Biopharmaceuticals

Tab. 16.6 Selected biopharmaceutical drugs.

Class	*Indication/ Therapeutic area*	*Market size 2000 ($ million)*	*Market size 2001 ($ million)*
Erythropoetin	Anemia	5787	6803
Insulin	Diabetes	3490	4017
Blood clotting factor	Hemophilia	2400	2585
Colony stimulating factor	Neutropenia	2083	2181
Interferon beta	Multiple sclerosis, hepatitis	1735	2087
Interferon alpha	Cancer, hepatitis	1769	1832
Monoclonal antibody	Cancer	1057	1751
Growth hormone	Growth disorders	1614	1706
Monoclonal antibody	Various	789	1152
Plasminogen activator	Thrombotic disorders	638	642
Interleukin	Cancer, immunology	195	184
Growth factor	Wound healing	98	115
Therapeutic vaccine	Various	31	50
Other various proteins	Various	1834	2006
Total		**23 520**	**27 111**

Biosimilars

Tab. 16.9 Biopharmaceutical drugs losing patent protection by 2006.

Brand name	*Generic name*	*Company*	*2001 global sales ($ m)*	*U.S. Patent expiration (year)*
Epogen/Procrit	Erythropoetin α	Amgen, Johnson & Johnson	6803	2004
Novolin	Human Insulin	Novo Nordisk	1829	2005
Humulin	Human Insulin	Elli Lilly	1061	2003
Neupogen	Filgrastim	Amgen	1380	2006
Avonex	Interferon beta-1a	Biogen	972	2003
Cerezyme/Ceredase	Alglucerase	Genzyme	570	2001
Synagis	Palivizumab	MedImmune	668	2004
Humatrope	Somatropin	Elli Lilly	311	2003
Activase	Alteplase	Genentech, Boehringer Ingelheim	267	2005
Nutropin	Somatropin	Genentech	250	2003
Protropin	Somatrem	Genentech	250	2005

Tab. 16.4 Biopharmaceuticals approved in the United States and/or Europe.

Product	*Organism utilized*	*Company*	*Therapeutic indication*	*Date approved*
Recombinant interferons and interleukins				
Pegasys (Peginterferon α-2a	*E. coli*	Roche	Hepatitis C	2002 (EU, US)
PegIntron A (PEGylated rIFN-α-2b	*E. coli*	Schering-Plough	Chronic hepatitis C	2000 (EU), 2001 (US)
Viraferon (rIFN-α-2 b)	*E. coli*	Schering-Plough	Chronic hepatitis B and C	2000 (EU)
ViraferonPEG (PEGylated rIFN-α-2 b)	*E. coli*	Schering-Plough	Chronic hepatitis C	2000 (EU)
Alfatronol (rh IFN-α-2b	*E. coli*	Schering-Plough	Hepatitis B, C and various cancers	2000 (EU)
Viraferon (rh IFN-α-2 b)	*E. coli*	Schering-Plough	Hepatitis B, C	2000 (EU)
Intron A (rIFN-α-eb	*E. coli*	Schering-Plough	Cancer, genital warts, hepatitis	1986 (US), 2000 (EU)
Alfatronol (rh IFN-α-2b	*E. coli*	Schering-Plough	Hepatitis B, C and various cancers	

Rebetron (combination of ribavirin and rh IFN-α-2 b)	*E. coli*	Schering-Plough	Chronic hepatitis C	1999 (EU)
Infergen (r IFN-α, synthetic type I IFN)	*E. coli*	Schering-Plough	Chronic hepatitis C	1997 (US), 1999 (EU)
Roferon A (rh IFN-α-2b	*E. coli*	Schering-Plough	Hairy cell leukemia	1986 (US)
Rebif (rh IFN-β-1 a)	CHO cells	Ares-Serono	Relapsing/remitting multiple sclerosis	1998 (EU), 2002 (US)
Avonex (rh IFN-β-1 a)	CHO cells	Biogen	Relapsing multiple sclerosis	1997 (EU), 1996 (US)

Tab. 16.4 (continued)

Product	*Organism utilized*	*Company*	*Therapeutic indication*	*Date approved*
Betaseron (rh IFN-β-1 b, differs from human protein by C17 → S)	*E. coli*	Berlex Labs/Chiron	Relapsing/remitting multiple sclerosis	1993 (US)
Betaferon/rh IFN-β-1 b, differs from human protein by C17 → S)	*E. coli*	Schering AG	Multiple sclerosis	1995 (EU)
Kineret (anakinra; rh IL-1 receptor antagonist)	*E. coli*	Amgen	Rheumatoid arthritis	2001 (US)
Neumega (r IL-11, lacks N-terminal proline of native molecule)	*E. coli*	Genetics Institute	Prevention of chemo-therapy-induced thrombocytopenia	1997 (US)
Proleukin (r IL-2, differs from human molecule in that it is devoid of an N-terminal alanine and contains C125 → S substitution	*E. coli*	Chiron	Renal cell carcinoma	1992 (US)
Actimmune (rh IFN-γ-1 b)	*E. coli*	Genentech	Chronic granulo-matous disease	1990 (US)

Recombinant vaccines				
Ambirix	*S. cerevisae*	GlaxoSmithKline	Immunization against Hepatitis A and B	2002 (EU)
Pediarix	*S. cerevisae*	SmithKline Beecham	Immunization against various conditions inducing Hepatitis B (children)	2002 (US)
HBVAXPRO	*S. cerevisae*	Aventis Pharma	Immunization against Hepatitis B	2001 (EU)
Twinrix	*S. cerevisae*	SmithKline Beecham (EU), GlaxoSmithKline (US)	Immunization against Hepatitis A and B	1996 (EU) (adult), 1997 (EU) (pediatric), 2001 (US)
Infanrix-Hexa	*S. cerevisae*	SmithKline Beecham	Immunization against diphtheria, tetanus, pertussis, *Haemophilus influenzae* type B, Hepatitis B and polio	2000 (EU)

Tab. 16.4 (continued)

Product	*Organism utilized*	*Company*	*Therapeutic indication*	*Date approved*
Infanrix-Penta	*S. cerevisae*	SmithKline Beecham	Immunization against diphtheria, tetanus, pertussis, Hepatitis B and polio	2000 (EU)
Hepcare	Mammalian (murine) cell line	Medeva Pharma	Immunization against hepatitis B	2000 (EU)
Hexavac	*S. cerevisae*	Aventis Pasteur	Immunization against diphtheria, tetanus, pertussis, *H. influenzae* type B, hepatitis B and polio	2000 (EU)
Procomvax	*S. cerevisae*	Aventis Pasteur	Immunization against *H. influenzae* type B and hepatitis B	1999 (EU)
Primavax	*S. cerevisae*	Aventis Pasteur	Immunization against diphtheria, tetanus and hepatitis B	1998 (EU)
Infanrix Hep B	*S. cerevisae*	SmithKline Beecham	Immunization against diphtheria, tetanus, pertussis and hepatitis B	1997 (EU)

Twinrix	*S. cerevisae*	SmithKline Beecham	Immunization against hepatitis A and B	1996 (EU) (adult), 1997 (EU)
Comvax	*S. cerevisae*	Merck	Vaccination of infants against *H. influenzae* type B and hepatitis B	1996 (US)
Tritanrix-HB	*S. cerevisae*	SmithKline Beecham	Vaccination against hepatitis B, diphtheria, tetanus and pertussis	1996 (US)
Recombivax	*S. cerevisae*	Merck	Hepatitis B prevention	1986 (US)
Lymerix	*E. coli*	SmithKline Beecham	Lyme disease vaccine	1998 (US)
Tricelluvax		Chiron SpA	Immunization against diphtheria, tetanus and pertussis	1999 (EU)

Tab. 16.4 (continued)

Product	*Organism utilized*	*Company*	*Therapeutic indication*	*Date approved*
Recombinant blood factors				
Helixate NexGen	BHK cells	Bayer	Hemophilia A	2000 (EU)
ReFacto	CHO cells	Genetics Institute/ Wyeth Europa	Hemophilia A	1999 (EU), 2000 (US)
Kogenate	BHK cells	Bayer	Hemophilia A	1993 (US), 2000 (EU)
Bioclate	CHO cells	Aventis Behring	Hemophilia A	1993 (US)
Recombinate	Animal cell line	Baxter Healthcare/ Genetics Institute	Hemophilia A	1992 (US)
NovoSeven	BHK cells	Novo Nordisk	Some forms of hemophilia	1996 (EU), 1999 (US)
Benefix	CHO cells	Genetics Institute	Hemophilia B	1997 (US, EU)

Recombinant anticoagulants

Tenecteplase	CHO cells	Boehringer Ingelheim	Myocardial infarction	2001 (EU)
TNKase	CHO cells	Genentech	Myocardial infarction	2000 (US)
Ecokinase	*E. coli*	Galenus Mannheim	Acute myocardial infarction	1996 (EU)
Rapilysin	*E. coli*	Roche	Acute myocardial infarction	1996 (EU)
Retavase	*E. coli*	Boehringer Mannheim/Centocor	Acute myocardial infarction	1996 (US)
Activase	CHO cells	Genentech	Acute myocardial infarction	1987 (US)
Refludan	*S. cerevisae*	Hoechst Marion Roussel/Behringwerke AG	Anticoagulation therapy for heparin-associated thrombocytopenia	1997 (EU), 1998 (US)
Revasc	*S. cerevisae*	Aventis	Prevention of venous thrombosis	1997 (EU)

Recombinant hormones

Insulin

Actrapid/Velosulin/ Monotard/Insulatard/ Protaphane/Mixtrad/ Actraphane/Ultratard	*S. cerevisae*	Novo Nordisk	Diabetes mellitus	2002 (EU)
Novolog	*S. cerevisae*	Novo Nordisk	Diabetes mellitus	2001 (US)
Novolog Mix 70/30	*S. cerevisae*	Novo Nordisk	Diabetes mellitus	2001 (US)
Novomix 30	*S. cerevisae*	Novo Nordisk	Diabetes mellitus	2000 (EU)
Lantus	*E. coli*	Aventis	Diabetes mellitus	2000 (EU, US)
Optisulin	*E. coli*	Aventis	Diabetes mellitus	2000 (EU)
NovoRapid	*E. coli*	Novo Nordisk	Diabetes mellitus	1999 (EU)
Liprolog	*E. coli*	Eli Lilly	Diabetes mellitus	1997 (EU)
Insuman	*E. coli*		Diabetes mellitus	1997 (EU)
Humalog	*E. coli*	Eli Lilly	Diabetes mellitus	1996 (EU, US)
Novolin	*E. coli*	Novo Nordisk	Diabetes mellitus	1991 (US)
Humulin	*E. coli*	Eli Lilly	Diabetes mellitus	1982 (US)

Human growth hormone (hGH)				
Somavert	*E. coli*	Pfizer	Treatment of acromegaly	2003 (US), 2002 (E()U)
Nutropin AQ	*E. coli*	Schwarz Pharma	Growth failure/ Turner's syndrome	1994 (US), 2001 (EU)
Serostim		Serono Laboratories	Treatment of AIDS-associated catabolism/ wasting	1996 (US)
Saizen		Serono Laboratories	hGH deficiency in children	1996 (US)
Genotropin	*E. coli*	Pharmacia & Upjohn	hGH deficiency in children	1995 (US)
Norditropin		Novo Nordisk	Treatment of growth failure in children due to inadequate growth hormone secretion	1995 (US)
BioTropin		Savient Pharmaceuticals	hGH deficiency in children	1995 (US)
Nutropin	*E. coli*	Genentech	hGH deficiency in children	1994 (US)
Humatrope	*E. coli*	Eli Lilly	hGH deficiency in children	1987 (US)
Protropin	*E. coli*	Genentech	hGH deficiency in children	1985 (US)

Product	*Organism utilized*	*Company*	*Therapeutic indication*	*Date approved*
Follicle-stimulating hormone				
Follistim	CHO cells	NV Organon	Infertility	1997 (US)
Puregon	CHO cells	NV Organon	Anovulation and superovulation	1996 (EU)
Gonal F	CHO cells	Ares-Serono	Anovulation and superovulation	1995 (EU), 1997 (US)
Other hormones				
Forsteo (human parathyroid hormone)	*E. coli*	Eli Lilly	Treatment of established osteoporosis in post-menopausal women	2003 (EU)
Forteo (human parathyroid hormone)	*E. coli*	Eli Lilly	Treatment of osteoporosis in some post-menopausal women	2002 (US)
Ovitrelle/Ovidrelle (choriogonadotropin)	CHO cells	Serono	Used in selected assisted reproductive techniques	2000 (US), 2001 (EU)

Tyrogen (human TSH)	CHO cells	Genzyme	Detection/treatment of thyroid cancer	1998 (US), 2000 (EU)
Luveris (human luteinizing hormone	CHO cells	Ares-Serono	Some forms of infertility	2000 (EU)
Forcaltonin (salmon calcitonin)	*E. coli*	Unigene	Paget's disease	1999 (EU)
Glucagen (human glucagon)	*S. cerevisae*	Novo Nordisk	Hypoglycemia	1998 (US)
Recombinant hematopoietic growth factors				
Erythropoietin				
Aranesp	CHO cells	Amgen	Treatment of anemia	2001 (US, EU)
Nespo	CHO cells	Dompe Biotech	Treatment of anemia	2001 (EU)
Neorecormon	CHO cells	Roche	Treatment of anemia	1997 (EU)
Procrit	Mammalian cell line	Ortho Biotech	Treatment of anemia	1990 (US)
Epogen	Mammalian cell line	Amgen	Treatment of anemia	1989 (US)

Product	*Organism utilized*	*Company*	*Therapeutic indication*	*Date approved*
Granulocyte-macrophage colony stimulating factor				
Neulasta		Amgen/Dompe Biotech	Chemotherapy-induced neutropenia	2002 (US, EU)
Leukine	*E. coli*	Immunex (now Amgen)	Autologous bone marrow transplantation	1991 (US)
Neupogen	*E. coli*	Amgen	Chemotherapy-induced neutropenia	1991 (US)
Monoclonal antibody-based products				
Bexxar (against CD20)	Mammalian cell line	Corixa/ GlaxoSmithKline	Treatment of CD20 positive follicular non-Hodgkin's lymphoma	2003 (US)
Xolair (binds IgE)	CHO cells	Genentech	Asthma	2003 (US)
Humira (against TNF)	Mammalian cell line	Abbott	Rheumatoid arthritis	2002 (US)
Zevalin (against CD20)	CHO cells	Idec Pharmaceuticals	Non-Hodgkin's lymphoma	2002 (US)

Mabcampth (EU), Campath (US) (against CD52)	Millenium, ILEX, Berlex	Chronic lymphocytic leukemia	2001 (EU, US)
Mylotarg (against CD33)	Wyeth	Acute myeloid leukemia	2000 (US)
Herceptin (against human epidermal growth factor receptor 2, HER2)	Genentech, Roche	Treatment of metastatic breast cancer, in case tumor over-expresses HER2 protein	1998 (US), 2000 (EU)
Remicade (against TNF-α)	Centocor	Treatment of Crohn's disease	1998 (US), 1999 (EU)
Synagis (against epitope on the surface of respiratory syntactical virus)	MedImmune, Abbott	Prophylaxis of lower-tract respiratory disease caused by syncytial virus in pediatric patients	1998 (US), 1999 (EU)
Mabthera (against CD20 surface antigen)	Hoffmann-La Roche	Non-Hodgkin's lymphoma	1998 (EU)
Rituxan (against CD20 surface antigen)	Genentech/IDEC Pharmaceuticals	Non-Hodgkin's lymphoma	1997 (US)
ReoPro (against the platelet surface receptor GPIIb/IIIa)	Centocor	Prevention of blood clots	1994 (US)
Orthoclone OKT3 (against CD3)	Ortho Biotech	Reversal of acute kidney transplant rejection	1986 (US)

Interleukin 6

Fusion strategy for E.coli production

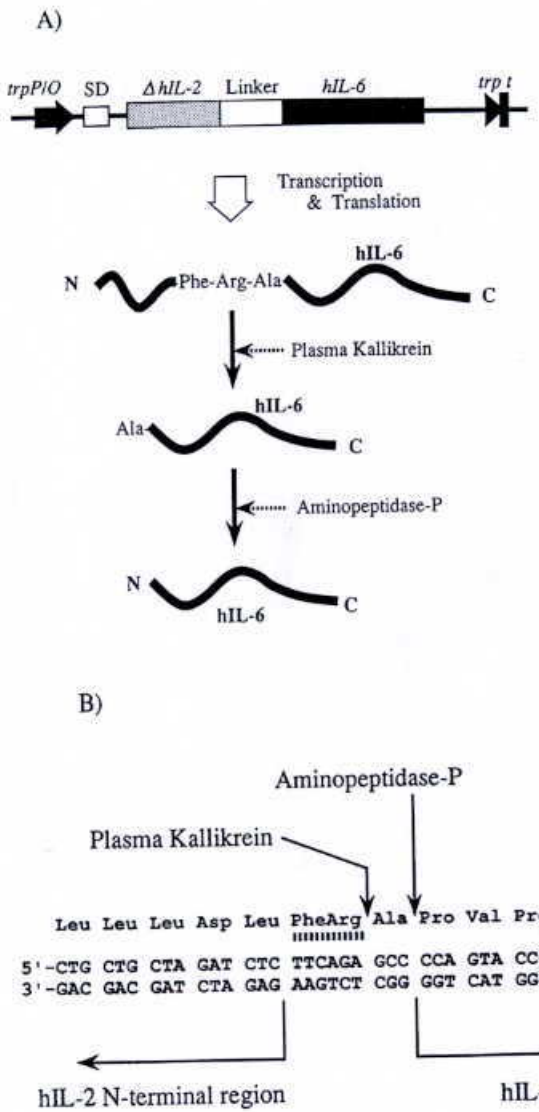


Figure 1 Fusion gene expression system for production of hIL-6. (A) Preparation strategy of the mature hIL-6 from the fusion protein by enzymatic cleavage processing. (B) Structure of the fusion protein at the junction point. (From Ref. 107)

