Production systems for recombinant antibodies

Thomas Schirrmann, Laila Al-Halabi, Stefan Dübel, Michael Hust

Technische Universität Braunschweig, Institut für Biochemie und Biotechnologie, Abteilung Biotechnologie, Spielmannstr. 7., 38106 Braunschweig

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Antibody Production in Prokaryotic Hosts
   3.1. Gram-negative bacteria
   3.2. Gram-positive bacteria
4. Antibody Production in Eukaryotic Hosts
   4.1. Yeast
   4.2. Filamentous fungi
   4.3. Insect cells
   4.4. Mammalian cells
   4.5. Transgenic plants
   4.6. Transgenic animals
5. Concluding remarks
6. References

1. ABSTRACT

Recombinant antibodies are the fastest growing class of therapeutic proteins. Furthermore, antibodies are key detection reagents in research and diagnostics. The increasing demand for antibodies with regards to amount and quality resulted in the development of a variety of recombinant production systems employing Gram-negative and Gram-positive bacteria, yeast and filamentous fungi, insect cell lines as well as mammalian cell lines. More recently, antibodies were also successfully produced in transgenic plants and animals. Currently, the production of recombinant antibodies for therapy is performed in mammalian cell lines to reduce the risk of immunogenicity caused by non-human post-translational modifications, in particular glycosylation. However, novel strategies already allow human-like glycosylation patterns in yeast, insect cell lines and transgenic plants. Furthermore, therapeutic strategies not requiring glycosylation of the Fc portion have been conceived, most prominently using bispecific antibodies or scFv fusion proteins, which can be produced in bacteria. Here, we review all current antibody production systems considering their advantages and limitations with respect to intended applications.

2. INTRODUCTION

Recombinant antibodies and antibody fragments are important tools for research, diagnostic and therapy. Currently, scientists and medics employ antibodies in their routine laboratory work for a large number of standard assays like immunoblot analysis, flow cytometry or immunohistochemistry. After completion and refinement of the human genome (1, 2), the demand for specific binders dramatically increased for systematic analysis of the huge number of proteins encoded by individual genes and open reading frames (ORFs) with respect to their function, cellular localization and regulation. Moreover, recombinant antibodies can be used for the diagnosis of diseases by detection of pathogens or toxins (e.g. (3)). For the last few decades, antibodies have been anticipated as molecules well suited for various therapeutic approaches (4, 5). The major therapeutic applications of antibodies are inflammatory (6) and tumour diseases (7). Over 30% of biopharmaceuticals in clinical trials are recombinant antibodies (8). The market for therapeutic antibodies is estimated at 30 billion US dollars in 2010 (9). Recombinant antibodies even have the potential to be used in consumer products, like in shampoos to prevent dandruff (10) or in
Figure 1. Nomenclature for a selection of recombinant antibody formats engineered for different applications, in comparison to native IgG (upper left corner). Variable regions are given in turquoise, constant regions in yellow, orange and red, artificial peptide linkers in purple. dHLX represents amphipathic helices used for dimerization of scFv fragments.

toothpaste to protect against tooth decay caused by caries (11,12), which will require very large quantities and appropriate antibody production systems.

Polyclonal antibodies are widely used as detection reagents in research and diagnostics but they are a batch-dependent limited resource and also contain antibodies with unknown specificities. In addition, polyclonal non-human antibodies are very immunogenic restricting their therapeutic application to acute intoxications, for example, after snake bites (13). A big milestone in antibody generation was the generation of monoclonal antibodies (mAbs) by hybridoma technology which is based on the fusion of antibody producing spleen cells from immunized mice or rats with immortal myeloma cell lines (14). However, the hybridoma technology has some limitations concerning the inefficient immune response to highly toxic or conserved antigens. In addition, today all novel therapeutic antibodies are of human origin or at least partially humanized to reduce the risk of immunogenicity. The generation of hybridomas from human B cells remains difficult but B cells of transgenic mice with the human IgG gene repertoire can be used for the generation of murine hybridomas producing human immunoglobulins (15-20). However, this method still requires immunization and a successful in vivo antibody response. In vitro antibody selection technologies such as antibody phage display and ribosomal display circumvent the in vivo immune response and allow the generation of human antibodies (21-25). Furthermore, antibody display technologies allow improvement of antibodies in terms of affinity, stability, solubility or even its “humanness”.

New recombinant antibody generation technologies increased the number of antibodies for different applications but also raised demands for appropriate production systems which are the focus of this review. The production of antibodies in high amounts is a challenge due to the high complexity of immunoglobulins. Immunoglobulin G (IgG) is a heterotetrameric molecule formed by the assembly of two different polypeptide chains connected by inter-chain disulfide bonds which form many domains with numerous non-covalent interactions and disulfide linkages. Antibody light chain (LC) and heavy chain (HC) consist of several “immunoglobulin fold” domains, each requiring an intra-domain disulfide bond for stabilization (26). Efficient and correct formation of the large number of disulfide bonds together with the folding and assembly of four chains to one IgG molecule requires an oxidizing environment and the assistance by a complex folding apparatus.

Many hosts for in vitro selection or production of antibodies do not have optimal conditions for the expression of full size IgGs. This problem was partially addressed by the use of smaller antibody fragments (Figure 1) which are still able to mediate antigen binding properties. These antibody fragments can be used for many research and diagnostic applications, and therapeutic treatments where binding to an epitope is sufficient for the therapeutic effect, for example, to achieve virus or toxin neutralization or receptor blocking. Furthermore, the smaller fragments are key elements for the success of most of the in vitro antibody selection systems, like phage display, cell surface display or ribosomal display (21-25, 25a).

