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Recombinant products

Table 1 Biopharmaceuticals approved in the United States and Europe (listed consecutively from the most recent approval in each class, with post-2010 registrations in bold and withdrawals in red)

Product	Company (location)	Therapeutic indication	Date approved
Recombinant blood factors			
Factor VIII			
Nuwiq (simoctocog alfa; rh blood factor VIII, produced in a human embryonic kidney cell line)	**Octapharma AB (Stockholm, Sweden)**	**Hemophilia A**	**2014 (EU)**
Eloctate (rh B-domain deleted factor VIII Fc fusion protein, produced in a HEK cell line)	**Biogen-Idec (Cambridge, MA, USA)**	**Hemophilia A**	**2014 (US)**
NovoEight (turoctocog alfa), rh factor VIII analog which, when activated, is structurally comparable to endogenous h factor VIIIa produced in a CHO cell line	**Novo Nordisk, (Bagsvaerd, Denmark and Plainsboro, NJ, USA)**	**Hemophilia A**	**2013 (EU & US)**
Xyntha (anti-hemophiliac factor), rh coagulation factor VIII produced in CHO cells	Pfizer/Wyeth (Philadelphia, PA)	Hemophilia A	2008 (US)
Advate (octocog α), rh factor VIII produced in CHO cells	Baxter (Vienna and Deerfield, IL, USA)	Hemophilia A	2004 (EU), 2003 (US)
Helixate NexGen (octocog α), rh factor VIII produced in BHK cells	Bayer (Berlin, Germany)	Hemophilia A	2000 (EU)
Refacto (Moroctocog-α), B-domain-deleted rh factor VIII produced in CHO cells)	Pfizer/Wyeth (Sandwich, UK)/ Genetics Institute (Cambridge, MA, USA)	Hemophilia A	1999 (EU), 2000 (US)
Kogenate/Helixate (anti-hemophiliac factor), rh factor VIII produced in BHK cells. Sold as Helixate by Aventis Behring through a license agreement	Bayer (Leverkusen, Germany, and Berkeley, CA, USA)	Hemophilia A	1993 (US), 2000 (EU)
Bioclate (anti-hemophiliac factor), rh factor VIII produced in CHO cells	Aventis Behring (King of Prussia, PA, USA)	Hemophilia A	1993 (US)
Recombinate (anti-hemophiliac factor), rh factor VIII produced in a CHO cell line)	Baxter Healthcare (Deerfield, IL, USA)/Genetics Institute	Hemophilia A	1992 (US)
Other blood factors			
Alprolix (rh factor IX fused to a human IgG_1 Fc domain), produced in a HEK cell line	**Biogen Idec**	**Hemophilia B**	**2014 (US)**
Rixubis (rh factor IX), produced in CHO cell line	**Baxter Healthcare**	**Hemophilia B**	**2013 (US)**
Tretten (USA); (NovoThirteen in EU); (Catridecog), rh factor XIII A-subunit, produced in *Saccharomyces cerevisiae*	**Novo Nordisk**	**Congenital factor XIII A-subunit deficiency**	**2012 (EU), 2013 (US)**
Recothrom (thrombin) rh factor IIa, produced in CHO cells	Bristol-Meyers Squibb (BMS; Princeton, NJ, USA)/ Zymogenetics (Seattle, WA, USA)	Control of minor bleeding during surgery	2008 (US)
NovoSeven (eptacog alfa, activated), rh factor VIIa, produced in BHK cells	Novo Nordisk	Some forms of hemophilia	1996 (EU), 1999 (US)
Benefix (nonacog alfa), rh Factor IX produced in CHO cells)	Pfizer/Wyeth	Hemophilia B	1997 (EU and US)
Recombinant thrombolytics, anticoagulants and other blood-related products			
Tissue plasminogen activator (tPA)			
Metalyse (tenecteplase), TNK-tPA, modified rh tPA produced in CHO cells	Boehringer Ingelheim (Ingelheim, Germany)	Myocardial infarction	2001 (EU)
TNKase (tenecteplase) modified rh tPA produced in CHO cells	Roche/Genentech (S. San Francisco, CA, USA)	Myocardial infarction	2000 (US)
Ecokinase (Reteplase) rh tPA produced in *Escherichia coli*; differs from human tPA in that 3 of its 5 domains have been deleted	Roche (Welwyn Garden City, UK)	Acute myocardial infarction	1996 (EU) Withdrawn 2000
Rapilysin (reteplase) rh tPA; see Ecokinase)	Actavis group PTC (Hafnarfjordur, Iceland)/Roche	Acute myocardial infarction	1996 (EU)
Retavase (Reteplase, rh tPA; see –Ecokinase)	Chiesi (Cary, NC, USA)	Acute myocardial infarction	1996 (US)

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Activase (Alteplase, rh tPA produced in CHO cells)	Roche/Genentech (S. San Francisco)	Acute myocardial infarction	1987 (US)
Hirudin			
Refludan (lepirudin) rh hirudin produced in *S. cerevisiae* (anticoagulant)	Bayer Healthcare (Leverkusen, Germany)	Anticoagulation therapy for heparin-associated thrombocytopenia	1997 (EU), 1998 (US) Withdrawn (EU) 2012
Revasc (desirudin), rh hirudin produced in *S. cerevisiae* (anticoagulant)	Canyon Pharmaceuticals, (London)	Prevention of venous thrombosis	1997 (EU)
Other			
Jetrea (ocriplasmin) recombinant truncated form of human plasmin, produced in *Pichia pastoris*	**ThromboGenics (Leuven, Belgium)**	**Symptomatic vitreomacular adhesion/vitreomacular traction**	**2013 (EU) 2012 (US)**
***Ruconest (conestat alfa), rh- complement C1 esterase inhibitor, produced in the milk of transgenic rabbits**	**Salix/Santarus (Raleigh, NC, USA), Pharming (Leiden, the Netherlands)**	**Acute angioedema**	**2014 US 2010 (EU)**
Atryn (rh antithrombin), from milk of transgenic goats	GTC Biotherapeutics London, UK), Ovation Pharmaceuticals (Deerfield, IL, USA)	Hereditary antithrombin deficiency	2009 (US), 2006 (EU)
Kalbitor (ecallantide), rh plasma kallikrein inhibitor, produced in *P. pastoris*	Dyax (Cambridge, MA, USA)	Hereditary angioedema	2009 (US)
Xigris (drotrecogin-α), rh activated protein C produced in a human cell line	Eli Lilly (Houten, the Netherlands)	Severe sepsis	2001 (US), 2002 (EU) Withdrawn 2011

Recombinant hormones			
Insulin			
Afrezza (rh insulin, produced in *E. coli*)	**MannKind (Danbury, CT, USA)**	**Diabetes mellitus**	**2014 (USA)**
Tresiba (insulin degludec), engineered long-acting human insulin analog, produced in *S. cerevisiae* (see also Ryzodeg entry)	**Novo Nordisk**	**Diabetes**	**2013 (EU)**
Ryzodeg (insulin degludec/insulin aspart), combination of 2 engineered insulins, produced in *S. cerevesiae*	**Novo Nordisk**	**Diabetes**	**2013 (EU)**
NovoLog mix (insulin aspart mix, a 50:50 mixture of engineered rh-insulin, produced in *S. cerevisiae* in soluble and protamine suspension forms)	Novo Nordisk	Diabetes mellitus	2008 (US)
Insulin Human Winthrop (rhInsulin produced in *E. coli*)	Sanofi (Frankfurt, Germany)	Diabetes mellitus	2007 (EU)
Exubera (inhalable rh insulin produced in *E. coli*)	Pfizer (Sandwich, UK)	Diabetes mellitus	2006 (EU and US) Withdrawn 2008
Levemir (insulin detemir), long-acting rh insulin produced in *S. cerevisiae*)	Novo Nordisk	Diabetes mellitus	2005 (US), 2004 (EU)
Apidra (insulin glulisine), rapid acting insulin analog, produced in *E. coli*)	Sanofi (Frankfurt, Germany)	Diabetes mellitus	2004 (EU and US)
Actrapid/Velosulin/Monotard/Insulatard/Protaphane/Mixtard/Actraphane/Ultratard (all contain rh insulin produced in *S. cerevisiae* formulated as short/intermediate/long-acting product)	Novo Nordisk	Diabetes mellitus	2002 (EU) Withdrawn (Monotard and Ultratard) 2006 (Velosulin) 2009
Novolog (insulin aspart) short-acting rh insulin analog, produced in *S. cerevisiae*	Novo Nordisk	Diabetes mellitus	2001 (US)
Novolog mix 70/30 (contains insulin aspart, short-acting rh insulin analog as one ingredient, produced in *S. cerevisiae* (see also Novomix)	Novo Nordisk	Diabetes mellitus	2001 (US)
Novomix 30 (contains insulin aspart, short-acting rh insulin analog, produced in *S. cerevisiae*, as one ingredient)	Novo Nordisk	Diabetes mellitus	2000 (EU)
Lantus (insulin glargine), long-acting rh insulin analog, produced in *E. coli*	Sanofi (Frankfurt, Germany)	Diabetes mellitus	2000 (EU and US)
Optisulin (insulin glargine), long-acting rh insulin analog, produced in *E. coli* (see also Lantus entry)	Sanofi (Frankfurt, Germany)	Diabetes mellitus	2000 (EU)
NovoRapid (insulin aspart), rh insulin analog, produced in *S. cerevisiae*	Novo Nordisk	Diabetes mellitus	1999 (EU)
Liprolog (Insulin lispro), insulin analog, produced in *E. coli*	Eli Lilly (Houten, the Netherlands)	Diabetes mellitus	2001 (EU)
Insuman (rh insulin), produced in *E. coli*	Sanofi (Frankfurt, Germany)	Diabetes mellitus	1997 (EU)
Humalog (insulin lispro, rh insulin analog), produced in *E. coli*	Eli Lilly (Houten, the Netherlands)	Diabetes mellitus	1996 (EU and US)

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Novolin (rh insulin), produced in *S. cerevisiae*)	Novo Nordisk	Diabetes mellitus	1991 (US) Withdrawn 2010
Humulin (rh insulin), produced in *E. coli*	Eli Lilly	Diabetes mellitus	1982 (US)
Human growth hormone			
Somatropin Biopartners (somatropin), rh growth hormone, produced in *S. cerevisiae*	**BioPartners (Reutlingen, Germany)**	**Growth failure/growth hormone deficiency**	**2013 (EU)**
Accretropin (somatropin) rhGH produced in *E. coli*	Emergent Biosolutions (Rockville, MD, USA)/Cangene (Winnipeg, MB, Canada)	Growth failure or short stature associated with Turner syndrome in pediatric patients	2008 (US)
Valtropin (somatropin) biosimilar r hGH produced in *S. cerevisiae*	Biopartners (Reutling, Germany), LG Life Sciences (Korea)	Certain forms of growth disturbance in children and adults	2007 (US), 2006 (EU) Withdrawn 2012 (EU)
Omnitrope (somatropin) biosimilar (in EU) r hGH produced in *E. coli*	Sandoz (Kundl, Austria)/Novartis (Princeton, NJ, USA)	Certain forms of growth disturbance in children and adults	2006 (EU and US)
Somavert (pegvisomant) PEGylated r hGH analog (antagonist) produced in *E. coli*	Pfizer (Sandwich, UK)/Nektar Therapeutics (San Francisco)	Acromegaly	2003 (US), 2002 (EU)
Nutropin AQ (r hGH produced in *E. coli*); different formulation of Nutropin—see later entry	Ipsen Pharma (Boulogne-Billiancourt, France)	Growth failure/Turner's syndrome	2001 (EU) Withdrawn (EU) 2008, (US) 1994
Serostim (somatropin), r hGH, produced in a mouse C127 cell line	EMD Serono (Geneva)	AIDS-associated catabolism/wasting	1996 (US)
Saizen (somatropin), r hGH, produced in a mouse C127 cell line	EMD Serono	hGH deficiency in children	1996 (US)
Genotropin (somatropin), r hGH produced in *E. coli*	Pfizer	hGH deficiency in children	1995 (US)
Norditropin (somatropin), r hGH, produced in *E. coli*	Novo Nordisk	Growth failure in children due to inadequate growth hormone secretion	1995 (US)
Tev-tropin/Bio-tropin (somatropin) (r hGH) produced in *E. coli*	Teva Pharmaceuticals USA (North Wales, PA, USA)	hGH deficiency in children	1995 (US)
Nutropin (somatropin), r hGH produced in *E. coli*	Roche/Genentech	hGH deficiency in children	1994 (US)
Humatrope (somatropin) r hGH produced in *E. coli*	Eli Lilly	hGH deficiency in children	1987 (US)
Protropin (somatrem), r hGH, differs from hGH only in containing an additional N-terminal methionine residue; produced in *E. coli*	Genentech	hGH deficiency in children	1985 (US) Withdrawn 2004

Follicle-stimulating hormone			
Ovaleap (follitropin alfa), biosimilar rh FSH, produced in a CHO cell line	**Teva Pharma (Utrecht, the Netherlands)**	**Infertility/subfertility**	**2013 (EU)**
***Elonva (corifollitropin alfa), a modified rh FSH in which the carboxy-terminal peptide of the β subunit of hCG is fused to the FSH β chain produced in CHO cells**	**Merck Sharp Dohme (MSD; Hoddesdon, UK)**	**Controlled ovarian stimulation**	**2010 (EU)**
Fertavid (follitropin β), rh FSH produced in CHO cells. Active identical to 'Puregon'	MSD (Hoddesdon, UK)	Infertility	2009 (EU)
Pergoveris (follitropin α/lutropin α), combination product containing rh FSH and rh LH, both produced in CHO cells	Merck Serono (London)	Stimulation of follicular development in women with severe LH and FSH deficiency	2007 (EU)
Follistim (follitropin-β), rh FSH produced in CHO cells)	Merck (Whitehouse Station, NJ, USA)	Infertility	1997 (US)
Puregon (follitropin-β) rh FSH produced in CHO cells)	N.V. Organon (Oss, the Netherlands)	Anovulation and superovulation	1996 (EU)
Gonal F (follitropin-α), rh FSH produced in CHO cells)	Merck Serono (London); EMD Serono (Rockland, MD, USA)	Anovulation and superovulation	1995 (EU), 1997 (US)
Other hormones			
Myalept (metreleptin), rh leptin analog, produced in *E. coli*	**AstraZeneca (London)/Amylin**	**Some forms of lipodystrophy**	**2014 (US)**
Gattex (in US)/Revestive (in EU); (teduglutide), rh GLP-2 analog, produced in *E. coli*	**NPS Pharma (Dublin)**	**Short bowel syndrome**	**2012 (US and EU)**
Victoza (liraglutide), a GLP-1 analog with attached fatty acid, produced in *S. cerevisiae	**Novo Nordisk**	**Type 2 diabetes**	**2010 (US), 2009 (EU)**
Preotact, rh parathyroid hormone, produced in *E. coli*	NPS Pharma (Dublin)	Osteoporosis	2006 (EU)
Fortical (r salmon calcitonin), produced in *E. coli*	Upsher-Smith Laboratories (Minneapolis, MN, USA)/Unigene (Fairfield, NJ, USA)	Postmenopausal osteoporosis	2005 (US)
Luveris (lutropin α) rh leutinizing hormone produced in CHO cells	EMD Serono	Some forms of infertility	2004 (US) 2000 (EU)

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Forsteo(EU)/Forteo (US) (teriparatide), r shortened human parathyroid hormone produced in *E. coli*	Eli Lilly (Houten, the Netherlands)	Established osteoporosis in some postmenopausal women	2003 (EU) 2002 (US)
Natrecor (nesiritide), rh natriuretic peptide produced in *E. coli*	Johnson & Johnson/Scios (Titusville, NJ, USA)	Acutely decompensated congestive heart failure	2001 (US)
Ovitrelle (EU)/Ovidrel (US) (choriogonadotropin-α) rhCG produced in CHO cells)	Merck/EMD Serono (London)	Selected assisted reproductive techniques	2001 (EU) 2000 (US)
Thyrogen (thyrotrophin-α), rhTSH produced in CHO cells)	Sanofi/Genzyme (Cambridge, MA, USA)	Thyroid cancer (detection and treatment)	1998 (US) 2000 (EU)
Forcaltonin (r salmon calcitonin), produced in *E. coli*	Unigene (Bushey Herne, UK)	Paget's disease	1999 (EU) Withdrawn 2008
Glucagen (rh glucagon), produced in *S. cerevisiae*	Novo Nordisk	Hypoglycemia	1998 (US)
Glucagon (glucagon, recombinant), rhGlucagon, produced in *E. coli*	Eli Lilly	Hypoglycemia	1998 (US)

Recombinant growth factors			
Erythropoietin			
Biopoin (epoetin theta), rhEPO produced in CHO cells	Teva (Ulm, Germany)	Anemia	2009 (EU)
Eporatio (epoetin theta), rhEPO produced in CHO cells	Teva	Anemia	2009 (EU)
Abseamed (epoietin-α), a biosimilar rhEPO produced in CHO cells	Medice Arzneimittel Putter (Iserlon, Germany)	Anemia associated with chronic renal failure	2007 (EU)
Binocrit (epoetin-α), a biosimilar rhEPO produced in CHO cells	Sandoz (Kundl, Austria)	Anemia associated with chronic renal failure	2007 (EU)
Epoetin α Hexal (epoietin-α), biosimilar a rhEPO produced in CHO cells	Hexal (Holzkirchen, Germany)	Anemia associated with chronic renal failure	2007 (EU)
Mircera (methoxy polyethylene glycol-epoetin β), PEGylated rh EPO produced in CHO cells	Roche (Welwyn Garden City, UK)	Anemia associated with chronic kidney disease	2007 (EU and US)
Retacrit (epoetin zeta), a biosimilar rh EPO produced in CHO cells	Hospira (Royal Leamington Spa, UK)	Anemia associated with chronic renal failure	2007 (EU)
Silapo (epoetin zeta), a biosimilar rh EPO produced in CHO cells	Stada (Bad Vibel, Germany)	Anemia associated with chronic renal failure	2007 (EU)
Aranesp (darbepoetin α), long-acting rEPO analog produced in CHO cells	Amgen (Breda, the Netherlands; (EU)	Anemia	2001 (EU and US)
Nespo (darbepoetin α; see also Aranesp) long-acting rEPO analog produced in CHO cells	Dompe Biotec (Milan, Italy)	Anemia	2001 (EU) Withdrawn 2008
Neorecormon (epoietin β), rh EPO produced in CHO cells	Roche (Welwyn Garden City, UK)	Anemia	1997 (EU)
Procrit (epoietin-α), rh EPO produced in a mammalian cell line	Janssen Biotech (Horsham, PA, USA)	Anemia	1990 (US)
Epogen (epoietin-α), rh EPO produced in a CHO cell line	Amgen	Anemia	1989 (US)
Colony-stimulating factors			
Grastofil (biosimilar filgrastim), rh G-CSF produced in *E. coli*	**Apotex (Leiden, the Netherlands)**	**Neutropenia**	**2013 (EU)**
Lonquex (lipegfilgrastim), PEGylated rh G-CSF produced in *E. coli*	**Teva Pharmaceuticals (Utrecht, the Netherlands)**	**Neutropenia**	**2013 (EU)**
Granix (tbo-filgrastim) (rh G-CSF produced in *E. coli*) (Note: this is identical to the product 'Tevagrastim', approved as a biosimilar in EU in 2008; see Tevagrastim entry below)	**Teva (Frazer, PA, USA)/Cephalon (Malvern, PA, USA)**	**Neutropenia**	**2012 (US)**
***Nivestim (biosimilar filgrastim, rhG-CSFproduced in *E. coli*)**	**Hospira (Lemington Spa, UK)**	**Neutropenia**	**2010 (EU)**
Filgrastim hexal biosimilar filgrastim, rh G-CSF produced in *E. coli*)	Hexal (Holzkirchen, Germany)	Neutropenia	2009 (EU)
Zarzio (biosimilar filgrastim, rh G-CSF produced in *E. coli*)	Sandoz (Kundl, Austria)	Neutropenia	2009 (EU)
Biograstim (biosimilar filgrastim, rh G-CSF produced in *E. coli*)	ABZ pharma (Ulm, Germany)	Neutropenia	2008 (EU)
Ratiograstim (biosimilar filgrastim; rh G-CSF produced in *E. coli*)	Ratiopharm (Ulm, Germany)	Neutropenia	2008 (EU)
Tevagrastim (biosimilar filgrastim, rh G-CSF produced in *E. coli*)	Teva (Radebeul, Germany)	Neutropenia	2008 (EU)
Filgrastim ratiopharm (biosimilar filgrastim; rh G-CSF produced in *E. coli*)	Ratiopharm (Ulm, Germany)	Neutropenia	2008 (EU) Withdrawn 2011
Neulasta (pegfilgrastim), PEGylated rh G-CSF. Also marketed in EU as Neupopeg	Amgen (Breda, the Netherlands)	Chemotherapy-induced neutropenia	2002 (EU and US) Withdrawn (EU, Neupopeg) 2008

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Leukine (sargramostim), rh GM-CSF, differs from the native human protein by one amino acid, R23→L; produced in *E. coli*	Sanofi/Berlex Laboratories	Autologous bone marrow transplantation	1991 (US) Withdrawn 2008 and reformulated without EDTA since 2008
Neupogen (filgrastim), rh G-CSF differs from human protein by containing an additional N-terminal methionine; produced in *E. coli*	Amgen	Chemotherapy-induced neutropenia	1991 (US)
Other growth factors			
Increlex (mecaserim), rh IGF-1 produced in *E.coli*	Ispen Pharma (Boulogne-Billancourt, France) (formerly Tercica, Brisbane, CA, USA)	Growth failure in children with IGF-1 deficiency or GH gene deletion (long-term treatment)	2007 (EU), 2005 (US)
IPlex (mecasermin rinfabate), a complex of rh IGF-1 and rh IGFBP-3 produced separately in *E. coli*	Insmed (Glen Allen, VA, USA)	Growth failure in children with severe primary IGF-1 deficiency or GH gene deletion (long-term treatment	2005 (US) Withdrawn 2007 for IGF-1 deficiency as per lawsuit filed by Genentech and Tercia
Kepivance (palifermin), a rh KGF produced in *E. coli*	Swedish Orphan Biovitrum (Stockholm, Sweden) (acquired from Amgen since last listed)	Severe oral mucositis in selected patients with hematologic cancers	2005 (EU) 2004 (US)
GEM 21S (growth factor enhanced matrix; contains rh PDGF-BB (Regranex—see entry below) and tricalcium phosphate)	BioMimetic Pharmaceuticals (Franklin, TN, USA)	Periodontally related defects	2005 (US)
Regranex (becaplermin), rh PDGF-BB produced in *S. cerevisiae*	Novartis/Johnson & Johnson (Raritan, NJ, USA)	Lower extremity diabetic neuropathic ulcers	1997 (US) 1999 (EU) Withdrawn (EU) 2012

Recombinant interferons, interleukins and tumor necrosis factors

Interferon-α			
PEGintron/ribetol combo pack (peginterferon-α), PEGylated rh IFNα-2b produced in *E. coli* and ribavirin	Schering Plough (Kenilworth, NJ, USA)	Chronic hepatitis C	2008 (US)
Pegasys (PEGinterferon α-2a), produced in *E. coli*	Roche/Genentech (Welwyn Garden City, UK)	Hepatitis C	2002 (EU and US)
PegIntron (PEG rIFN-α-2b), produced in *E. coli*	Merck Sharp & Dohem (MSD, Hoddesdon, UK)	Chronic hepatitis C	2000 (EU) 2001 (US)
Viraferon (rIFN-α-2b), produced in *E. coli*)	Schering Plough (Brussels)	Chronic hepatitis B, C	2000 (EU) Withdrawn 2008
ViraferonPeg (PEG rIFN-α-2b), produced in *E. coli*	MSD (Hoddesdon, UK)	Chronic hepatitis C	2000 (EU)
Intron A (also known as Alfatronol) (rIFN-α-2b), produced in *E. coli*	MSD (Hoddesdon, UK)	Cancer, genital warts, hepatitis	1986 (US) 2000 (EU)
Rebetron (combination of ribavirin and rh IFN-α2b) produced in *E. coli*	Schering Plough	Chronic hepatitis C	1999 (US)
Infergen (interferon alficon-1), r IFN-α, synthetic type I IFN produced in *E. coli*	InterMune/Amgen	Chronic hepatitis C	1997 (US) 1999 (EU) Withdrawn (EU) 2006
Roferon A (rh IFN-α2a), produced in *E. coli*	Roche	Hairy cell leukemia	1986 (US) Withdrawn 2007
Interferons β & γ			
Plegridy (rh peginterferon beta 1a), produced in a CHO cell line	**Biogen Idec (Berkshire, UK)**	**Multiple sclerosis**	**2014 (EU)**
Extavia (interferon beta-1b), rh IFN-β1b produced in *E. coli*	Novartis	Multiple sclerosis	2009 (US) 2008 (EU)
Rebif (interferon-β1a), rh IFN-β1a, produced in CHO cells	EMD Serono (London)	Relapsing/remitting multiple sclerosis	2002 (US) 1998 (EU)
Avonex (interferon-β1a), rh IFN-β1a, produced in CHO cells	Biogen-IDEC (Maidenhead, UK)	Relapsing multiple sclerosis	1997 (EU) 1996 (US)
Betaferon (interferon-β-1b), r IFN-β1b, differs from human protein by C17→S, produced in *E. coli*	Bayer Pharma (Berlin)	Multiple sclerosis	1995 (EU)
Betaseron (rIFN-β1b), differs from human protein by C17→S, produced in *E. coli*	Bayer/Berlex Labs (Richmond, CA, USA)/Chiron (Emeryville, CA, USA)	Relapsing/remitting multiple sclerosis	1993 (US)
Actimmune (rh IFN-γ1b, produced in *E. coli*)	Vidara Therapeutics (Dublin)	Chronic granulomatous disease	1990 (US)