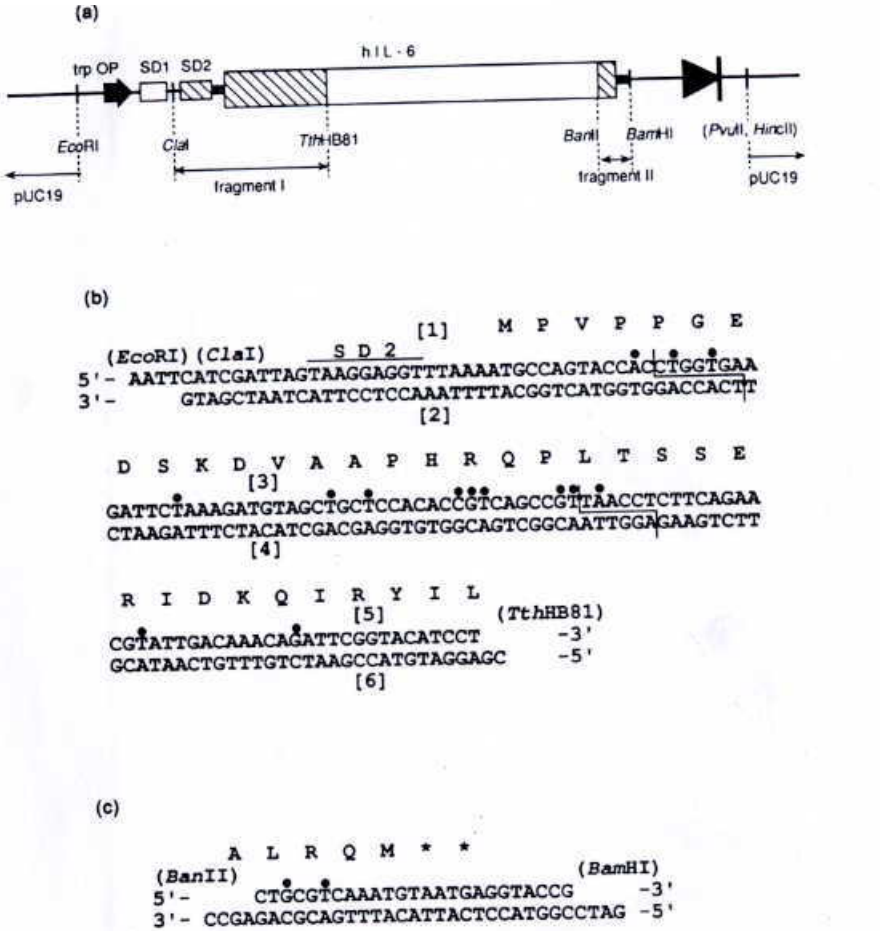


Figure 2 Structure of pBSF2-SD7 used for the high-level expression of hIL-6. (a) Schematic diagram of t structure of the expression system. The hatched boxes represent chemically synthesized DNA fragments. Nucleotide sequences of the fragment I, and (c) the fragment II. The dots above the nucleotides indicate exchanged bases. (From Ref. 61)

hGH

Human Growth Hormone - HGH

hGH

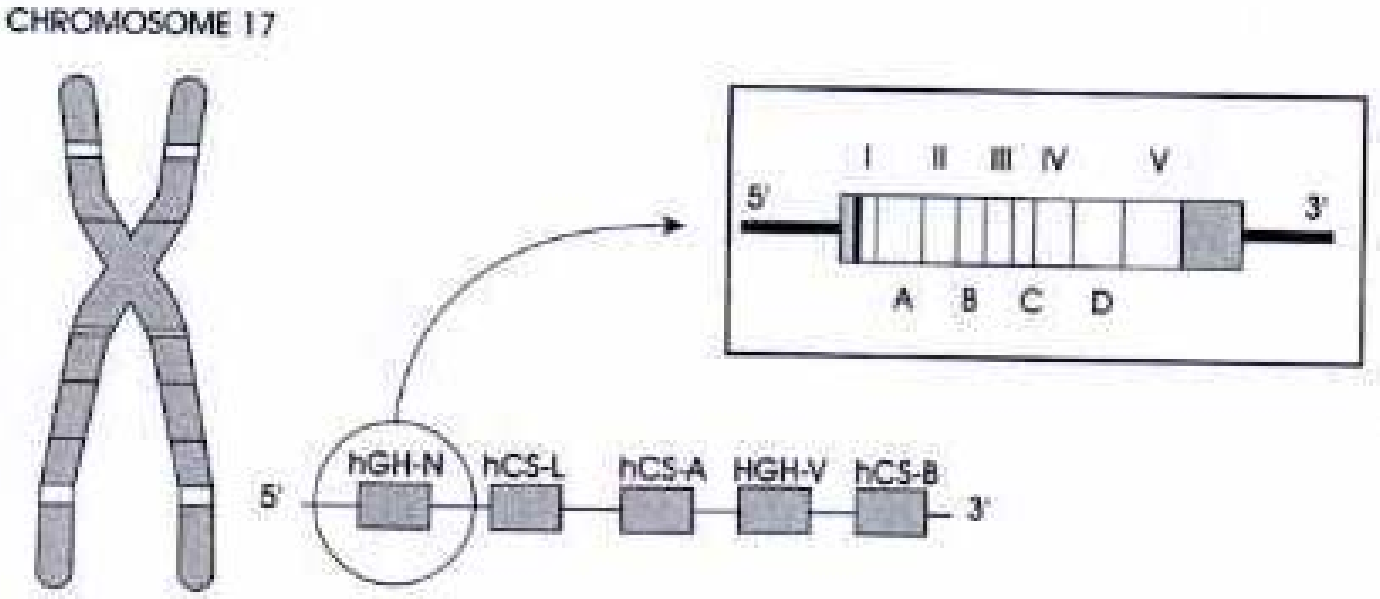


Figure 2. Gene responsible for the synthesis of GH. HGH-N: human growth hormone normal, hGH-V human growth hormone variant, hCS-L: human chorionic somatomammotropin like, hCS-A and hCS-B human chorionic somatomammotropin.

hGH

Growth hormone (GH) is the most abundant anterior pituitary hormone that accounts for 4-10 % of the wet weight of the anterior pituitary in the human adult amouting to about 5-10 mg per gland.

There are several forms of GH, but the predominant form secreted under physiological conditions has 191 amino acids (aa), a molecular weight of 22,650 Da and is synthesized by the acidophil cells (somatotrophic cells) in the pars distalis. The hormone derives from a prohormone and is converted to GH by proteolysis (Figure 1).

Figure 1. Structure of GH.

hGH

Intracellular Production

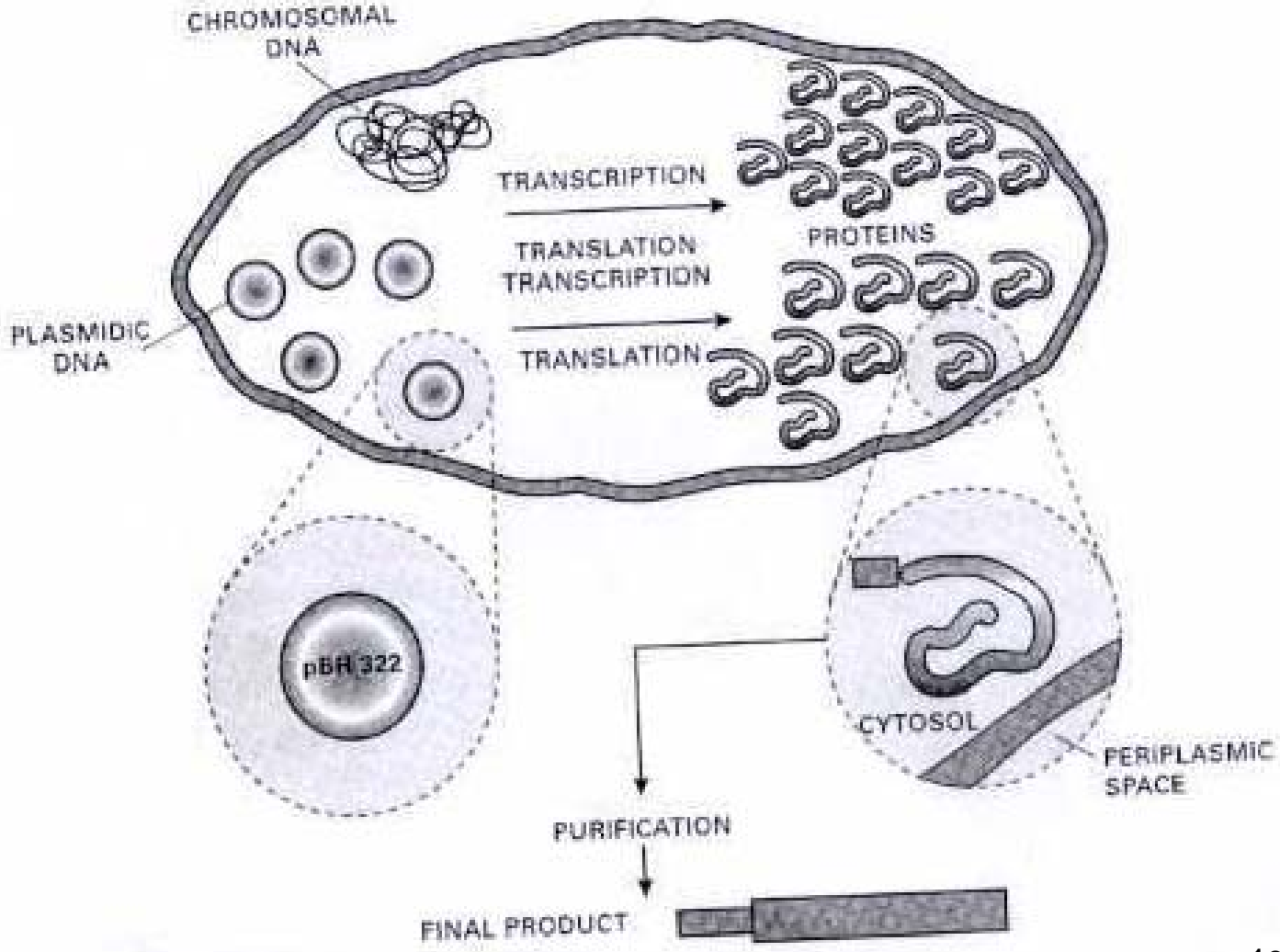


Figure 9. First GH production technique by *E. coli*.

1st aa replaced by Met

Met

hGH

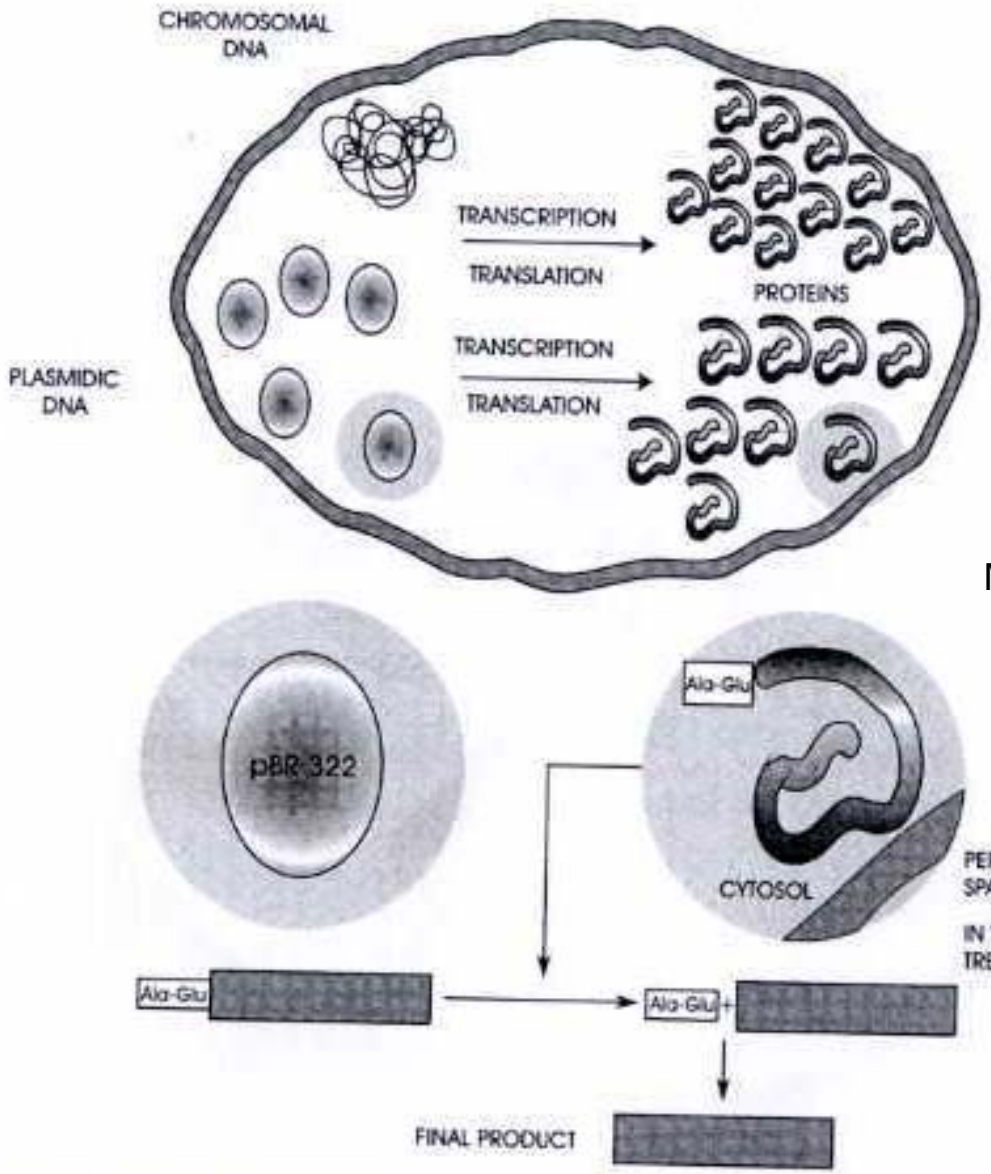
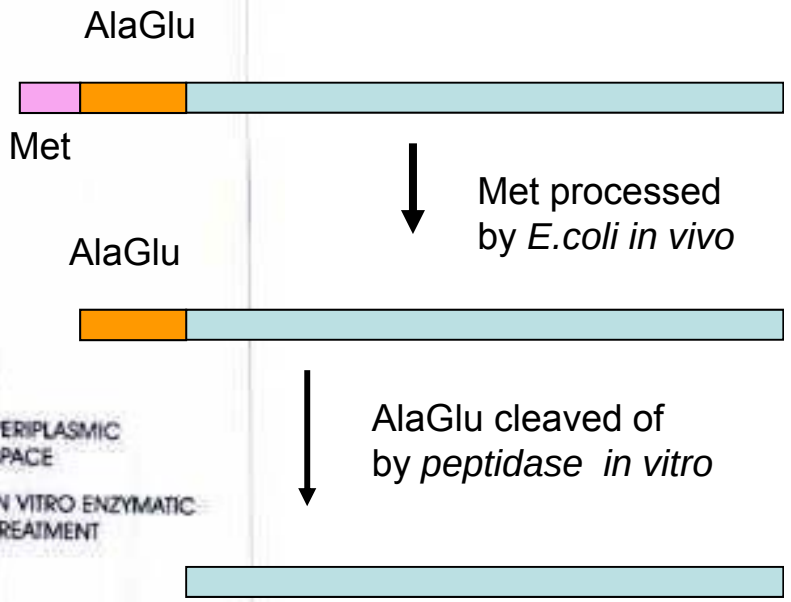


Figure 10. Second GH production technique by *E. coli*.

Intracellular Production



hGH

Secretory Expression

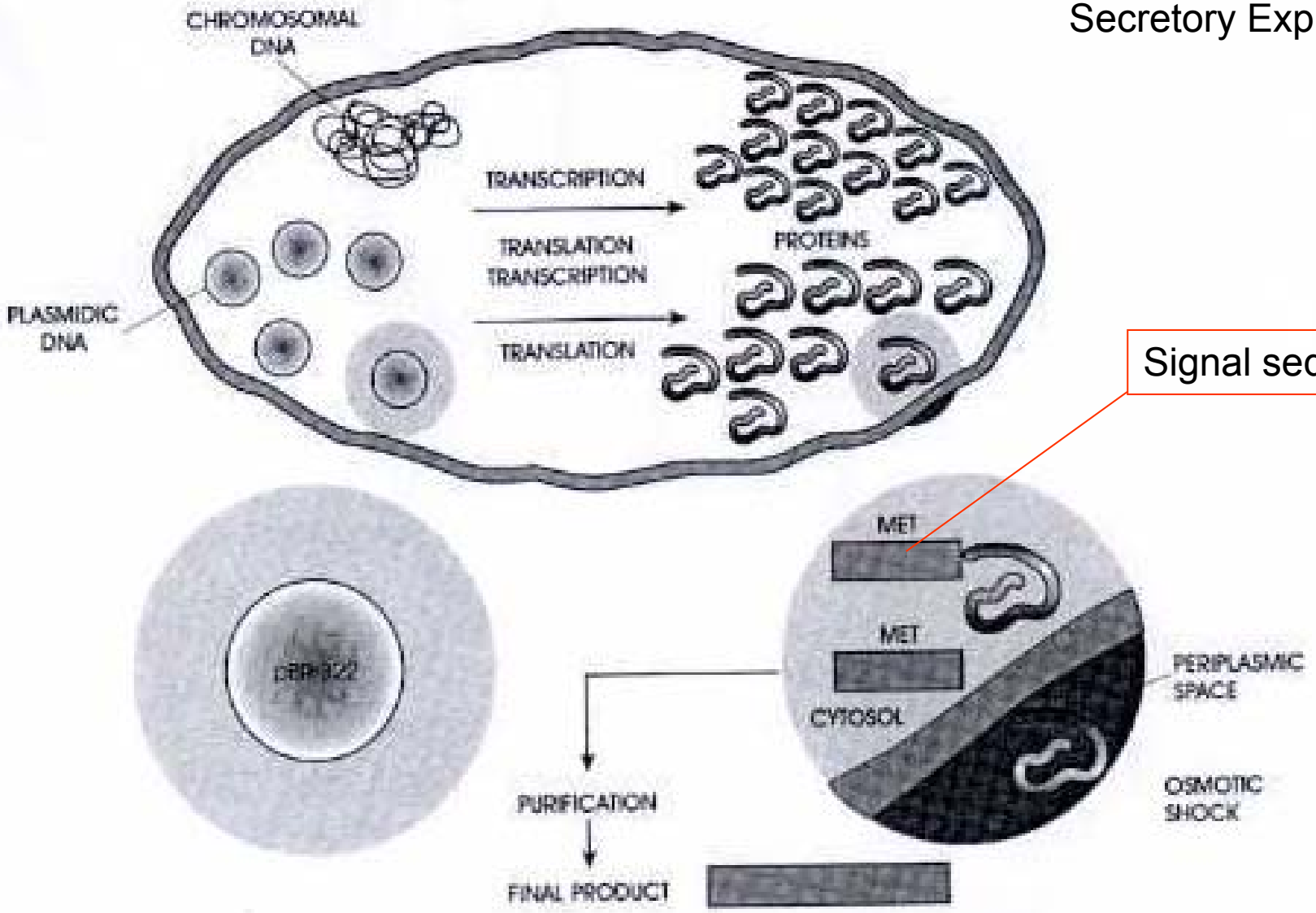


Figure 11. Third synthetic procedure for GH synthesis in *E. coli*.