The Fv fragment is the smallest antigen binding fragment of immunoglobulins retaining its complete antigen binding site, and consists only of the variable (V) regions. To overcome the low stability of Fv fragments, a soluble and flexible 15 - 25 amino acid peptide linker is used to connect the V regions to form a scFv fragment (27), or the constant domains CL and CH1 are added to the V regions to obtain a Fab fragment (see Fig 1). Currently, scFv and Fab are the most widely used antibody fragments, in particular of those produced in prokaryotes. Other antibody formats have been produced in prokaryotic and eukaryotic cells, for example, disulfide-bond stabilized scFvs (ds-scFv) (28), single chain Fab fragments (scFab) combining scFv and Fab properties (29), multimeric antibody formats such as dia- tria- or tetrabodies generated by reducing the linker length in scFvs (30-33), minibodies (miniAbs) comprising different formats consisting of scFvs linked to oligomerization domains such as immunoglobulin CH3 domain (30), leucin zipper, helix turn helix motif or streptavidin, or scFv-scFv tandems to enhance the apparent affinity by avidity effect (34, 34b, 35). Moreover, there are bispecific antibody formats
Production systems for recombinant antibodies

combining two different antigen binding domains in one molecule which can be used to cross-link two different targets (36, 37). The smallest antibody fragments are V\textsubscript{HH} of camelid heavy chain antibodies (38) and similar single domain antibodies (dAb) (39).

Production levels of up to gram per liter amounts were achieved for smaller antibody fragments even in bacteria (40, 41). However, for many therapeutic applications, the immunoglobulin Fc moiety is essential, particularly, if antibody mediated effector functions or enhanced serum half life are required. For example, antibody dependent cellular cytotoxicity (ADCC) requires specific N-linked glycosylation in the Fc domain which can only be processed in eukaryotic hosts. The fusion of antibody fragments to the Fc domain (e.g. V\textsubscript{HH}-Fc, scFv-Fc) is used to gain effector functions and avidity almost indistinguishable from IgGs (38, 42-44) whilst requiring the production of only a single polypeptide.

3. ANTIBODY PRODUCTION IN PROKARYOTIC HOSTS

3.1. Gram-negative bacteria

The Gram-negative bacterium Escherichia coli is the most widely used production system for recombinant proteins, reaching volumetric yields of more than 10 g/L for cytoplasmic expression. Key to the success for the first expression of a functional antibody fragment in E. coli described by Skerra and Plückthun in 1988 (45) was the translocation of both V chains into the periplasmic space of E. coli where the oxidizing environment allowed the correct formation of disulfide bonds and the assembly to a functional Fv fragment. This strategy also allowed the first expression of functional Fab fragments in E. coli (46).

The expression of recombinant antibodies in the reducing cytoplasmic compartment mostly results in non-functional aggregates (47). Recovery of functional antibody fragments from cytoplasmic inclusion bodies by complete denaturation and refolding (48) is not very efficient. Only heavily engineered cystein free variants of a few scFvs were successfully produced in the cytoplasm of E. coli, not requiring refolding (49, 50). E. coli strains with mutations in the glutathione and thioredoxin reductase in combination with coexpression of cytoplasmic chaperones GroEL/ES, trigger factor, DnaK/J as well as signal sequence-less variants of periplasmic chaperones DsbC and Skp increased yield of correctly folded Fab with Skp showing the most significant effect. A maximum yield of 0.8 mg functional Fab per liter per O.D\textsubscript{600} was achieved (51).

Despite these efforts, most antibody fragments are produced in the periplasm of E. coli using N terminal leader sequences targeting the periplasmic Sec pathway, for example, signal peptides derived from outer membrane protein A (OmpA), alkaline phosphatase A (PhoA) or pectate lyase B (PelB) (52-55). After expression, recombinant antibodies are usually isolated from the periplasmic fraction (56, 57), and in some cases from culture supernatant (58, 59). Periplasmic expression and yield of functional scFv fragments could be improved by co- or overexpression of GroES/L or of the peptidylprolyl-cis,trans-isomerase FkPa (60-62).

The Fab format requires the expression, periplasmic transport, correct folding and assembly of two different polypeptide chains. Among the different vector formats and arrangements, bicistronic vectors with the first cistron encoding the light chain and the second cistron encoding the Fd fragment (consisting of Vh and CH1) were optimal (56). Recently, even aglycosylated full size IgG were successfully produced in E. coli (63). Interestingly, the production of full size IgG in E. coli was only possible after fine adjustment of the translation strength of both IgG chains by introduction of silent mutations into the translation initiation region of the leader sequence to achieve optimal periplasmic transport (63, 64).

Since antibody expression in E. coli is related to host toxicity resulting in very low yields, vector mutation and plasmid loss, it must be well repressed before production phase and requires an adjustable induction during the production. These critical parameters can be addressed by the promoter system, plasmid copy number and other parameters (65, 65a, 65b). Periplasmic antibody production is usually performed at 20 to 30°C rather than at 37°C to prevent overloading of E. coli’s secretory pathway and intracellular aggregation. However, very high yields of antibody fragments produced in E. coli are mainly provided by high cell density fermentation in bioreactors. The high yield of 1.2 g/L for a hapten-specific scFv produced in a bioreactor (65) compared to 16.5 mg/L obtained by optimized shake flask production (66) can be mostly addressed to the over 100-fold higher cell density in the bioreactor.

Cell-wall-less L-forms of the Gram-negative bacterium Proteus mirabilis were used for the production of miniAbs and scFvs (35, 67), with total and functional scFv yields of 83-127 mg/L and 9 -12 mg/L, respectively (67).

3.2. Gram-positive bacteria

Gram-positive bacteria directly transfer secretory proteins into the medium due to the lack of an outer membrane, which may facilitate the production and purification of antibody fragments. The Gram-positive bacteria Bacillus brevis (68, 69) and Bacillus subtilis (70, 71) have been successfully used for the production of different antibody fragments. Recently, Bacillus megaterium, which does not produce alkaline proteases and provides high stability of plasmid vectors during growth (72) and which allows stable transgene expression during long term cultivation in bioreactors, was employed for the production of antibody fragments. Functional scFv fragments could be directly isolated from the supernatant (73).
Production systems for recombinant antibodies

Lactobacilli are “generally regarded as safe” (GRAS) microorganisms and have also been tested for antibody production. To date, two lactobacillus strains were used for the production of scFvs, Lactobacillus zaeae / casei (74, 75) and Lactobacillus paracasei (38, 76). The GRAS status of lactobacilli allows their direct use for oral application, for example, for the production of anti-Streptococcus mutans antibody fragments to prevent tooth decay (74).