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Others			
Kineret (anakinra), rh IL-1 receptor antagonist produced in *E. coli*	Swedish Orphan Biovitrum/ Amgen	Rheumatoid arthritis	2001 (US)
Beromun (tasonermin), rh TNF-α, produced in *E. coli*	Boehringer Ingelheim (Ingelheim, Germany)	Adjunct to surgery for subsequent tumor removal, to prevent or delay amputation	1999 (EU)
Neumega (oprelvekin), rh IL-11, lacks N-terminal proline of native molecule produced in *E. coli*	Pfizer/Genetics Institute	Prevention of chemotherapy-induced thrombocytopenia	1997 (US)
Proleukin (aldesleukin), rh IL-2, differs from human molecule in absence of an N-terminal alanine and contains C125→S substitution, produced in *E. coli*	Prometheus Therapeutics and Diagnostics (San Diego)/Chiron	Renal cell carcinoma	1992 (US)

Recombinant vaccines			
Hepatitis B			
Hexacima/Hexyon (multicomponent vaccine containing r HBsAg produced in Hansenula polymorpha as one component	Sanofi Pasteur (Lyon, France)	Immunization against several pathogens/toxins	2013 (EU)
Ambirix (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	GlaxoSmithKline (GSK, Rixensart, Belgium)	Immunization against hepatitis A and B	2002 (EU)
Pediarix (combination vaccine containing rHBsAg produced in *S. cerevisiae* as one component)	GSK	Immunization of children against various conditions inducing hepatitis B	2002 (US)
HBVAXPRO (rHBsAg produced in *S. cerevisiae*)	Sanofi (Lyon, France)	Immunization of children and adolescents against hepatitis B	2001 (EU)
Twinrix (adult and pediatric forms in EU; combination vaccine containing rHBsAg produced in *S. cerevisiae* as one component)	GSK (Rixensart, Belgium)	Immunization against hepatitis A and B	2001 (US) 1996 (EU adult) 1997 (EU pediatric)
Infanrix Hexa (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	GSK (Rixensart, Belgium)	Immunization against diphtheria, tetanus, pertussis, *Haemophilus influenzae* b and hepatitis B and polio	2000 (EU)
Infanrix Penta (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	GSK (Rixensart, Belgium)	Immunization against diphtheria, tetanus, pertussis, polio and hepatitis B	2000 (EU) Withdrawn 2013
Hepacare (r S, pre-S & pre-S2 HBsAg produced in a murine cell line)	Evans Vaccines (Liverpool, UK)	Immunization against hepatitis B	2000 (EU) Withdrawn 2002
Hexavac (combination vaccine, containing rHBsAg produced *S. cerevisiae* as one component)	Sanofi Pasteur (Lyon, France)	Immunization against diphtheria, tetanus, pertussis, hepatitis B, polio and *H. influenzae* type b	2000 (EU) Withdrawn 2012
Procomvax (combination vaccine, containing r HBsAg as one component)	Sanofi Pasteur (Lyon, France)	Immunization against *H. influenzae* type B and hepatitis B	1999 (EU) Withdrawn 2009
Primavax (combination vaccine, containing r HBsAg produced in *S. cerevisiae* as one component)	Sanofi Pasteur (Lyon, France)	Immunization against diphtheria tetanus and hepatitis B	1998 (EU) Withdrawn 2000
Engerix B (r HBsAg) produced in *S. cerevisiae*	GSK	Immunization against hepatitis B	1998 (US)
Infanrix Hep B (combination vaccine containing rHBsAg produced in *S. cerevisiae* as one component)	GSK (Rixensart, Belgium)	Immunization against diphtheria, tetanus, pertussis and hepatitis B	1997 (EU) Withdrawn 2005
Comvax (combination vaccine, containing HbsAg produced in *S. cerevisiae*, as one component)	Merck (Whitehouse Station, NJ, USA)	Immunization of infants against *H. influenzae* type B and hepatitis B	1996 (US)
Tritanrix-Hep B (combination vaccine, containing r HBsAg produced in *S. cerevisiae* as one component)	GSK (Rixensart, Belgium)	Immunization against hepatitis B, diphtheria, tetanus and pertussis	1996 (EU) Withdrawn 2014
Recombivax (r HBsAg produced in *S. cerevisiae*)	Merck	Immunization against hepatitis B	1986 (US)
Other			
Bexsero (meningococcal group B vaccine, rDNA component, absorbed). Multicomponent subunit vaccine, produced in *E. coli*.	**Novartis (Siena, Italy)**	**Immunization against invasive meningococcal disease**	**2013 (EU)**
Flublok (recombinant hemagglutinin proteins from 3 influenza strains), produced in an *Spodoptera frugiperda* cells using baculovirus	**Protein Sciences (Meriden, CT, USA)**	**Immunization against influenza**	**2013 (US)**
***Provenge (sipuleucel-T, autologous peripheral blood mononuclear cells in combination with rh prostatic acid phosphatase–GM-CSF produced in an insect cell line)**	**Dendreon (London)**	**Prostate cancer**	**2013 (EU) 2010 (US)**

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Cervarix (r, C-terminally truncated major caspid L 1 proteins from human papillomavirus types 16 and 18 produced in a baculovirus-based expression system)	GSK (Rixensart, Belgium)	Prevention of cervical cancer	2009 (US) 2007 (EU)
Gardasil (EU & US). Also marketed as Silgard in EU, (quadrivalent human papillomavirus (HPV) r vaccine; contains major capsid proteins from four HPV types, produced in *S. cerevisiae*)	In EU: Sanofi-Pasteur, Lyon France; (Gardisil), Merck	Therapeutic indication: vaccination against diseases caused by HPX	2006 (EU and US)
Dukoral (*Vibrio cholerae* and r cholera toxin B subunit)	Crucell Sweden (Stockholm, Sweden)	Immunization against disease caused by *V. cholerae* subunit O 1	2004 (EU)
Lymerix (r OspA, a lipoprotein found on the surface of *B. burgdorferi*, produced in *E. coli*.)	GSK	Immunization against Lyme disease	1998 (US) Withdrawn 2002
Triacelluvax (combination vaccine containing r modified pertussis toxin as one component)	Chiron (Siena, Italy)	Immunization against diphtheria, tetanus and pertussis	1999 (EU) Withdrawn 2002

Monoclonal antibody (mAb)-based products			
Entyvio (vedolizumab), humanized IgG targeting the human α4β7 integrin, produced in CHO cells	Takeda Pharma/Millennium (Deerfield, IL, for USA; Taastruup, Denmark, for EU)	Ulcerative colitis, Crohn's disease	2014 (US & EU)
Sylvant (siltuximab), chimeric mAb that binds human interleukin-6, produced in a CHO cell line	Janssen Biotech (Horsham, PA, USA)	Multicentric Castleman's disease	2014 (US & EU)
Cyramza (ramucirumab), human mAb that binds the VEGF-2 receptor, produced in NS0 cell line	Eli Lilly	Gastric cancer	2014 (US)
Gazyva(US)/Gazyvaro (EU) (obinutuzumab), humanized glycoengineered mAb specific for CD20 expressed on B lymphocytes, produced in a CHO cell line	Roche (Genentech)/ Roche, Welwyn Garden City, UK (EU)	Chronic lymphocytic leukemia	2014 (EU) 2013 (US)
Inflectra/Remsima (infliximab), biosimilar, chimeric mAb specific for TNF-α, produced in Sp2/0 cell line	Hospira (Royal Leamington Spa, UK; Inflectra) Celltrion (Budapest, Hungary (Remsima)	Arthritis, colitis, Crohn's, psoriasis, ankylosing spondylitis	2013 (EU)
Kadcyla (trastuzumab emtansine), humanized mAb specific for HER-2 antigen, produced in CHO cell line and conjugated to the small-molecule cytotoxin, DM1	Roche/Genentech (Welwyn Garden City, UK)	Breast cancer	2013 (EU and US)
Simponi Aria (golimumab), identical active to that in Simponi (see below); different active strength and mode of administration	Janssen Biotech	Rheumatoid arthritis	2013 (US)
Perjeta (pertuzumab), human mAb specific for HER2, produced in a CHO cell line	Roche/Genentech (Welwyn Garden City, UK)	Breast cancer	2013 (EU) 2012 (US)
Abthrax (raxibacumab), human IgG mAb raised against the protective antigen (PA) of Bacillus anthracis, produced in a NS0 cell line	GSK/Human Genome Sciences	Inhalational anthrax	2012 (US)
Adcetris (brentuximab vedotin), chimeric mAb conjugate specific for human CD 30 (expressed on the surface of lymphoma cells), produced in a CHO cell line	Takeda Pharma (Roskilde, Denmark)/Seattle Genetics	Lymphoma	2012 (EU) 2011 (US)
Benlysta (belimumab), human mAb which targets human B-lymphocyte stimulator (BLyS), a B-cell survival factor, produced in an NS0 cell line	Glaxo Group (Greenford, UK/ Human Genome Sciences (USA)	Lupus	2011 (US and EU)
Xgeva (denosumab) (see Prolia entry)	Amgen (Breda, the Netherlands)	Treatment of bone loss associated with cancer	2011 (EU) 2010 (US)
Yervoy (ipilimumab), human mAb. Binds to CTLA-4 (a negative regulator of T-cell activation), thereby enhancing T-cell activation and proliferation, produced in CHO cell line	Bristol-Myers Squibb (Uxbridge, UK)	Melanoma	2011 (US and EU)
Actemra (US)/RoActemra (EU) (tocilizumab), humanized mAb specific for IL-6, produced in a mammalian cell line	Roche (Welwyn Garden City, UK)	Rheumatoid arthritis	2010 (US) 2009 (EU)
Arzerra (ofatumumab), human mAb specific for CD20, produced in NS0 hybridoma cells	Novartis/Genmab (Greenford, UK)	Chronic lymphocytic leukemia	2010 (EU) 2009 (US)
Prolia (denosumab), human mAb specific for RANK ligand, produced in CHO cells	Amgen (Breda, the Netherlands)	Osteoporosis in postmenopausal women	2010 (EU and US)
Scintimun (besilesomab), murine mAb specific for NCA-95 found on surface of granulocytes, produced in hybridoma cells	CIS Bio International (Gif sur Yvette Cedex, France)	*In vivo* diagnosis/investigation of sites of inflammation/infection via scintigraphic imaging	2010 (EU)
Cimzia (certolizumab pegol), anti-TNFα humanized and PEGylated antibody Fab′ fragment produced in *E. coli*	UCB Pharma (Brussels, Belgium)	Crohn's disease, rheumatoid arthritis	2009 (EU) 2008 (US)
Ilaris (canakinumab), human mAb specific for interleukin-1β, produced in murine Sp2/0 cells	Novartis (Horsham, UK)/ Regeneron (Tarrytown, NY, USA)	Cryopyrin-associated periodic syndromes (CAPS)	2009 (EU and US)
Removab (catumaxomab), a bispecific engineered antibody produced in hybrid hybridoma cells	Neovii Biotech (Graefelfing, Germany)	Malignant ascites in patients with EpCAM positive carcinomas	2009 (EU)
Simponi (golimumab) human mAb specific for TNF-α, produced in Sp2/0 cells	Janssen Biotech (Beerse, Belgium)	Rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis	2009 (EU and US)

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Stelara (ustekinumab), human mAb specific for the p40 subunit of IL-12 and IL-23, produced in Sp2/0 cells)	Janssen Biotech (Beerse, Belgium)	Moderate to severe plaque psoriasis	2009 (EU and US)
Lucentis (ranibizumab), humanized IgG fragment that binds and inactivates VEGF-A, produced in *E. coli*	Roche/Genentech	Neovascular (wet) age-related macular degeneration	2007 (EU) 2006 (US)
Soliris (eculizumab), a humanized IgG that binds human C5 complement protein, produced in a murine myeloma cell line	Alexion (Cheshire, CT, USA, Paris)	Paroxysmal nocturnal hemoglobinuria	2007 (US and EU)
Vectibix (panitumumab), human mAb that binds to hEGFR, produced in a CHO cell line	Amgen (Breda, the Netherlands)/ Abgenix	EGFR-expressing colorectal carcinoma	2007 (EU) 2006 (US)
Tysabri (natalizumab), a humanized mAb raised against selected leukocyte alpha4 beta1/7 integrins, produced in a murine myeloma cell line	Biogen Idec (Maidenhead, UK)/ Elan	Relapsing forms of multiple sclerosis	2006 (EU) 2004 (US); 2005 suspended, 2006 resumed
Xolair (omalizumab), humanized mAb that binds IgE at the site of high-affinity IgE receptor binding, produced in CHO cells	Roche/Genentech	Moderate to severe persistent asthma in adults and adolescents	2005 (EU) 2003 (US)
Zevalin (ibritumomab tiuxetan), murine mAb targeted against the CD20 antigen, produced in CHO cells	Spectrum Pharmaceuticals (Amsterdam, the Netherlands)	Non-Hodgkin's lymphoma	2004(EU) 2002 (US)
Erbitux (cetuximab), chimeric mAb against human EGF receptor, produced in Sp2/0 cells	Merck/BMS/Lilly/Imclone Systems (New York)	EGF receptor-expressing metastatic colorectal cancer	2004 (EU and US)
Raptiva (efalizumab), humanized mAb that binds to LFA-1, which is expressed on all leukocytes; produced in CHO cells	Serono (London) Genentech (US)	Chronic moderate to severe plaque psoriasis in adults	2004 (EU) 2003 (US) Withdrawn 2009
Avastin (bevacizumab), humanized mAb raised against VEGF; produced in CHO cells	Roche/Genentech (Welwyn Garden City, UK)	Metastatic colorectal cancer, glioblastoma, metastatic renal carcinoma	2005 (EU) 2004 (US)
NeutroSpec (fanolesomab) murine mAb raised against CD 15 surface antigen of selected leukocytes, produced in hybridoma cells	Palatin Technolgies (Cranbury, NJ, USA)/Mallinckrodt (Hazelwood, MO, USA)		2004 (US) Withdrawn 2005
Humira (EU and US) was also sold as Trudexa in EU (adalimumab) (anti-TNF) human mAb produced in a CHO cell line	AbbVie (Maidenhead, UK)	Rheumatoid arthritis	2003 (EU) 2002 (US) Withdrawn (EU, Trudexa) 2003
Bexxar (tositumomab), radiolabeled mAb directed against CD20, produced in a murine hybridoma cell line	GSK	CD 20 positive follicular non-Hodgkin's lymphoma	2003 (US) Withdrawn 2014
Mabcampath (EU) or Campath (US) (alemtuzumab), humanized mAb directed against CD52 surface antigen of B-lymphocytes, produced in a CHO cell line	Genzyme (Naarden, the Netherlands), Millennium (Cambridge, MA, USA)	Chronic lymphocytic leukemia	2001 (EU and US) Withdrawn (EU) 2012
Mylotarg (gemtuzumab zogamicin) a humanized antibody-toxic antibiotic conjugate targeted against CD33 antigen found on leukemic blast cells, produced in an NSO cell line	Wyeth (Madison, NJ, USA)	Acute myeloid leukemia	2000 (US) Withdrawn 2010
Herceptin (trastuzumab), humanized mAb directed against HER 2, produced in a murine cell line	Roche/Genentech (Welwyn Garden City, UK)	Treatment of metastatic breast cancer if tumor overexpresses HER2 protein	1998 (US) 2000 (EU)
Remicade (infliximab), chimeric mAb directed against TNF-α, produced in an Sp2/0 cell line	Janssen Biotech (Leiden, the Netherlands)	Crohn's disease	1998 (US) 1999 (EU)
Synagis (palivizumab), humanized mAb directed against an epitope on the surface of respiratory syncytial virus, produced in a murine myeloma cell line	MedImmune (Gaithersburg, MD, USA) /AbbVie (London) AstraZeneca	Prophylaxis of lower respiratory tract disease caused by syncytial virus in pediatric patients	1998 (US) 1999 (EU)
Zenapax (daclizumab), humanized mAb directed against the α-chain of the IL-2 receptor), produced in an NSO cell line	Roche/(Welwyn Garden City, UK)/ Protein Design Labs	Prevention of acute kidney transplant rejection	1997 (US) 1999 (EU) Withdrawn (EU) 2009
Humaspect (votumumab), human mAb directed against cytokeratin tumor-associated antigen, produced in a human lymphoblastoid cell line	KS Biomedix (Farnham, UK)	Detection of carcinoma of the colon or rectum	1998 (EU) Withdrawn 2004
Mabthera (EU)/Rituxan (US) (rituximab), chimeric mAb directed against CD20 surface antigen of B lymphocytes, produced in a CHO cell line	Roche (Welwyn Garden City, UK)/ Biogen-Idec	Non-Hodgkin's lymphoma	1998 (EU) 1997 (US)
Simulect (basiliximab), chimeric mAb directed against the α-chain of the IL-2 receptor, produced in a murine myeloma cell line	Novartis (Horsham, UK)	Prophylaxis of acute organ rejection in allogeneic renal transplantation	1998 (EU)
LeukoScan (sulesomab), murine mAb fragment (Fab) directed against NCA 90, a surface granulocyte nonspecific cross-reacting antigen, produced in an Sp2/0 cell line	Immunomedics (Darmstadt, Germany)	Diagnostic imaging for infection and inflammation in bone of patients with osteomyelitis	1997 (EU)

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Verluma (nofetumomab), murine mAb fragments (Fab) directed against carcinoma-associated antigen, produced in a murine cell line	Boehringer Ingelheim/NeoRx (Seattle)	Detection of small-cell lung cancer	1996 (US) Withdrawn 1999
Tecnemab KI, murine mAb fragments (Fab/Fab_2 mix) directed against HMW-MAA, produced in murine ascites culture	Amersham Sorin (Milan, Italy)	Diagnosis of cutaneous melanoma lesions	1996 (EU) Withdrawn 2000
ProstaScint (capromab pentetate), murine mAb-directed against the prostrate-specific membrane antigen (PSMA), produced in a murine cell line	EUSA Pharma/Cytogen (Princeton, NJ, USA)	Detection, staging and follow-up of prostate adenocarcinoma	1996 (US)
MyoScint (imiciromab-pentetate), murine mAb fragment directed against human cardiac myosin, produced in a murine cell line	Centocor	Myocardial infarction imaging agent	1996 (US) Withdrawn 1999
CEA-scan (arcitumomab), murine mAb fragment (Fab), directed against human carcinoembryonic antigen, CEA, produced in mice ascites	Immunomedics (Darmstadt, Germany)	Detection of recurrent/metastatic colorectal cancer	1996 (EU and US) Withdrawn 2005
Indimacis 125 (igovomab), murine mAb fragment (Fab_2) directed against the tumor-associated antigen CA 125, produced in a murine cell line	CIS Bio (Gif-sur-Yvette, France)	Diagnosis of ovarian adenocarcinoma	1996 (EU) Withdrawn 2009
ReoPro (abciximab), Fab fragments derived from a chimeric mAb, directed against the platelet surface receptor GPIIb/IIIa, produced in a mammalian cell line	Janssen Biologics (Leiden, the Netherlands)/Centocor	Prevention of blood clots	1994 (US)
OncoScint CR/OV (satumomab pendetide), murine mAb directed against the tumor-associated glycoprotein, TAG-72, produced in a murine cell line	Cytogen	Detection, staging and follow-up of colorectal and ovarian cancers	1992 (US) Withdrawn 2002
Orthoclone OKT3 (muromomab CD3), murine mAb directed against the T-lymphocyte surface antigen CD3, produced in a murine cell line	Janssen-Cilag/Ortho Biotech	Reversal of acute kidney transplant rejection	1986 (US)
Other recombinant products			
Bone morphogenetic proteins			
Opgenra (eptotermin α), rhBMP-7 produced in CHO cells	Olympus Biotech (Limerick, Ireland)	Posterolateral lumbar spinal fusion	2009 (EU)
Infuse bone graft (contains dibotermin-alfa, a rh BMP-2 produced in CHO cells placed on an absorbable collagen sponge. Note: this is the same active ingredient present in the product Infuse)	Wyeth (Madison, NJ, USA)	Acute open tibial shaft fracture	2004 (US)
Inductos (dibotermin alfa); rhBMP-2 produced in CHO cells	Medtronic BioPharma (Heerlen, the Netherlands); Genetics Institute (Cambridge, MA)	Acute tibia fractures	2002 (EU)
Infuse (rh BMP2 produced in CHO cells)	Medtronic Sofamor Danek (Memphis, TN, USA)	Promotes fusion of lower spine vertebrae	2002 (US)
OP-1 implant (US)/Osigraft (EU) (eptotermin alfa), rh BMP-7, produced in CHO cells	Olympus Biotech (Limerick, Ireland); Stryker Biotech (Hopkington, MA, USA)	Non-union of tibia	2001 (EU and US)
Recombinant enzymes			
Vimizim (elosulfase alfa), rh *N*-acetylgalactosamine-6-sulfatase, produced in a CHO cell line	**BioMarin (London)**	**Mucopolysaccharidosis IVA (Morquio A syndrome)**	**2014 (US and EU)**
Krystexxa (pegloticase), r urate oxidase, PEGylated post synthesis, produced in *E. coli*	**Savient (Dublin)/Crealta Pharmaceuticals (Lake Forest, IL, USA)**	**Gout**	**2013 (EU) 2010 (US)**
Elelyso (taliglucerase alfa) rh glucocerebrosidase, produced in engineered carrot root cell culture	**Pfizer/Protalix (Karmiel, Israel)**	**Gaucher disease**	**2012 (US)**
Voraxaze (glucarpidase) r carboxypeptidase, produced in *E. coli*	**BTG International**	**Treatment of toxic plasma methotrexate concentrations in patients with delayed methotrexate clearance due to impaired renal function**	**2012 (US)**
***Lumizyme (alglucosidase alfa), rh acid- α-glucosidase, produced in a CHO cell line**	**Sanofi/Genzyme**	**Pompe disease (glycogen storage disease type II)**	**2010 (US)**
***VPRIV (velaglucerase alfa), rh-glucocerebrosidase, produced in a human fibroblast cell line**	**Shire Human Genetics (Danderyd, Sweden)**	**Gaucher disease**	**2010 (US and EU)**
Elaprase (idursulfase), rh iduronate-2-sulfatase, produced in a human cell line	Shire Human Genetic Therapies (Danderyd, Sweden)	Mucopolysaccharidosis II (Hunter's syndrome)	2007 (EU) 2006 (US)
Naglazyme (galsulfase), rh *N*-acetylgalactosamine 4 sulfatase, produced in a CHO cell line	BioMarin (London)/ Novato, CA, USA	Long-term enzyme replacement therapy in patients suffering from Mucopolysaccharidosis VI	2006 (EU) 2005 (US)

Table 1 (continued)