hGH

Secretory Production in Eucaryotic Cell Line

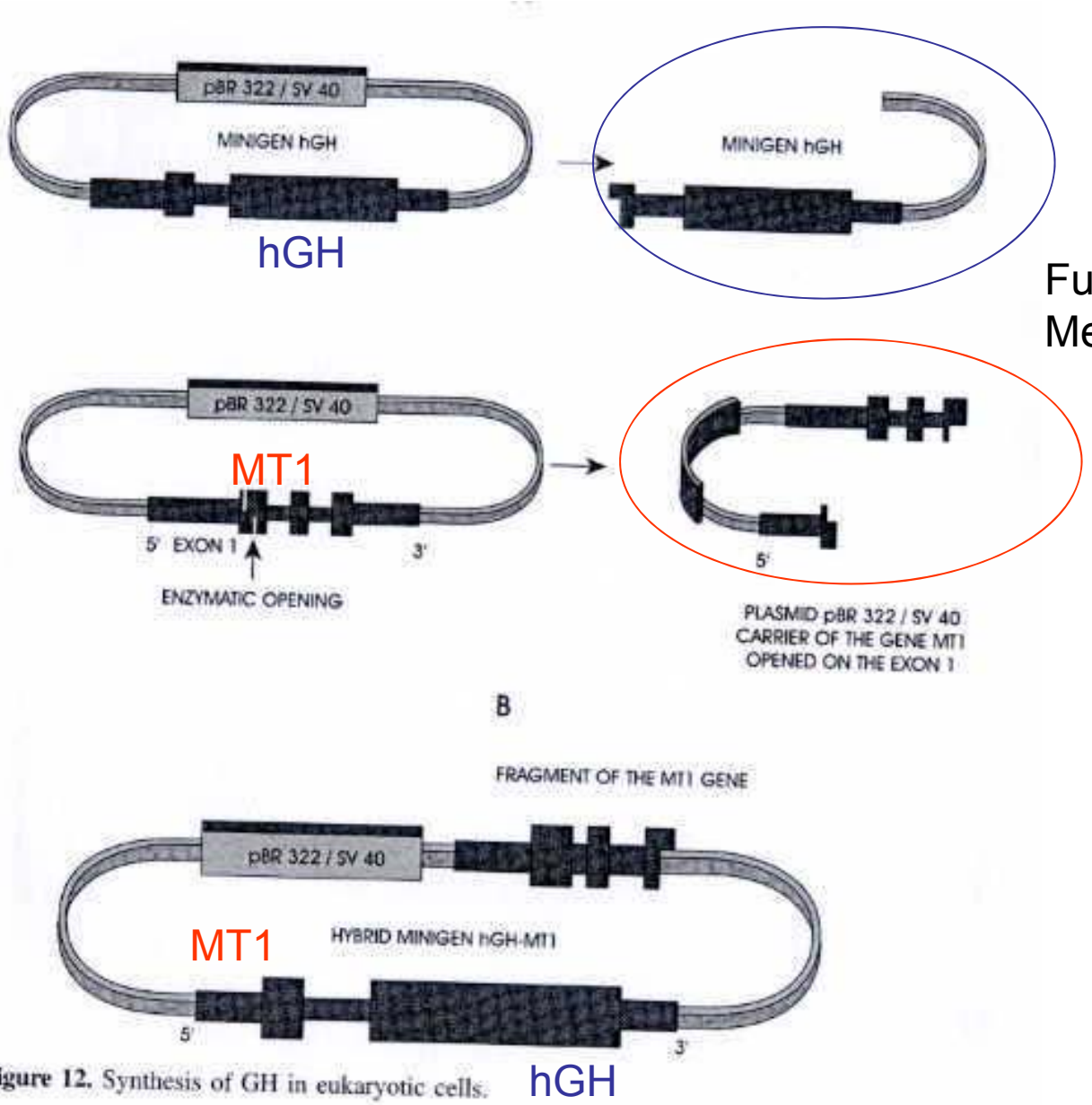


Figure 12. Synthesis of GH in eukaryotic cells.

Fusion of hGH Gene to Metallothionein Promoter

hGH

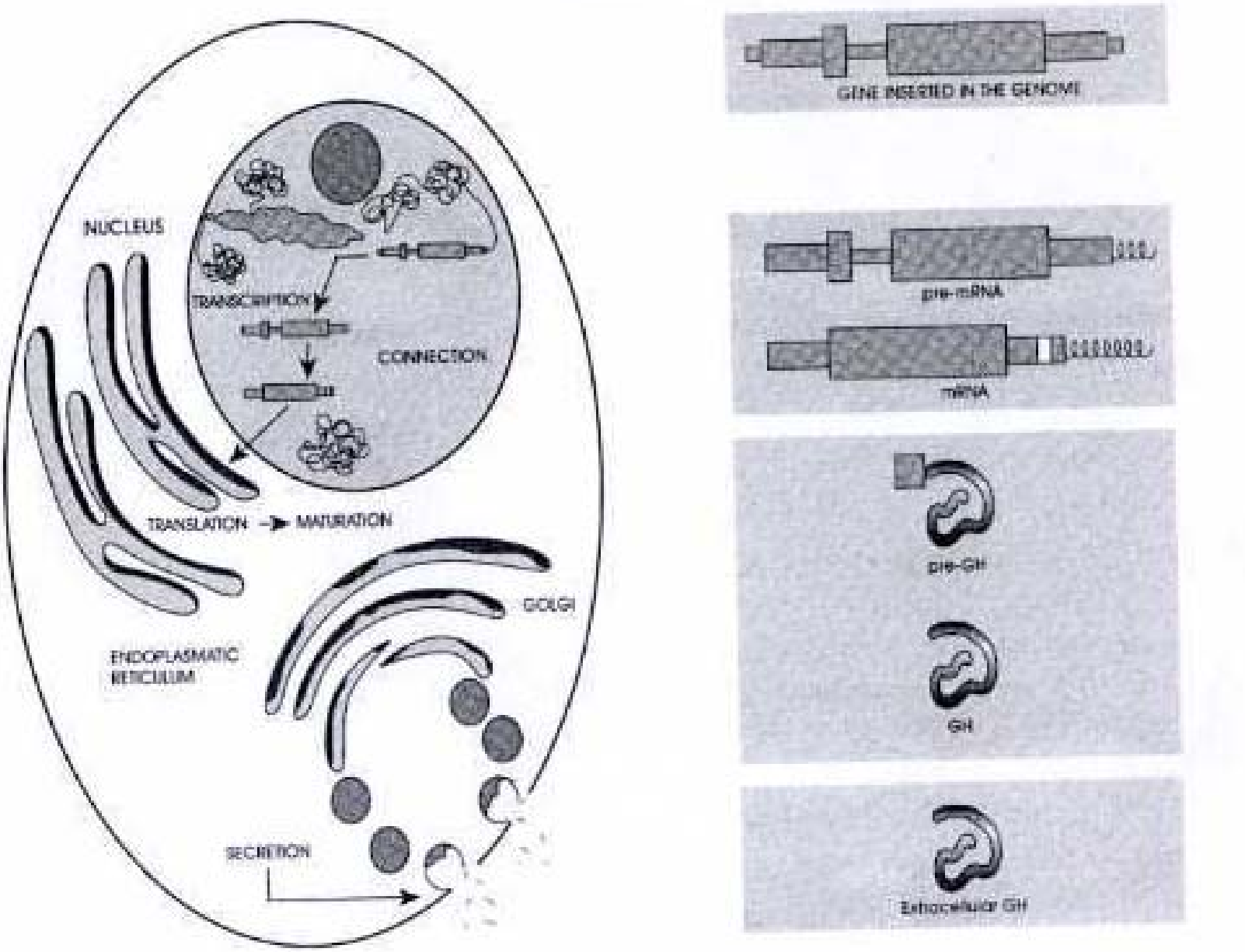
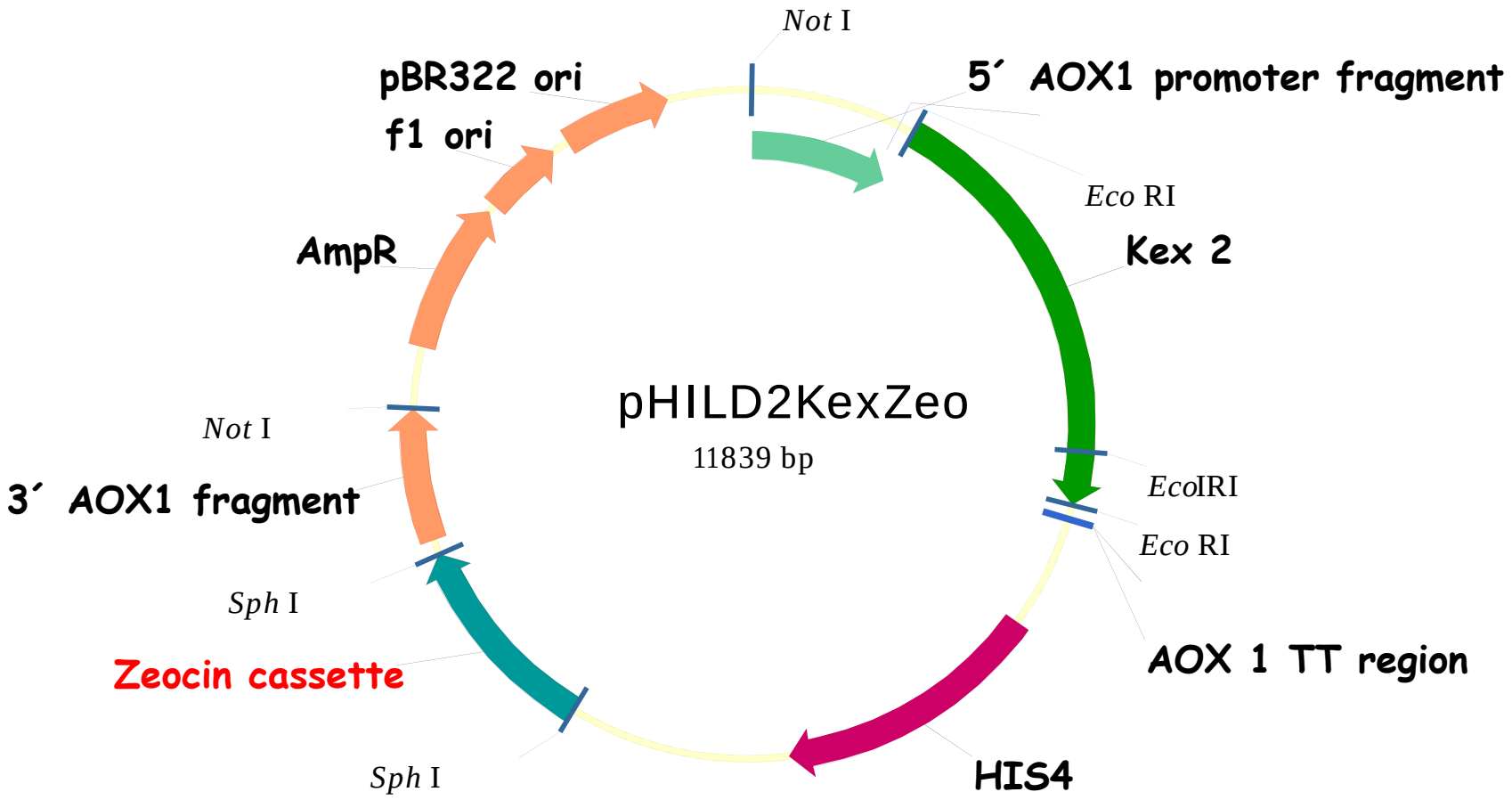


Figure 13. Secretion of GH to the medium from an eukaryotic cell.

Secretory systems → Engineeering

Construction of pHILαHZeo

Co-Expression of KEX2



Integration in HIS4 locus is possible with selection for Zeozin resistence

Secretory systems → Engineeering

Co-expression of Processing enzymes → KEX2

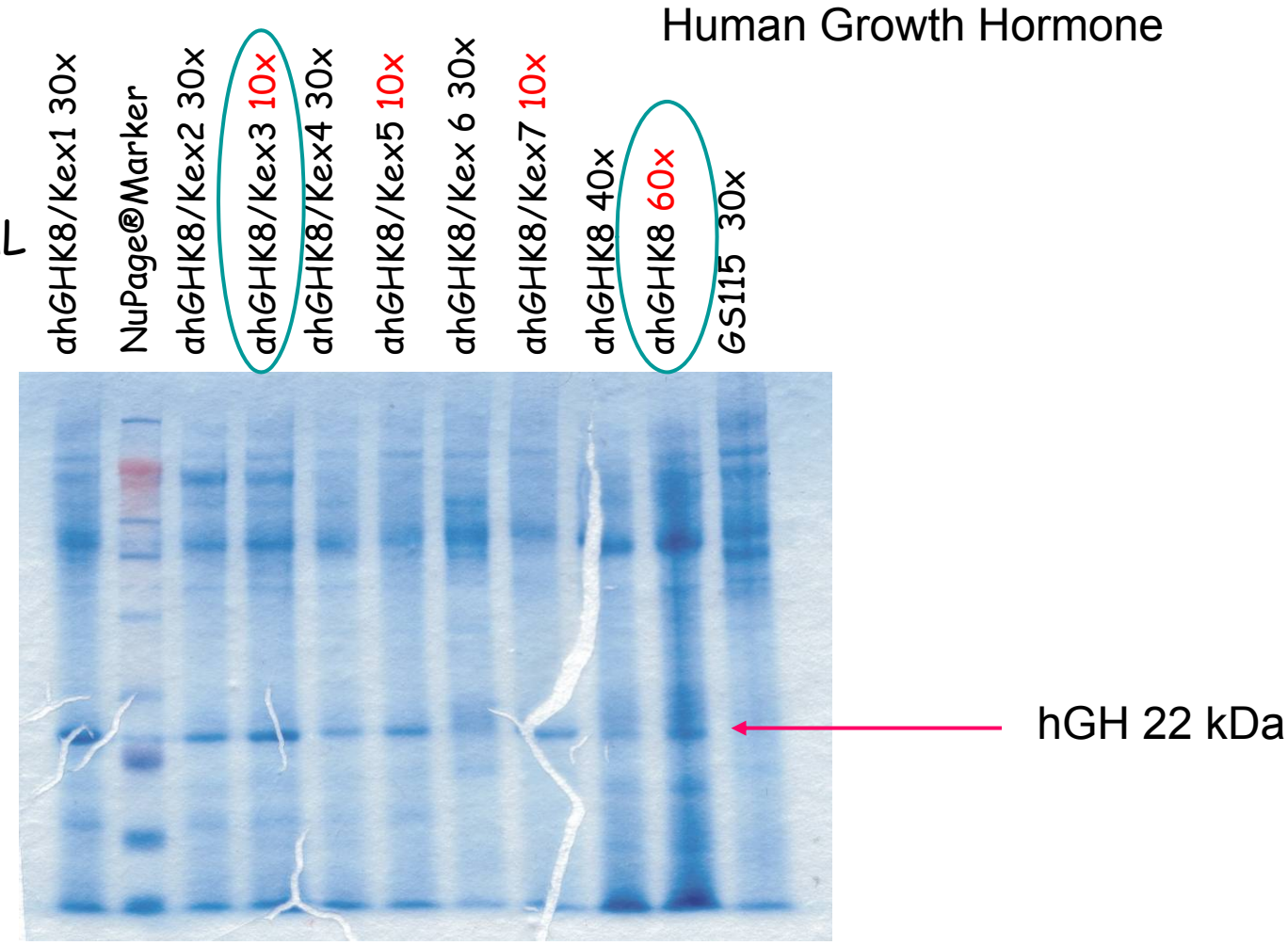
Fermentation: 250 mL wide necked baffled flasks

concentrated media 4 days after harvesting, stored at 4°C

conditions:

BMG, 140rpm,

29°C



hFSH

Folicle Stimulating Hormone - FSH

Table 1. Use of Preparations with FSH Activity in Clinical Practice

1945	First treatments to induce ovulation with pregnant mare serum gonadotropin obtained from the urine of pregnant mares. Extracts contained non-human heterologous proteins
1950s	Preparations of human pituitary gonadotropins with FSH and LH activity
1962	Extracts from the urine of postmenopausal women (human menopausal gonadotropin) with FSH and LH activity
1983	Preparations of urinary FSH, lacking LH in practice, but with scant purity (active ingredient 1-2 % of the product)
1993	Urinary FSH highly purified by immunochromatography (active ingredient > 95 % of product)
1995	Recombinant human FSH (follitropin-α) obtained from mammalian cells (Chinese hamster ovary) (Gonal F®)

hFSH

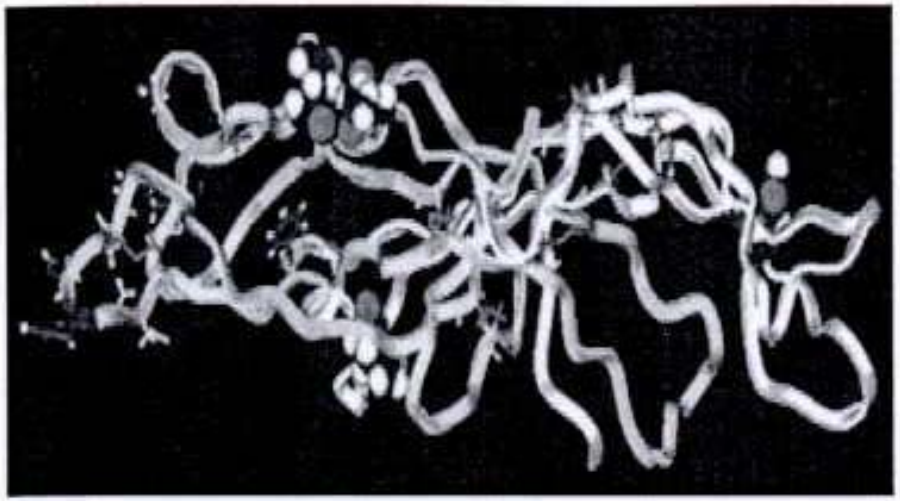


Figure 1. Three-dimensional diagram of the human FSH molecule (see color plates, page XXIII).