4. ANTIBODY PRODUCTION IN EUKARYOTIC HOSTS

Eukaryotic hosts provide a more sophisticated protein folding and secretion apparatus than prokaryots. Correct post-translational processing of IgG, particularly glycosylation, is only provided by eukaryotic cells. However, their capacity to generate human glycosylation patterns shows great variations (5). The immunoglobulin Fc part contains specific N-linked glycosylations which are important for the interaction with other components of the immune system, like the recognition by Fc receptor bearing immune cells. The IgG glycosylation pattern can vary which influences effector properties of IgGs (77). Elimination of core fucosylation from IgG improved binding to Fc-gamma receptors and ADCC activity (78). Therefore, the capacity of the host system to achieve the required glycosylation is an important parameter for a number of therapeutic antibodies and their application.

4.1. Yeast

The heterologous production of recombinant proteins in yeasts is attractive because it combines the advantages of short generation time, ease of genetic manipulation, robustness and simple medium requirements of unicellular microbial hosts with enhanced post-translational folding, processing and secretion capacity of eukaryots. Many yeast strains have GRAS status and do not produce endotoxins which could facilitate the admittance for the production of heterologous proteins for in vivo diagnostics and therapy. Another advantage of yeasts are the low number of secreted proteins, facilitating the downstream processing of secretory produced heterologous proteins.

Beside the well-characterised baker’s yeast Saccharomyces cerevisiae, non–Saccharomyces yeast strains are available for recombinant polypeptide production including Pichia pastoris, Hansenula polymorpha, Schizosaccharomyces pombe (78), Schwanniomyces occidentalis, Kluyveromyces lactis and Yarrowia lipolytica (79). However, mostly S. cerevisiae and P. pastoris have been studied for recombinant antibody production. P. pastoris shows a better overall capacity of producing and secreting heterologous proteins than S. cerevisiae. It also prefers respiratory growth leading to higher cell densities of more than 100 g/L dry weight (80). One of the most prominent features of P. pastoris is the metabolism of methanol as sole carbon source. The alcohol oxidase 1 (AOX1) promoter is tightly controllable by methanol and commonly used for recombinant protein expression in P. pastoris.

The first expression of a scFv fragment in P. pastoris was shown by Ridder et al. in 1995 (81). In P. pastoris, different scFvs were produced with a yield of 70 mg/L (82) to 250 mg/L (83). Under optimized fermentation conditions even up to 1.2 g/L functional scFv were obtained (84). In S. cerevisiae over 100 mg/L of a llama VHH were produced in shake flask culture (85). DNA shuffling of three homologous VHH clones did not only improve affinity and thermal stability but also expression yield (86).

The secretory production of recombinant antibodies in yeast requires a N terminal signal sequence targeting yeast’s secretory pathway. Initially, the secretory signal sequence derived from the S. cerevisiae mating factor alpha (alpha-factor) prepro-peptide has been used. Proteolytic cleavage sites (KEK2 often in combination with STE13 sites) introduced after the secretory signal sequence led to incomplete or incorrect proteolytic processing (87), but simple signal peptides could be used instead.

In the case of Fab fragments or IgGs, both antibody chains must be fused to the alpha-factor prepropeptide leader sequence and co-transformed into yeast. The yield of Fabs produced in yeast ranged from 1 to 50 mg/L in shake flasks to 0.5 g/L in bioreactors. There are only rare data about IgG expression in yeast. In an early study, a mouse-human chimeric antibody and its Fab fragment were produced in S. cerevisiae with yields of 50 – 80 μg/L and 0.2 mg/L, respectively. The chimeric IgG mediated tumor specific binding and ADCC but no CDC (88). In contrast, scFv-Fc fusion proteins were produced with more than 10-30 mg/L in P. pastoris (89).

Overexpression of the chaperone immunoglobulin binding protein BiP or protein disulfide isomerase PDI in S. cerevisiae increased scFv secretion titers 2 to 8 fold for five scFvs with an average yield of 20 mg/L in shake flask culture (90).

Reported disadvantages of yeasts include lower transformation frequencies than E. coli, failure to express AT-rich genes due to premature transcriptional termination, inefficient secretion of some larger proteins (>30 kDa), proteolysis of secreted proteins during high-cell density fermentation, and inappropriate glycosylation of human glycoproteins. Yeasts also tend to hyperglycosylate heterologous proteins even at positions not glycosylated in the native host, and immunogenicity and different pharmacokinetics can be caused by carbohydrate structures not not observed in higher eucaryotes (216). However, P. pastoris mediates much lower hyperglycosylation than S. cerevisiae and often exhibits N-linked carbohydrate structures similar the mammalian high-mannose core unit Man5GlcNAc2 (91). Also, there are strong efforts to accomplish humanized glycosylation pattern in P. pastoris (92-95).

Recently, the production of a human IgG showing humanized N-linked glycosylation in P. pastoris has been reported (96). Unlike full size IgGs produced in
Production systems for recombinant antibodies

wildtype yeast, those produced in glycoengineered yeast had functional antibody-mediated effector functions.

4.2. Filamentous fungi

Filamentous fungi of the genera Trichoderma and Aspergillus have a high capacity to secrete large amounts of protein and metabolites into the medium (97). They have been widely used in food industry, for example, A. niger for the citric acid production. Furthermore, A. niger (subgenus A. awamori) and A. oryzae are classified as GRAS for some of their products and, thus, are suitable hosts for the production of recombinant proteins. In A. niger yields of up to 1.2 g/L IgG were obtained. Two promoters are used for the expression of antibodies, the glucoamylase promoter g1A (98) and the endoxyanlase A promoter exIA (99). After fusion of the antibody chains to the C terminus of glucoamylase, which is highly secreted by Aspergillus, the yield was increased five fold to 50 mg/L (100). To release the antibody, protease cleavage sites like KexB are introduced between antibody chain and glucoamylase (98). In Trichoderma reesei, 150 mg/L of a Fab fused to cellobiohydrolase I was produced, whereas without a fusion partner the yield was only 1 mg/L (101). A. awamori was used for the production of scFs, llama VHH and antibody enzyme fusion proteins (97, 99, 102). Some proteolytic degradation was observed. Chrysosporium lucknowense C1 is a triple protease deletion mutant (Delta-alp1, Delta-pep4, Delta-alp2) which is has been used for small scale production, for screening, as well as for large scale protein production in bioreactors (103).