Product	Company (location)	Therapeutic indication	Date approved
Myozyme (alglucosidase α), rh acid glucosidase produced in CHO cells	Sanofi/Genzyme (Naarden, the Netherlands)	Pompe disease	2006 (EU and US)
Aldurazyme (laronidase), r-α-L-iduronidase produced in CHO cells	BioMarin	Long-term replacement in patients with mucopolysaccharidosis I	2003 (EU and US)
Hylenex (rh hyaluronidase), produced in CHO cells	Baxter/ Halozyme Therapeutics (San Diego)	Adjuvant to increase absorption and dispersion of other drugs	2005 (US)
Fabrazyme (agalsidase beta), rh α-galactosidase, produced in CHO cells	Sanofi/Genzyme (Naarden, the Netherlands)	Fabry disease (α-galactosidase A deficiency)	2003 (US) 2001 (EU)
Replagal (agalsidase alfa), rh α-galactosidase, produced in a human cell line	Shire Human Genetic Therapies (Danderyd, Sweden)/TKT Europe	Fabry disease (α-galactosidase A deficiency)	2001 (EU)
Fasturtec (Elitex in US) (rasburicase), r urate oxidase, produced in *S. cerevisiae*	Sanof (Paris)	Hyperuricemia	2001 (EU) 2002 (US)
Cerezyme (imiglucerase), rh-β-glucocerebrosidase, produced in a CHO cell line	Sanofi/Genzyme (Naarden, the Netherlands)	Gaucher disease	1997 (EU) 1994 (US)
Pulmozyme (dornase-α), r DNase produced in CHO cells	Roche/Genentech	Cystic fibrosis	1993 (US)
Fusion proteins			
Eperzan (in EU)/ Tanzeum (in USA) (albiglutide), GLP-1 receptor agonist, a fusion protein consisting of two tandem copies of modified human GLP-1 to human albumin, produced in *S. cerevisiae*	**GSK (Cork, Ireland)**	**Type 2 diabetes**	**2014 (EU & US)**
Zaltrap (aflibercept), a combination drug containing a fusion protein, consisting of the extracellular ligand binding domains of VEGF receptors 1 and 2 fused to an IgG Fc, produced in a CHO cell line	**Regeneron/Sanofi (Paris)**	**Metastatic colorectal cancer**	**2013 (EU) 2012 (USA)**
Eylea (aflibercept). Same active biopharmaceutical active as in Zaltrap	**Regeneron/Bayer (Berlin)**	**Neovascular (wet) age-related Macular degeneration**	**2012 (EU) 2011 (USA)**
Nulojix (belatacept), a fusion protein consisting of the extracellular domain of human CTLA4 fused to IgG Fc. It binds CD80 and CD86 on antigen-presenting cells, thereby inhibiting T-cell activation, produced in a CHO cell line	**Bristol-Myers Squibb (Uxbridge, UK)**	**Prophylaxis of organ rejection following kidney transplant**	**2011 (USA and EU)**
Arcalyst (US)/Rilonacept (EU) (rilonacept) dimeric fusion protein with each monomer consisting of the ligand-binding domains of the hIL-1 receptor and the IL-1 receptor accessory protein, and the Fc region of h IgG-1, produced in CHO cells	Regeneron	Cryopyrin-associated periodic syndromes (CAPS)	2009 (EU) 2008 (US) Withdrawn (EU) 2012
Nplate (romiplostim), a dimeric fusion protein with each monomer consisting of two thrombopoietin receptor binding domains and the Fc region of hIgG-1, produced in *E. coli*	Amgen (Breda, the Netherlands)	Thrombocytopenia	2009 (EU), 2008 (US)
Orencia (abatacept), fusion protein which links the extracellular domain of human cytotoxic T-lymphocyte associated antigen-4 with modified Fc region of IgG1, produced in a mammalian cell line	BMS (Uxbridge, UK)	Rheumatoid arthritis	2007 (EU), 2005 (US)
Amevive (alefacept), dimeric fusion protein consisting of the extracellular CD2-binding portion of the human LFA-3 linked to the Fc region of human $IgG1_1$ produced in CHO cells	Astellas Pharma/Biogen-Idec	Moderate to severe chronic plaque psoriasis in adults	2003 (US) Withdrawn 2011
Enbrel (etanercept), rTNF receptor–IgG fragment fusion protein, produced in CHO cells	Amgen (Immunex)/Pfizer/Takeda	Rheumatoid arthritis	1998 (US) 2000 (EU)
Ontak (denileukin diftitox), r IL-2–diphtheria toxin fusion protein that targets cells displaying a surface IL-2 receptor, produced in *E. coli*	Eisai (Tokyo)/Ligand Pharmaceuticals (San Diego)	Cutaneous T-cell lymphoma	1999 (US)
Gene therapy and nucleic acid-based			
Kynamro (mipomersen sodium) an 2′-*O*-(2-methoxy) ethyl–modified ribose antisense oligonucleotide)	**Sanofi/Isis (Carlsbad, CA, USA)**	**Familial hypercholesterolemia**	**2013 (USA)**
Glybera (alipogene tiparvovec), h *LPL* gene housed in an engineered AAV1 vector	**uniQure (Amsterdam, the Netherlands)**	**Lipoprotein lipase deficiency**	**2012 (EU)**
Macugen (pegaptanib sodium injection), a synthetic PEGylated oligonucleotide aptamer that specifically binds VEGF protein	Eyetech (New York)/Pfizer (EU)/Valeant Pharmaceuticals (Montreal)	Treatment of neovascular, age-related macular degeneration	2006 (EU) 2004 (USA)
Vitravene (fomivirsen), an antisense oligonucleotide	Isis Pharmaceuticals/Novartis	Cytomegalovirus retinitis in AIDS patients	1998 (US) 1999 (EU) Withdrawn (EU)

Table 3 The 20 top-selling biopharmaceutical products in 2013

Ranking	Product	Sales ($ billions)[a]	Year first approved	Company	Patent expiry (EU)	Patent expiry (US)
1	Humira (adalimumab; anti-TNF)	11.00	2002	AbbVie & Eisai	2018	2016
2	Enbrel (etanercept; anti-TNF)	8.76	1998	Amgen, Pfizer, Takeda Pharmaceuticals	2015	2028
3	Remicade (infliximab; anti-TNF)	8.37	1998	J&J, Merck & Mitsubishi Tanabe Pharma	2015	2018
4	Lantus (insulin glargine)	7.95	2000	Sanofi	2014	2014
5	Rituxan/MabThera (rituximab; anti CD20)	7.91	1997	Biogen-IDEC, Roche	2013	2016
6	Avastin (bevacizumab; anti-VEGF)	6.97	2004	Roche/Genentech	2019	2017
7	Herceptin (anti-HER2)	6.91	1998	Roche/Genentech	2014	2019
8	Neulasta (pegfilgrastim)	4.39	2002	Amgen	2015	2014
9	Lucentis (ranibizumab; anti-VEGF)	4.27	2006	Roche/Genentech, Novartis	2016	2016
10	Epogen/Procrit/Eprex/ESPO (epoetin alfa)	3.35	1989	Amgen, J&J, KHK	Expired	2013
11	Novolog/Novorapid (insulin aspart)	3.13	1999	Novo	2015	2015
12	Avonex (IFN-β-1a)	3.00	1996	Biogen Idec	2015	2015
13	Humalog mix 50:50 (insulin lispro)	2.61	1996	Lilly	2015	2014
14	Rebif (IFN-β-1a)	2.59	1998	Merck Serono	2015	2013
15	Aranesp/Nesp (darbepoetin α)	2.42	2001	Amgen, KHK	2016	2024
16	Advate/Recombinate (Octocog α)	2.37	1992	Baxter		
17	Levemir (insulin detemir)	2.15	2004	Novo	[Levemir]	2014
18	Actrapid/Novolin (insulin)	2.02	1991	Novo	2017	
19	Erbitux (cetuximab; anti-EGF)	1.92	2004	Bristol-Myers Squibb, Merck Serono	2014	2016
20	Eylea (aflibercept; anti-VEGF)	1.88	2011	Regeneron, Bayer	2020	2021

[a]Financial data from LaMerie Business Intelligence. J&J, Johnson & Johnson

Table 4 Biosimilar products that have gained European Marketing Authorization within the EU.

Product type	Biosimilar brand	Reference product	Year approved	Marketing authorization sponsor	Manufacturer of active substance
Somatropin (hGH)	Omnitrope	Genotropin	2006	Sandoz (Kundl, Austria)	Sandoz (Kundl, Austria)
	Valtropin	Humatrope	2006 (withdrawn 2012)	Biopartners (Reutlingen, Germany)	LG Life Sciences (Jeonbuk-do, South Korea)
Epoetin alfa (EPO)	Binocrit	Eprex/Erypo	2007	Sandoz (Kundl, Austria)	Rentschler (Laupheim, Germany) & Lek (Menges, Slovenia)
	Epoetin alfa hexal		2007	Hexal (Holzkirchen, Germany)	
	Abseamed		2007	Medice Arzneimittel (Iserlohn, Germany)	
Epoetin zeta (EPO)	Retacrit		2007	Hospira (Warwickshire, UK)	Norbitec (Uetersen, Germany)
	Silapo		2007	Stada (Vilbel, Germany)	
Filgrastim (G-CSF)	Ratiograstim	Neupogen	2008	Ratiopharm (Ulm, Germany)	Sicor (Vilnius, Lithuania)
	Filgrastim ratiopharm		2008 (withdrawn 2011)	Ratiopharm (Ulm, Germany)	
	Biograstim		2008	AbZ pharma (Ulm, Germany)	
	Tevagrastim		2008	Teva (Radebeul, Germany)	
	Zarzio		2009	Sandoz (Kundl, Austria)	Sandoz (Kundl, Austria)
	Filgrastim hexal		2009	Hexal (Holzkirchen, Germany)	
	Nivestim		2010	Hospira (Warwickshire, UK)	Hospira (Zagreb, Croatia)
	Grastofil		2013	Apotex (Leiden, the Netherlands)	Intas Biopharmaceuticals (Gujarat, India)
Follitropin alfa (FSH)	Ovaleap	Gonal F	2013	Teva (Utrecht, the Netherlands)	Merckle Biotech, (Ulm, Germany)
	Bemfola		2014	Finox Biotech (Balzers, Liechtenstein)	Polymun Scientific Immunbiologische Forschung (Klosterneuburg, Austria)
mAb	Remsima	Remicade	2013	Celltrion Hungary Budapest, Hungary	Celltrion (Incheon, Korea)
	Inflectra		2013	Hospira (Warwickshire, UK)	

hGH, human growth hormone

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Product type	Biosimilar brand	Reference product	Year approved	Marketing authorization sponsor	Manufacturer of active substance
Somatropin (hGH)	Omnitrope	Genotropin	2006	Sandoz (Kundl, Austria)	Sandoz (Kundl, Austria)
	Valtropin	Humatrope	2006 (withdrawn 2012)	Biopartners (Reutlingen, Germany)	LG Life Sciences (Jeonbuk-do, South Korea)
Epoetin alfa (EPO)	Binocrit	Eprex/Erypo	2007	Sandoz (Kundl, Austria)	Rentschler (Laupheim, Germany) & Lek (Menges, Slovenia)
	Epoetin alfa hexal		2007	Hexal (Holzkirchen, Germany)	
	Abseamed		2007	Medice Arzneimittel (Iserlohn, Germany)	
Epoetin zeta (EPO)	Retacrit		2007	Hospira (Warwickshire, UK)	Norbitec (Uetersen, Germany)
	Silapo		2007	Stada (Vilbel, Germany)	
Filgrastim (G-CSF)	Ratiograstim	Neupogen	2008	Ratiopharm (Ulm, Germany)	Sicor (Vilnius, Lithuania)
	Filgrastim ratiopharm		2008 (withdrawn 2011)	Ratiopharm (Ulm, Germany)	
	Biograstim		2008	AbZ pharma (Ulm, Germany)	
	Tevagrastim		2008	Teva (Radebeul, Germany)	
	Zarzio		2009	Sandoz (Kundl, Austria)	Sandoz (Kundl, Austria)
	Filgrastim hexal		2009	Hexal (Holzkirchen, Germany)	
	Nivestim		2010	Hospira (Warwickshire, UK)	Hospira (Zagreb, Croatia)
	Grastofil		2013	Apotex (Leiden, the Netherlands)	Intas Biopharmaceuticals (Gujarat, India)
Follitropin alfa (FSH)	Ovaleap	Gonal F	2013	Teva (Utrecht, the Netherlands)	Merckle Biotech, (Ulm, Germany)
	Bemfola		2014	Finox Biotech (Balzers, Liechtenstein)	Polymun Scientific Immunbiologische Forschung (Klosterneuburg, Austria)
mAb	Remsima	Remicade	2013	Celltrion Hungary Budapest, Hungary	Celltrion (Incheon, Korea)
	Inflectra		2013	Hospira (Warwickshire, UK)	

hGH, human growth hormone

Interleukin 6

Fusion strategy for E.coli production

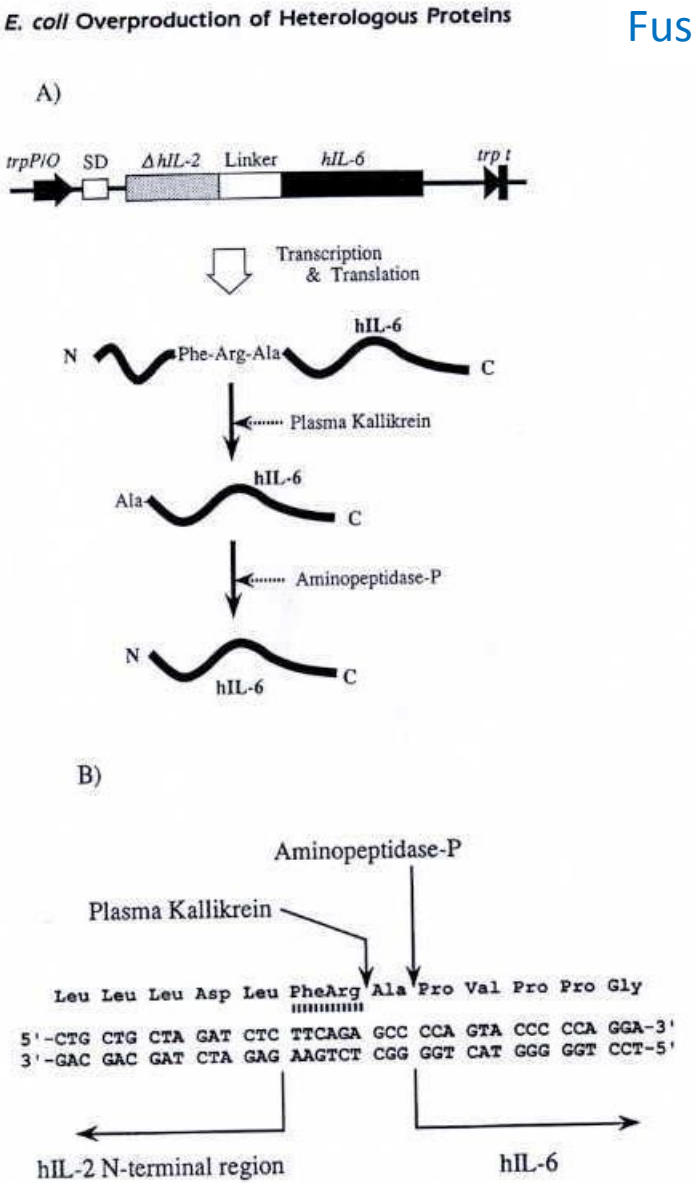

Figure 1 Fusion gene expression system for production of hIL-6. (A) Preparation strategy of the mature hIL-6 from the fusion protein by enzymatic cleavage processing. (B) Structure of the fusion protein at the junction point. (From Ref. 107)

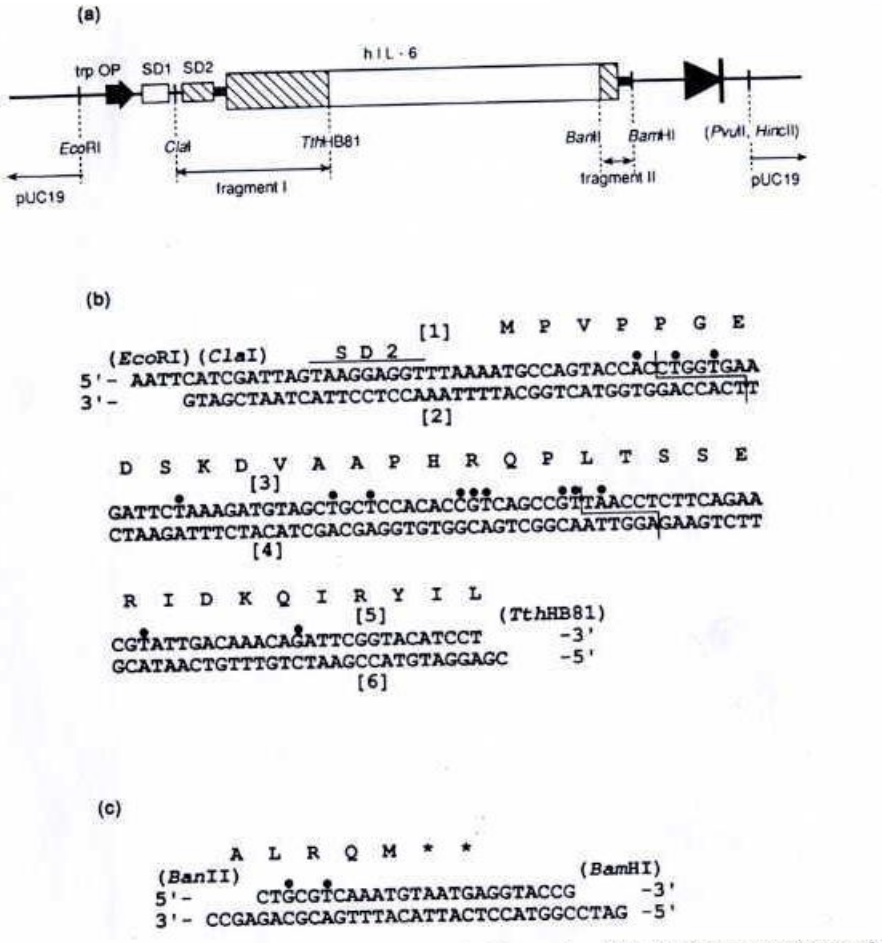

Figure 2 Structure of pBSF2-SD7 used for the high-level expression of hIL-6. (a) Schematic diagram of t structure of the expression system. The hatched boxes represent chemically synthesized DNA fragments. (Nucleotide sequences of the fragment I, and (c) the fragment II. The dots above the nucleotides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

Recombinant Production of Human Interleukin 6 in *Escherichia coli*

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1 Department of Agrobiotechnology, Agricultural and Environmental Faculty, University of Rostock, Rostock, Germany, **2** Bioserv GmbH, Rostock, Germany

Abstract

In this study, we compared basic expression approaches for the efficient expression of bioactive recombinant human interleukin-6 (IL6), as an example for a difficult-to-express protein. We tested these approaches in a laboratory scale in order to pioneer the commercial production of this protein in *Escherichia coli* (*E. coli*). Among the various strategies, which were tested under Research and Development (R&D) conditions, aggregation-prone IL6 was solubilized most effectively by co-expressing cytoplasmic chaperones. Expression of a Glutathion-*S*-Transferase (GST) fusion protein was not efficient to increase IL6 solubility. Alteration of the cultivation temperature significantly increased the solubility in both cases, whereas reduced concentrations of IPTG to induce expression of the T7lac-promotor only had a positive effect on chaperone-assisted expression. The biological activity was comparable to that of commercial IL6. Targeting the expressed protein to an oxidizing environment was not effective in the generation of soluble IL6. Taken together, the presence of chaperones and a lowered cultivation temperature seem effective to isolate large quantities of soluble IL6. This approach led to *in vivo* soluble, functional protein fractions and reduces purification and refolding requirements caused by downstream purification procedures. The final yield of soluble recombinant protein averaged approximately 2.6 mg IL6/liter of cell culture. These findings might be beneficial for the development of the large-scale production of IL6 under the conditions of current good manufacturing practice (cGMP).

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Competing Interests: The authors have the following interests. Roswitha Koslowski and Udo Meyer are employed by BIOSERV GmbH. The experiments for this publication were done in cooperation BIOSERV GmbH. The company provided the equipment for the Bioassay in order to test the biological activity of plant-made C5a. There are no further patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

* E-mail: inge.broer@uni-rostock.de

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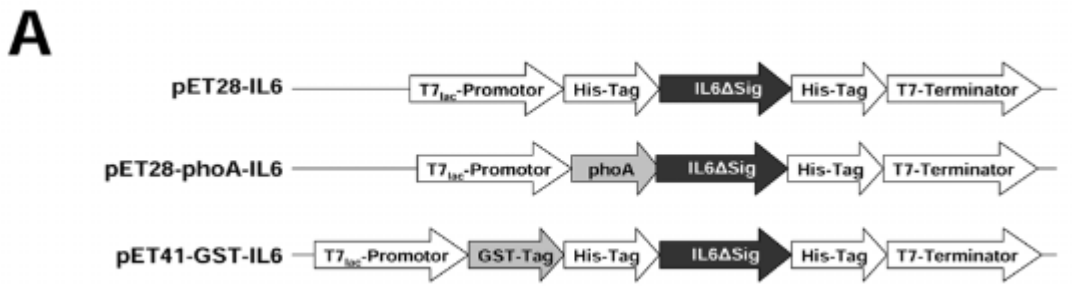


Table 1. Recombinant strains used in this work.

Host strain	Recombinant plasmids	Fusion Tag
Origami 2	pET28-IL6ΔSig	n- & c-terminal His Tag
		T7-Tag
BL21	pET28-IL6ΔSig	n- & c-terminal His Tag
		T7-Tag
BL21	pET28-phoA-IL6ΔSig	c-terminal His-Tag
BL21	pET28-IL6ΔSig	n- & c-terminal His Tag
	pBB540/pBB542	T7-Tag
BL21	pET28-phoA-IL6ΔSig	c-terminal His-Tag
	pTUM4.1	
BL21	pET41-GST-IL6ΔSig	n-terminal GST-Tag
		n- & c-terminal His-Tag

doi:10.1371/journal.pone.0054933.t001

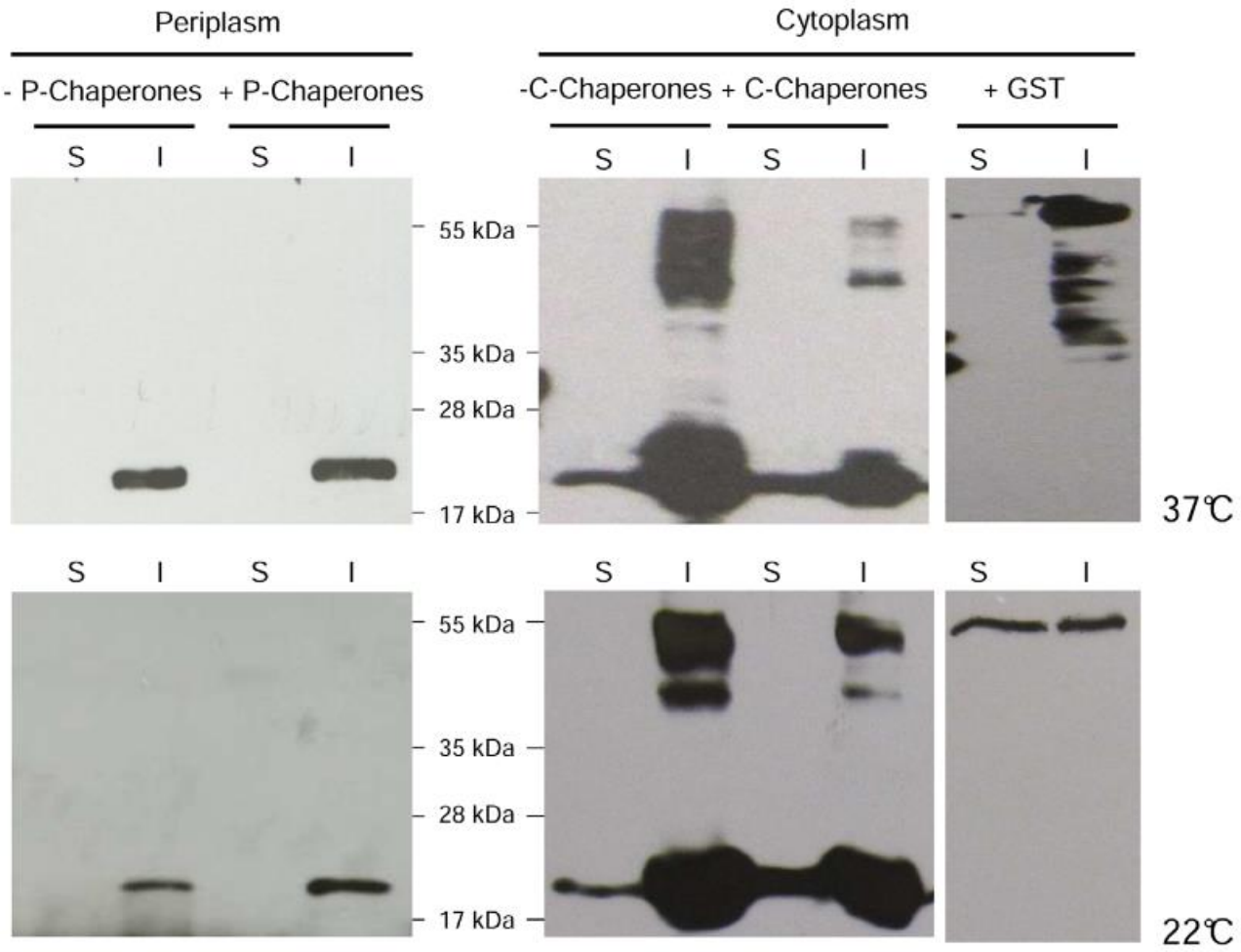


Figure 3. Western blotting analysis of the IL6 expression in the cytoplasm and periplasm of *E. coli* BL21. (S) soluble and (I) insoluble fraction of IL6, expressed with and without the concomitant overexpression of endogenous cytoplasmic chaperones DnaK, DnaJ, GrpE, GroES, GroEL (C-Chaperones) or periplasmic chaperones DsbA, DsbC, SurA, FkpA (P-Chaperones) at 37°C and 22°C, respectively. Additionally, IL6 was expressed fused to GST in the cytoplasm.
doi:10.1371/journal.pone.0054933.g003

Human Growth Hormone - **hGH**

hGH

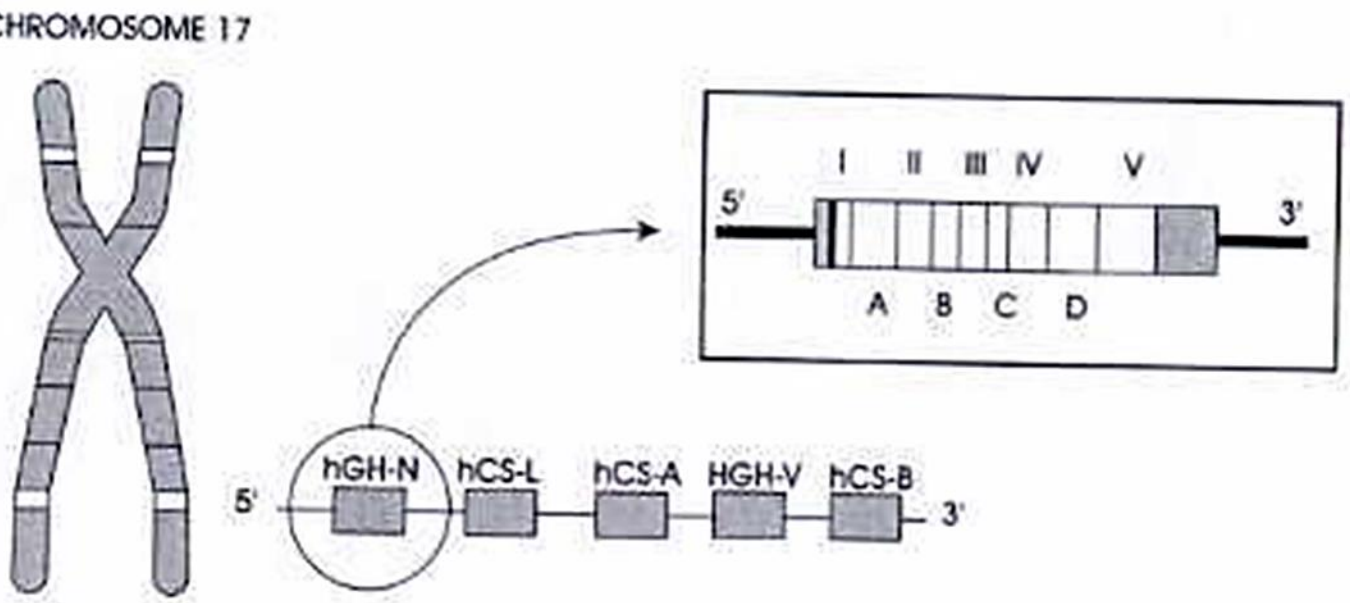


Figure 2. Gene responsible for the synthesis of GH. HGH-N: human growth hormone normal, hGH-V human growth hormone variant, hCS-L: human chorionic somatomammotropin like, hCS-A and hCS-B human chorionic somatomammotropin.