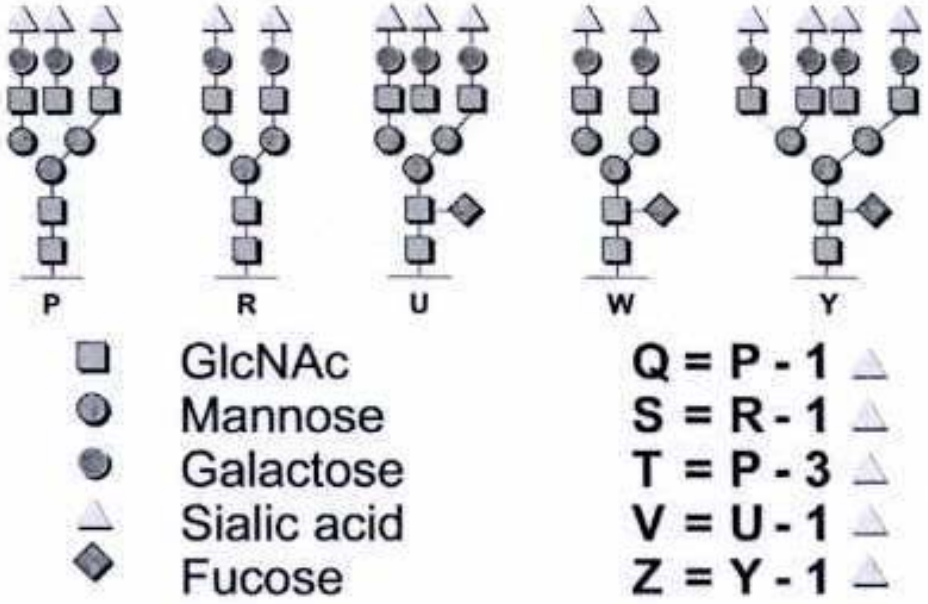


Figure 2. Structure of the lateral glycidic chains linked to the α and β subunits of human FSH (see colo plates, page XXIII).

hFSH

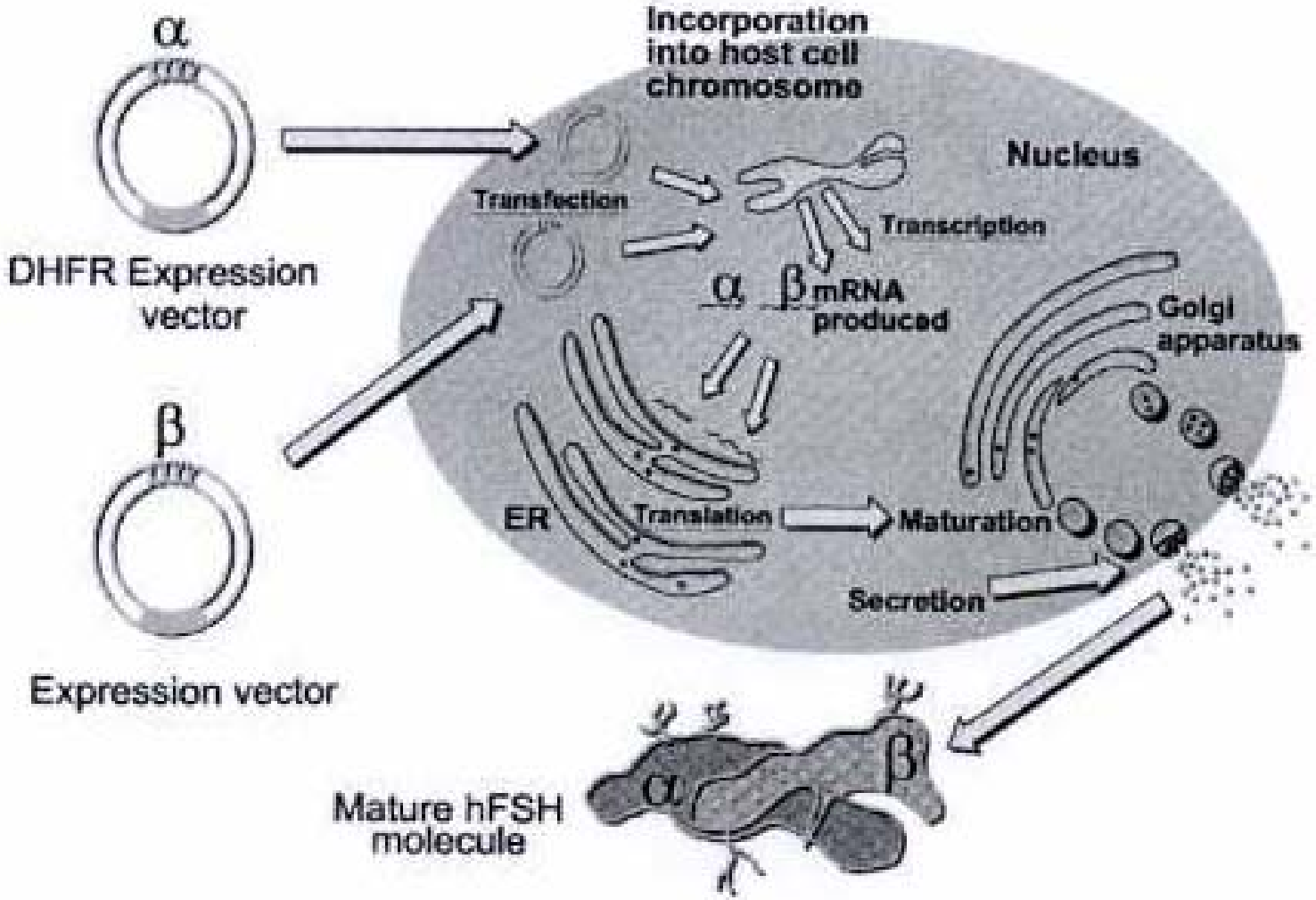


Figure 4. Expression of rhFSH in eukaryotic cells (CHO) (see color plates, page XXIV).

hFSH

Table 3. Physicochemical Analysis and Product Specifications of Urinary and Recombinant Gonadotropin Preparations

	Older Preparations	**Highly Purified Urofollitropin (u-FSH)**	**Recombinant Human FSH (rhFSH; Gonal-F®)**
Potency	*in vivo* bioassay	*in vivo* bioassay	*in vivo* bioassay
Specific activity (IU mg^{-1} protein)	40-150	approximately 9,000	> 10,000
Protein content 75 IU (µg)	370-750	6-11	5
Active protein content in bulk (% FSH)	< 3 %	> 95 %	> 99.9 %
Residual LH activity	0.7 IU per 75 IU FSH	Negligible	None
Isoelectric point	?	3-5.5	3.5-6.1

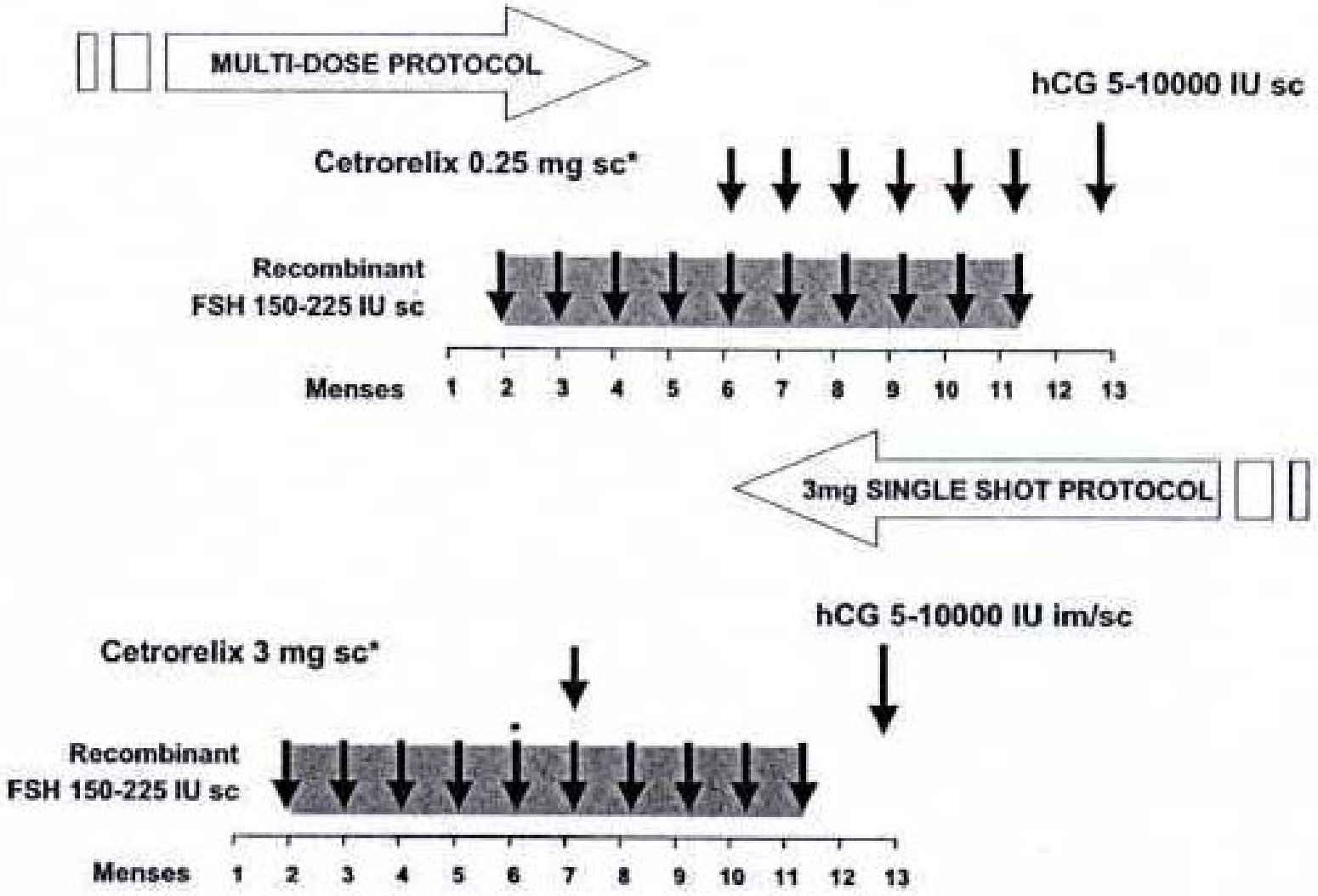


Figure 9. Superovulation regimes for IVF-ICSI with recombinant FSH and GnRH antagonist (cetrorelix, Cetrotide®) administration.

HBV

Hepatitis B Vaccine

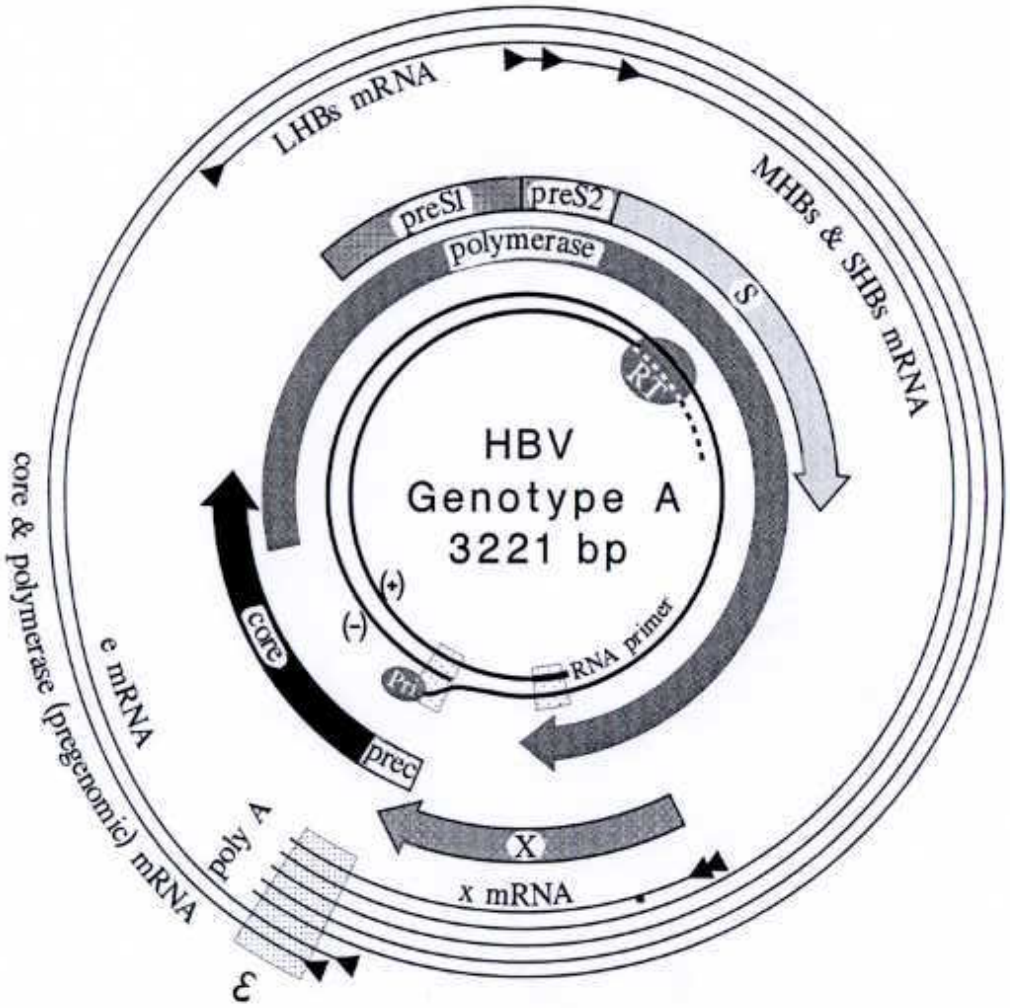


Figure 2. Schematic diagram of the HBV genome and its genetic organization. The inner circle represents the viral DNA as found in virions. The arrows represent the 4 different ORFs. Outer circles represent the coterminal viral mRNAs as found in infected cells. The 5'end of (−) strand DNA is linked with the priming domain (Pri), the 3' end of the (+) strand DNA is associated with the reverse transcriptase domain (RT) of the viral polymerase (modified from [33]).

HBV

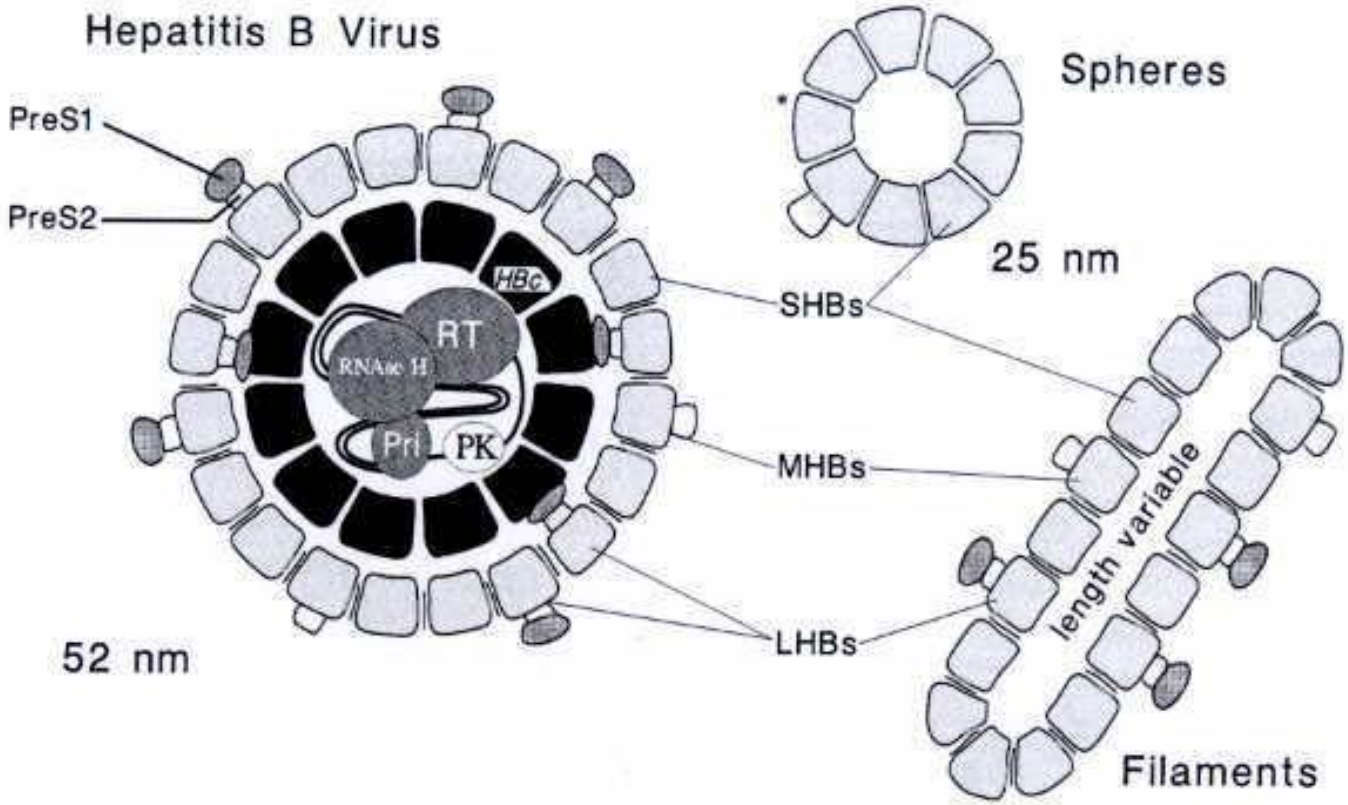


Figure 3. Schematic diagram of hepadnavirus particles. The virus particles contain an internal nucleocapsid (HBc), the viral genome, the polymerase consisting of domains with reverse transcriptase activity (RT), RNaseH and a domain serving as primer for the synthesis of (−) strand DNA (Pri). The subviral particles shown on the right, are made up only of surface proteins in different compositions (modified from [33]).