4.3. Insect cells

Insect cells are a versatile eukaryotic system for heterologous protein expression. Insect-specific viruses from the family of Baculoviridae, particularly the Autographa californica nuclear polyhedrosis virus (AcNPV), are origin for most used insect expression vectors. Baculoviruses are highly species-specific and are considered as safe for humans, mammals and plants. Therefore, no special safety precautions must be considered. A Baculovirus based Hepatitis virus C vaccine is promising as the first human therapeutic produced in insect cells (104). Non-essential Baculovirus genes in the tissue culture life cycle, like Polyhedrin, P10 or Basic can be replaced even by large heterologous genes. Heterologous proteins can be produced at levels ranging between 0.1% and 50% of the total insect cell protein. Recombinant Baculoviruses are usually used for the infection of insect cell lines like SF-9 and SF-21 of Spodoptera frugiperda or High Five™ (BTI-TN-5B1-4) of Trichoplusia ni. Important parameters for optimising the production are the multiplicity of infection (m.o.i.) and the protein production time which seems to be limited to 96 h after infection due to the start of cell lysis. Protease inhibitors are recommended because cell lysis is associated with the release of viral proteases. Yields of up to 32 mg/L of secreted monomeric anti-phOx scFv were obtained in a 6 L bioreactor with 10^7 cells per liter after 72 h with m.o.i. of 1 (105). The production of various IgGs reached yields of 6 – 18 mg/L (106). IgGs produced in High Five cells showed terminal galactosyl residues beta (1,4)-linked to the biantennary GlcNAc residues but the absence of sialylation, formation of paucimannosidic structures and the presence of potentially allergenic alpha1,3-fucose linkages were the differences observed relative to mammalian glycosylation pattern (107). However, IgG produced in insect cells were able to mediate effector functions like complement binding (108, 109). Ten µg/mL of an anti-Rhesus D antibody produced in SF9 cells mediated lysis of Rh” red blood cells by ADCC (110). Insect cell protein expression systems were improved using protease deficient Baculovirus strains or insect cell lines with additional glycosyltransferase gene modifications to obtain glycosylation pattern comparable to mammalian cell lines (111).

After expression of IgG in insect cells driven by the strong Polyhedrin promoter an extensive aggregation was observed probably due to the overloading of the cellular folding and post-translational processing apparatus (112). Recombinant overexpression of the ER resident chaperone BiP significantly enhanced levels of soluble and secreted IgG in T. ni cells (113). Enhanced secretion of IgG as well as IgG recovery from insoluble aggregates was also achieved by co-expression of PDI. Overexpression of the human cytosolic chaperone hsp70 increased solubility and secretion of murine IgG in T. ni cells by enhancing the solubility of the light chain precursor (114).

As an alternative to the Baculovirus system which often suffers from a strong intracellular protein aggregation and from strong use of cellular metabolism resulting in a high diversity of post-translational modified products, insect cell expression plasmids can be transiently or stably transfected into Schneider 2 (S2) cells of Drosophila melanogaster. Secretory production requires a signal sequence like the honeybee melittin leader. Stable transfection of Drosophila cell lines with monomeric and dimeric antibody fragments resulted in yields of up to 25 µg/mL (115).

4.4. Mammalian cells

Despite high production costs due to expensive medium and cultivation requirements as well as difficult handling, today 60-70 % of recombinant protein pharmaceutics and all currently approved therapeutic antibodies are produced in mammalian cell lines. The success of mammalian production systems is due to their advanced folding, secretion and post-translational apparatus which leads to producing antibodies which are indistinguishable from those produced in the human body. Moreover, the still continuing progress of the mammalian cell culture technology has already reached antibody production levels of about 5 g/L, 10-fold higher than what they were some years ago (116). Major parameters responsible for this development are improved generation of high producer cell lines and prolonged
cultivation at very high cell densities during production. The productivity of mammalian cells increased from 10 pg antibody per cell per day in 1986 to about 90 pg/cell/d in 2004 which is still in the scale of earlier reports for a myeloma cell line producing 20 to 80 pg/cell/d (117). However, improved production processes increased cell densities from 2 x 10^6 cells/mL in 1986 to 10^7 cells/mL in 2004. In addition, improved cell viability allows production times at high cell densities for almost 3 weeks. To reduce costs as well as risks of contamination by pathogens or bovine spongiform encephalopathy (TSE/BSE) agents, media without supplementation of animal sera or serum components had been developed (118). Moreover, research is still progressing in process optimization and genetic engineering of production cell lines regarding glycosylation homogeneity as well as improved metabolism, reduced apoptosis and inducible cell cycle arrest for prolonged production (119,120).

However, mammalian cell lines are still under critical observation regarding to unknown viral contamination (121,122) and release of factors with oncogenic or pathogenic potential. Therefore, most mammalian cell lines used for recombinant protein production are derived from normal diploid mammalian cells by a known transformation mechanism but not from tumor tissues. For recombinant protein production, Chinese hamster ovary cells (CHO) cells, mouse myeloma NS0 cells, baby hamster kidney (BHK) cells and two cell lines generated from human embryonal tissue by transformation with Adenovirus 5 (Ad5) DNA, the human embryonic kidney cell line HEK-293 and the human retinal cell line Per.C6 (Crucell, NL) gained regulatory approval for recombinant protein production (123). More recently, the HKB11 cell clone, derived from a hybrid cell fusion of HEK 293S with the Burkitt's lymphoma line 2B8, showed 10-fold higher product yield than CHO cells in serum free media (124, 125). Although glycosylation pattern of mammalian glycoproteins is very similar to that in humans (126), even small differences can influence pharmacokinetics and effector functions of antibodies.

Large scale antibody production usually needs long term production stability which requires chromosomal integration of antibody genes into the host cell genome. That demands a long term screening of large numbers of clones to identify stable high producer clones. There are many strategies to overcome negative effects of the integration site by introduction of protective cis-regulatory elements including insulators, boundary elements, scaffold / matrix attachment regions (S/MARs) (127), chromatin opening elements (128) and antirepressor elements (129) into the vector which reduce influence of heterochromatin and stabilize transgene expression (130, 131). Silencing can also be blocked by inhibition of histone deacetylation using butyrate (132) which could enhance the protein expression levels of the cells (133) but can also induce apoptosis. Recombination enzymes, like bacteriophage P1 Cre recombinase, lambda phage integrase, or yeast Flp recombinase can efficiently catalyse the site specific integration in chromosomal recombination site (134). However, cell lines with chromosomal recombination cassettes optimized for high level protein expression have not been available, so far.