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).

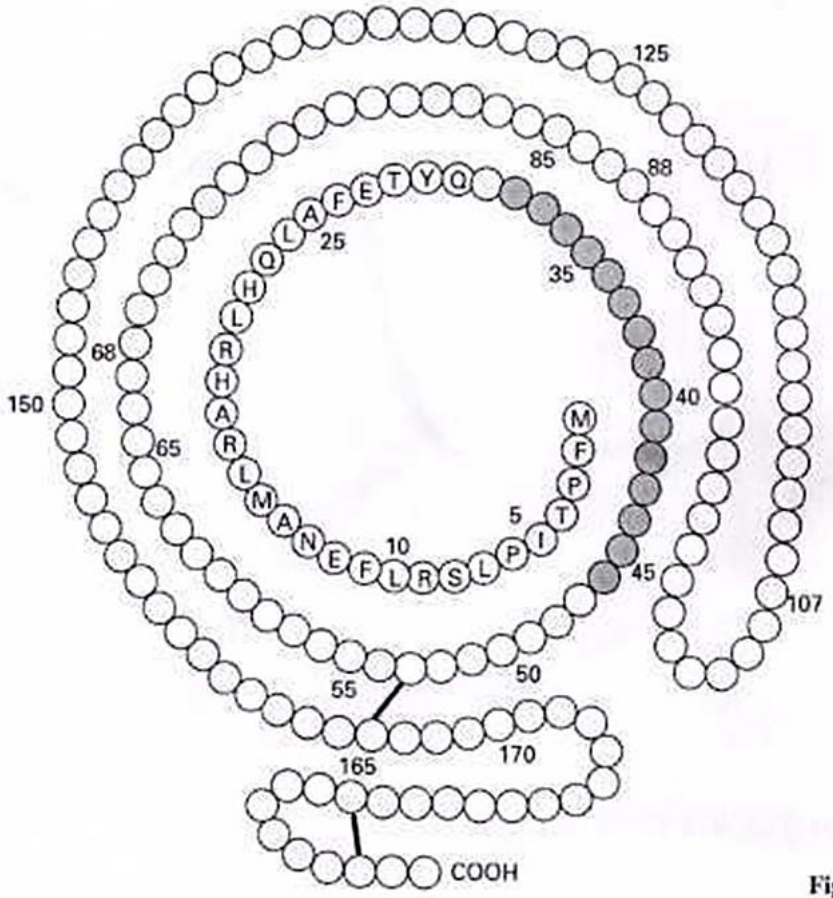


Figure 1. Structure of GH.

hGH

Intracellular Production

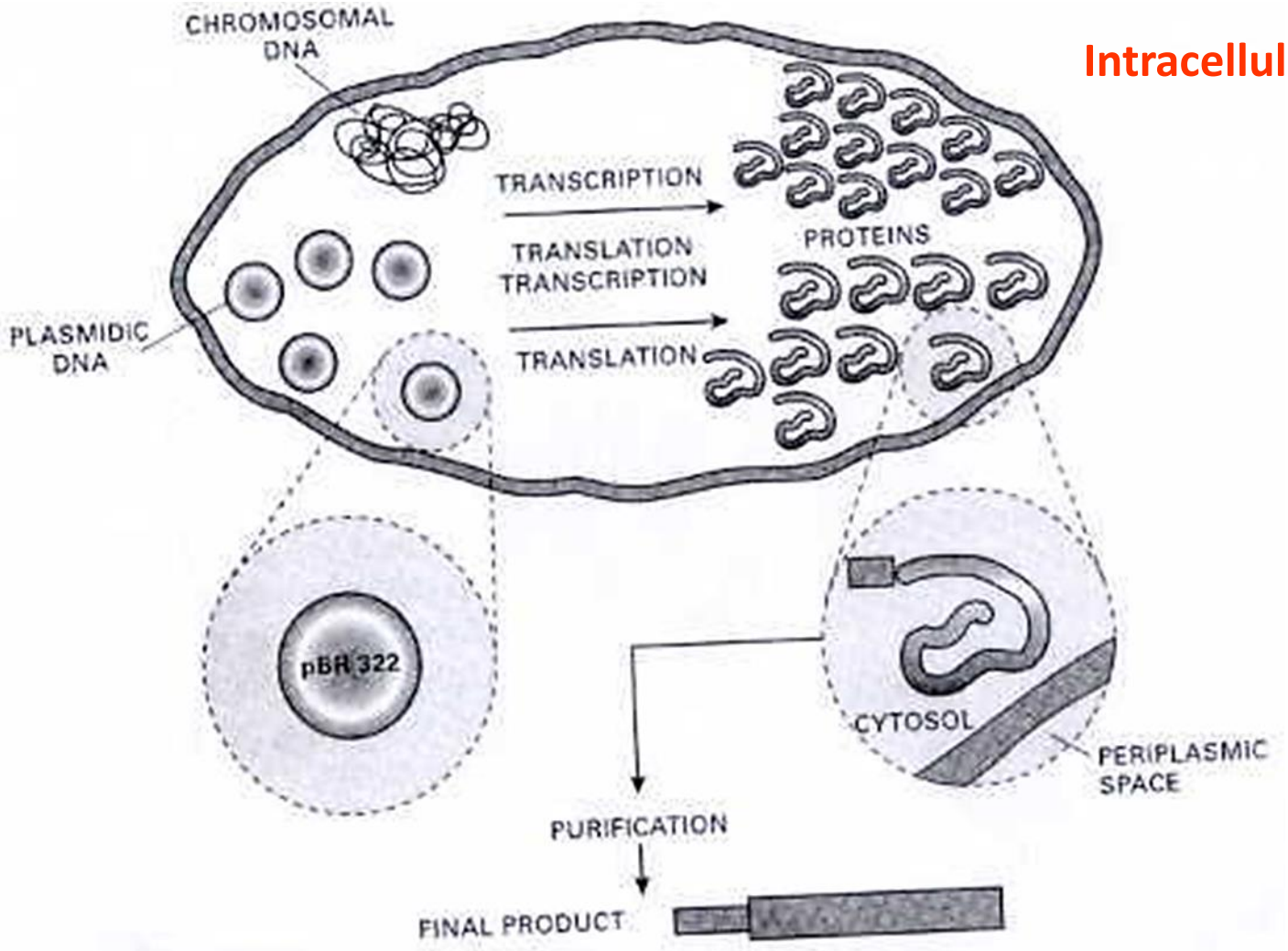


Figure 9. First GH production technique by *E. coli*.

1st aa replaced by Met

Met

hGH

Intracellular Production

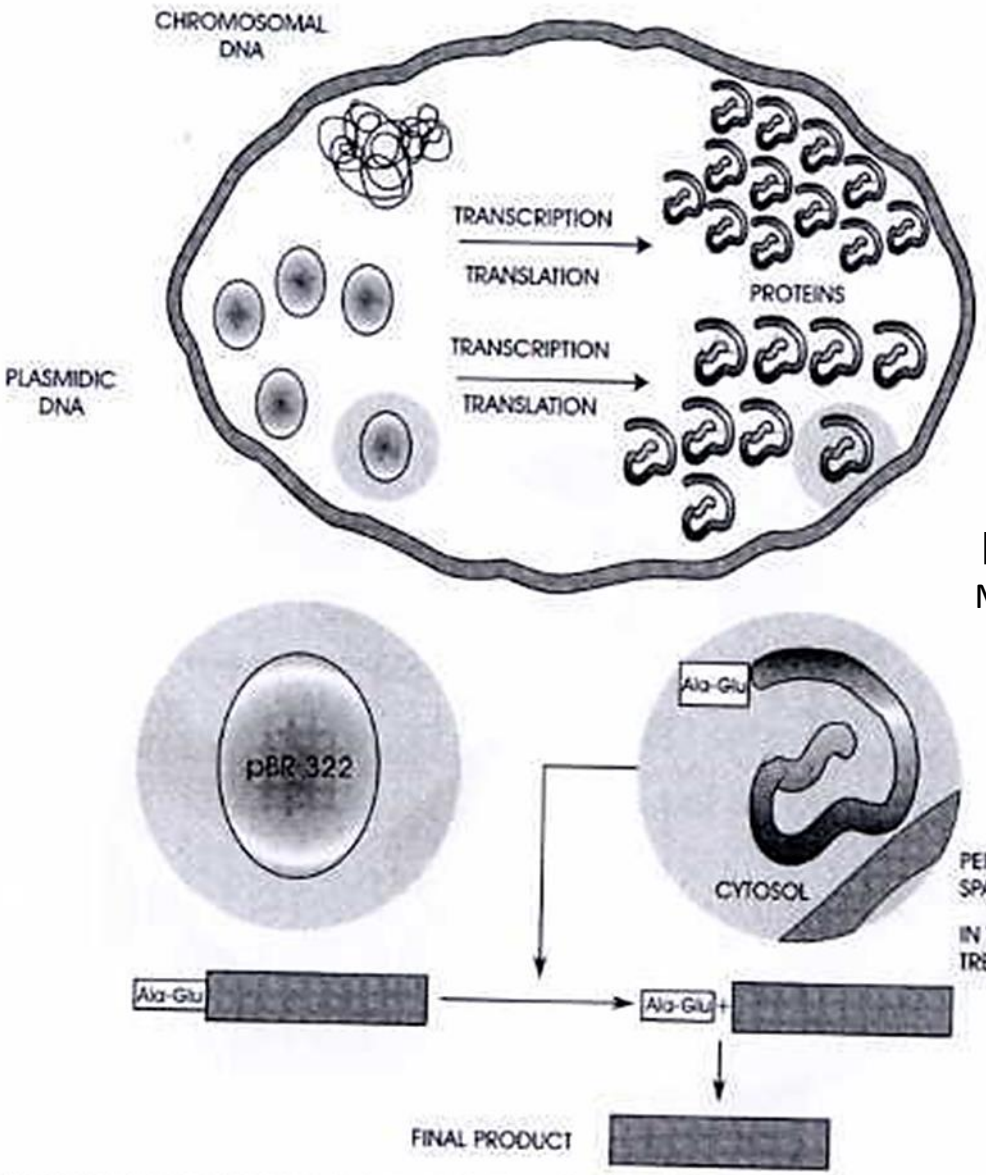
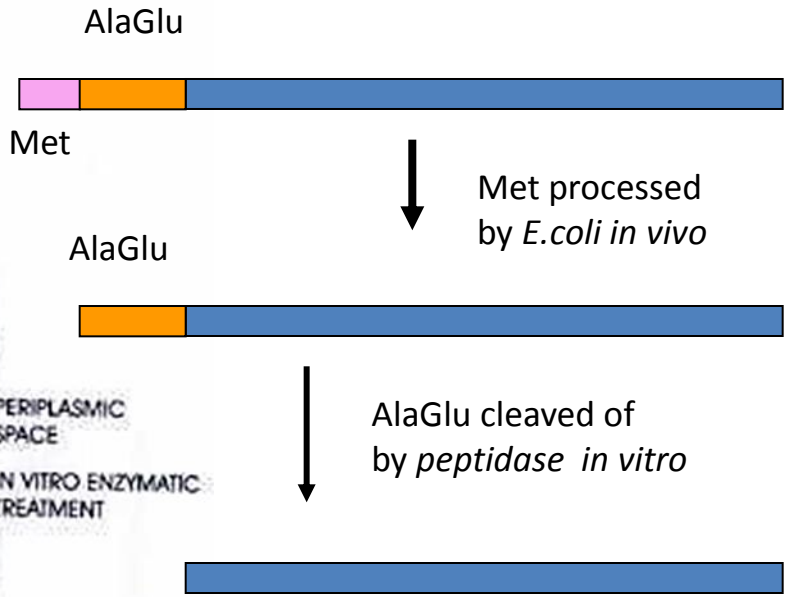


Figure 10. Second GH production technique by *E. coli*.



hGH

Secretory Expression

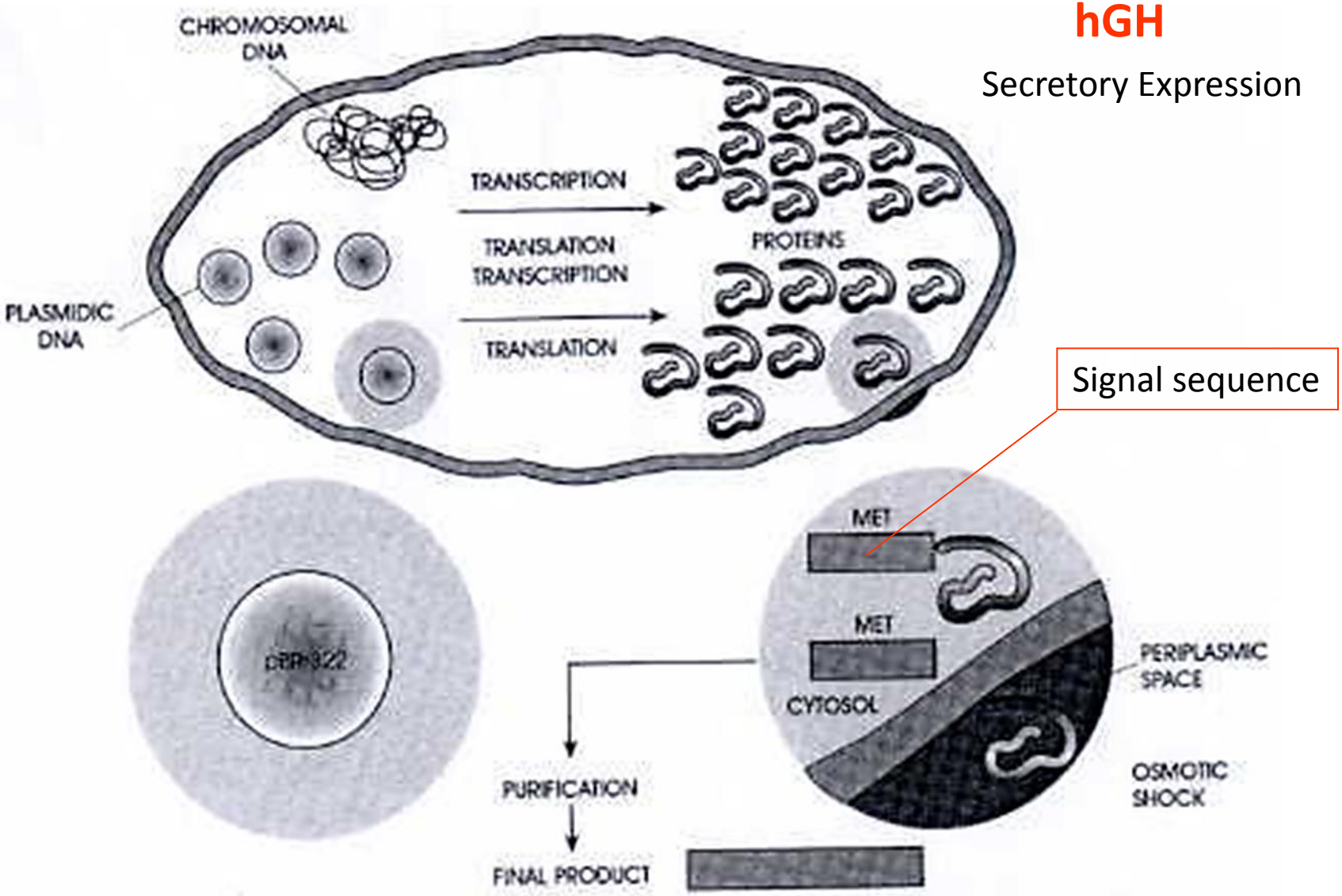


Figure 11. Third synthetic procedure for GH synthesis in *E. coli*.

Secretory Production in Eucaryotic Cell Line

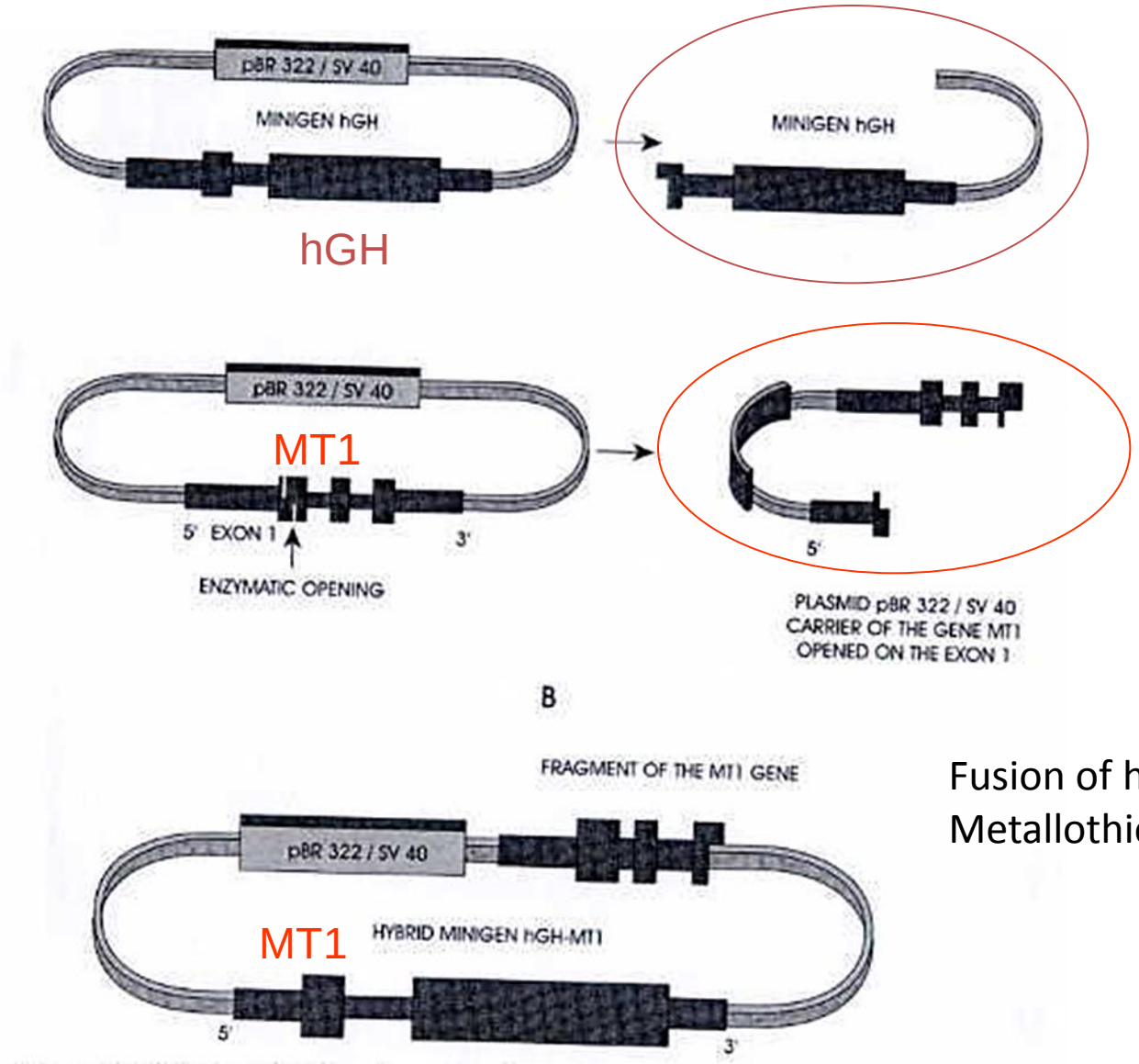


Figure 12. Synthesis of GH in eukaryotic cells.