HBV

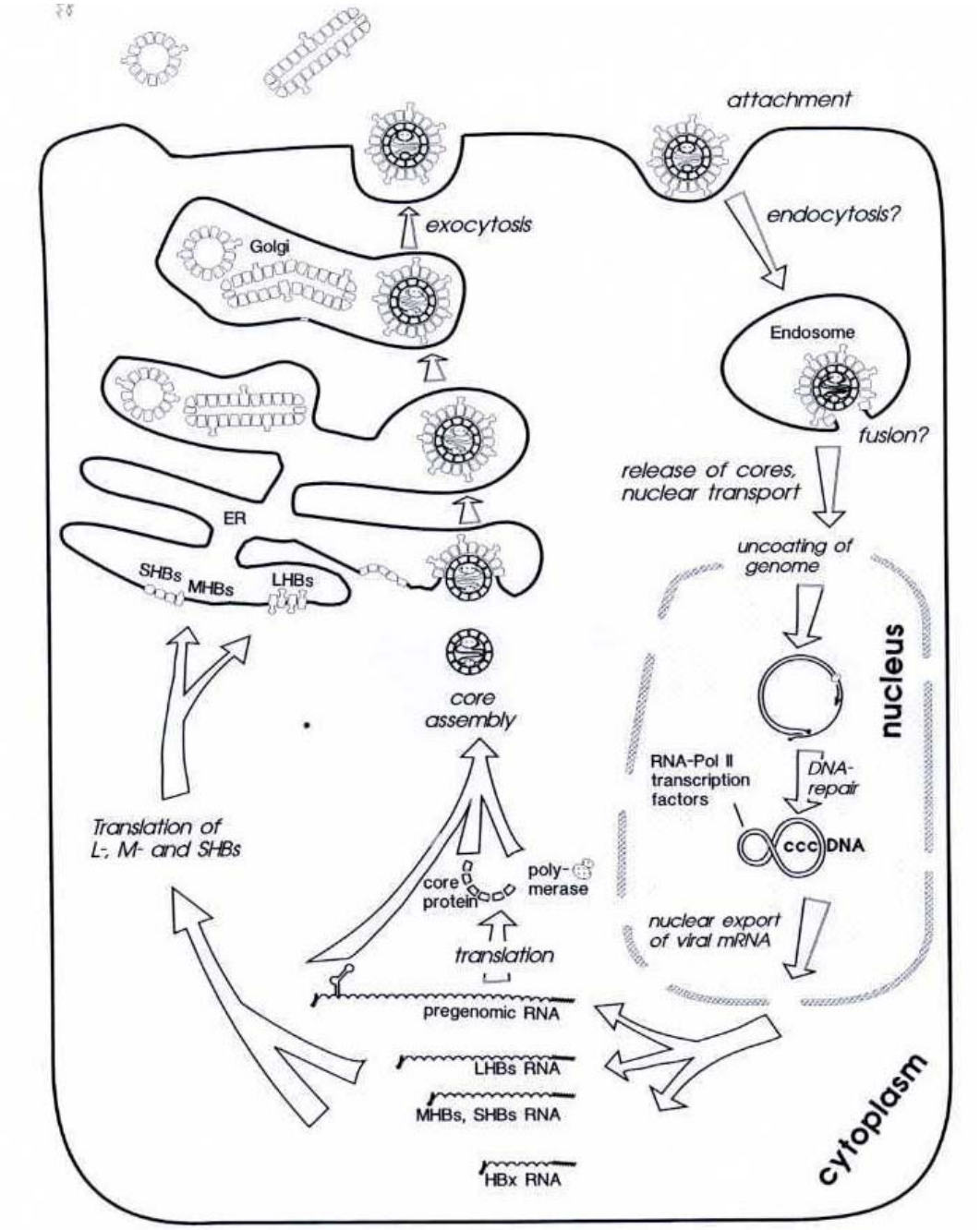
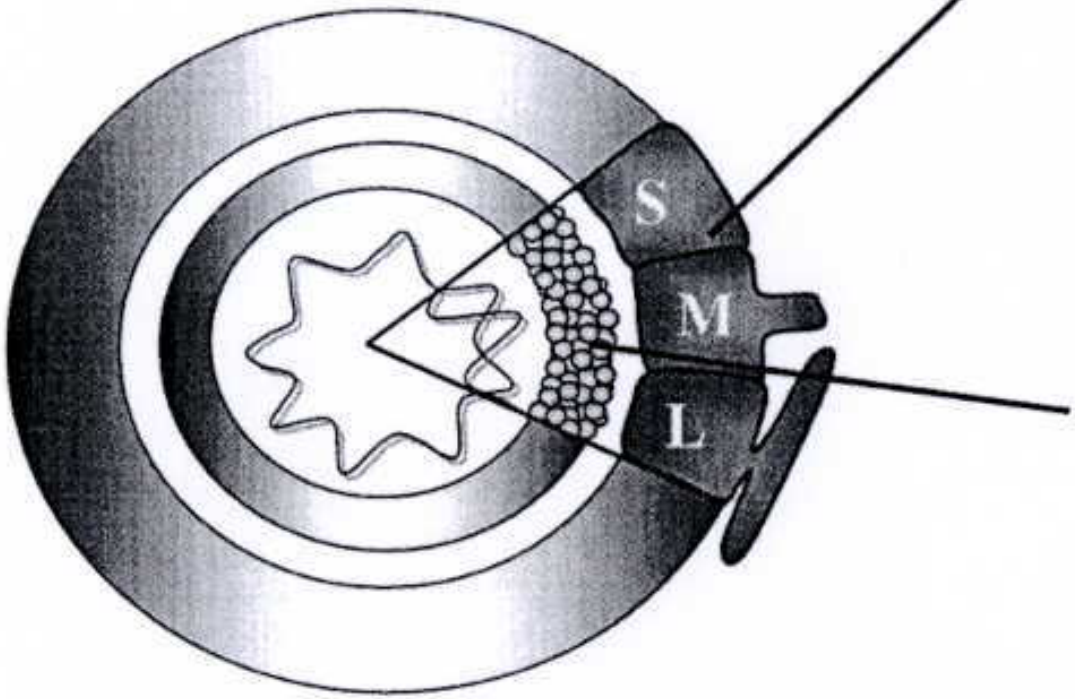


Figure 4. Simplified model of the hepadnaviral life cycle; for details, see text.

HBV



Surface Antigens

Salmonella	S/M/L[a]
E. coli	S[b], M[c], L[c]
S. cerevisiae	S[d], L[e], S/M[f]
H. polymorpha	S[g], M[h], S/L[g]
P. pastoris	S[i]
Mammalian cells	S[j], L[k], S/M[l], M/L[m], S/M/L[n]
Insect cells	S[o]
Potato	S[p], M[p]
Lupin	S[q]
Lettuce	S[q]
Tobacco	S[r]

Core Antigens

Salmonella[s]
E. coli[t]
S. cerevisiae[u]
Mammalian cells[v]
Tobacco[w]

Core/Surface Combinations

Salmonella	M/L/core[x]
E. coli	S/core[y]
S. cerevisiae	S/M/L/core[z]

Figure 7. Expression of hepatitis B genes. The various recombinant antigens produced so far are shown in a schematic drawing of the virus. They are produced in the expression system indicated. References are as follows: [a] [62], [b] [63], [c] [64], [d] [4], [e] [65], [f] [66], [g] [67], [h] [68], [i] [69], [j] [70], [k] [71], [l] [72], [m] [73], [n] [74], [o] [75], [p] [76], [q] [77], [r] [78], [s] [79], [t] [80], [u] [81], [v] [82], [w] [83], [x] [84], [y] [85], [z] [86]. Commercially available *S. cerevisae*- and *H. polymorpha*-derived hepatitis B vaccines are listed in Table 3.

HBV

Table 3. Commercially Available *S. cerevisiae*- and *H. polymorpha*-Derived Hepatitis B Vaccines

Product	Trade Name	Company	Approval, Date	Recombinant Host Organism
HBsAg vaccine	Recombivax®	Merck and Co., Inc.	FDA, Jul. 1986	*S. cerevisiae*
HBsAg vaccine	Engerix B®	SmithKline Beecham Biologicals	FDA, Sep. 1989	*S. cerevisiae*
HBsAg vaccine	AgB®	Laboratorio Pablo Cassará (LPC)	Argentina, Sep. 1995	*H. polymorpha*
HBsAg vaccine	Hepavax-Gene®	Korea Green Cross (KGCC)	WHO, 1997	*H. polymorpha*

HBV

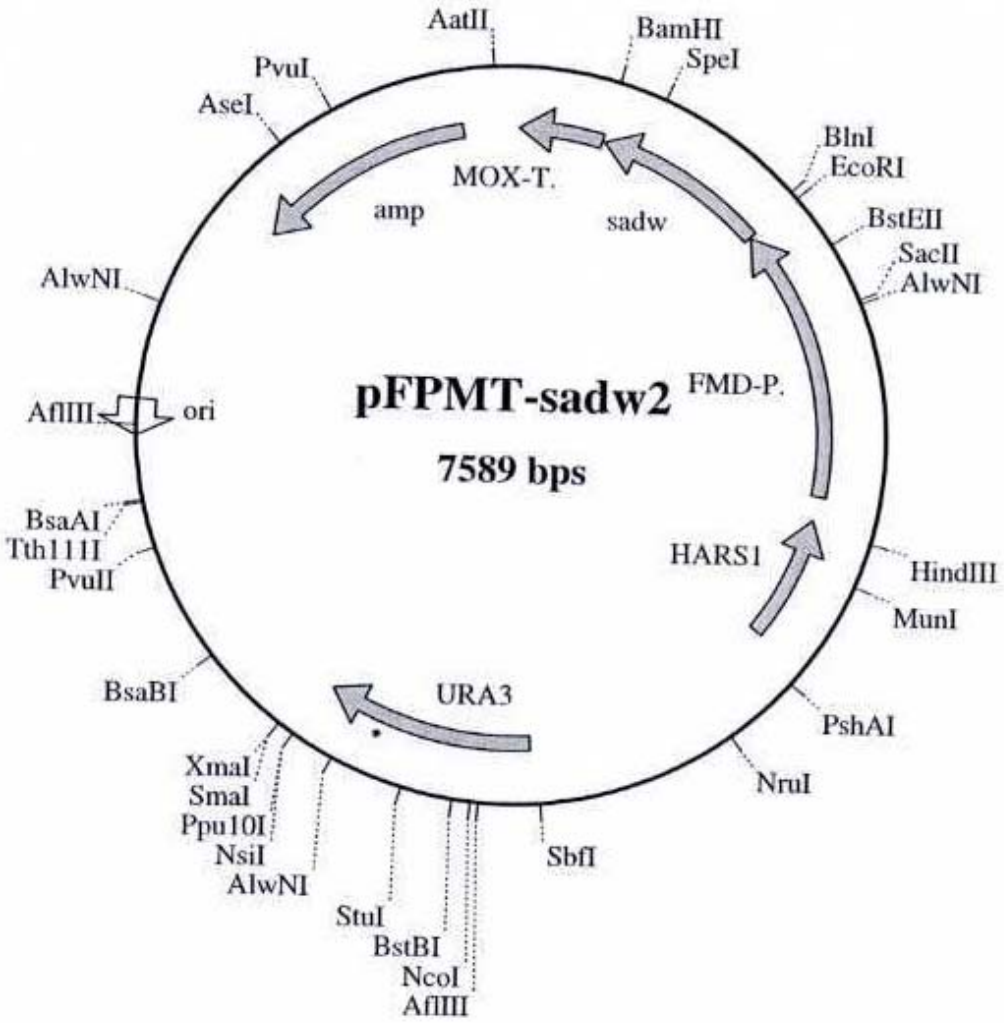


Figure 9. Map of plasmid vector pFPMT-sadw2 containing a *FMD*-promoter/HBsAg(adw2)/ *MOX*-terminator expression cassette. pFPMT-sadw2 is composed of the following DNA fragments, starting from the unique *Hind*III site in a counter-clockwise direction: the *FMD* promoter, a fragment coding for HBsAg (subtype adw2), a *MOX* sequence for transcriptional termination, a sequence containing a gene for ampicillin resistance and an origen of replication for propagation in *E. coli*, the *URA*3 gene as a transformation marker in *ura*3 mutants of *H. polymorpha* and a *Hansenula* autonomously replicating sequence (*HARS*1).

HBV

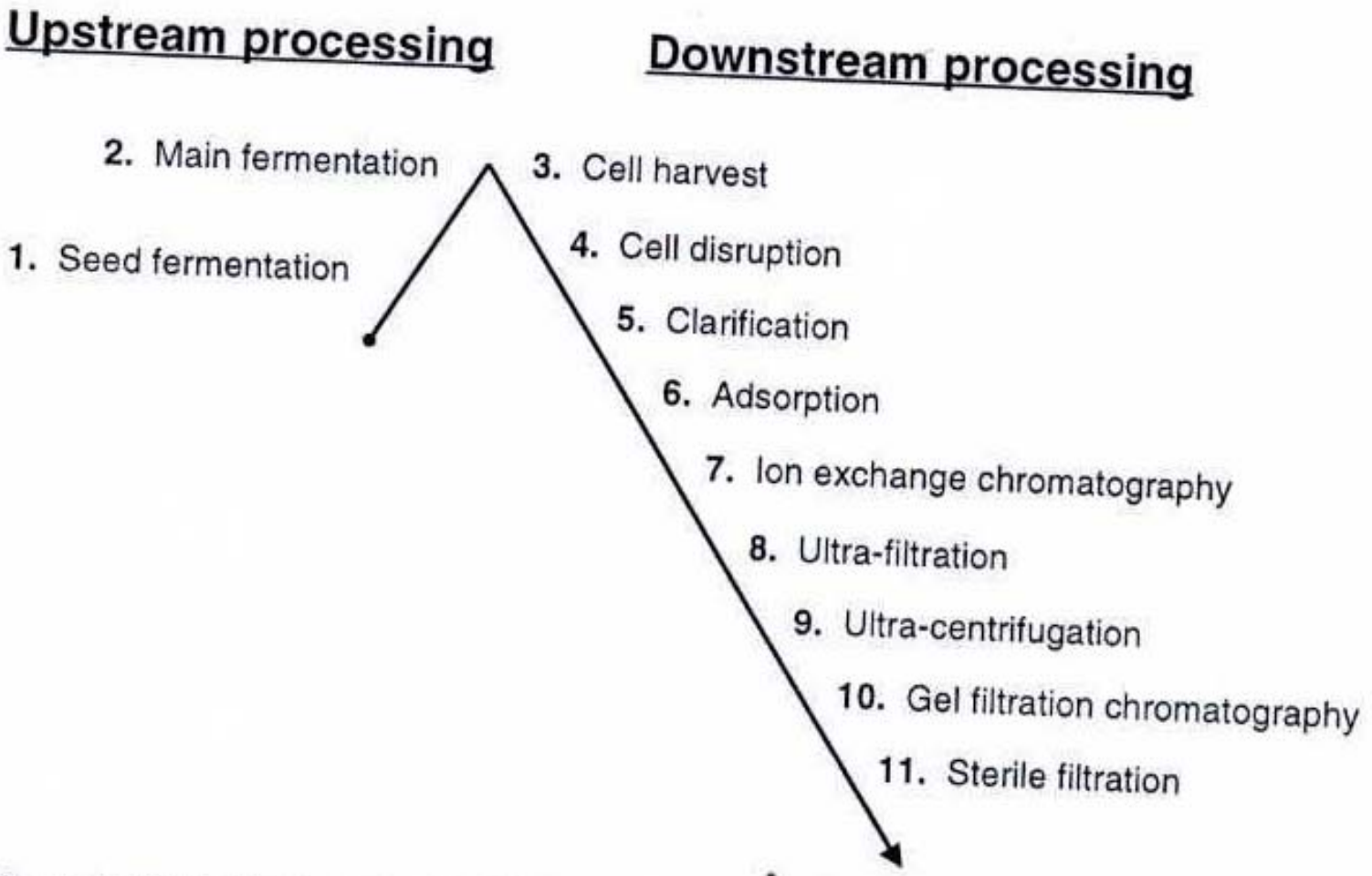


Figure 11. Production process for HBsAg particles in recombinant *H. polymorpha*. Recombinant strains of *H. polymorpha* expressing HBsAg are fermented and the antigen is purified as described in the text (see Sect. 3.4). The process yields purified HBsAg integrated onto yeast-derived membrane particles which may then be adsorbed to aluminum hydroxide for administration as a vaccine.

