selective agent	Action of selective agent	Marker gene	Action of marker gene protein
Xyl-A	Damages DNA	Adenine deaminase *(ada)*	Deaminates Xyl-A
Blasticidin S	Inhibits protein synthesis	Blasticidin S deaminases *(Bsr, BSD)*	Deaminates blasticidin S
Bleomycin	Breaks DNA strands	Bleomycin-binding protein *(Ble)*	Binds to bleomycin
G-418 (Geneticin)	Inhibits protein synthesis	Neomycin phosphotransferase *(neo)*	Phosphorylates G-418
Histidinol	Produces cytotoxic effects	Histidinol dehydrogenase *(hisD)*	Oxidizes histidinol to histidine
Hygromycin B	Inhibits protein synthesis	Hygromycin B phosphotransferase *(Hph)*	Phosphorylates hygromycin B
MSX	Inhibits glutamine synthesis	Glutamine synthetase *(GS)*	Cells that produce excess glutamine synthetase survive.
MTX	Inhibits DNA synthesis	Dihydrofolate reductase *(dhfr)*	Cells that produce excess dihydrofolate reductase survive.
PALA	Inhibits purine synthesis	Cytosine deaminase *(codA)*	Lowers cytosine levels in the medium by converting cytosine to uracil
Puromycin	Inhibits protein synthesis	Puromycin *N*-acetyltransferase *(Pac)*	Acetylates puromycin

MSX, methionine sulfoximine; MTX, methotrexate; PALA, *N*-(phosphonacetyl)-L-aspartate; Xyl-A, 9-β-D-xylofuranosyl adenine.