hGH

Fusion of hGH Gene to Metallothionein Promoter

hGH

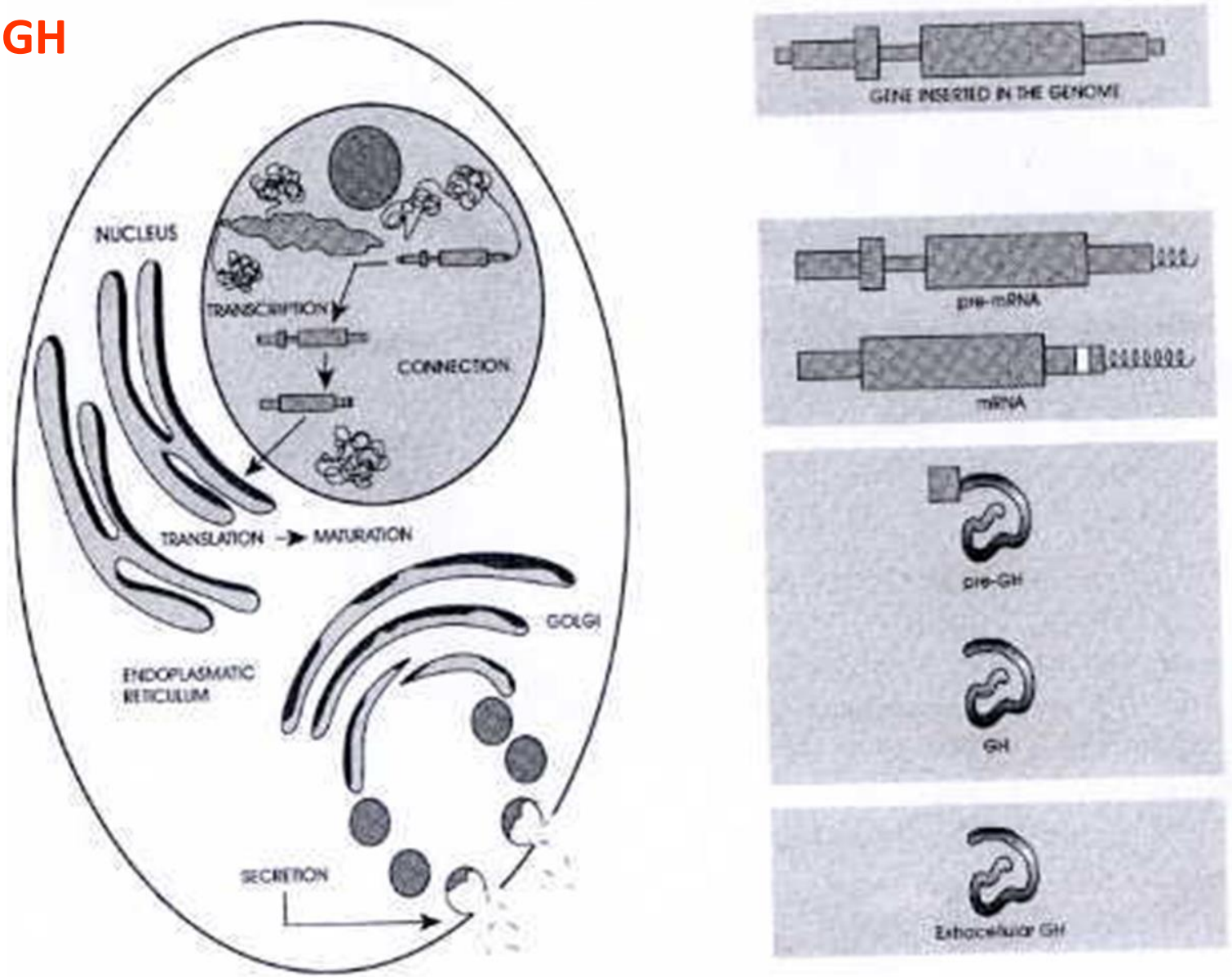
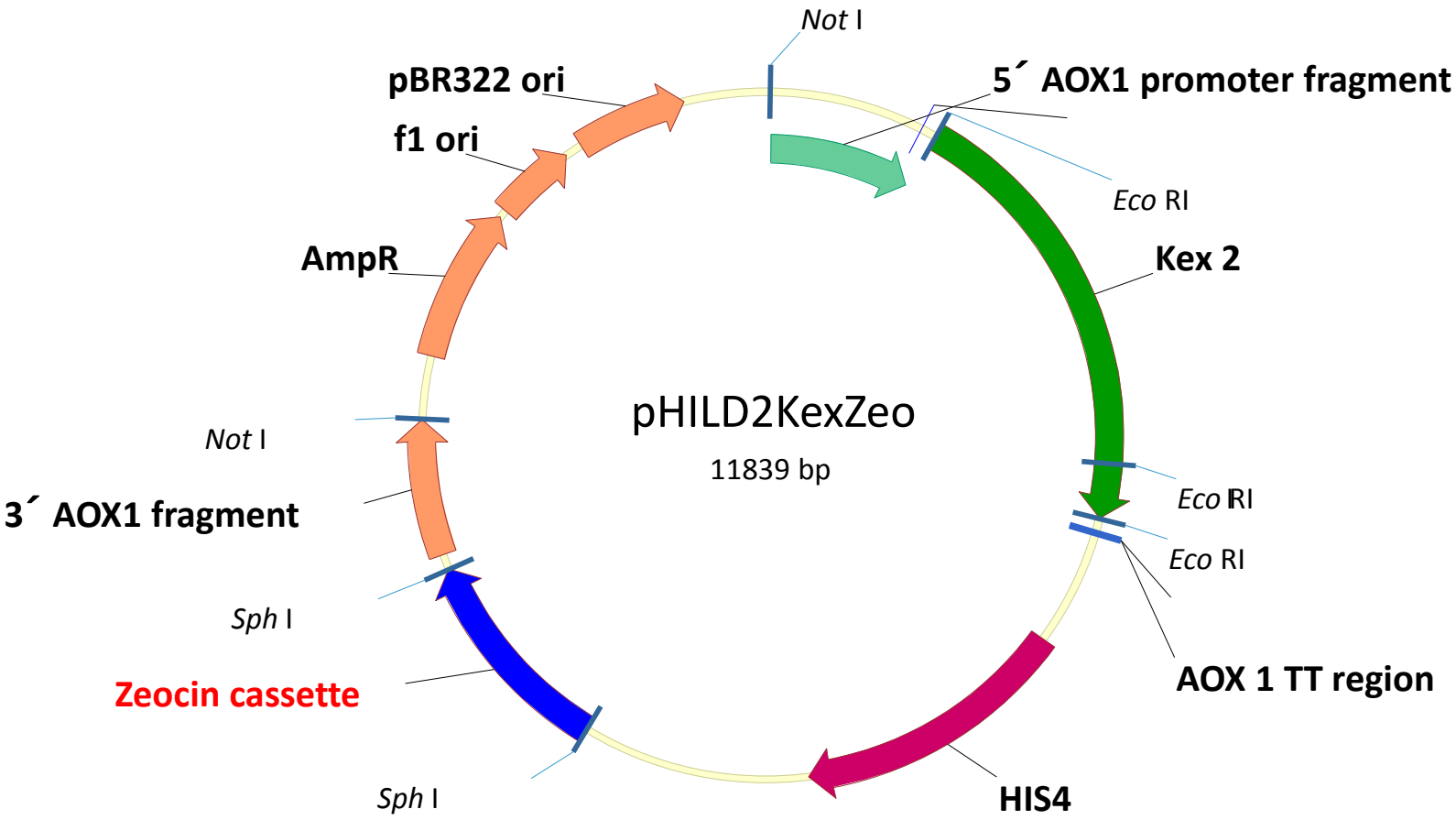


Figure 13. Secretion of GH to the medium from an eukaryotic cell.

Secretory systems → Engineering

Construction of pHILαHZeo

Co-Expression of KEX2



Integration in HIS4 locus is possible with selection for Zeozin resistance

Secretory systems → Engineering

Co-expression of Processing enzymes → KEX2

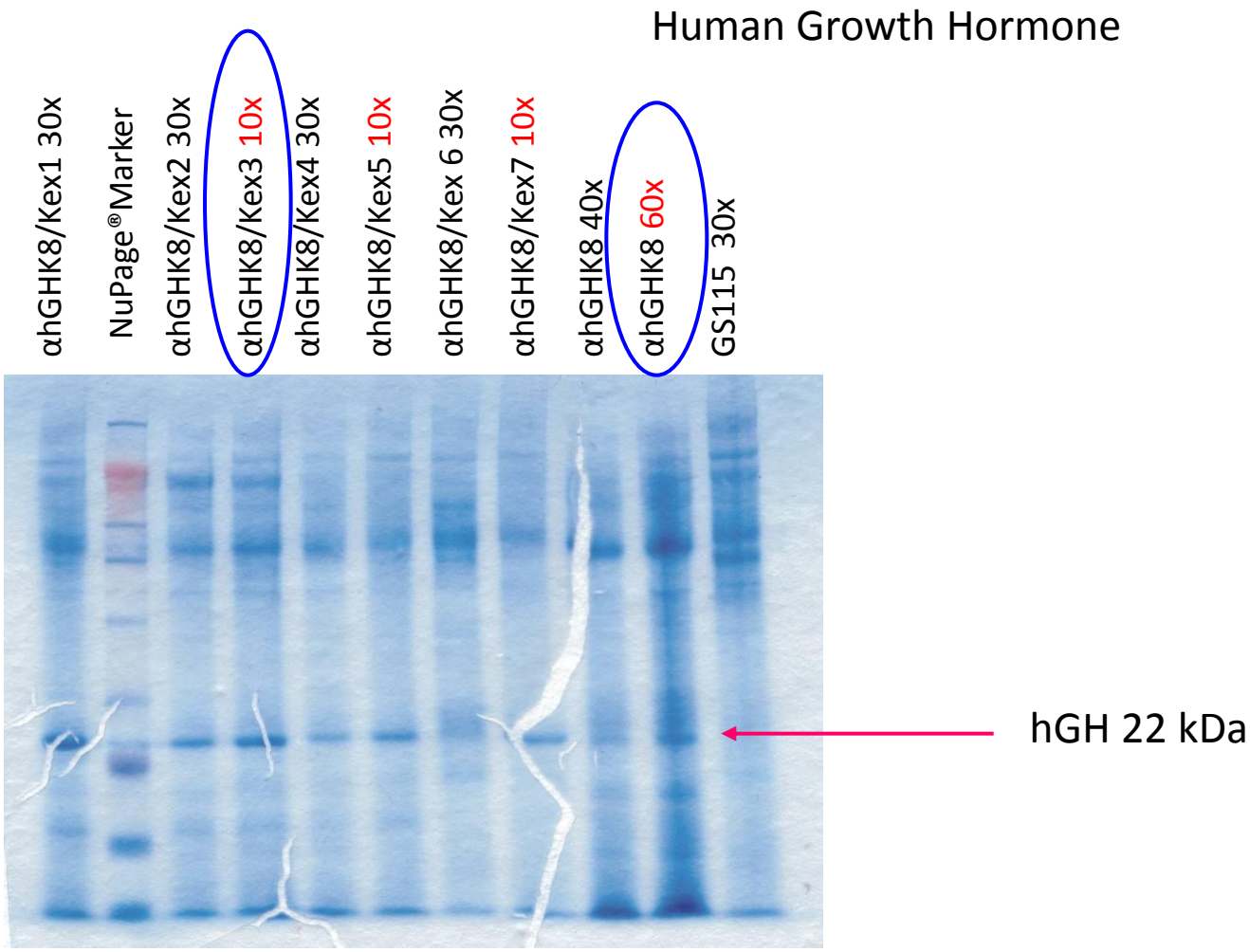
Fermentation: 250 mL wide necked baffled flasks

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

conditions:

BMG, 140rpm,

29° C



Follicle 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

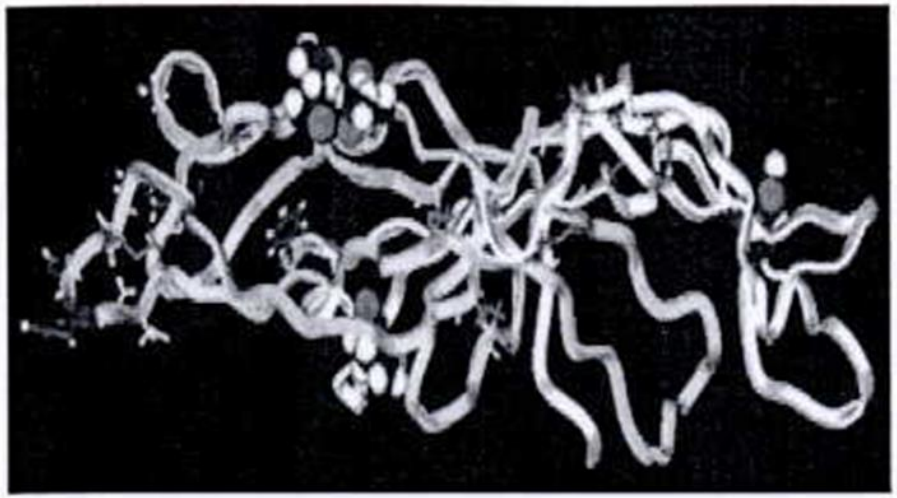


Figure 1. Three-dimensional diagram of the human FSH molecule (see color plates, page XXIII).

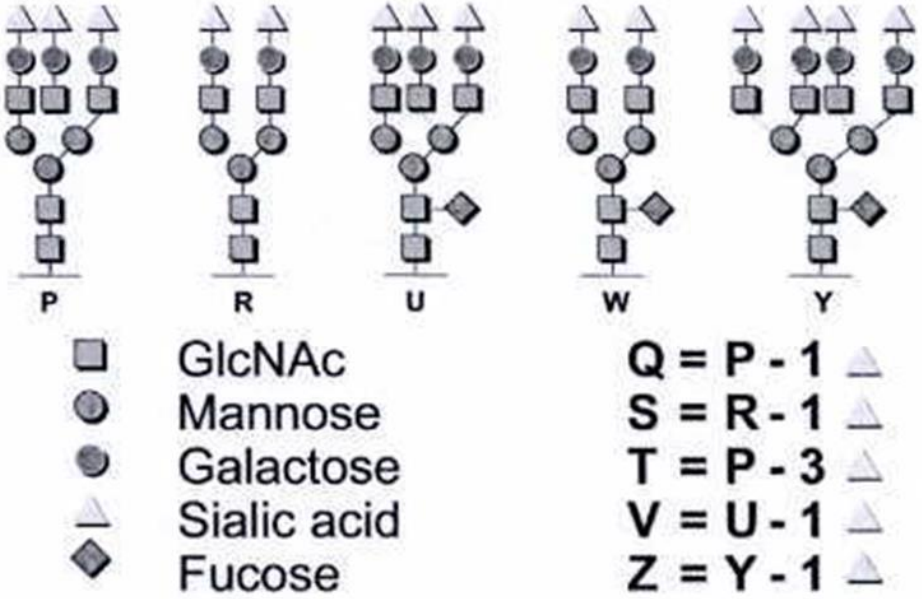


Figure 2. Structure of the lateral glycidic chains linked to the α and β subunits of human FSH (see colo plates, page XXIII).

hFSH

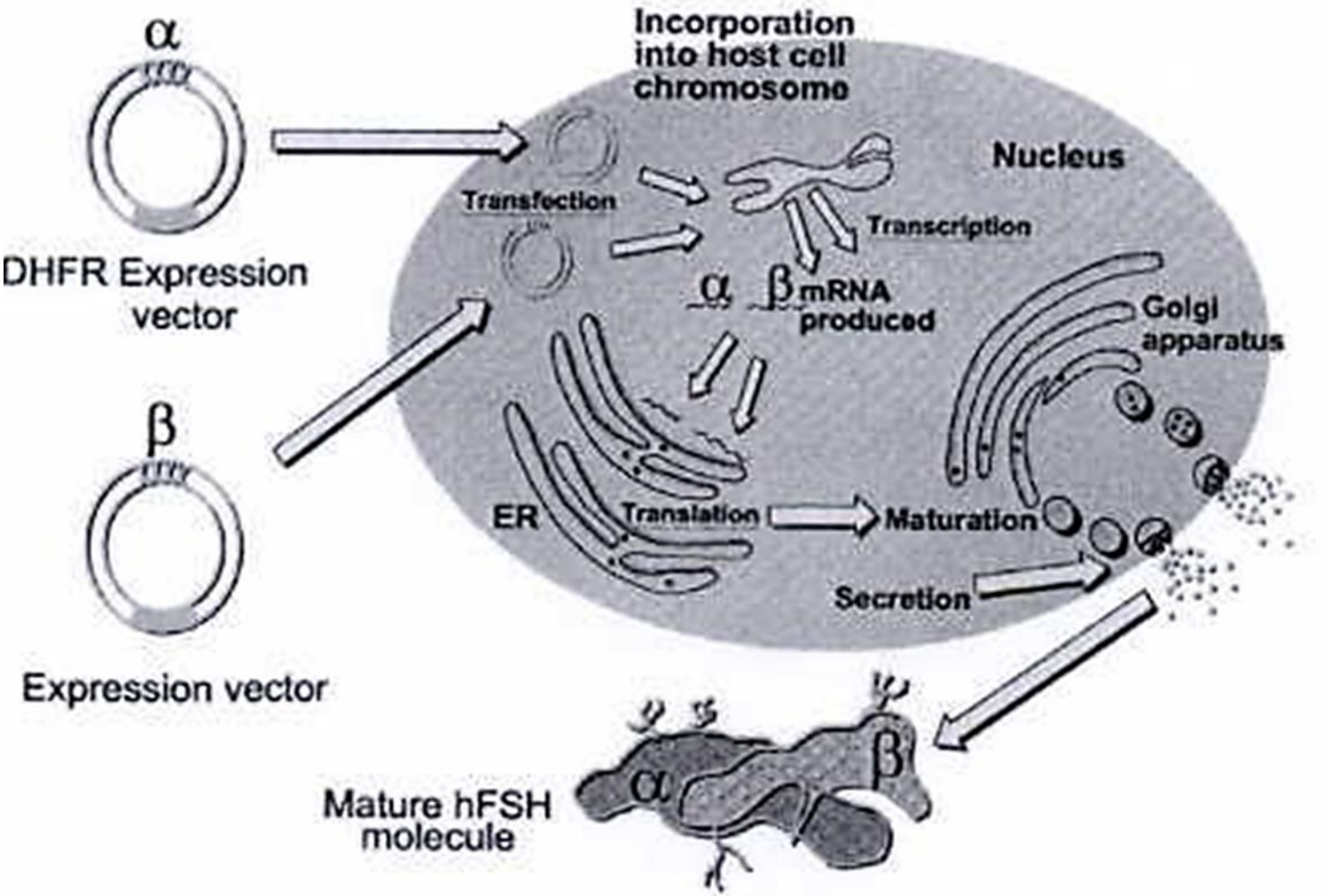


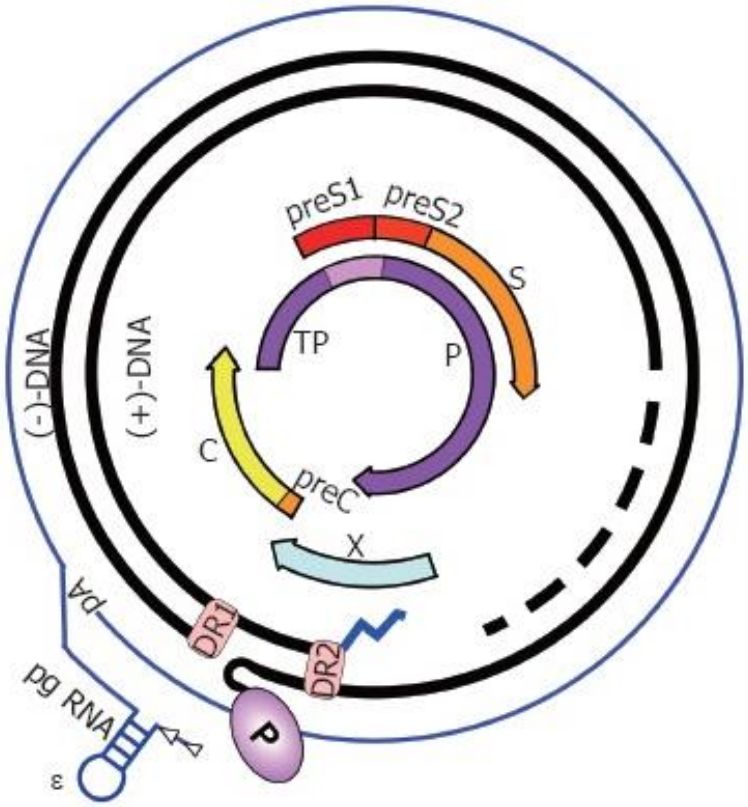
Figure 4. Expression of rhFSH in eukaryotic cells (CHO) (see color plates, page XXIV).

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

Hepatitis B Virus HBV



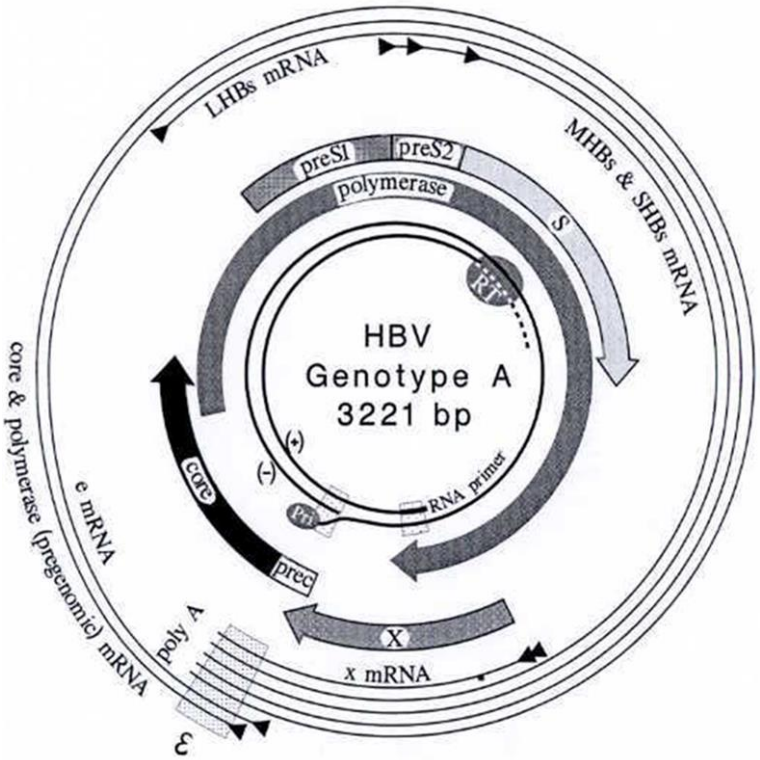
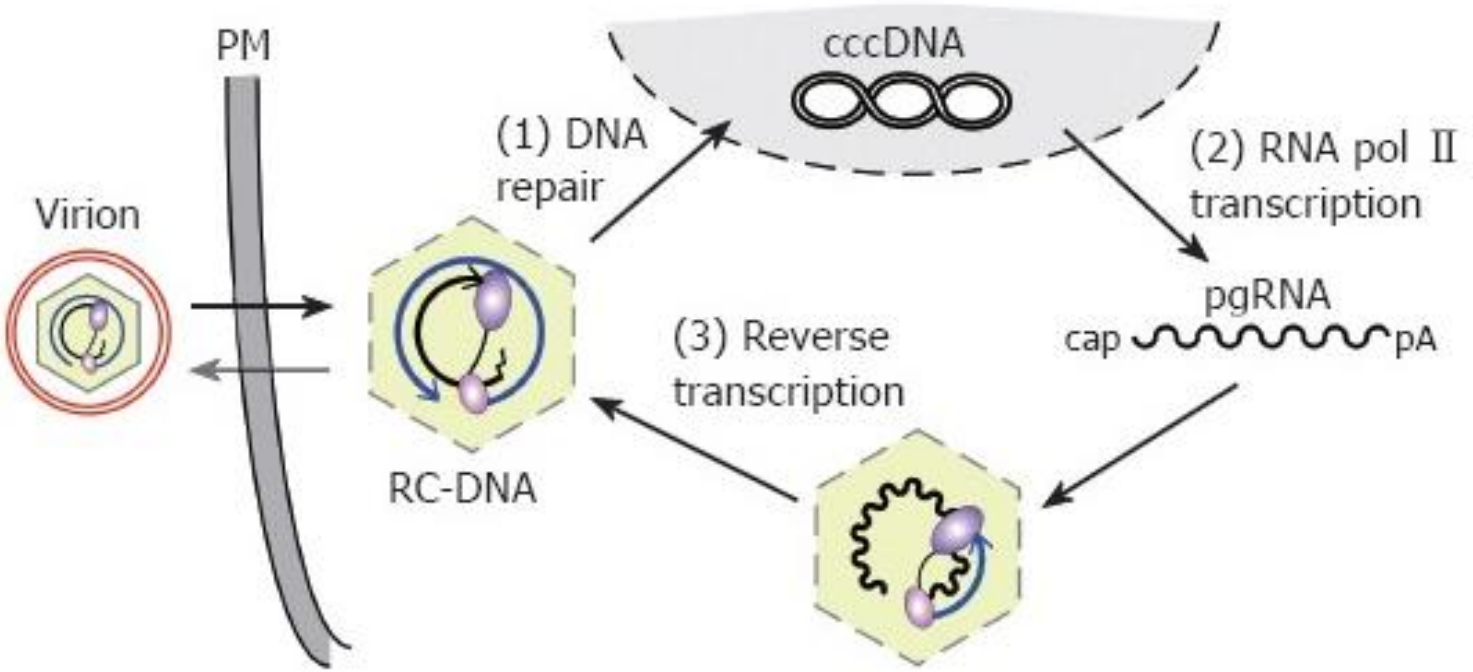


Figure 2. Schematic diagram of the HBV genome and its genetic organization. The inner circle represents the viral DNA as found in virions. The arrows represent the 4 different ORFs. Outer circles represent the coterminal viral mRNAs as found in infected cells. The 5'end of (−) strand DNA is linked with 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 genome organization. The partially double-stranded, circular RC-DNA is indicated by thick black lines, with P covalently linked to the 5´ end of the (-)-DNA, and the RNA primer (zigzag line) at the 5´ end of (+)-DNA. The dashed part symbolizes the heterogeneous lengths of the (+)-strands. DR1 and DR2 are the direct repeats. The outer circle symbolizes the terminally redundant pgRNA with ε close to the 5´ end, and the poly-A tail at the 3´ end. The precore mRNA is nearly identical, except it starts slightly upstream. The relative positions of the open reading frames for core (C), P, preS/S, and X are shown inside. TP, Terminal protein domain of P.