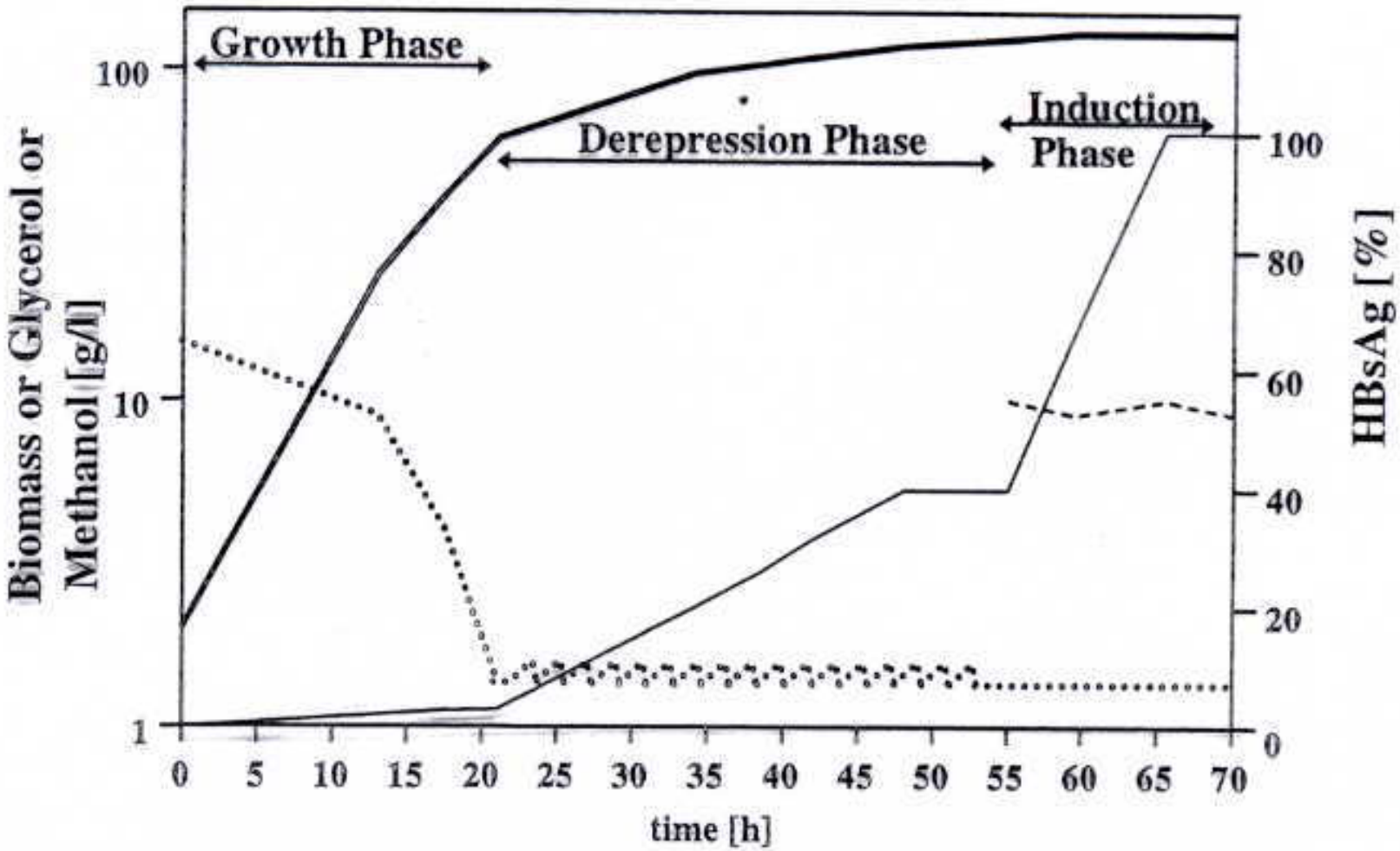


Figure 12. Fermentation of a HBsAg-producing *H. polymorpha* strain (schematic). The fermentation procedure follows the description provided in the text (see Sect. 3.4.1).
—— biomass; —— HBsAg; ----- methanol; ..... glycerol

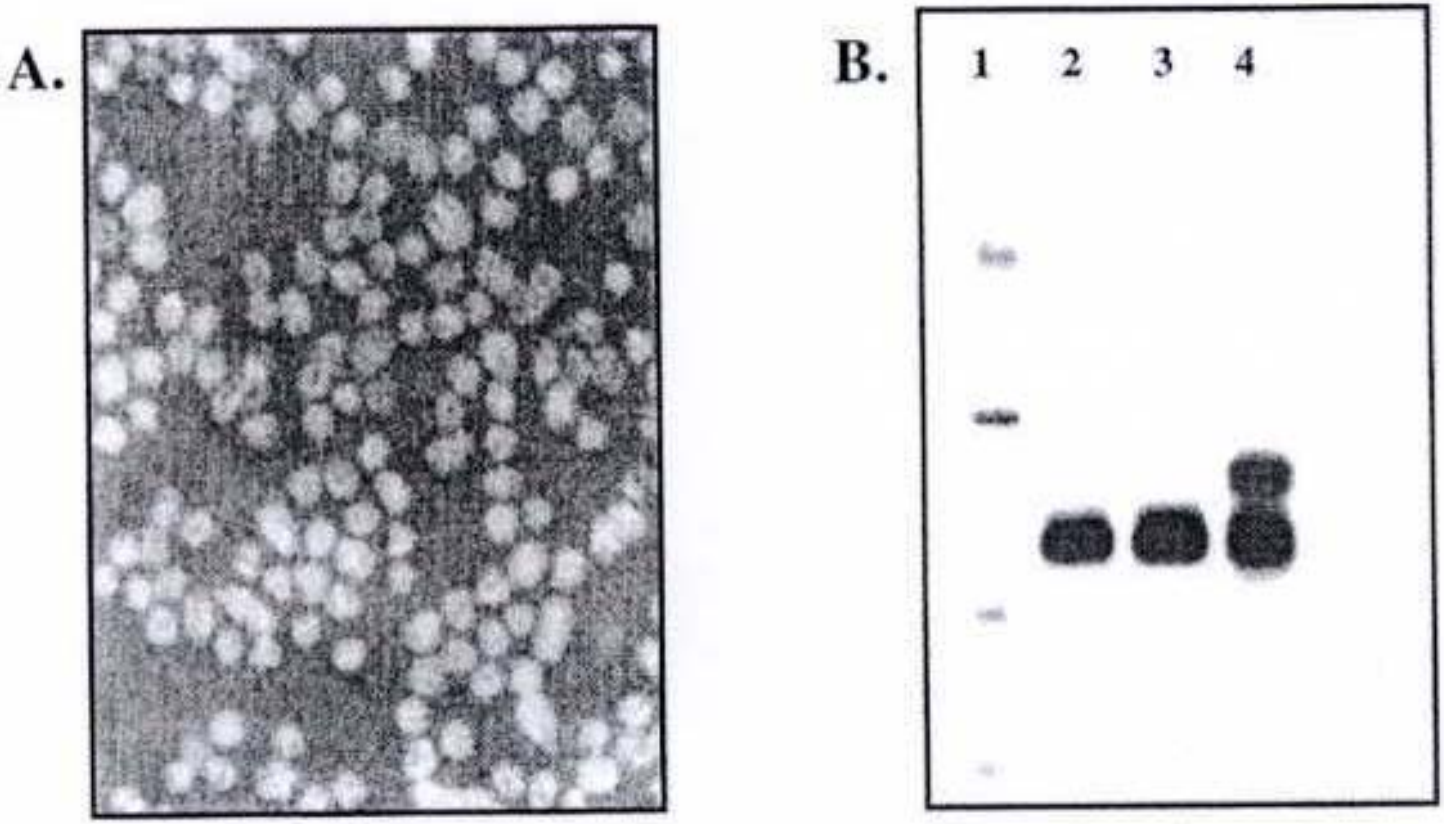


Figure 10. Characterization of recombinant HBsAg-particles produced in *H. polymorpha*. HbsAg particles were purified and analyzed as described in the text (see Sect. 3.3.3). **A.** Electron microscopy analysis (142,000X) **B.** SDS-PAGE analysis of purified HBsAg. Two batches of HBsAg were separated on 12 % SDS gels and visualized by silver staining. Lane 1: MW marker; lanes 2 and 3: two batches of purified r-HBsAg; lane 4: commercial serum-derived HBsAg.

11.12.14

tPA

Tissue Plasminogen Activator tPA

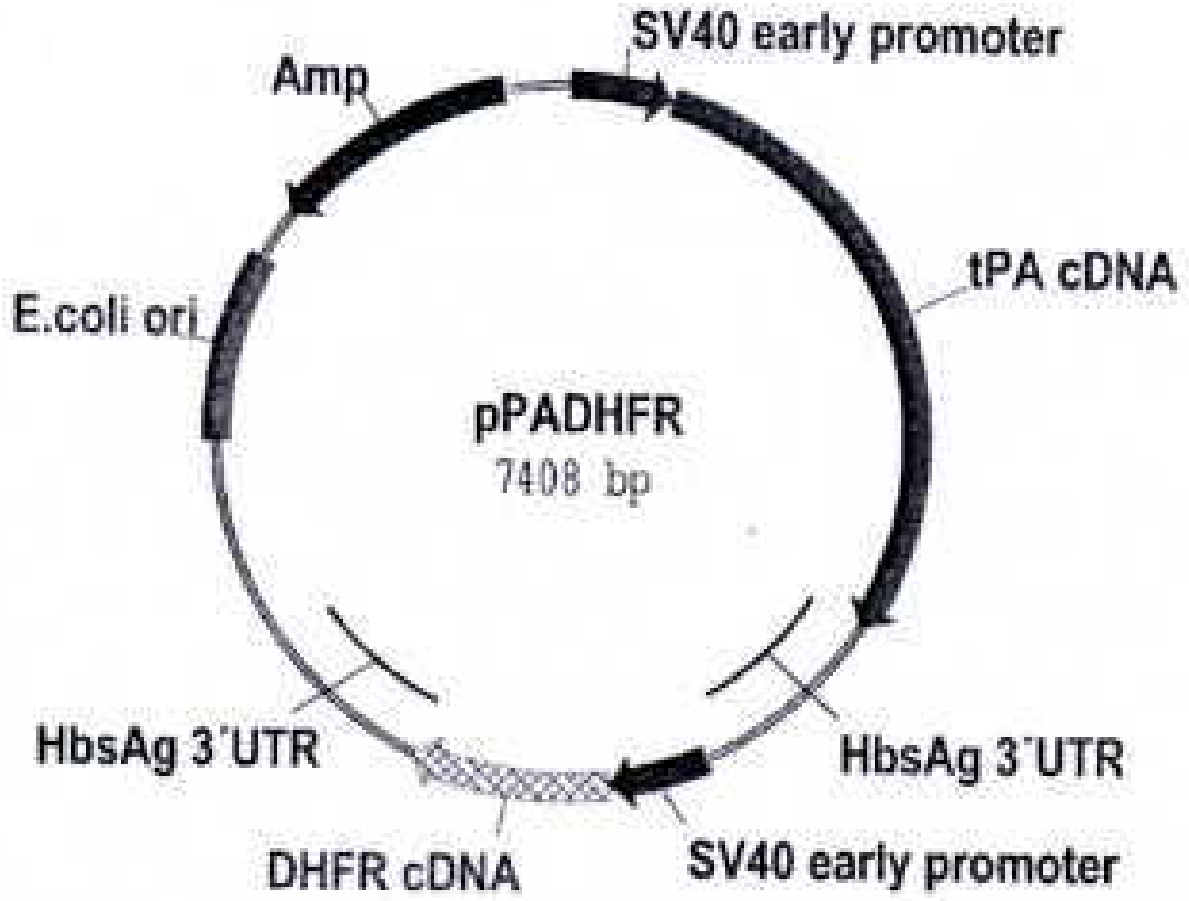


Figure 4. Expression vector for t-PA.

tPA

CHO
Chinese Hamster Ovary

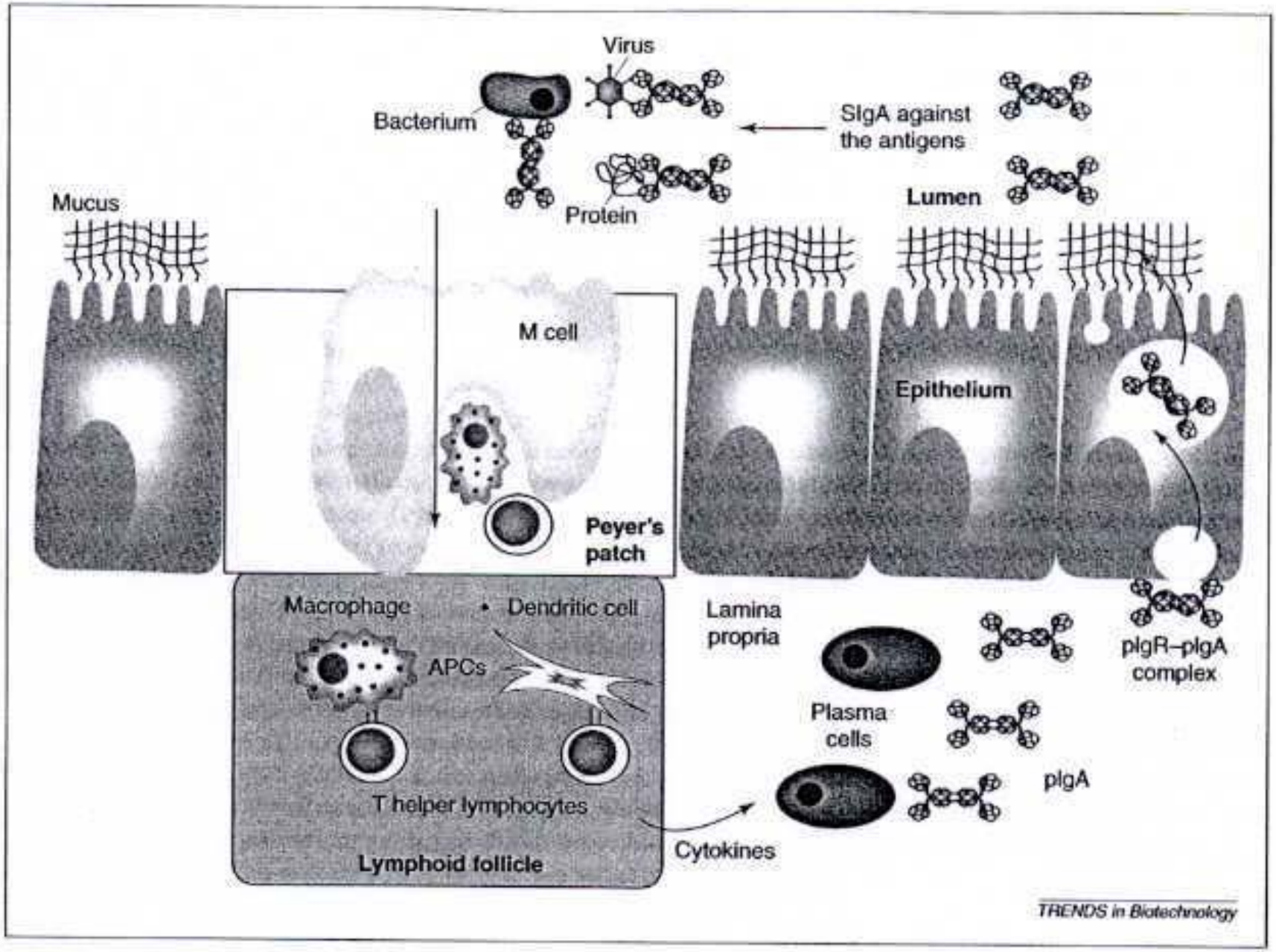
8.6.2 Production Cell Line

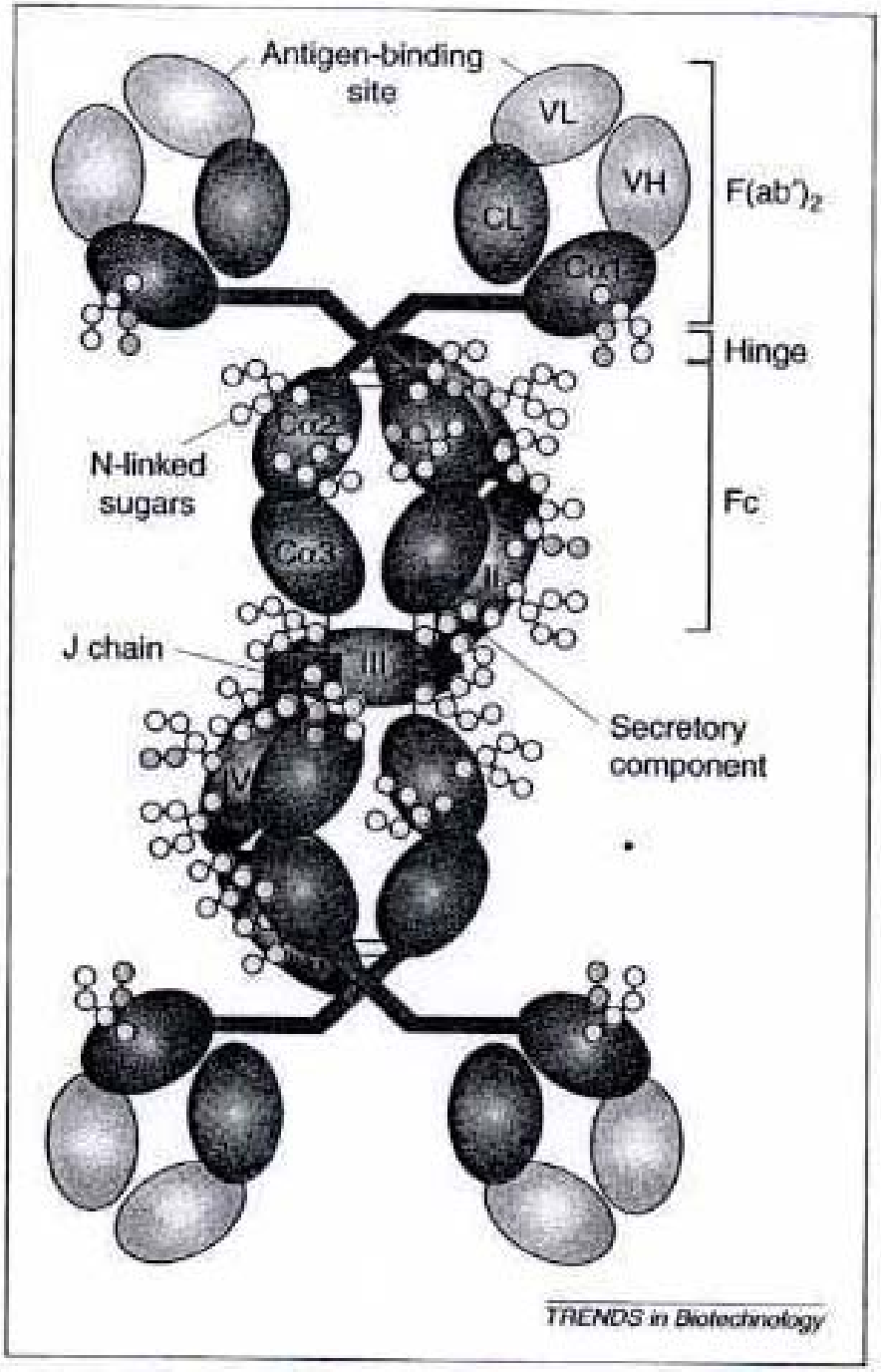
The host cell for the plasmid pPADHFR is a CHO cell line, which was derived from biopsy material in 1957 and which has been distributed since 1970 through the American Type Culture Collection (ATCC) who designated the original cell line CHO-K1 as CCL-61. This cell line has undergone hundreds of serial subcultures and is considered to be a continuous cell line of indefinite life span *in vitro*.