In contrast to the chromosomal integration site, genetic elements driving the transcription seem to play a minor role for a stable high transgene expression. In most cases, strong promoters like the immediate early cytomegalovirus (CMV) or the cellular elongation factor (EF) 1-alpha promoter are implemented. Polyadenylation sites usually derived from the simian virus (SV) 40 or the bovine growth hormone (BGH) improve mRNA stability and enhance translation efficiency. Furthermore, splicing of mRNA is known to promote mRNA packaging and transfer into cytosol to stabilize and enhance gene expression as well as to reduce silencing of heterologous transgenes (135, 136). For IgG expression, two different genes must be stably transfected into one cell clone, either by co-transfection or by using bicistronic expression vectors. Bicistronic vectors employing internal ribosomal entry sites (IRES) allow the translation of two or more cistrons from the same transcript (137). The encephalomyelitis virus (ECMV) IRES has shown the highest efficiency in various mammalian cell lines. Mutated IRES derivatives even allow the control of translation efficiency in relation to the cap dependent cistron. The ratio between light and heavy chain has great impact on the secretion level of functional IgG (137b). The long term stability of ECMV IRES containing bicistronic constructs has been demonstrated even in the absence of selection pressure over months (137).

Production cell lines are usually the result of a year-long, tedious process of repeated cycles of gene amplification. Here, two systems are used with most success, the dihydrololate reductase (DHFR) and the glutamyl synthetase (GS) selection systems (138). Yield and functionality of an IgG1 produced in dhfr- CHO and GS-NSO were equivalent (139). Prolonged fed-batch production of three to four weeks using GS-NS0 cells yields up to 1.8 g/L antibody (140) or even up to 2.7 g/L final antibody concentration after improved feeding with NS0 cells containing 3 vector copies per cell (141). Anti-p185HER-2 scFv-CH3 minibodies (10H8) were expressed with up to 60 mg/L in NS0 cells (142). Particularly in research and development, transient or semi-stable production systems are often used to circumvent the time consuming selection of single stable expression cell clones requiring several months. Transient productions in scales of hundreds of milligrams are possible in mammalian cells (143). Derivatives of the human embryonic kidney (HEK) cell line 293 transformed either with the simian virus 40 (SV40) large T antigen (HEK293T) or the Epstein Barr virus (EBV) nuclear antigen EBNA-1 (HEK293-EBNA) combine high transfection efficiency with the semi-stable episomal propagation of expression plasmids containing the origin of replication of SV40 or EBV.
respectively, allowing transient production for more than two weeks. Transient transfection of HEK293T cells can be efficiently performed using cationic liposomes (Lipofectamine, HEKfectin, etc.) or polyethylenimines (PEI) (144). Calcium phosphate (CaPi) transfection is also efficient for HEK293 cells and inexpensive (145). Vectors with compatible restriction cassettes allow one step cloning of scFv fragments from phage display libraries into the scFv-Fc format. Small scale production in HEK293T cells was also used for the screening of functional IgG clones after two-step subcloning of Fab gene fragments immediately after phage display selection (146). Transient productions in tissue culture plates using adherent HEK293T cells yielded 1 and 20 mg/L of IgG and scFv-Fc, respectively (137 and our own unpublished results). Transient productions in liter scales are mostly performed in stirred-tank bioreactors (STR) but with an increasing degree in disposable plastic bioreactors like WAVE™ (147).

4.5. Transgenic plants

Particularly, if very large amounts of biopharmaceuticals are required and production costs are to be considered, plants are an attractive alternative even for recombinant antibody production. All other production systems, notably mammalian cell systems, can not be up-scaled without limitation. Moreover, up-scaling of biofermentation processes often does not proportionally reduce production costs. Against that, the growing of plants can be easily expanded simply by using larger fields without much higher costs. Theoretically, the costs of an IgA produced in transgenic plants are only 1 to 10 % in comparison to hybridoma production, even acknowledging the more expensive downstream process in plants (148).

Dicotyledonous plants, like tobacco, are mainly transformed using the soil bacterium Agrobacterium tumefaciens. T-DNA vectors derived from the tumor inducing (Ti) plasmid of A. tumefaciens are used for the gene transfer. Under control of a viral plant promoter, mostly the constitutive cauliflower mosaic virus (CaMV) 35S promoter, the antibody gene is cloned into a region termed as T-DNA which is flanked by two 25 base pair long imperfect repeats. The T-DNA is transferred to the nucleus of the plant cell by the combined action of virulence (vir) genes provided by the T-DNA vector or a separate plasmid and subsequently integrated into the plant genome by non-homologous recombination. Transformed cells are selected by resistance markers and complete transgenic plants are regenerated from transformed calli (149). Rapid transient antibody productions in plants has also been performed using modified plant viral vectors (150, 151) with production levels of up to 0.7 % of total protein. Monocotyledonous plants are mostly transfected by particle gun (152).

Recombinant production of antibodies in plants has been mostly performed in tobacco, in Nicotiana tabacum (153-160) and Nicotiana benthamiana (161). The first IgG was produced in tobacco by Hiatt et al. in 1989 (153). The production of an anti-cutinase scFv in tobacco could be increased from 0.01 % to 1 % of total soluble protein by fusion with the endoplasmic reticulum (ER) retention signal KDEL (156, 157). IgGs were also produced in suspension cultures of tobacco (162). Antibody production in plant seeds immediately offers excellent storage conditions until downstream processing. A scFv produced in the seed of tobacco reached protein levels from 0.43 % to 0.67 % of total soluble protein (163). Alternatively, antibody encoding genes were also introduced into the chloroplast genome allowing high copy numbers, increased expression levels and prevention of expression crossing by pollen count (164). In potato plants (Solanum tuberosum) antibody genes were transferred into chloroplasts as well as amyloplasts (165) and in Arabidopsis thaliana Fab fragments were produced in appostals (166). Other plants successfully used for antibody production include soybean (Glycine max) (167), rice (Oryza sativa), wheat (Triticum aestivum) (168), lettuce (Lactuca sativa) (169), petunia (Petunia hybrida) (170) and alfalfa (Medicago sativa) (171) (Tab. 1). Furthermore, there are antibody production systems using aquatic plants like the algea Chlamydomonas reinhardtii (172) and the common duckweed (Lemma minor). In addition, Lemna minor has been stably modified to process human N-linked core glycosylation by RNA interference targeting expression of the endogenous alpha-1,3-fucosyltransferase and beta-1,2-xyllosyltransferase transcripts (173). Large Scale Biology (U.S.A.) produces anti-idiotype antibodies in tobacco which are already in clinical phase II for the treatment of Non-Hodgkin lymphoma (174, 175). CaroRX developed by Planet Biotechnology (Hayward, CA, U.S.A.) is produced in transgenic tobacco. It is a complex secretory IgA-like molecule consisting of a hybrid antibody comprising C-gammal and -2 domains of the heavy chain of an IgG fused to C-alpha2 and -3 of IgA, plus a J chain and a secretory component analog. It binds to the streptococcal antigen I/II of Streptococcus mutans, the major causative agent of bacterial tooth decay. CaroRX already demonstrated prevention of S. mutans adherence to tooth enamel in clinical phase II studies. Additionally, topical oral treatment in human subjects was safe and effective (11, 12).