World J Gastroenterol. 2007 Jan 7; 13(1): 48–64.
Published online 2007 Jan 7. doi: 10.3748/wjg.v13.i1.48

HBV



Replication cycle of the hepadnaviral genome. Enveloped virions infect the cell, releasing RC-DNA containing nucleocapsids into the cytoplasm. RC-DNA is transported to the nucleus, and repaired to form cccDNA (1). Transcription of cccDNA by RNA polymerase II (2) produces, amongst other transcripts (not shown), pgRNA. pgRNA is encapsidated, together with P protein, and reverse transcribed inside the nucleocapsid (3). (+)-DNA synthesis from the (-)-DNA template generates new RC-DNA. New cycles lead to intracellular cccDNA amplification; alternatively, the RC-DNA containing nucleocapsids are enveloped and released as virions. PM, plasma membrane.

World J Gastroenterol. 2007 Jan 7; 13(1): 48–64.
Published online 2007 Jan 7. doi: 10.3748/wjg.v13.i1.48

HBV

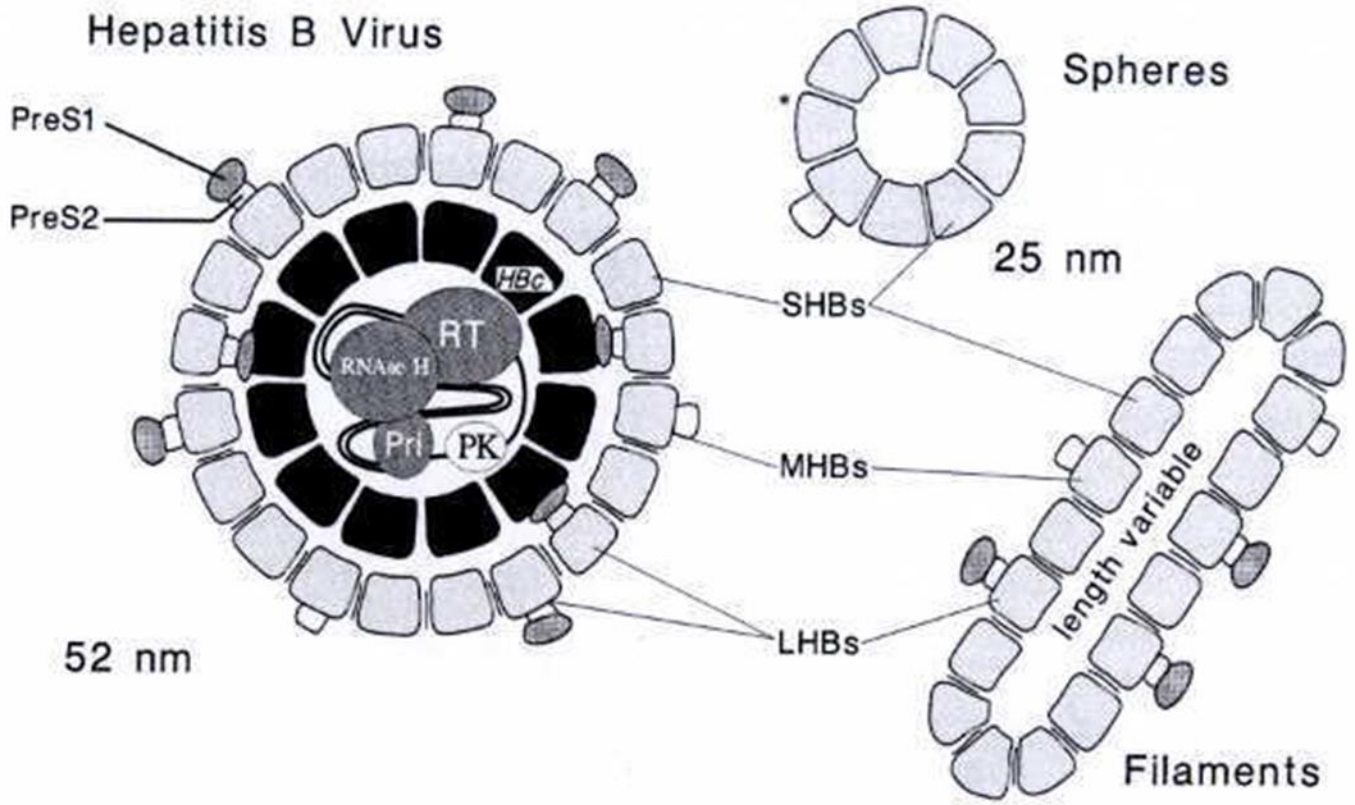
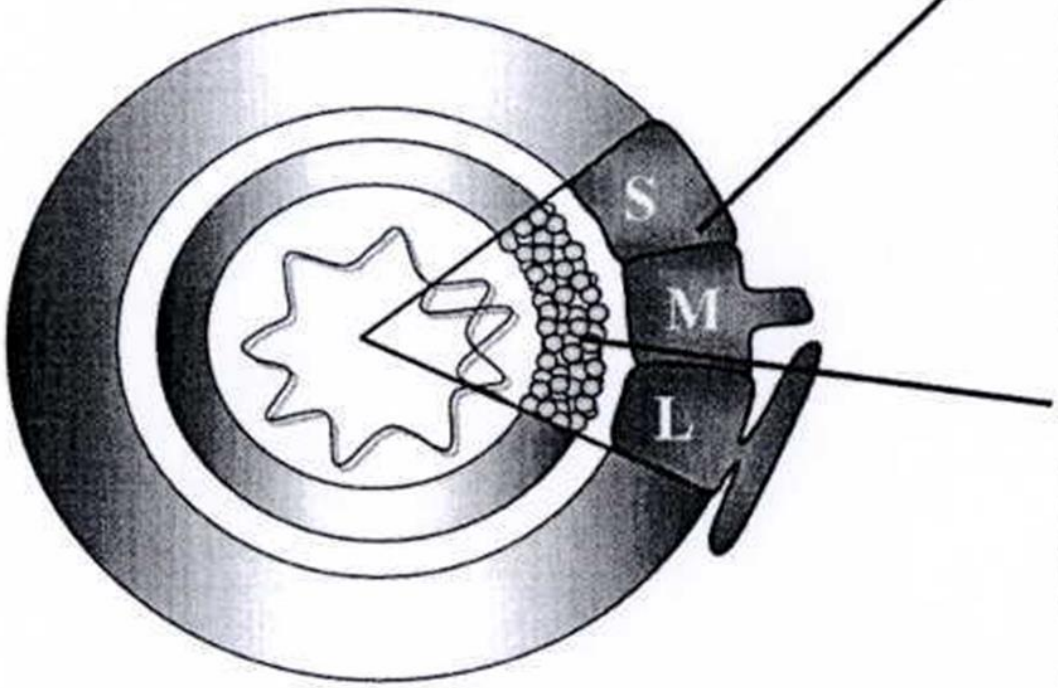


Figure 3. Schematic diagram of hepadnavirus particles. The virus particles contain an internal nucleocapsid (HBc), the viral genome, the polymerase consisting of domains with reverse transcriptase activity (RT), RNaseH and a domain serving as primer for the synthesis of (−) strand DNA (Pri). The subviral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

HBV



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

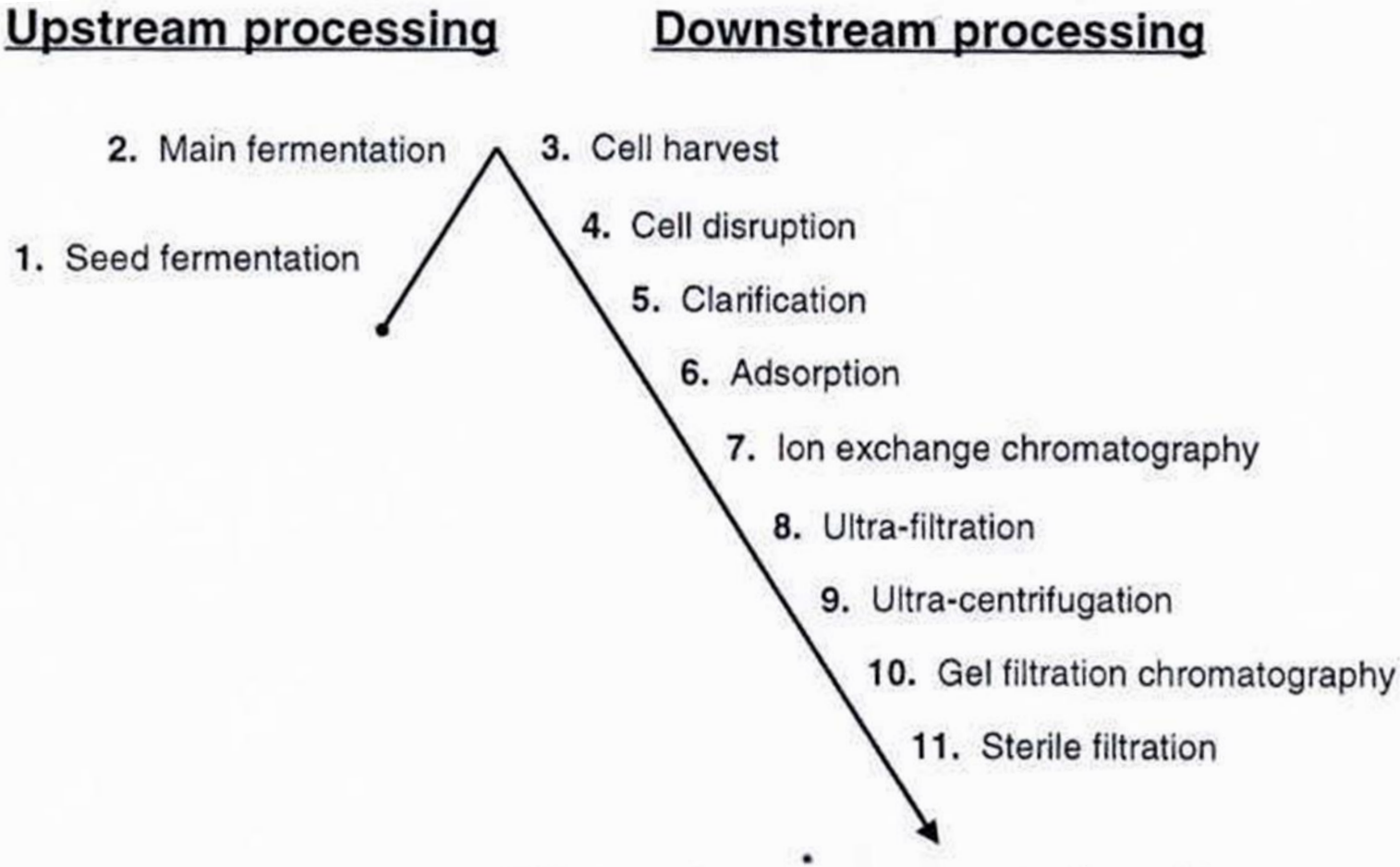


Figure 11. Production process for HBsAg particles in recombinant *H. polymorpha*. Recombinant strains of *H. polymorpha* expressing HBsAg are fermented and the antigen is purified as described in the text (see Sect. 3.4). The process yields purified HBsAg integrated onto yeast-derived membrane particles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

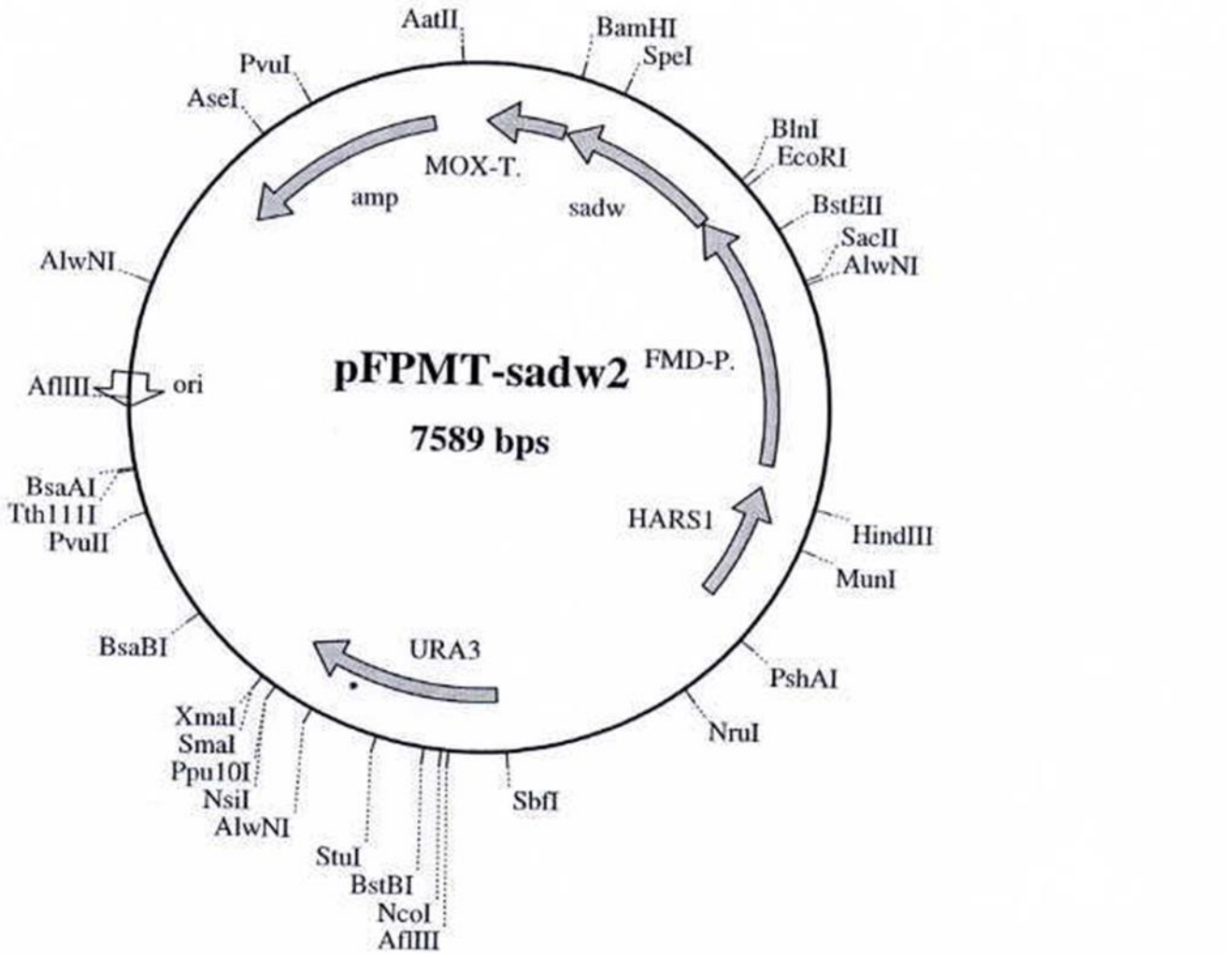


Figure 9. Map of plasmid vector pFPMT-sadw2 containing a *FMD*-promoter/HBsAg(adw2)/ *MOX*-terminator expression cassette. pFPMT-sadw2 is composed of the following DNA fragments, starting from the unique *Hind*III site in a counter-clockwise direction: the *FMD* promoter, a fragment coding for HBsAg (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).

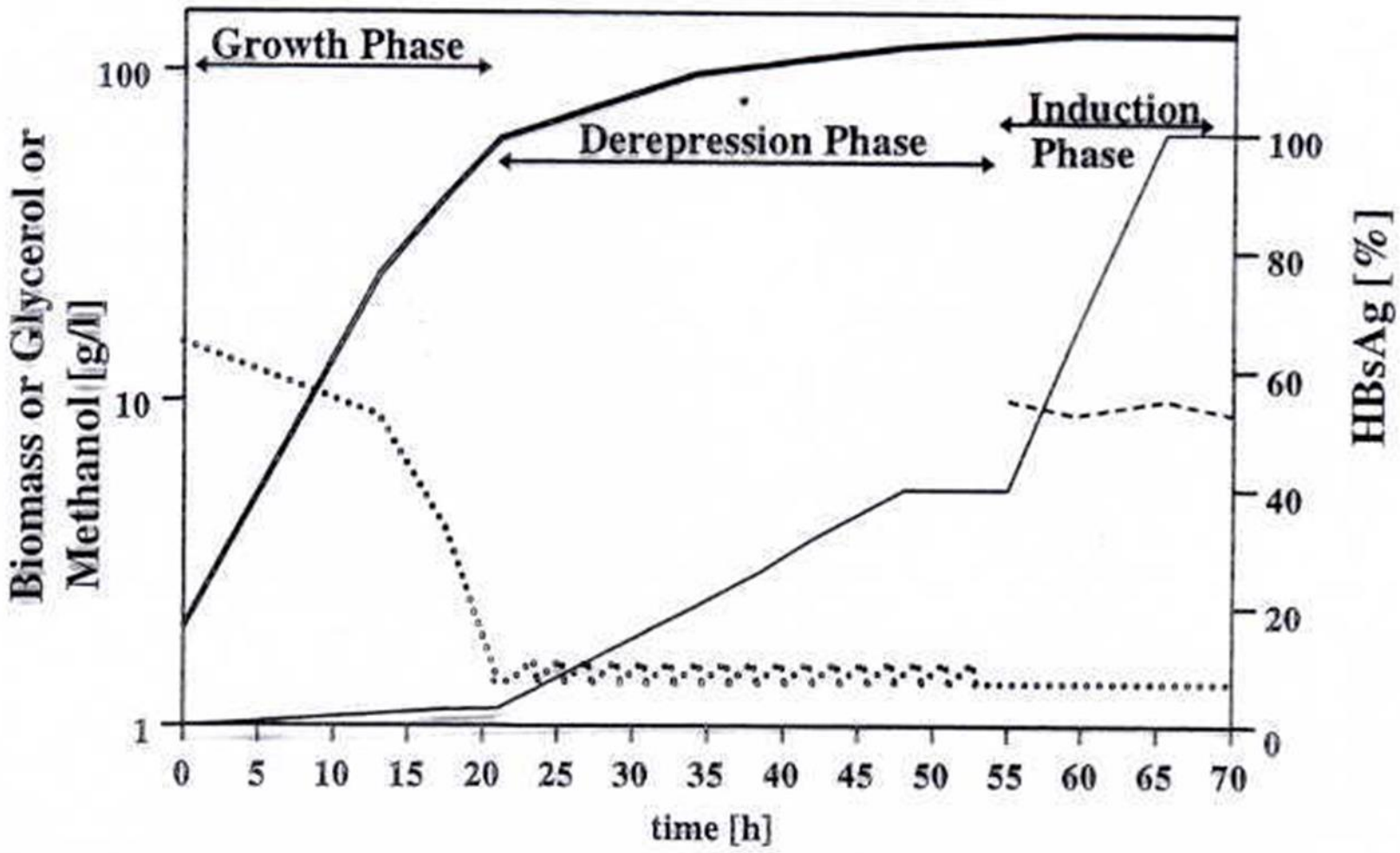


Figure 12. Fermentation of a HBsAg-producing *H. polymorpha* strain (schematic). The fermentation procedure follows the description provided in the text (see Sect. 3.4.1).
___ biomass; ___ HBsAg; ----- methanol; ..... glycerol

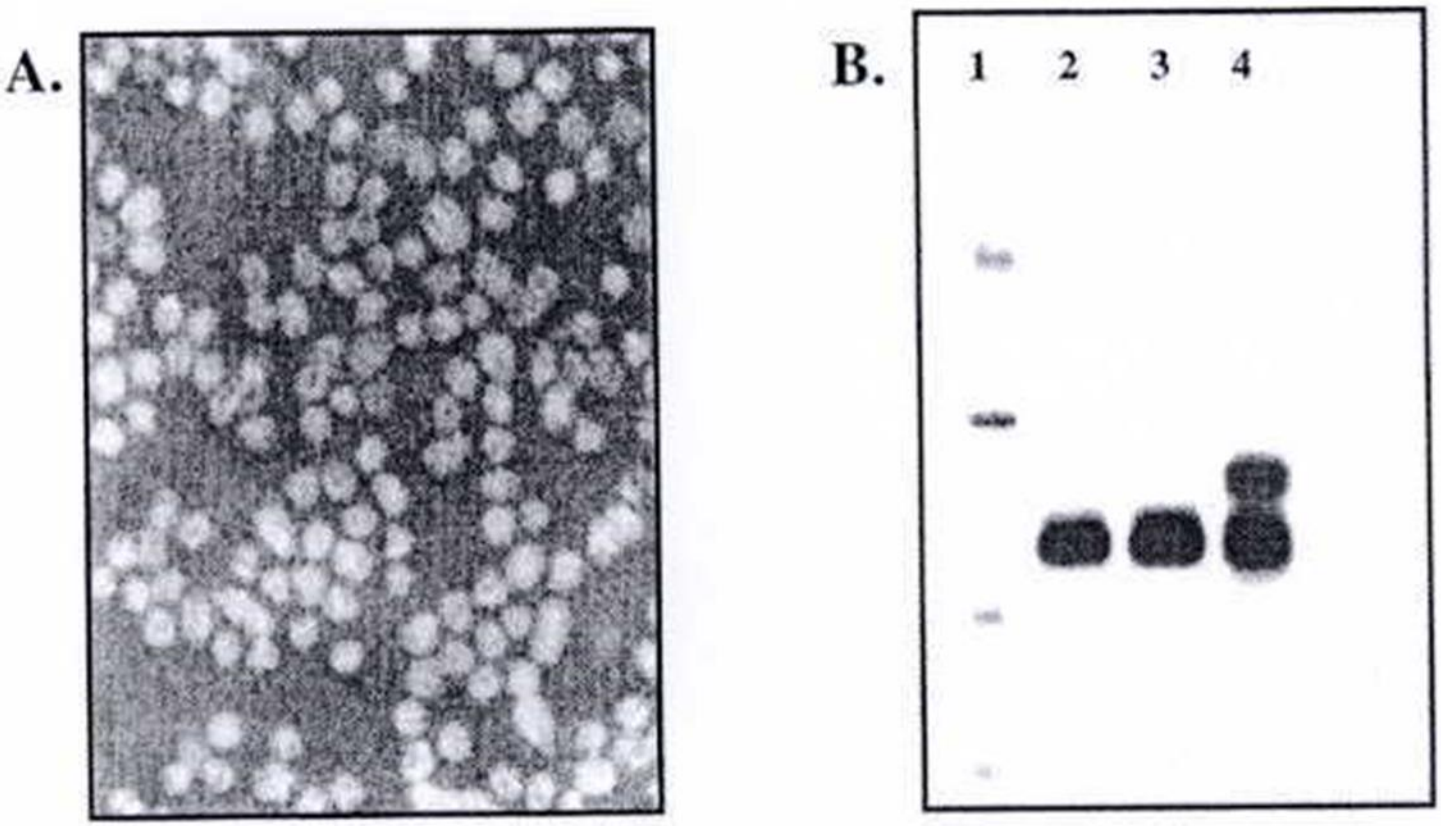


Figure 10. Characterization of recombinant HBsAg-particles produced in *H. polymorpha*. HbsAg particles were purified and analyzed as described in the text (see Sect. 3.3.3). **A.** Electron microscopy analysis (142,000X) **B.** SDS-PAGE analysis of purified HBsAg. Two batches of HBsAg were separated 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.