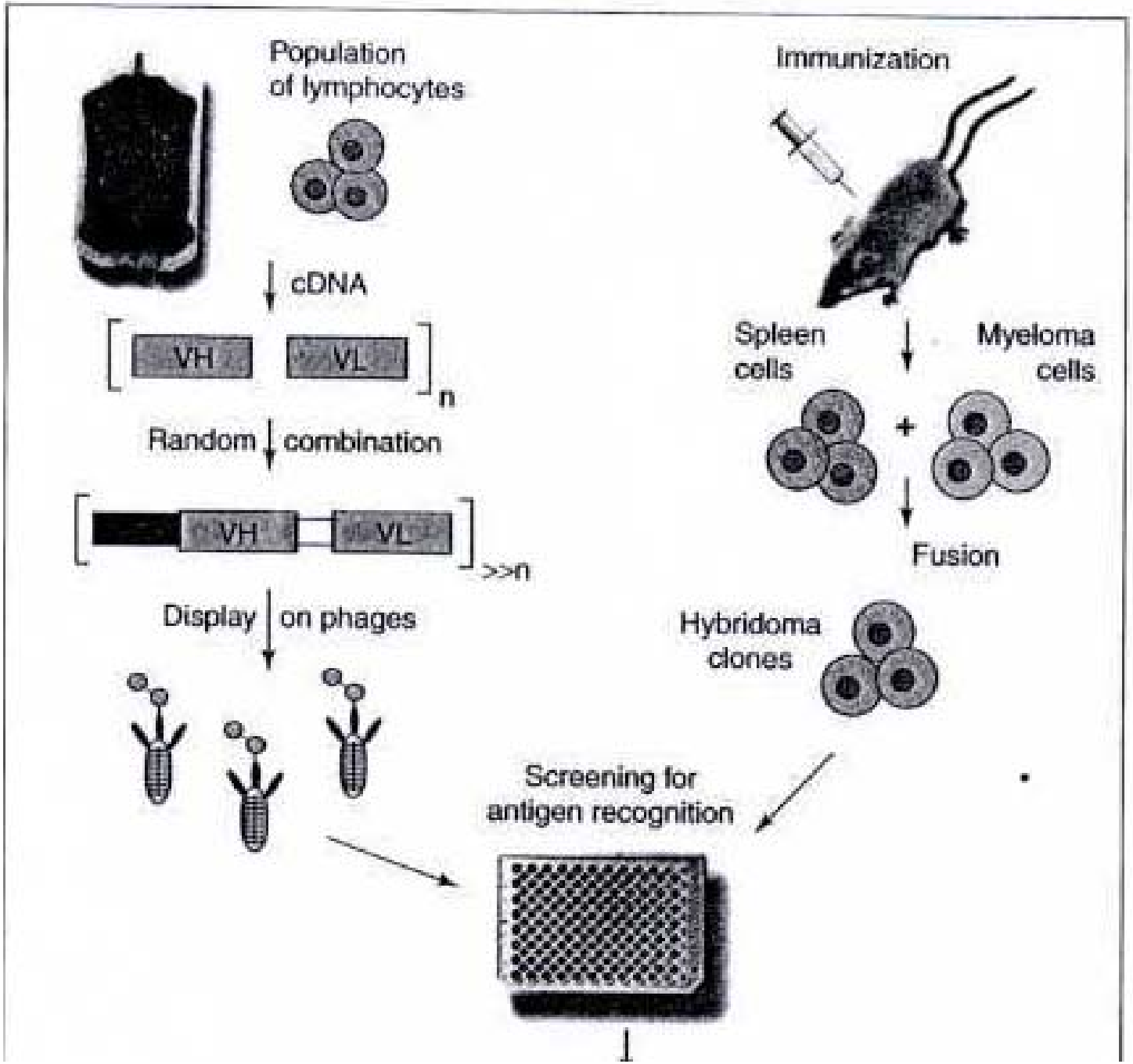
Recombinant immunoglobulin A: powerful tools for fundamental and applied research

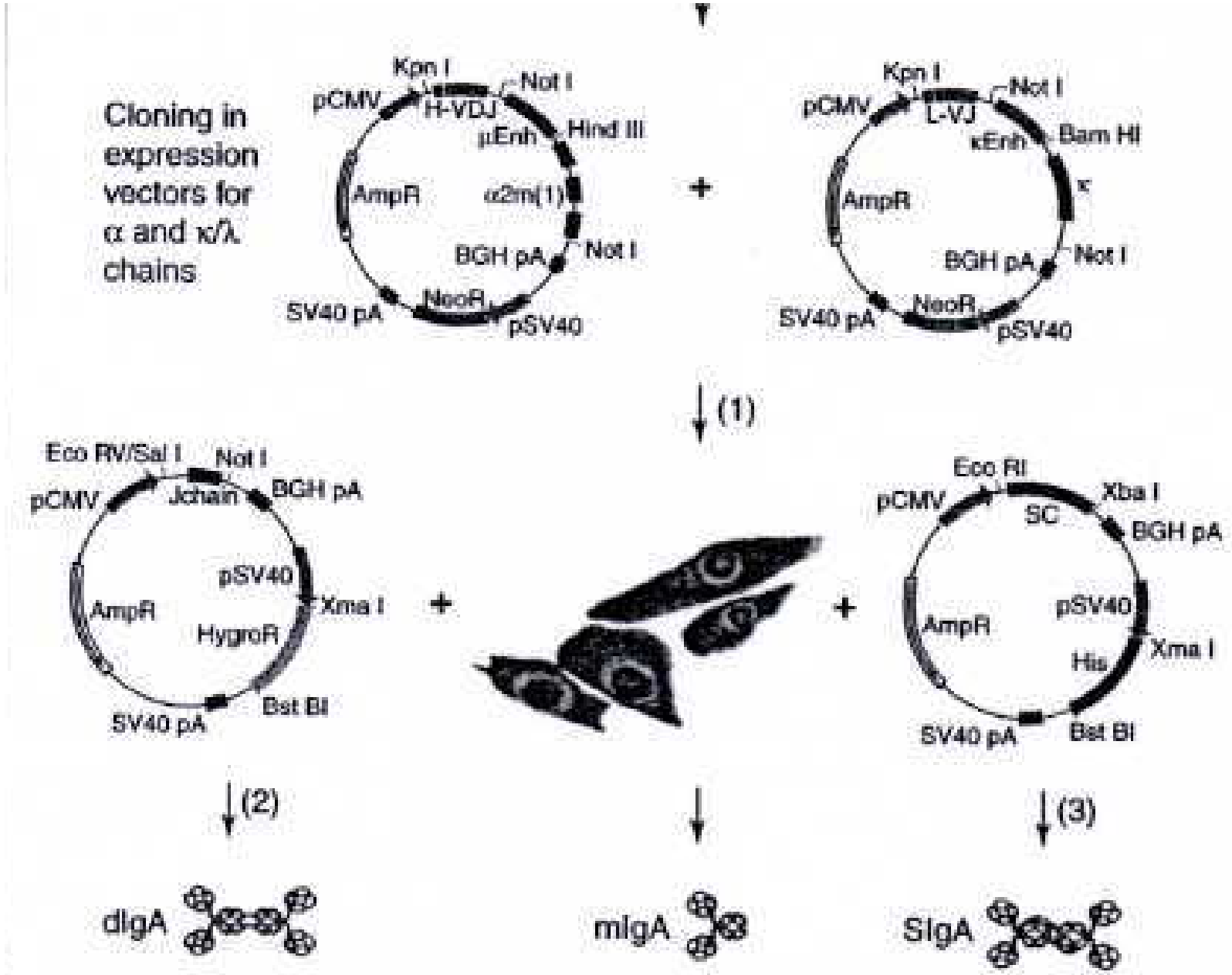
Blaise Corthésy

Trends Biotechnol


Virus
Bacterium
SIgA against
the antigens
Protein
Mucus
Lumen
M cell
Epithelium
Peyer's
patch
Macrophage
Dendritic cell
Lamina
propria
APCs
pIgR–pIgA
complex
Plasma
cells
pIgA
T helper lymphocytes
Cytokines
Lymphoid follicle
TRENDS in Biotechnology


Antigen-binding site
VL
VH
CL
Cγ1
F(ab')2
Hinge
Fc
N-linked sugars
Cγ2
Cγ3
J chain
Secretory component
TRENDS in Biotechnology


Population
of lymphocytes
cDNA
VH
VL
n
Random
combination
VH
VL
>>n
Display
on phages
Immunization
Spleen
cells
Myeloma
cells
+
Fusion
Hybridoma
clones
Screening for
antigen recognition


Cloning in expression vectors for α and κ/λ chains
Kpn I
Not I
pCMV
H-VDJ
μEnh
Hind III
AmpR
α2m(1)
BGH pA
Not I
SV40 pA
NeoR
pSV40
Kpn I
Not I
pCMV
L-VJ
κEnh
Bam HI
AmpR
κ
BGH pA
Not I
SV40 pA
NeoR
pSV40
(1)
Eco RV/Sal I
Not I
pCMV
Jchain
BGH pA
pSV40
AmpR
Xma I
HygroR
SV40 pA
Bst BI
Eco RI
pCMV
SC
Xba I
BGH pA
AmpR
pSV40
His
Xma I
SV40 pA
Bst BI
(2)
(3)
dIgA
mIgA
SIgA
TRENDS in Biotechnology

Enzymes

Most commercial Enzymes are produced as recombinant enzymes

Main Hosts:

Escherichia coli
Bacillus amyloliquefaciens
Saccharomyces cerevisiae
Kluyveromyces lactis
Pichia pastoris
Aspergillus niger/awamori

Class of enzyme - Reaction profile

1: Oxidoreductases: catalyze oxidation reactions, involve the movement of electrons from one molecule to another.
Dehydrogenases: removal of hydrogen
Oxidases: acceptor oxygen
Peroxidases: acceptor hydrogen peroxide

2: Transferases: catalyse the transfer of groups of atoms (radicals) from one molecule to another. (Aminotransferases or transaminases)

3: Hydrolases: catalyse reactions between a substrate and water
e.g.: cleavage of peptide bonds in proteins,
glucosidic bonds in carbohydrates
and ester bonds in lipids.

4: Lyases: catalyse the addition of groups to double bonds or the formation of double bonds through the removal of groups.
e.g.Pectate lyases: split the glycosidic linkages by beta-elimination.

5: Isomerases: catalyse the transfer of groups from one position to another on the same molecule.
change the structure of a substrate by rearranging its atoms.

6: Ligases: join molecules together with covalent bonds.
reactions require energy in the form of cofactors such as ATP.

Table 1

Impact of enzyme technology in industry.

	Keywords	Comments on publication	References
Agriculture	Feed additives	Positive effects on environment, animal health, and efficiency	[1,3,7]
	Heterologous enzyme production	Laccase and trypsin productions in plants	[9]
Chemicals	Biocatalysis	Review on preparative biotransformations	[39]
	Polymers	Polymer synthesis by *in vitro* enzyme catalysis	[40•]
	Bulk organic compounds	Review on pathway engineering	[32]
Cleaning	New detergent enzymes	Increased competition and lower prices	[7]
Energy	Fuel alcohol from biomass	Genencor and Novozymes contract with DOE	[10]
		logen biomass-to-ethanol demonstration plant	[11•]
Food	Enzymes used in food preparation	Editorial on new enzyme applications in food	[6]
	Nutraceuticals	Increased carotene content of tomato	[8]
Pharma	Chiral compounds	Enantioselective biocatalysis	[13]
	Glycoprotein engineering	*In vitro* protein glycosylation	[41]
	Enzymes as pharma targets	Several reviews in edited book	[5•]
Materials	Paper, textile, leather treatment	New enzymes from extremophiles	[21]
	Biosteel (silk)	Heterologous expression of spider silk	[42]

Note, only a limited selection of new developments in established fields is shown.

J. van Beilen, Current Opinion in Biotechnology 2002, **13**:338–344

Typical enzymes used in industrial processes.

1: Oxidoreductases

- Catalases
- Glucose oxidases
- Laccases
- Peroxidases
- Dehydrogenases - Reductases

2: Transferases

- Fructosyl-transferases
- Glucosyl-transferases

3: Hydrolases

- Amylases
- Cellulases
- Lipases, Esterases
- Pectinases
- Proteases
- Pullulanases

4: Lyases

- Pectate lyases
- (Alpha-acetolactate) decarboxylases

5: Isomerases

- Glucose isomerase

6: Ligases

- emerging field

Enzymes In Biocatalysis

Enzyme	Substrate	Product	Application
Nitrile hydratase	3-Cyano-pyridine	Nicotinamide	Pharmaceutical intermediate
Nitrile hydratase	Acrylonitrile	Acrylamide	Intermediate for water-soluble polymers
D-amino acid oxidase & glutaric acid acylase	Cephalosporin C salt	7-Amino-cephalosporanic acid	Intermediate for semisynthetic antibiotics
Penicillin acylase	7-Amino-deacetoxy-cephalosporanic acid	Cephalexin	Antibiotics
Penicillin G acylase	Penicillin G	6-Amino-penicillanic acid	Intermediate for semisynthetic antibiotics
Ammonia lyase	Fumaric acid + ammonia	L-Aspartic acid	Intermediate for aspartame
Thermolysine	L-Aspartic acid + D,L-phenylalanine	Aspartame	Artificial sweetener
Dehalogenase	(R,S)-2-Chloro-propionic acid	(S)-2-Chloro-propionic acid	Intermediate for herbicides
Lipase	(R,S)-Glycidyl-butyrate	(S)-Glycidyl-butyrate	Chemical intermediate
Lipase	Isosorbide diacetate	Isosorbide 2-acetate	Pharmaceutical intermediate
Lipase	(R,S)-Naproxen ethyl ester	(S)-Naproxen	Drug
Lipase	Racemic 4-methoxy-phenylmethyl glycidate	(2R,3S)-4-methoxy-phenylmethyl glycidate	Pharmaceutical intermediate
Acylase	D,L-Valine + acetic acid	L-Valine	Pharmaceutical intermediate
Acylase	Acetyl-D,L-methionine	L-Methionine	Pharmaceutical intermediate

Source: Novozymes

Enzyme applications in the food industry

Enzymes used in baking

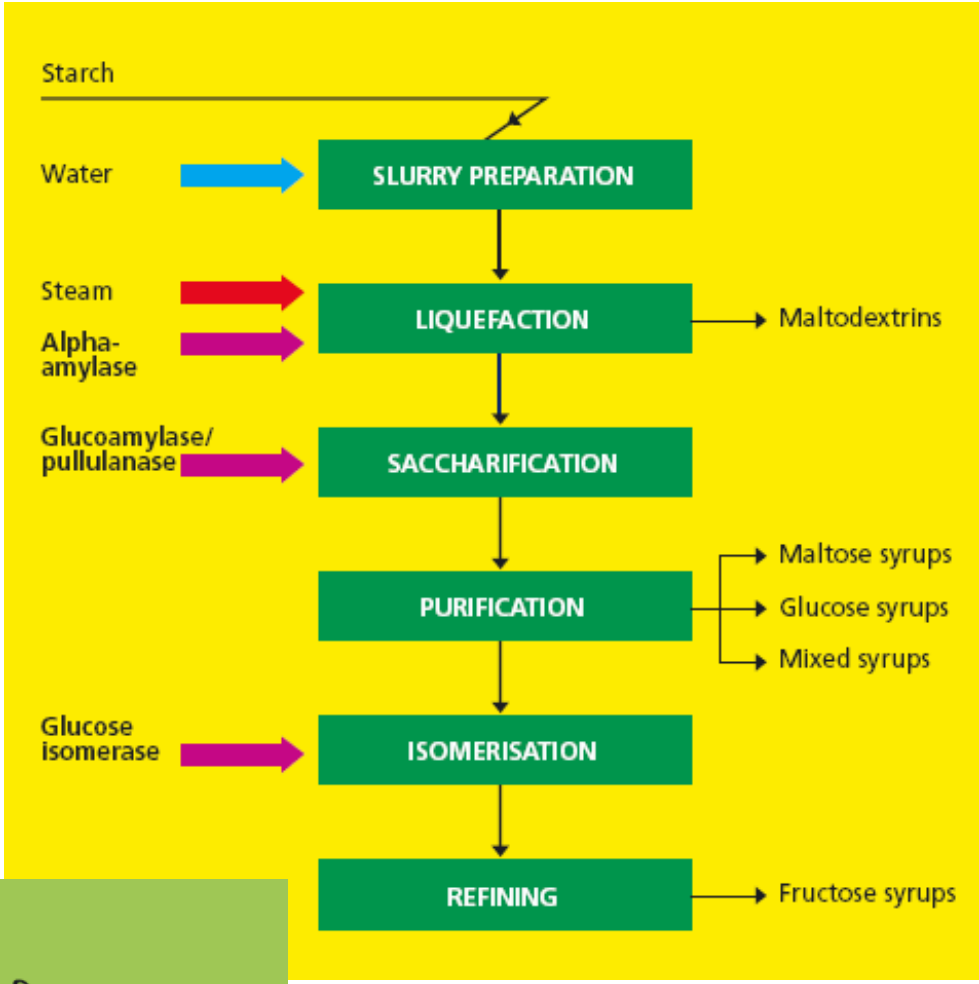
Enzyme	Effect
Amylase	Maximises the fermentation process to obtain an even crumb structure and a high loaf volume.
Maltogenic alpha-amylase	Improves shelf life.
Glucose oxidase	Oxidises free sulphydryl groups in gluten to make weak doughs stronger and more elastic.
Lipase	Oxidises free sulphydryl groups in gluten to make weak doughs stronger and more elastic.
Lipoxygenase	Bleaching and strengthening dough.
Xylanase	Dough conditioning. Easier dough handling and improved crumb structure.
Protease	Weakens the gluten to give the plastic properties required in doughs for biscuits.