4.6. Transgenic animals

The generation of transgenic animals is very difficult but allows the production of recombinant antibodies in milk or eggs. The production is highly scalable and downstream processing is less expensive than from plants. The oral administration of milk from transgenic mice secreting IgG1 or IgA derivatives of the corona virus neutralizing mAb 6A.C3 provided protection against coronavirus infections. Both antibody chains were under control of the whey acidic protein and beta-lactoglobulin promoter. Neutralizing activity corresponded to immunoglobulin concentrations of up to 5 to 6 mg per mL (176). Moreover, 0.8 g of immonoenzyme fusion (177) and 0.5 mg of a chimeric anti-CD19 IgG per mL milk were produced in...
transgenic mice (178). However, there are concerns relating to potential product immunogenicity caused by different glycosylation patterns characteristic for proteins produced in transgenic milk in comparison to native human serum proteins. The presence of N-glycolyl neuraminic acid could potentially trigger immunological complication or other side effects in man (179).

Another technology for recombinant protein production in transgenic animals is the targeted expression in the naturally sterile egg white of transgenic chicken. Each egg contains about 3 to 4 g protein with half of it expressed by both 

**Ovalbumin** alleles. The production is scalable and regulatory paths to current GMP have been paved by companies producing vaccines in eggs. Recently, transgenic chicken were generated expressing immunoglobulin chain genes under control of the 

**Ovalbumin** gene promoter including all known regulatory elements responsible for its oviduct specificity and steroid dependent induction in laying hens (180). Up to 3 mg per egg of fully assembled IgGs were obtained. Antigen binding was not altered and ADCC was even enhanced whereas half-life in mouse serum was half of that of natural antibodies. Further technical advances and detailed product characterisation, particularly antigenicity, remain to be assessed (181).

Another unique possibility is the immunization of transgenic large animals containing human chromosomal immunoglobulin loci as a novel source for human polyclonal antibodies (hPABs) for therapy. Transgenic calves containing a human artificial chromosomes with the entire unarranged sequences of the human immunoglobulin HC and lambda LC locus were already generated producing functional human immunoglobulins (182). Furthermore, there are first issues to knock out bovine immunoglobulin µ heavy chain locus and bovine prion gene to address the potential BSE risk in cattle (183).

An overview of recombinant antibodies produced in different hosts is shown in Table 1. It must be discriminated between the yield of functional antibodies after purification and the total yield. Furthermore, intrinsic properties of the individual antibody molecule can dramatically influence its expression.

**5. CONCLUDING REMARKS**

Although at present, mammalian cell lines are the only system used for the production of therapeutic antibodies, alternative expression hosts like yeasts, insect cells as well as transgenic plants are under development which can produce antibodies with human-like glycosylations suitable for therapeutic application at much lower production costs. For some therapeutic applications, mostly in **in vitro** diagnosticians and strategies that rely on antigen neutralization, “correct” post-translational modifications are irrelevant and production systems employing hosts like bacteria, yeasts, filamentous fungi and insect cells can be used. They have many advantages over mammalian cells, such as easier handling, higher robustness and reduced production costs. Transgenic plants and animals have the potential for the production of antibodies in theoretically unlimited amounts. A comparison of key features of the different production systems is given in Table 2.

The Gram-negative bacterium 

**E. coli** is the best genetically examined organism and provides a large set of molecular-biological tools for genetic engineering. Antibody fragments like scFvs or Fabs are usually produced in 

**E. coli** by periplasmic expression. Particularly, in combination with antibody phage display 

**E. coli** allows fast, robust and reliable small scale antibody production of the selected antibodies. High yields of 1 - 2 g/L of functional scFv or Fab produced in 

**E. coli** are strongly dependent on the individual antibody fragment and require optimized high cell density fermentation.

Gram-positive bacteria are well suited for biotechnological processes because of their powerful secretion apparatus and lack of outer membrane. However, antibody production systems employing Gram-positive bacteria are still in the developmental stage.

Yeast combine short generation time, ruggedness and low nutrient requirements of microbial prokaryots with the eukaryotic folding and secretion capacity. The simple generation of stable transgenic yeast clones has capacity for through-put processes, but far beyond 

**E. coli**. Despite the availability of modified yeast strains which make human-like glycosylations possible, production of complex full size IgGs still remains a challenge.

Filamentous fungi require higher efforts to generate transformed clones in comparison to yeast. However, they are promising candidates for biotechnological production because of their long biotechnological tradition and their high secretory capacity. 

**A. niger** has already been used to produce large amounts of functional IgG with great potential for improvement.

Insect cells have a better suited protein folding and secretion apparatus than prokaryots and lower eukaryots, as well as higher robustness combined with less sophisticated requirements for fermentation than mammalian cells. However, development of stable insect cell lines and process technology is not as far developed as for mammalian cells.