Tissue Plasminogen Activator tPA

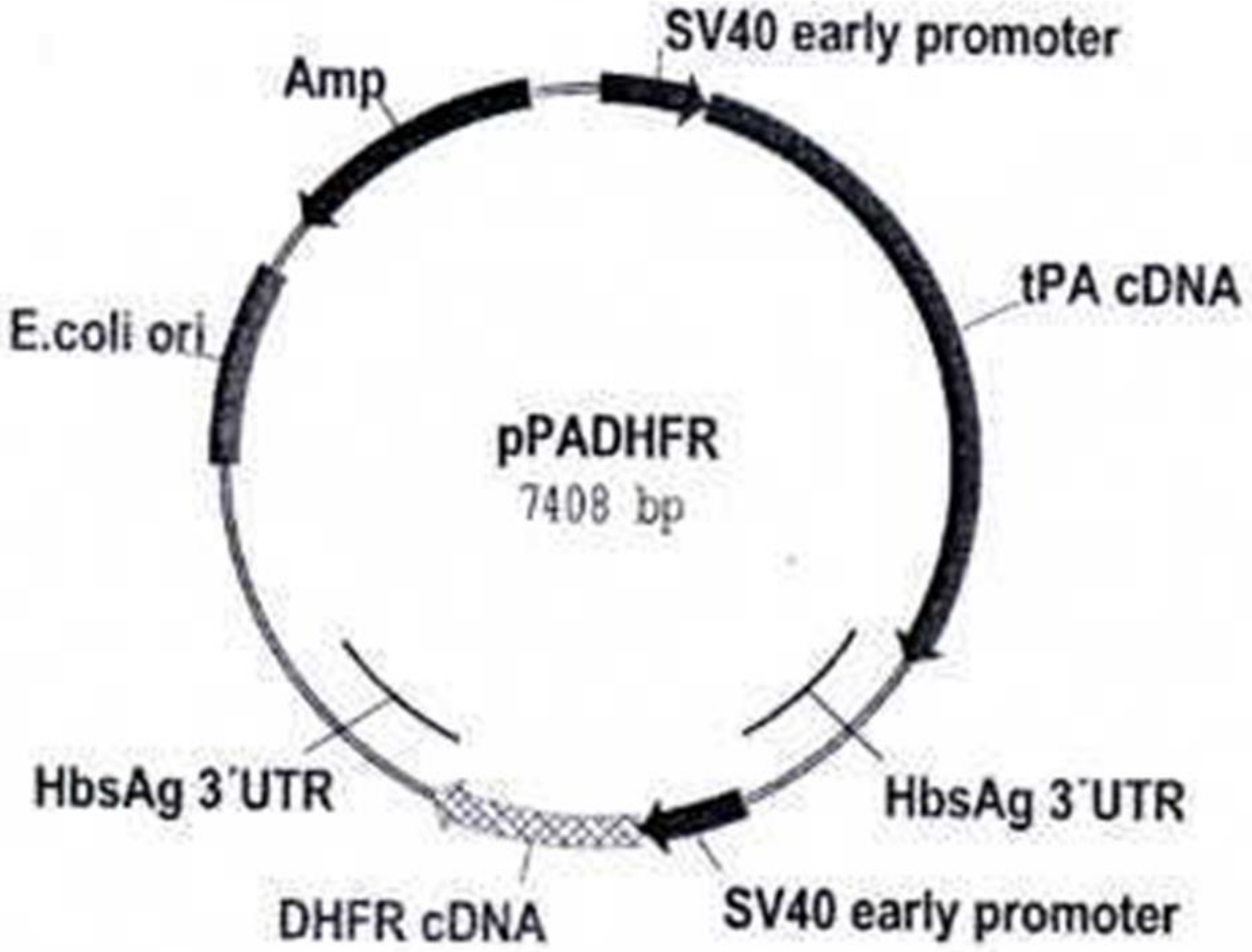


Figure 4. Expression vector for t-PA.

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*.

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Recombinant immunoglobulin A: powerful tools for fundamental and applied research

Blaise Corthésy

The use of monoclonal antibodies has become routine in research and diagnostic laboratories but the potential level of antibodies in use in public health and medical applications is still far from its maximum. From a clinical perspective, topical immunotherapy of mucosal surfaces with monoclonal antibodies can block entry and transmission of bacteria, viruses, fungi and parasites that infect humans, and defeat some key strategies, evolved by many pathogens, to evade the host immune system. The chief antibody at mucosal surfaces is secretory immunoglobulin A (SIgA), a multi-polypeptide complex originating from two cell types. The recent design of heterologous expression systems, coupled with modern biotechnology processes, should form a sound basis for studying the functional properties of SIgAs and evaluate their value as biotherapeutics. Here, we discuss the principles underlying mucosal immunity and review the application of recombinant SIgA to the dissection of mechanisms in passive and active protection at mucosal surfaces.

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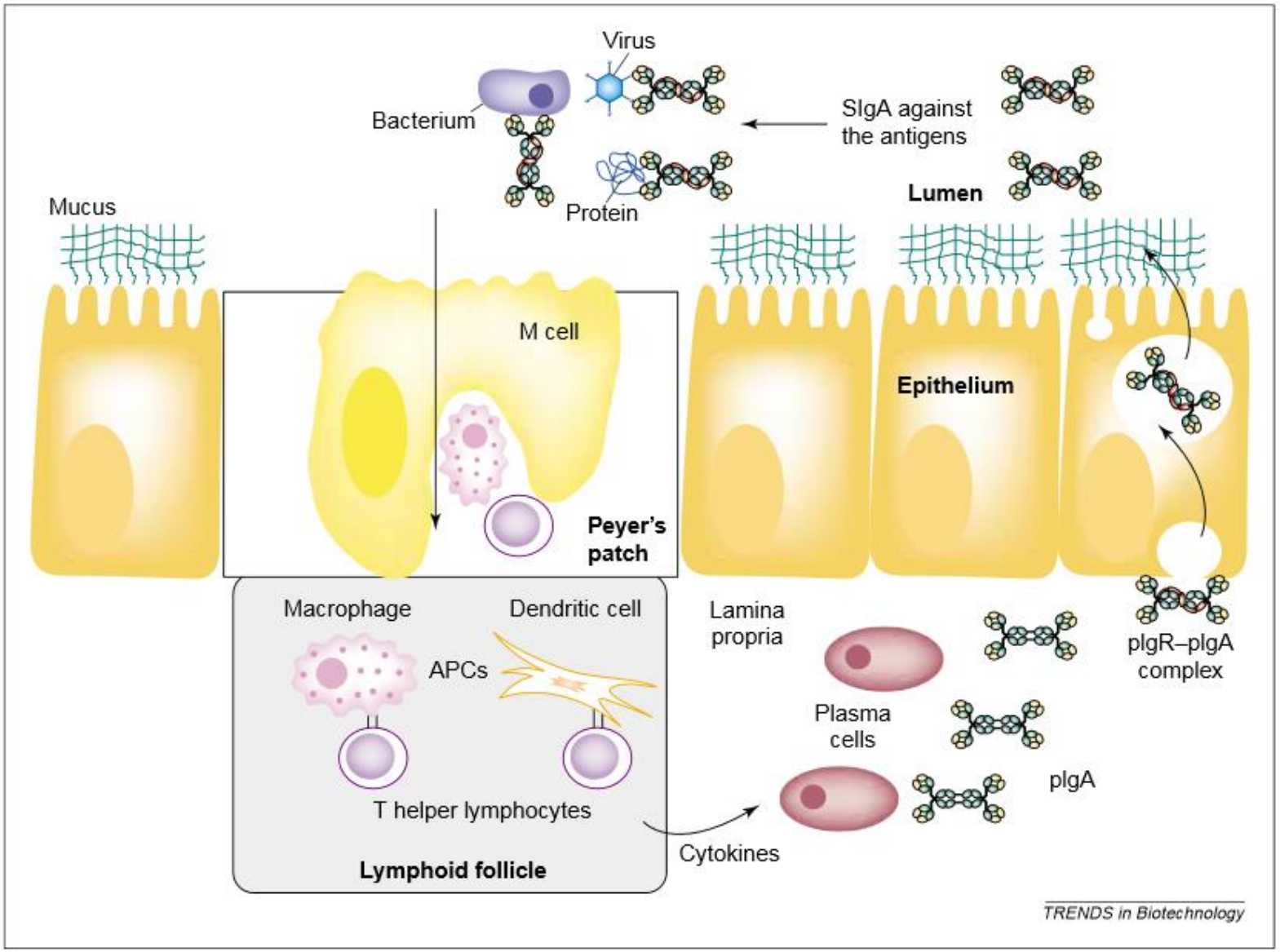


Fig. 1. A scheme for induction of intestinal immune responses. Luminal antigens are sampled by M cells in the Peyer's patch (shown for simplification as a white rectangle contiguous to the epithelium) and delivered to antigen-presenting cells (APCs) including macrophages and dendritic cells present in the dome of the lymphoid follicle. This triggers activation of T helper lymphocytes, which produce cytokines necessary for the maturation of B cells into plasma cells secreting polymeric IgA (pIgA) antibodies that carry the J chain. pIgA is selectively transported by a mechanism called transcytosis to the lumen by the polymeric immunoglobulin receptor (pIgR), and after cleavage at the apical side of the epithelial cell, is released as secretory IgA (SIgA).

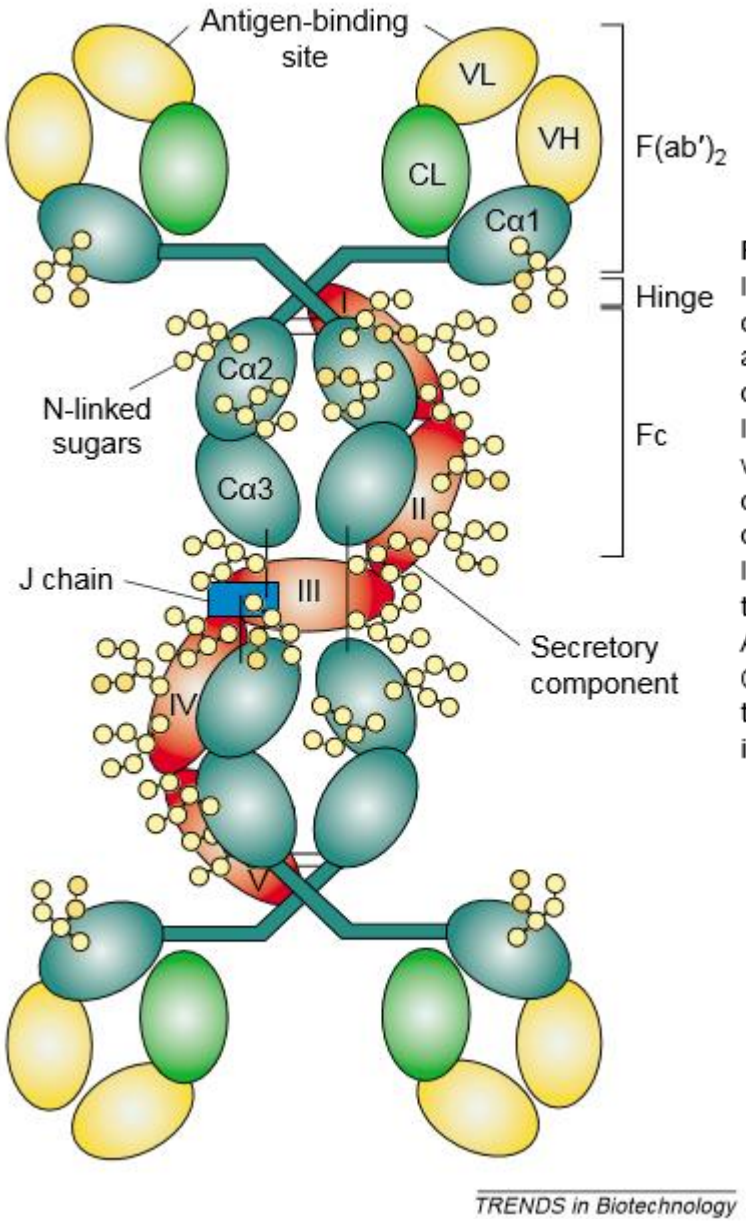


Fig. 2. A schematic representation of a human dimeric secretory IgA2m(1) with N-glycosylation sites drawn as connected yellow blocks on polypeptides. Two IgA monomers are depicted in a tail-to-tail arrangement, with a J chain (blue box) covalently linked through disulfide bridges to the tailpiece of the heavy chains of two monomers. In IgA2m(1), the light and heavy chains are not disulfide bridged, whereas the light chains are disulfide-bonded to each other (not drawn). Secretory component (SC) is made of five immunoglobulin-like domains (red ellipsoids) derived from the extracellular portion of pIgR. In human SIgA, up to 80% of SC is covalently linked with IgA dimers through a disulfide bridge connecting Cα3 and SC domain V. Abbreviations: Cα1, α2, α3, the constant domains of the heavy chain; CL, the constant domain of the light chain; VH, the variable domain of the heavy chain; VL, the variable domain of the light chain; I–V, the five immunoglobulin-like domains that constitute SC.

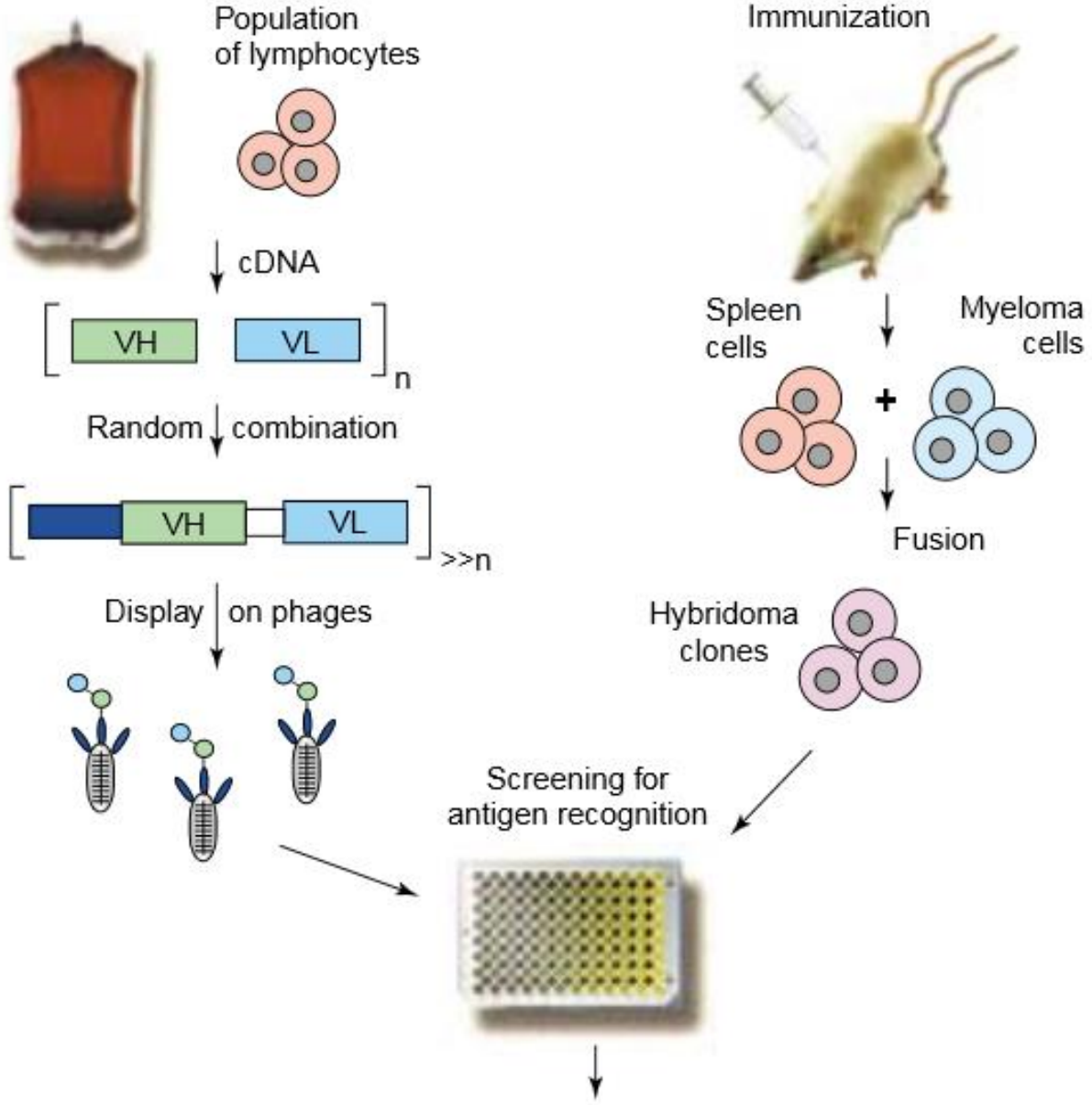
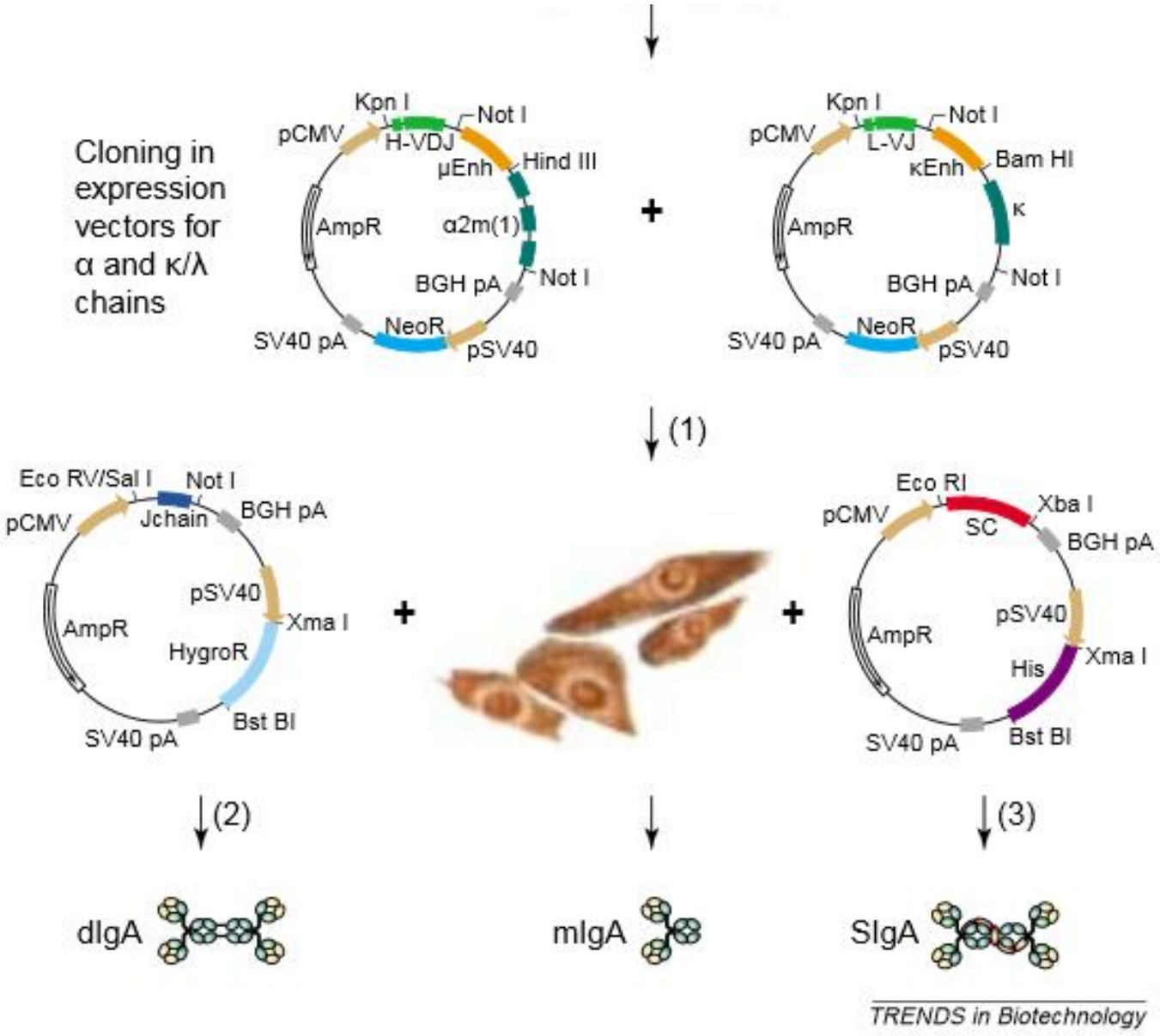


Fig. 3. Strategies to produce monoclonal recombinant secretory IgA. Rearranged genomic sequences coding for the variable domains of heavy (VH) and light (VL) immunoglobulin chains can be isolated from naïve B cells, and randomly combined before insertion into phage expression vectors. Fragments (Fv) displayed on phages, carrying the best combination of VH and VL domains for a specific antigen, are identified by multiple screening steps (e.g. ELISA). Variable regions (Fv) sequences can alternatively be isolated from a hybridoma clone showing adequate antigen specificity. Fv fragments can then be converted into IgA immunoglobulin following insertion into expression vectors for the heavy (α) and light (κ/λ) chains. Polymerization and assembly into SIgA require two other expression vectors coding for the J chain and the secretory component. Successive rounds of transfection into CHO cells allow recovery of (1) monomeric, (2) dimeric, polymeric (not shown) and (3) SIgA.

Fig. 3. Strategies to produce monoclonal recombinant secretory IgA. Rearranged genomic sequences coding for the variable domains of heavy (VH) and light (VL) immunoglobulin chains can be isolated from naïve B cells, and randomly combined before insertion into phage expression vectors. Fragments (Fv) displayed on phages, carrying the best combination of VH and VL domains for a specific antigen, are identified by multiple screening steps (e.g. ELISA). Variable regions (Fv) sequences can alternatively be isolated from a hybridoma clone showing adequate antigen specificity. Fv fragments can then be converted into IgA immunoglobulin following insertion into expression vectors for the heavy (α) and light (κ/λ) chains. Polymerization and assembly into SIgA require two other expression vectors coding for the J chain and the secretory component. Successive rounds of transfection into CHO cells allow recovery of (1) monomeric, (2) dimeric, polymeric (not shown) and (3) SIgA.


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
κ
AmpR
BGH pA
Not I
SV40 pA
NeoR
pSV40
(1)
Eco RV/Sal I
Not I
pCMV
Jchain
BGH pA
pSV40
AmpR
Xma I
HygroR
Bst BI
SV40 pA
Eco RI
Xba I
pCMV
SC
BGH pA
pSV40
AmpR
Xma I
His
Bst BI
SV40 pA
(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
Trichoderma reesei

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.

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

Principle Enzymatic Activity	Host Organism (production organism)	Donor Organism	Application Examples	Price* $/Kg
α-Acetolactate decarboxylase	Bacillus amyloliquefaciens or subtilis	Bacillus sp.	Beverages	50-60
α-Amylase (Thermal)	Bacillus amyloliquefaciens Bacillus licheniformis	Bacillus sp. Bacillus sp.	Cereal, Beverages Sugar, Bakery	1500-10.000
Catalase	Aspergillus niger	Aspergillus sp.	Milk, Egg	1000-10.000
Chymosin	Aspergillus niger var. awamori/ Kluyveromyces lactis	Calf stomach	Cheese	460-500
Cyclodextrin glucano trans-ferase	Bacillus licheniformis	Thermoanaero-bacter sp.	Cereal	N/A*
ß-Glucanase	Bacillus amyloliquefaciens/ subtilis/ Trichoderma reesei or longibrachiatum	Bacillus sp. Trichoderma sp.	Cereal, Beverages Cereal, Dietary food	N/A
Glucose isomerase	Streptomyces lividans/ Streptomyces rubiginosus	Actinoplanes sp. Streptomyces sp.	Cereal	N/A
Glucose oxidase	Aspergillus niger	Aspergillus sp.	Egg, Beverages, Bakery, Salads	182-186
Hemicellulase	Bacillus amyloliquefaciens or subtilis	Bacillus sp.	Bakery	N/A
Lipase yt	Aspergillus oryzae	Candida sp./ Rhizomucor sp./ Thermomyces sp.	Fats Fats, Bakery	202-206
Maltogenic amylase	Bacillus amyloliquefaciens or subtilis	Bacillus sp.	Cereal, Beverages, Bakery	50-1500
Protease (Neutral)	Aspergillus oryzae/ Bacillus amyloliquefaciens or subtilis. Bacillus licheniformis	Rhizomucor sp. Bacillus sp. Bacillus sp.	Cheese, Meat, Fish, Cereal Beverages, Bakery Salads Meat, Fish	3-30
Pullulanase	Bacillus licheniformis/ Klebsiella planticola	Bacillus sp. Klebsiella sp.	Cereal Cereal, Beverages, Bakery	15-30
Xylanase	Aspergillus oryzae Aspergillus niger var. awamori/ Bacillus amyloliquefaciens or subtilis/ Bacillus licheniformis/ Trichoderma reesei or longibrachiatum	Aspergillus sp. Thermomyces sp./ Bacillus sp./ Bacillus sp./ Trichoderma sp.	Cereal Cereal, Bakery Bakery, Cereal, Beverage Cereal Cereal, Beverages	10-80

**Range of prices is based on values given by the manufacturer/seller in the site: http://www.alibaba.com/*
N/A: not available.

Table 2: List of commercial enzymes from genetically modified microorganisms used in food industry, adapted from [14]. Table 2: List of commercial enzymes from genetically modified microorganisms used in food industry, adapted from [14].