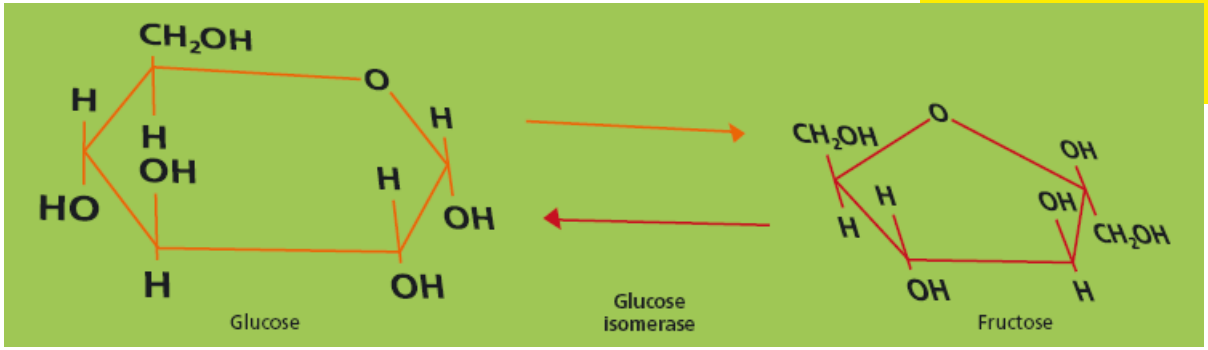
Source:Novozymes

Enzyme applications in the food industry

Sweetener production

Enzymes for starch modification glucose syrups





Enzyme applications in the food industry

Dairy products

Rennet and rennet substitutes
- **Recombinant calf chymosin**
- **Microbial rennets**

Cheese ripening
- **Lipases**

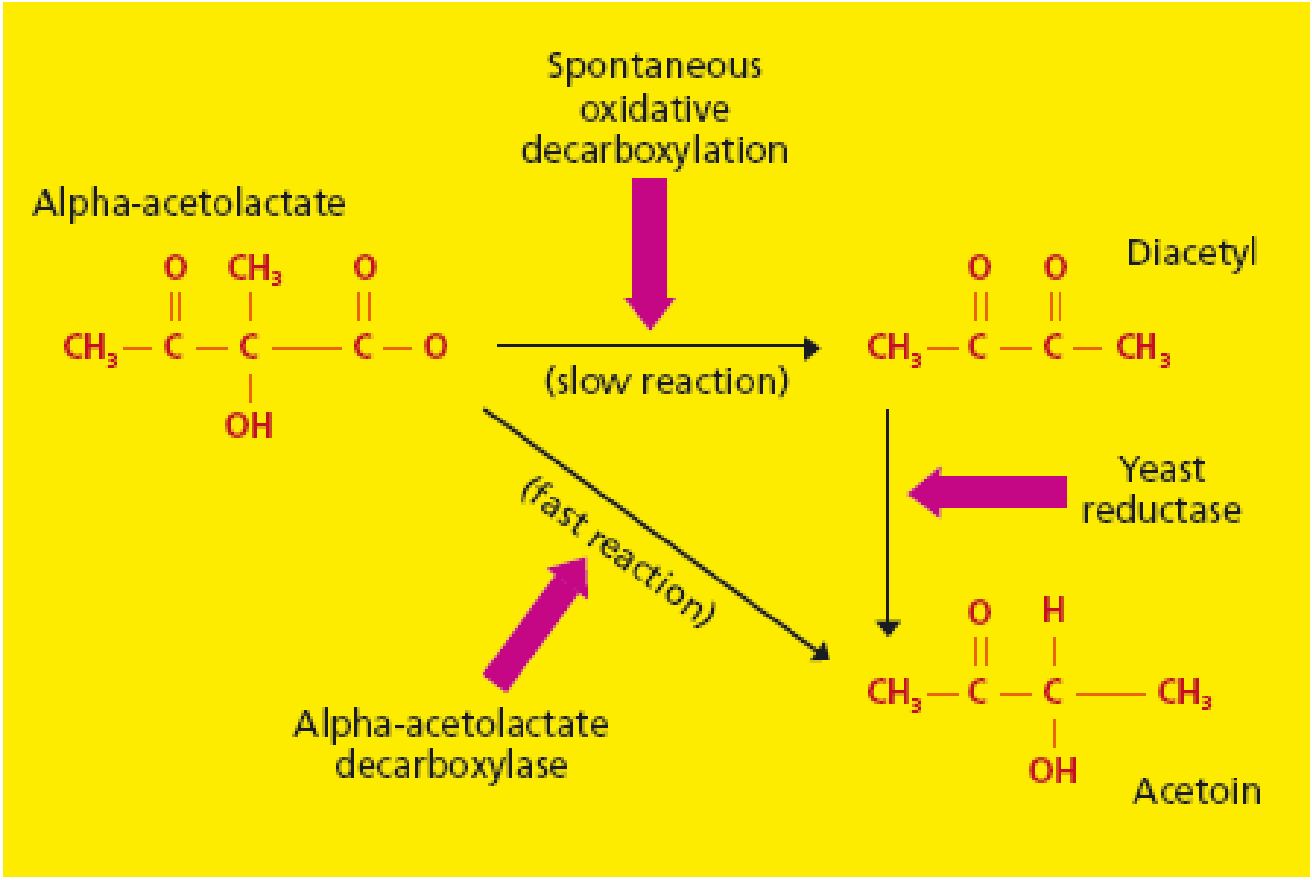
Infant milk formulas
- **Proteases**
- **(allergy problem cow milk)**

Enzyme applications in the food industry

Brewing

alpha-amylase
beta-glucanase
protease
pentosanase

Diacetyl

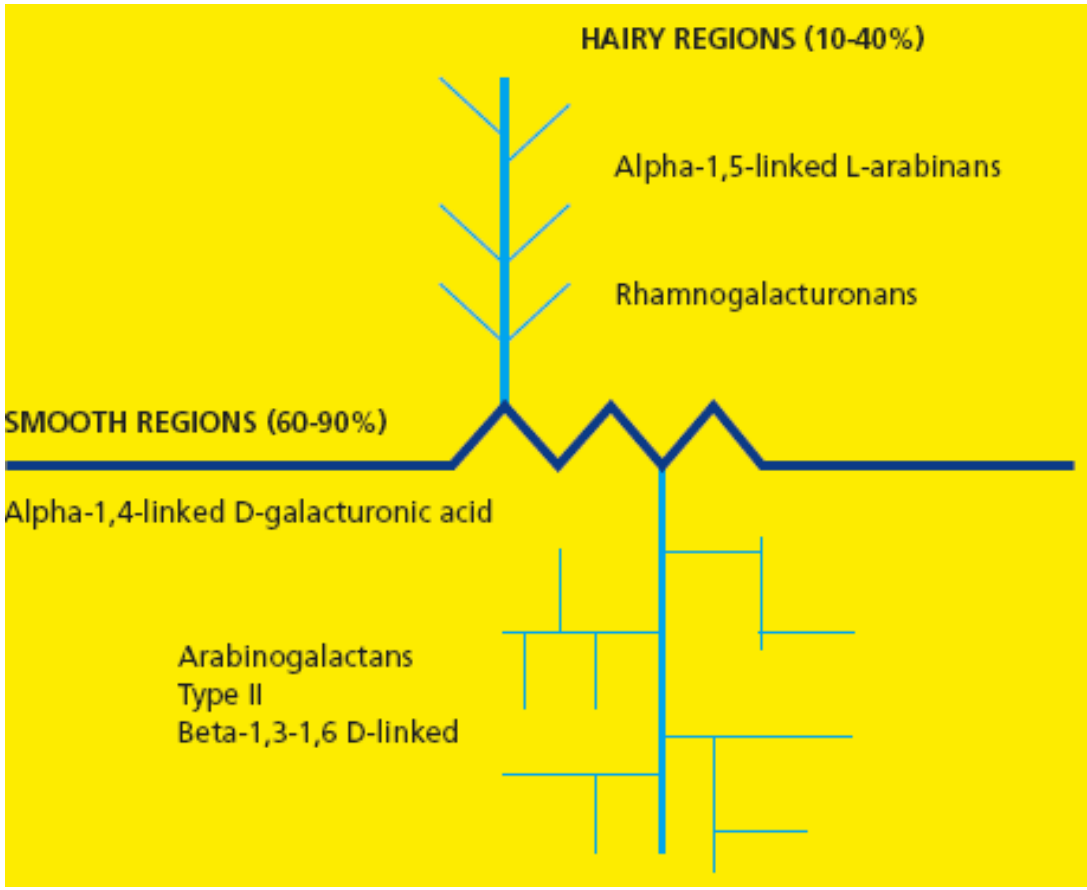


Enzyme applications in the food industry

Extraction of plant material

Wine making
Fruit Juices
Oil Extraction

Pectin degradation



Enzyme applications in the food industry

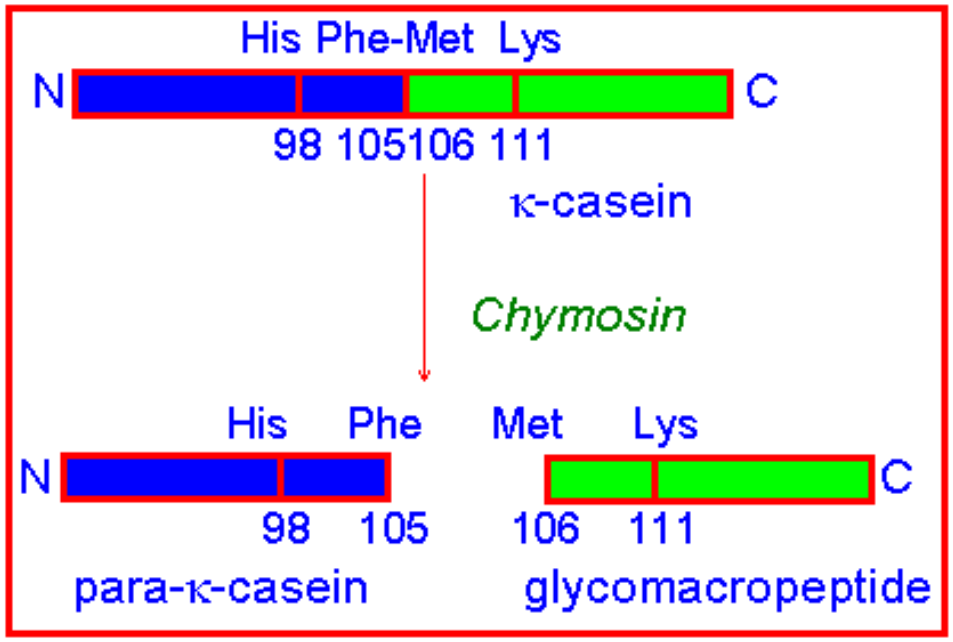
Enzymatic modification of lipids

Enzymatic modification of lipids
Lipases, Esterases

Enzymatic degumming
phospholipase

Enzyme applications in the food industry

Chymosin



Preprochymosin is shortened by 16 amino acids during secretion- appears in the stomach as prochymosin → is activated to chymosin by cleavage of an additional 42 amino acids.

Recombinant Chymosin:

(1) chymosin A from *Escherichia coli* K-12
(2) chymosin B from *Kluyveromyces lactis*
(3) chymosin B from *Aspergillus niger* var. *awamori.*

Table 2 Secreted Chymosin Production From *A. awamori*

Details	Yield of chymosin (mg/L) in shake-flasks[a]
Glucoamylase signal–prochymosin	1–5
Chymosin signal–prochymosin	2–7
Chymosin signal–prochymosin *pepA* deletion	10–15
Glucoamylase–prochymosin *pepA* deletion	ca. 250
Glucoamylase–prochymosin nitrosoguanidine mutagenesis and screening; *pepA* deletion	270–650
As above, deoxyglucose resistance	500–1200
As above, extra copies of expression cassette	0–1350

[a]Production levels of chymosin from a production run are not given.

Source: Refs. 60, 120.

Proteins for Research

Enzymes
Human Proteins
Antibodies

Restriction Endonucleases

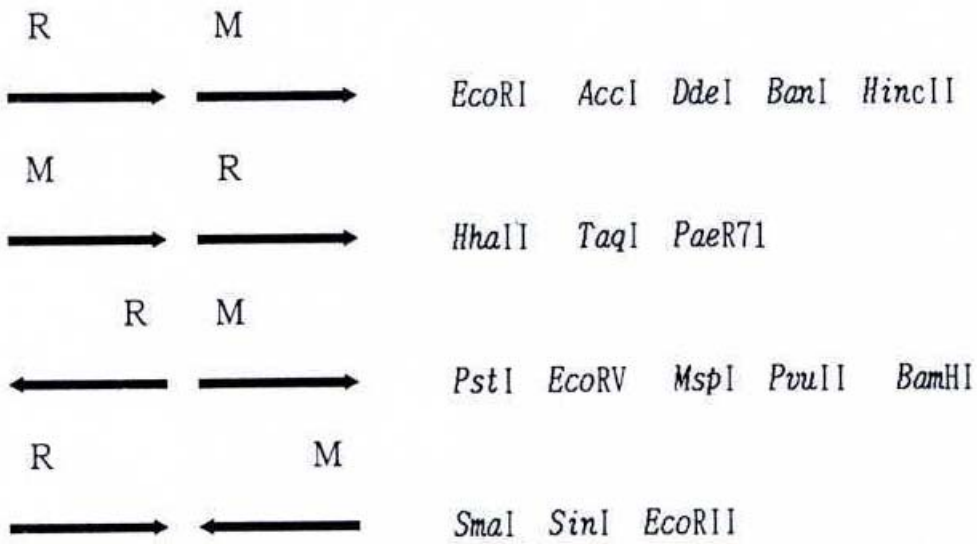


Figure 3 Gene organization of various restriction–modification genes. Genes are indicated as arrows; the directions indicate transcriptional orientation.

Table 1 Main Type II R–M Enzymes That Have Been Cloned

R–M enzyme	Donor	Recognition sequence[a]	Cloning method[b]	Host	Refs.
Acc I	*Acinetobacter calcoaceticus*	GTMKAC	(3)	*E. coli*	31
*Bam*HI	*Bacillus amyloliqufaciens* H	GGATCC	(3)	*B. subtilis*	23
			(4)	*E. coli*	15
Ban I	*B. aneurinolyticus*	GRGCYC	(3)	*E. coli*	29
Ban III	*B. aneurinolyticus*	ATCGAT	(3)	*E. coli*	11, 30
Dde I	*Desulfovibrio desulfuricans*	CTNAG	(4)	*E. coli*	14
*Eco*RI	*Escherichia coli* RY13	GAATTC	(1)	*E. coli*	5, 6
*Eco*RV	*E. coli* J62 (pLG74)	GATATC	(1)	*E. coli*	8
Hha II	*Haemophilus haemolyticus*	GANTC	(2)	*E. coli*	3
*Hinc*II	*H. influenzae* Rc	GTYRAC	(4)	*E. coli*	16
*Hind*III	*H. influenzae* Rd	AAGCTT	(3)	*E. coli*	4
Kpn I	*Klebsiella pneumoniae*	GGTACC	(4)	*E. coli*	17
Msp I	*Moraxella* species	CCGG	(3)	*E. coli*	51
*Pae*R7I	*Pseudomonas aeruginosa* (pMG7)	CTCGAG	(1)	*E. coli*	9
Pst I	*Providencia stuartii*	CTGCAG	(2)	*E. coli*	12
Pvu I	*Proteus vulgaris*	CGATCG	(3)	*E. coli*	52
Pvu II	*P. vulgaris*	CAGCTG	(1)	*E. coli*	10
Sal I	*Streptomyces albus*	GTCGAC	(2)	*S. lividans*	24
Sin I	*Salmonella infantis*	GGWCC	(3)	*E. coli*	53
Sma I	*Serratia marcescens*	CCCGGG	(3)	*E. coli*	54
Taq I	*Thermus aquaticus* YT1	TCGA	(3)	*E. coli*	55
Xba I	*Xanthomonas badrii*	TCTAGA	(3)	*E. coli*	4

[a]Only one strand of the recognition sequence is shown, printed 5' to 3'. The standard abbreviations for alternative nucleotide are: M, A or C; K, G or T; R, A or G; Y, C or T; W, A or T.

[b]Cloning methods are divided into four groups: (1) subcloning of natural plasmid; (2) cloning based on phage restriction; (3) cloning based on vector modification; and (4) two-step cloning.

Influence of host features on expresssion of R-endonucleases

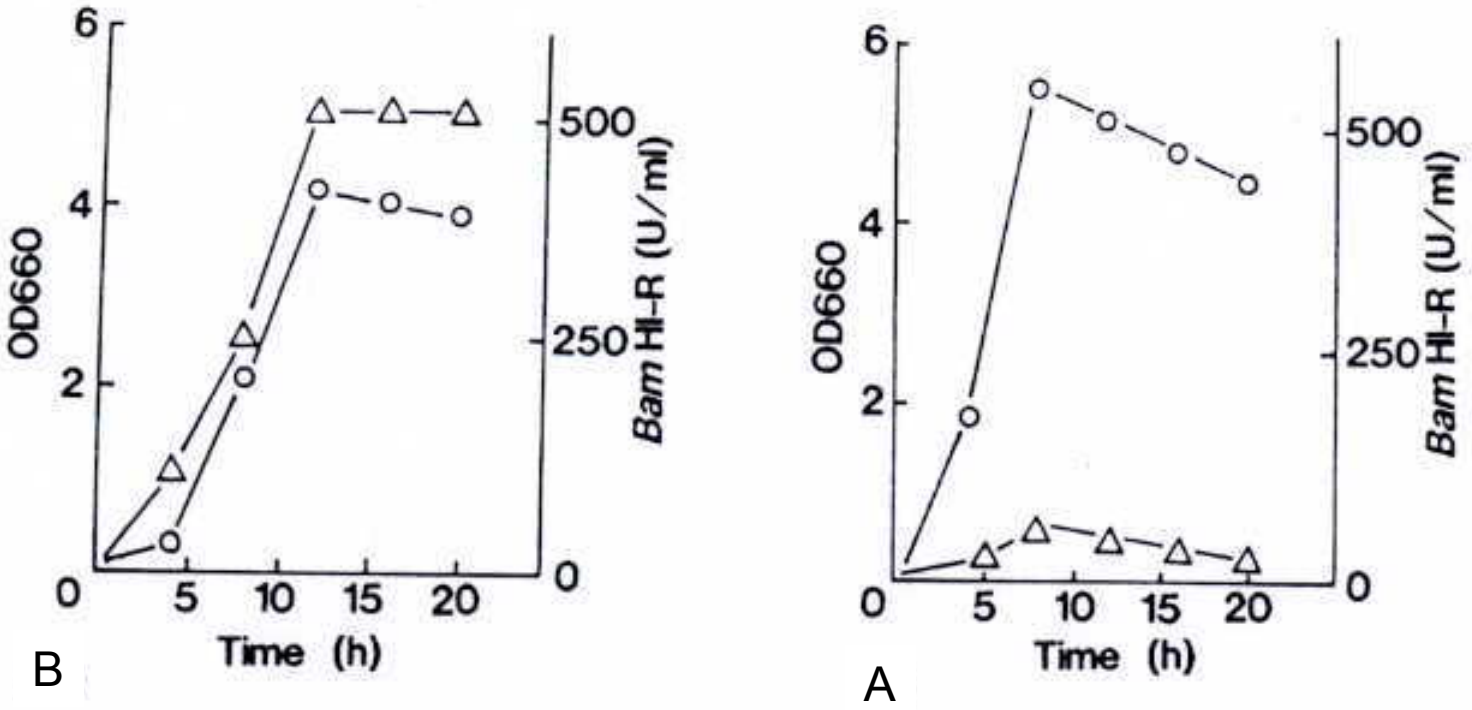


Figure 2 Comparison of the bacterial growth and *Bam*HI-R production between (A) *B. subtilis* (p*Bam*HIRM22 and (B) *B. amyloliquefaciens* H. *B. subtilis*(pBamHIRM22) and *B. amyloliquefaciens* H were cultured in a 500-ml flask at 30°C on a reciprocal shaker. Bacterial growth (OD_{660}, ○) and *Bam* HI-R activity (△) were measured.

B.amyloliquefaciens naturally expresses *Bam*H1 Methylase

Co-Expression of Methylase → Protection against toxic effects of R-endonuclease