Mammalian cell lines are still the system of choice for the production of therapeutic antibodies, because they have the best equipped folding and secretion apparatus for antibodies and are capable to produce human glycosylations, provide easy appoints.
Production systems for recombinant antibodies

Table 1. Production of recombinant antibodies in different hosts

<table>
<thead>
<tr>
<th>Host</th>
<th>Antigen</th>
<th>Antibody format (clone)</th>
<th>Production system</th>
<th>Yield</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>digoxin</td>
<td>Fab (Z2-10)</td>
<td>shake flask</td>
<td>0.8 mg/L; O.D. 600</td>
<td>51</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>CD18</td>
<td>F (ab')2</td>
<td>fermentor</td>
<td>2.5 g/L</td>
<td>41</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>lysozyme</td>
<td>scFv (D1.3)</td>
<td>400 mL, shake flask</td>
<td>0.3 – 1.0 mg/L</td>
<td>73, 184</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>MUC1</td>
<td>scFv</td>
<td>100 mL, shake flask</td>
<td>10 mg/L</td>
<td>185</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>p815</td>
<td>Fab</td>
<td>10 L fermentor</td>
<td>2.2 mg/L</td>
<td>80</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>PPL</td>
<td>VL Dab</td>
<td>1.5 L fermentor</td>
<td>35-65 mg/L</td>
<td>186</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>phOx</td>
<td>scFv</td>
<td>50 mL shake flask</td>
<td>0.2 mg/L</td>
<td>66</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>scorpion toxin Cn2</td>
<td>scFv (BCF2)</td>
<td>n.d.</td>
<td>0.3 mg/mL; 1.0 mg/L</td>
<td>187</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>tissue factor</td>
<td>IgG</td>
<td>10 L fermentor</td>
<td>30-150 mg/L</td>
<td>83</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>TAG-72</td>
<td>Fv (B72.3)</td>
<td>shake flask, fermentor</td>
<td>40 mg/L; 450 mg/L</td>
<td>188</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>FAP</td>
<td>scFv</td>
<td>30 mL shake flask</td>
<td>1-2 mg/L</td>
<td>87</td>
</tr>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus brevis</em></td>
<td>uPA</td>
<td>Fab</td>
<td>2 L shake flask</td>
<td>100 mg/L</td>
<td>68</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>lysozyme</td>
<td>scFv (D1.3)</td>
<td>400 mL, shake flask</td>
<td>0.41 mg/L</td>
<td>73</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>digoxin</td>
<td>scFv</td>
<td>n.d.</td>
<td>2 mg/L</td>
<td>70</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em></td>
<td>rotavirus</td>
<td>Vh</td>
<td>n.d.</td>
<td>1 mg/L</td>
<td>58</td>
</tr>
<tr>
<td><em>Streptomyces lividans</em></td>
<td>lysozyme</td>
<td>Fv</td>
<td>n.d.</td>
<td>1 mg/L</td>
<td>190</td>
</tr>
<tr>
<td><strong>Eukaryots</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pichia pastoris, Kluyveromyces</em></td>
<td>catalytic antibody</td>
<td>scFv (4B2)</td>
<td>n.d.</td>
<td>1.3-4 mg/L</td>
<td>191</td>
</tr>
<tr>
<td><em>Kluyveromyces</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Yarrowia lipolytica, Kluyveromyces lactis</em></td>
<td>human Ras</td>
<td>scFv</td>
<td>shake flakes</td>
<td>30-20 mg/L</td>
<td>192</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>HCG; RR6</td>
<td>V HH (H-14; R2)</td>
<td>100 mL shake flask</td>
<td>100 mg/L ; 200 mg/L</td>
<td>203</td>
</tr>
<tr>
<td><em>Trichoderma reesei</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger var. awamori</em></td>
<td>ErbB2</td>
<td>hlgG; Fab (trastuzumab)</td>
<td>50 mL, shake flask</td>
<td>0.9 g/L; 1.2 g/L</td>
<td>68</td>
</tr>
<tr>
<td><em>Aspergillus niger var. awamori</em></td>
<td>lysozyme</td>
<td>scFv (D1.3)/D1.3-glucosylase</td>
<td>n.d.</td>
<td>10/50 mg/L</td>
<td>100</td>
</tr>
<tr>
<td><em>Aspergillus niger var. awamori</em></td>
<td>R66 azo-dye hapten</td>
<td>Vh (12)</td>
<td>shake flask</td>
<td>7.5 mg/L</td>
<td>102</td>
</tr>
<tr>
<td><strong>Filamentous Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseuduphila melanogaster, DS2</em></td>
<td>ACMV</td>
<td>scFv, scFv-γ2mM (S20)</td>
<td>25-30 mg/L</td>
<td>80 mg/L</td>
<td>115</td>
</tr>
<tr>
<td><em>Drosophila melanogaster, SC2</em></td>
<td>E-selectin</td>
<td>scFv</td>
<td>n.d.</td>
<td>1-0.4 mg/L</td>
<td>204</td>
</tr>
<tr>
<td><em>S. frugiperda, Sf9</em></td>
<td>phOx</td>
<td>scFv</td>
<td>n.d.</td>
<td>10 mg/L</td>
<td>105</td>
</tr>
<tr>
<td><em>S. frugiperda, Sf9</em></td>
<td>avasnote</td>
<td>IgG1</td>
<td>n.d.</td>
<td>10 mg/L</td>
<td>112</td>
</tr>
<tr>
<td><em>Trichoplusia ni</em></td>
<td>lipoprotein I</td>
<td>Murine IgG2a</td>
<td>150 mL shake flask</td>
<td>100 mg/L</td>
<td>110</td>
</tr>
<tr>
<td><strong>Mammalian cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hek293F</td>
<td>various</td>
<td>IgG and scFv-Fc</td>
<td>L, HEK293, tissue culture plates</td>
<td>1-20 mg/L</td>
<td>137, unpubl.</td>
</tr>
<tr>
<td>Hek293E</td>
<td>n. d.</td>
<td>hlgG</td>
<td>PEI, 10 L shake bottle</td>
<td>80 mg/L</td>
<td>143</td>
</tr>
<tr>
<td>CHO-K1</td>
<td>several antigens</td>
<td>IgG</td>
<td>L, 1 L spinner flask</td>
<td>10 mg/L</td>
<td>206</td>
</tr>
<tr>
<td>CHO-K1-LS</td>
<td>TAG72</td>
<td>Chimeric IgG4 (B72.3)</td>
<td>PEI, 10 L shake flask</td>
<td>50 mg/L</td>
<td>207</td>
</tr>
<tr>
<td>Stable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>TAG-72</td>
<td>Fv (B72.3)</td>
<td>roller bottle</td>
<td>4 mg/L</td>
<td>188</td>
</tr>
<tr>
<td>GS-N90</td>
<td>TAG-72</td>
<td>clgG4 (cB72.3)</td>
<td>fermentor, fed-batch, air-lift</td>
<td>960 mg/L</td>
<td>208</td>
</tr>
<tr>
<td>N90</td>
<td>p185</td>
<td>scFv-CH3 (10H8)</td>
<td>n.d.</td>
<td>60 mg/L</td>
<td>142</td>
</tr>
</tbody>
</table>