Citation: Sarrouh B, Santos TM, Miyoshi A, Dias R, Azevedo V (2012) Up-To-Date Insight on Industrial Enzymes Applications and Global Market. J Bioprocess Biotechniq S4:002 doi:10.4172/2155-9821.S4-002

Review Article

Enzymes in Food Processing: A Condensed Overview on Strategies for Better Biocatalysts

Enzyme Research
Volume 2010, Article ID 862537
doi:10.4061/2010/862537

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Food and feed is possibly the area where processing anchored in biological agents has the deepest roots. Despite this, process improvement or design and implementation of novel approaches has been consistently performed, and more so in recent years, where significant advances in enzyme engineering and biocatalyst design have fastened the pace of such developments. This paper aims to provide an updated and succinct overview on the applications of enzymes in the food sector, and of progresses made, namely, within the scope of tapping for more efficient biocatalysts, through screening, structural modification, and immobilization of enzymes. Targeted improvements aim at enzymes with enhanced thermal and operational stability, improved specific activity, modification of pH-activity profiles, and increased product specificity, among others. This has been mostly achieved through protein engineering and enzyme immobilization, along with improvements in screening. The latter has been considerably improved due to the implementation of high-throughput techniques, and due to developments in protein expression and microbial cell culture. Expanding screening to relatively unexplored environments (marine, temperature extreme environments) has also contributed to the identification and development of more efficient biocatalysts. Technological aspects are considered, but economic aspects are also briefly addressed.

Table 1: An overview of enzymes used in food and feed processing (adapted from [10, 12, 13, 68]).

Class	Enzyme	Role
Oxidoreductases	Glucose oxidase	Dough strengthening
	Laccases	Clarification of juices, flavor enhancer (beer)
	Lipoxygenase	Dough strengthening, bread whitening
Transferases	Cyclodextrin Glycosyltransferase	Cyclodextrin production
	Fructosyltransferase	Synthesis of fructose oligomers
	Transglutaminase	Modification of viscoelastic properties, dough processing, meat processing
Hydrolases	Amylases	Starch liquefaction and sachcarification
		Increasing shelf life and improving quality by retaining moist, elastic and soft nature
		Bread softness and volume, flour adjustment, ensuring uniform yeast fermentation
		Juice treatment, low calorie beer
	Galactosidase	Viscosity reduction in lupins and grain legumes used in animal feed, enhanced digestibility
	Glucanase	Viscosity reduction in barley and oats used in animal feed, enhanced digestibility
	Glucoamylase	Saccharification
	Invertase	Sucrose hydrolysis, production of invert sugar syrup
	Lactase	Lactose hydrolysis, whey hydrolysis
	Lipase	Cheese flavor, in-situ emulsification for dough conditioning, support for lipid digestion in young animals, synthesis of aromatic molecules
	Proteases (namely, chymosin, papain)	Protein hydrolysis, milk clotting, low-allergenic infant-food formulation, enhanced digestibility and utilization, flavor improvement in milk and cheese, meat tenderizer, prevention of chill haze formation in brewing
	Pectinase	Mash treatment, juice clarification
	Peptidase	Hydrolysis of proteins (namely, soy, gluten) for savoury flavors, cheese ripening
	Phospholipase	In-situ emulsification for dough conditioning
	Phytases	Release of phosphate from phytate, enhanced digestibility
	Pullulanase	Saccharification
	Xylanases	Viscosity reduction, enhanced digestibility, dough conditioning
Lyases	Acetolactate decarboxylase	Beer maturation
Isomerases	Xylose (Glucose) isomerase	Glucose isomerization to fructose

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Table 2: Some examples of strategies undertaken to improve the performance of enzymes with applications in food and feed.

Enzyme	Role	Targeted improvement	Strategy/comments	Reference
α-amylase	Starch liquefaction	Thermostability	Protein engineering through site-directed mutagenesis. Mutant displayed increased half-life from 15 min to about 70 min (100°C).	[70]
	Starch liquefaction	Activity	Directed evolution. After 3 rounds the mutant enzyme from *S. cerevisiae* displayed a 20-fold increase in the specific activity when compared to the wild-type enzyme.	[71]
	Baking	pH-activity profile	Protein engineering through site-directed mutagenesis	[72]
L-arabinose isomerase	Tagatose production	pH-activity profile	Protein engineering through directed evolution	[73]
Glucoamylase	Starch saccharification	Substrate specificity, thermostability and pH optimum	Protein engineering through site-directed mutagenesis	[74]
Lactase	Lactose hydrolysis	Thermostability	Immobilization	[75]
Pullulanase	Starch debranching	Activity	Protein engineering through directed evolution	[76]
Phytase	Animal feed	pH-activity profile	Protein engineering through site-directed mutagenesis	[77]
Xylose (glucose) isomerase	Isomerization/epimerization of hexoses, pentoses and tetroses	pH-activity profile	Protein engineering through directed evolution. The turnover number on D-glucose in some mutants was increased by 30%–40% when compared to the wild type at pH 7.3. Enhanced activities are maintained between pH 6.0 and 7.5.	[78]
		Substrate specificity	Protein engineering through site-directed mutagenesis. The resulting mutant displayed a 3-fold increase in catalytic efficiency with L-arabinose as substrate.	[79]

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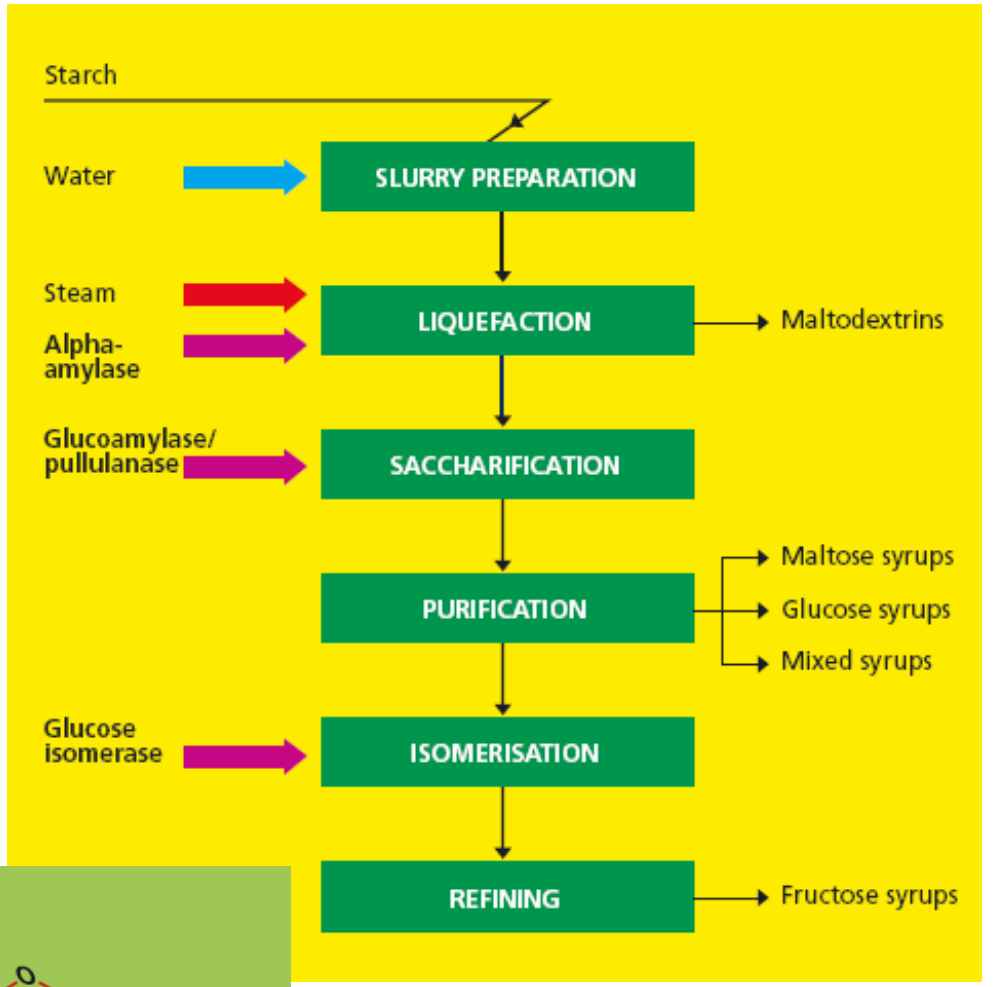
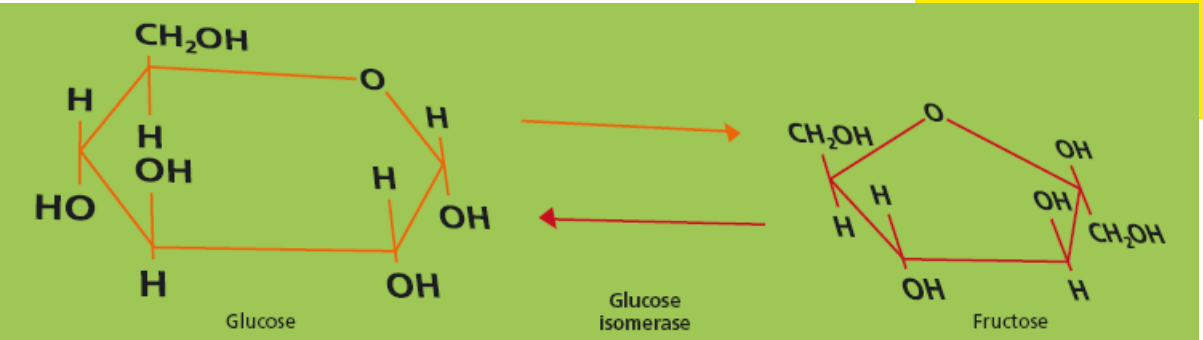
Enzyme applications in the food industry

Sweetener production

Enzymes for starch modification glucose syrups



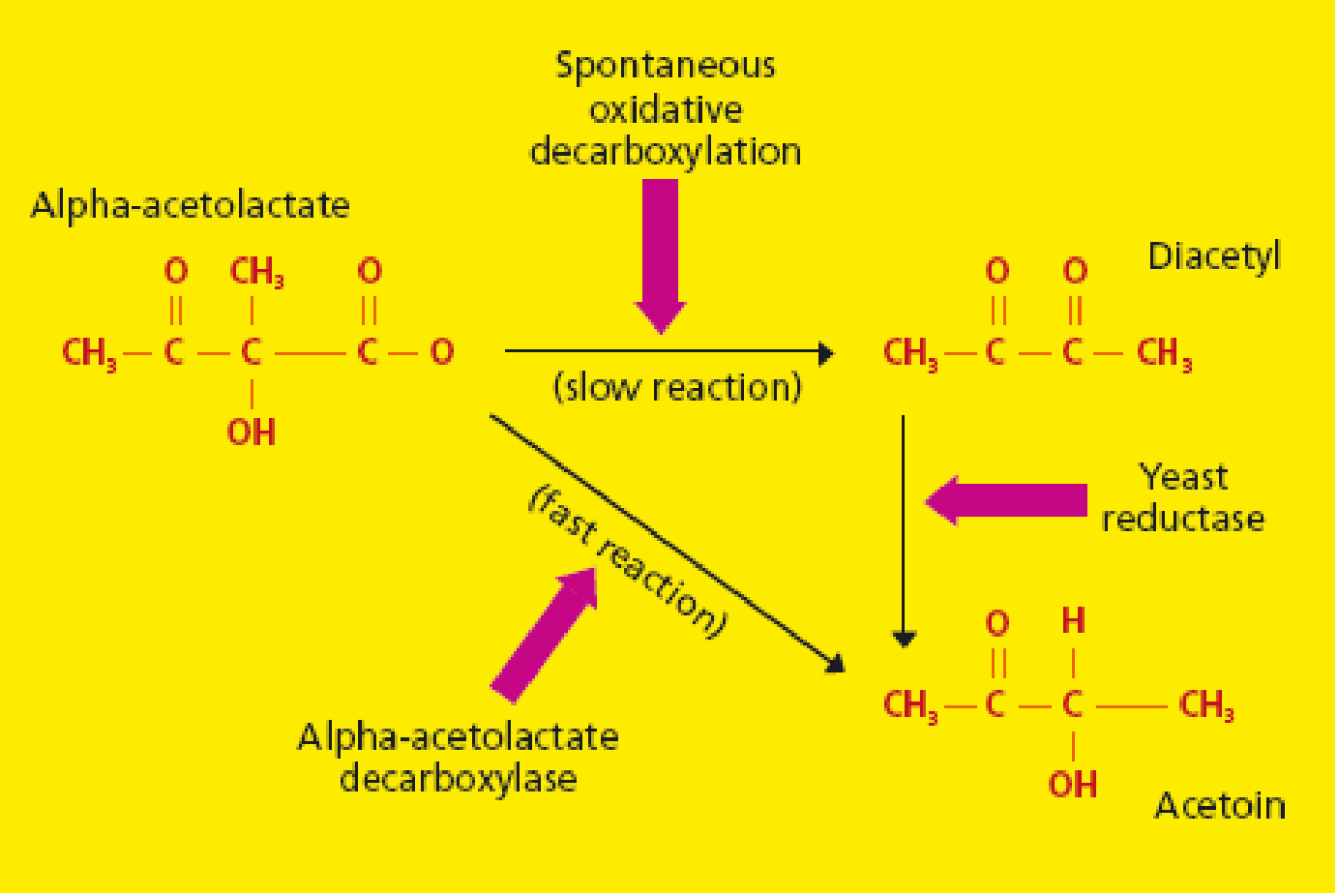


Enzyme applications in the food industry

Diacetyl

Brewing

alpha-amylase
beta-glucanase
protease
pentosanase

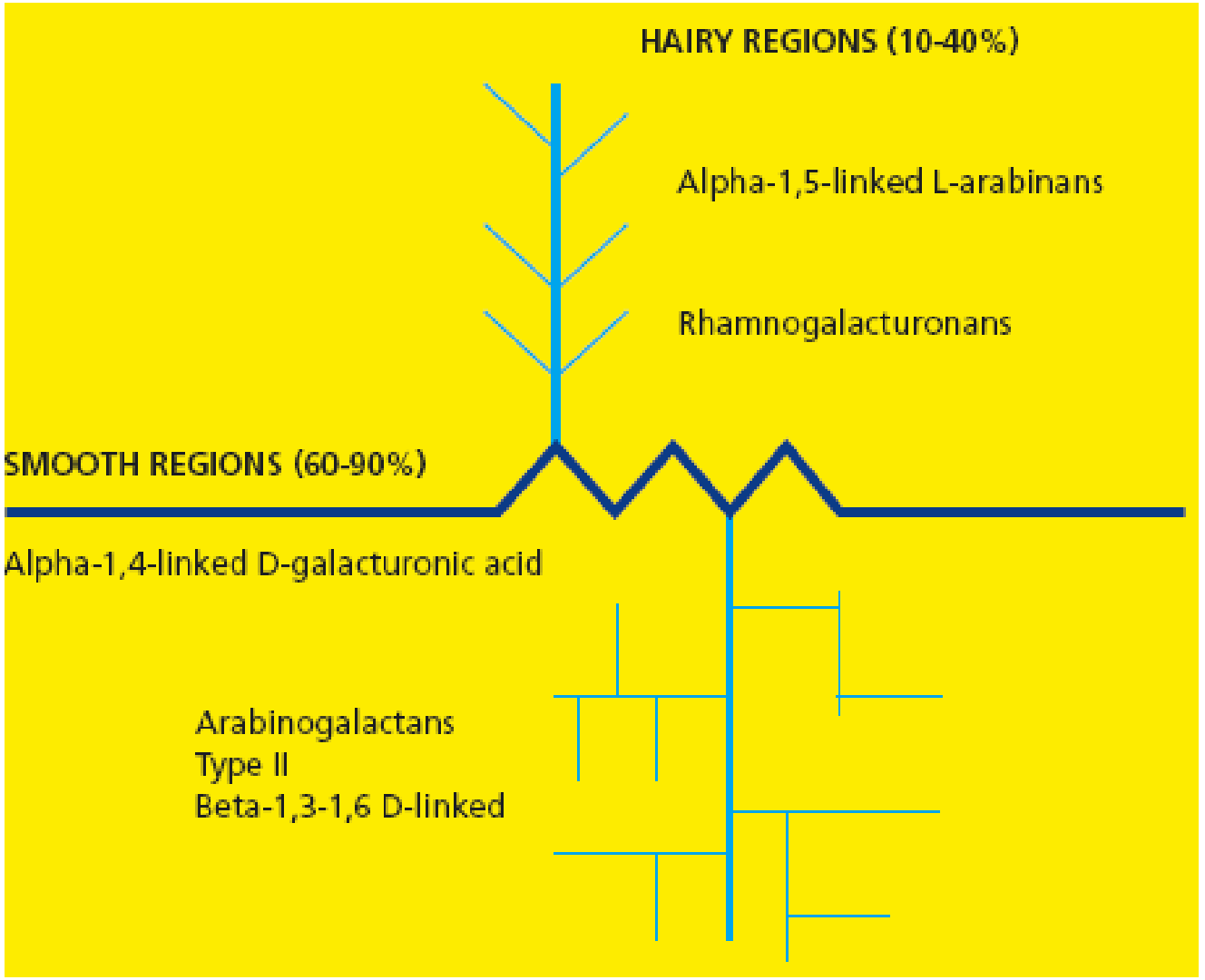


Enzyme applications in the food industry

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

Dairy products

Rennet and rennet substitutes

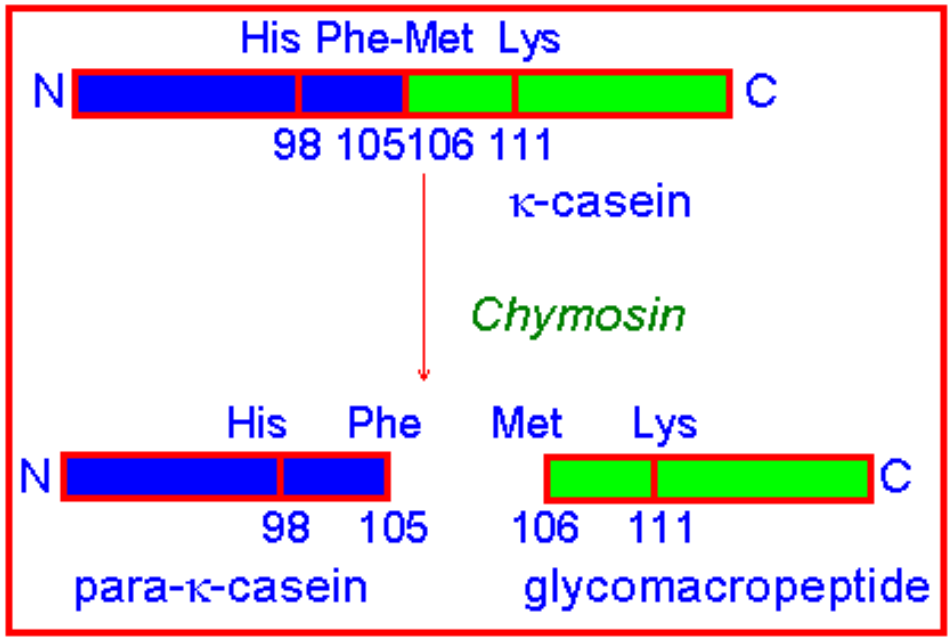
- Recombinant calf chymosin
- Microbial rennets

Cheese ripening

- Lipases

Infant milk formulas

- Proteases
- (allergy problem cow milk)



Enzyme applications in the food industry

Chymosin

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

http://dwb4.unl.edu/Chem/CHEM869N/CHEM869NLinks/www.fst.rdg.ac.uk/courses/fs560/topic1/t1g/t1g.htm

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

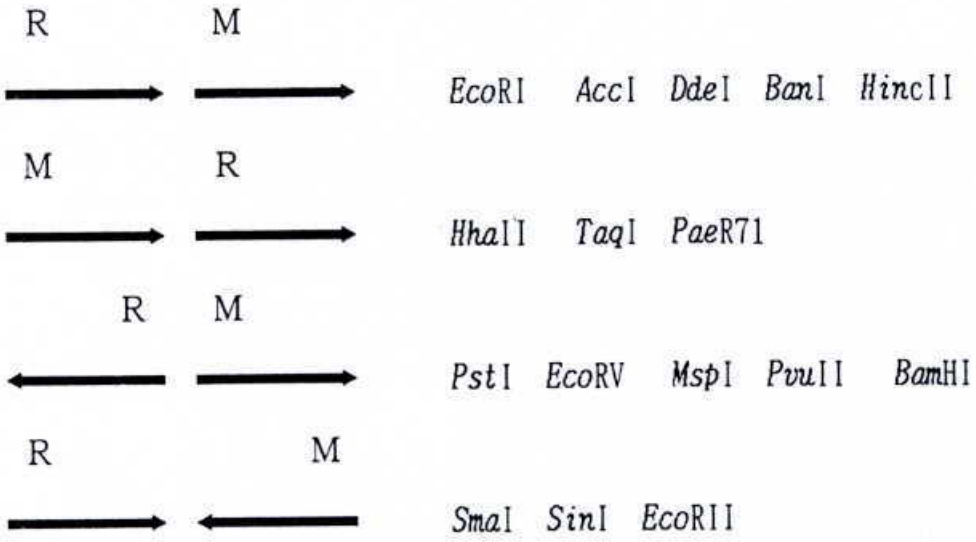


Figure 3 Gene organization of various restriction–modification genes. Genes are indicated as arrows; the directions indicate transcriptional orientation.

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 expression of R-endonucleases

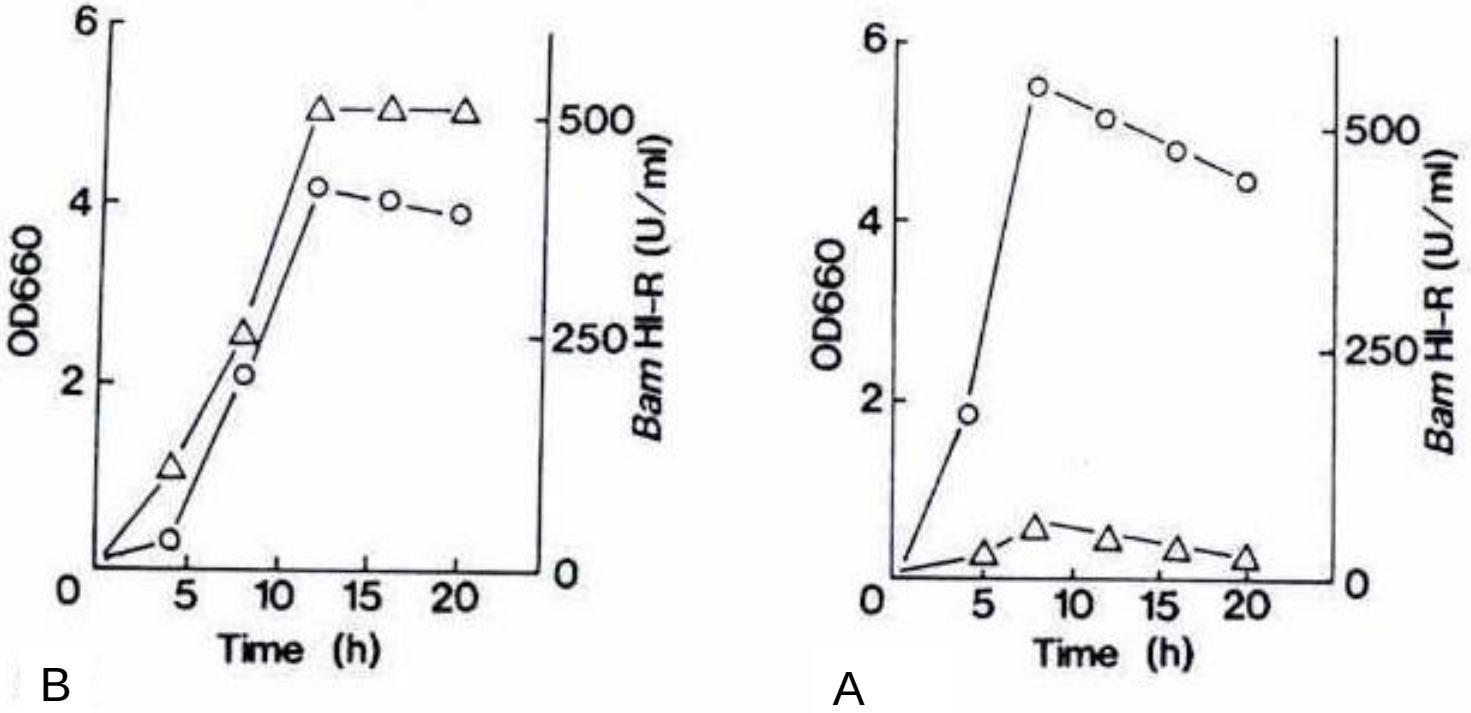


Figure 2 Comparison of the bacterial growth and *Bam*HI-R production between (A) *B. subtilis* (p*Bam*HIRM22 and (B) *B. amyloliquefaciens* H. *B. subtilis*(pBamHIRM22) and *B. amyloliquefaciens* H were cultured in a 500-ml flask at 30°C on a reciprocal shaker. Bacterial growth (OD_{660}, ○) and *Bam*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

B.amyloliquefaciens naturally expresses *Bam*H1 Methylase

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