4584
Production systems for recombinant antibodies

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Generation of cells</th>
<th>Yield</th>
<th>Glycosylation</th>
<th>Whole Ig (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>reticulocyte lysate</td>
<td>not necessary</td>
<td>not necessary</td>
<td>very low</td>
</tr>
<tr>
<td>Prokaryotic organisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td></td>
<td>Cytoplasm</td>
<td>very fast</td>
<td>simple</td>
<td>high/ req. S-S refolding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Periplasm, soluble</td>
<td>very fast</td>
<td>simple</td>
<td>low/medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Periplasm, inclusion bodies</td>
<td>very fast</td>
<td>simple</td>
<td>high/ req. refolding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other Gram-negative</td>
<td>very fast</td>
<td>simple</td>
<td>low/medium</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
<td>fast</td>
<td>simple</td>
<td>low/medium</td>
<td>-</td>
</tr>
<tr>
<td>Eukaryotic organisms</td>
<td></td>
<td>Yeasts</td>
<td>medium</td>
<td>1-2 months</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>filamentous fungi</td>
<td>medium</td>
<td>&gt; 1 month</td>
<td>low/medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>baculovirus / insect cells</td>
<td>medium</td>
<td>&gt; 1 month</td>
<td>low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mammalian cells</td>
<td>transient</td>
<td>medium</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stable</td>
<td>medium</td>
<td>ca. 1 year</td>
<td>high</td>
</tr>
<tr>
<td></td>
<td>Transgenic plants</td>
<td>slow</td>
<td>6 mo -&gt; 1 year</td>
<td>high</td>
<td>different</td>
</tr>
</tbody>
</table>

Table 2. Recombinant production systems

Abbreviations: ACMV, African cassava mosaic virus; CaPi, Calcium phosphate transfection; CEA, carcinoembryonic antigen; FAP, fibroblast activation protein alpha; GS, glutamyl synthetase amplification system; HCG, human pregnancy hormone; RR6, azo-dye hapten; HCG, human chorionic gonadotropin hCK, human creatin kinase; HIV, human immunodeficiency virus; LF, lipofectamine; lipoprotein I, lipoprotein I of P. aeruginosa; PEI, polyethylenimine transfection; phOx, 2-phenyloxazoline-5-on; PPL, peptostreptococcal protein L; PSA, prostate-specific antigen; PSMA, prostate specific membrane antigen; serpins, serpins of Mamestra configurata; SP2/0, murine myeloma cell line; STR, stirred-tank bioreactor; TFR, transferin receptor; TMV, tobacco mosaic virus; uPA, urokinase type plasminogen activator.

downstream processing, highest product quality as well as lowest immunogenicity. However, large scale protein production in mammalian cells requires very high technological efforts and is very expensive and time consuming. To date, up to 5 g per liter functional IgG production have been reported which has not been achieved by any other biofermentation system and, notwithstanding this, there are still efforts to increase even further the yield of mammalian antibody production. However, up-scaling of mammalian production does not dramatically reduce the production costs.

Transgenic plants represent the most scalable production system with least production costs. Growing of plants can be easily expanded by simply using larger fields without significant higher costs, only downstream processing is connected with higher effort. Still, the
Production systems for recombinant antibodies

growing of transgenic plants is politically not accepted in several countries.

Currently, the generation of transgenic animals is very complex and difficult. However, antibody production in milk or eggs is also highly scalable. Down-stream processing of antibodies is much easier than that of transgenic plants and might not be required for topical and oral applications.

There is no 'universal' production system, that can guarantee high yields of recombinant antibody, particularly since every antibody-based molecule itself will cause its own issues in terms of expression as a result of quite different folding efficiencies of individual antibodies. In the very near future, recombinant antibodies from other production systems than mammalian cell culture will surely find their way into the therapeutic application. Agrarian mass production systems like transgenic plants or animals may even allow reductions in production cost to a level allowing the use of recombinant antibodies for consumer applications.

6. REFERENCES

Production systems for recombinant antibodies


Production systems for recombinant antibodies


Production systems for recombinant antibodies


131. M. Zahn-Zabal, M. Kobr, P. A. Girod, M. Imhof, P. Chatellard, M. de Jesus, F. Wurm and N. Mermod: Development of stable cell lines for production or regulated
Production systems for recombinant antibodies

135. H. Le Hir, A. Nott and M. J. Moore: Splicing enhanced by sodium butyrate. Expression of recombinant plasmids in mammalian cells is
87 (1), 29-42 (2001)
138. T. Omasa: Gene amplification and its application in mammalian expression of recombinant IgG
antibody fragments produced in transgenic plants against the organic herbicides atrazine and paraquat. *Biochem Biophys Acta* 1381 (2), 147-60 (1998)


166. K. Peeters, C. De Wilde and A. Depicker: Highly efficient targeting and accumulation of a F (ab) fragment within the secretory pathway and apoplast of Arabidopsis thaliana. *Eur J Biochem* 268 (15), 4251-60 (2001)


**Abbreviations:** IgG: immunoglobulin G; mAb: monoclonal antibody; Fv: fragment variable; scFv: single chain Fv; Fab: fragment antigen binding; Fc: fragment crystallizable

**Key Words:** Recombinant Antibody, Production Systems, *Escherichia coli*, Gram positive Bacteria, Yeasts, Filamentous Fungi, Insect Cells, Mammalian Cells, Transgenic Plants, Transgenic Animals, Review

**Send correspondence to:** Professor Dr. Stefan Dübel, Institute of Biochemistry and Biotechnology, Department of Biotechnology, Spielmannstr. 7, 38106 Braunschweig, Germany, Tel: 490531-391-5731, Fax: 490531-391-5763, E-mail: s.duebel@tu-bs.de

http://www.bioscience.org/current/vol13.htm