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Changes during subclone development and ageing of human antibody-producing recombinant CHO cells☆

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Abstract

Some of the problems encountered with human or human-mouse heterohybridomas, such as low growth rates and high serum requirements, have led to the increased use of recombinant cell lines for production of human antibodies. To evaluate the suitability of such alternative cell lines for the production of human antibodies we have analysed several subclones with differing specific production rates of a recombinant CHO cell line. Gene copy number and site of chromosomal integration for the light and heavy chain and the *dhfr* gene were determined by in-situ hybridisation. Specific mRNA content was analysed by Northern blot. In addition the intracellular content in light and heavy chain was measured by flow cytometry and the specific secretion rates were determined. The stability of gene expression was followed in the highest producing subclone for over a year. As previously seen in heterohybridoma cells a high expression rate of light chain is beneficial in speeding up secretion rates of whole antibody. When grown in the presence of G418 and methotrexate the amplified gene copies in the genome of recombinant CHO cells were stable over more than 100 passages. However, the expression of light chain, and with it the secretion rate, decreased with time. The low intracellular concentration of light chain resulted in accumulation of heavy chain in the endoplasmic reticulum due to retention by chaperones. The specific secretion rate decreased by 50% after 100 passages. When no G418 or methotrexate were present 75% of the gene copies were lost after 100 passages.

Keywords: Antibody production; Stability; Recombinant Chinese hamster ovary cells; Light and heavy chain

Abbreviations: CHO, Chinese hamster ovary cells; *dhfr*/DHFR, dihydrofolate reductase gene/protein; G418, neomycin; MTX, methotrexate.

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1. Introduction

The increased demand for human antibodies for therapy has led to studies concerning the optimal cell line for high yield production of such complex molecules. Human or human-mouse heterohybridomas often have a series of limitations such as low growth rates and high serum requirements. This, coupled with some general concern about the use of cells of human origin for pharmaceutical production, has led to the alternative use of recombinant cells for production purposes. Recombinant cell lines offer several advantages such as the possibility to select cell lines for transfection that grow serum-free and the option to control the antibody isotype. Apart from recombinant myeloma cells using glutamine synthetase as amplifiable marker (Bebbington et al., 1992) the dihydrofolate reductase (DHFR) amplification system using a dihydrofolate reductase negative Chinese hamster ovary (CHO) cell line is the most frequently employed. This system was established by Alt et al. (1978) and first used for foreign gene co-amplification by Urlaub and Chasin (1980), and later by Kaufman and Sharp (1982). Page and Sydenham (1991) were the first to report production of human antibodies in CHO cells. In this system the genes for the light and heavy chain are transfected on plasmids bearing the neomycin (G418) resistance gene as a selectable marker. A plasmid bearing the *dhfr* gene is co-transfected. After selection of plasmid bearing genes with G418, the gene copy number is amplified by culturing the cells under increasing concentrations of methotrexate (MTX). Only cells that produce increased amounts of DHFR will survive this treatment. When the *dhfr* genes are amplified other neighbouring genes often are co-amplified, so that after each round of amplification cells are subcloned to search for clones with increased production rates.

In recombinant CHO cells the expression rate of DHFR was shown to correlate to the used MTX concentration (Gu et al., 1992). However the expression of the co-transfected product protein varied within the populations analysed. Apart from inefficient co-transfection, it has been suggested that cells have different possibilities to acquire MTX resistance which lead to increased MTX tolerance without the desired increase in gene copy number (Gu et al., 1992; Pendse et al., 1992). This emphasises the need to screen for high producers after each amplification step. Wurm (1990) and Wurm and Petropoulos (1994) have described the integration, amplification and stability of plasmids integrated into the genome of CHO cells. In summary, it was shown that the co-amplification of *dhfr* and product genes works sufficiently well and that there is a correlation between gene copy number, mRNA levels and secretion rate of product (Kaufman et al., 1985; Gu et al., 1992; Pendse et al., 1992). However, problems did arise due to the overburdening of the post-translational pathway and resulting intracellular degradation of already translated product (Kaufman et al., 1988; Pendse et al., 1992; Schröder and Friedl, 1997). In the previously mentioned studies proteins were produced that were encoded by a single gene sequence. In the case of antibodies additional complications can be expected due to the necessary balanced transcription of two different genes and the assembly of four polypeptide chains. This assembly process involves several of the chaperones and helper proteins found in the quality control system of the endoplasmic reticulum (ER) (Bole et al., 1986) and has been identified as one of the rate limiting steps in antibody synthesis in mouse hybridoma cells (Bibila and Flickinger, 1992). Gebert and Gray (1994), describing the production of human follicle stimulating hormone consisting of two subunits, also found a significant influence of post-transcriptional processing on secretion rates. Previous work on human-mouse heterohybridoma cells has shown the importance of the intracellular concentration of light chain for efficient assembly (Borth et al., 1999).

In the present study we have analysed a recombinant CHO cell line producing the IAM2F5 antibody genetically modified from IgG_3 to IgG_1 (Kunert et al., 1998). A total of three subclones with different production rates were analysed as to their specific mRNA content, intracellular content and specific secretion rates of light and heavy chain. In addition the site of chromosomal integration and degree of amplification of the exoge-

nous genes were analysed by in-situ hybridisation. These studies were performed to compare antibody production by recombinant CHO cells to production in native human × mouse heterohybridoma cells and to give information about any additional factors that might affect the evolution of high producing subclones in a recombinant system. Furthermore we have analysed the stability of gene expression of the highest producing subclone in the presence and absence of G418 and MTX for more than 1 year.

2. Material and methods

2.1. Cell lines and culture media

CHO2F5 cells were obtained by transfection of CHO dhfr − cells (ATCC CRL9096) with the genes for the light and heavy chain of antibody IAM2F5 (changed from subtype IgG_3 to IgG_1) under the control of the cytomegalovirus (CMV) immediate early promoter together with the *dhfr* gene (Kunert et al., 1998). The gene copy number was amplified by increasing the methotrexate (MTX) concentration and subcloning. The genealogy of the subclones and the MTX concentrations used are shown in Fig. 1. The cells were cultivated in DMEM supplemented with 10% dialysed fetal calf serum, 4 mM glutamine, 0.5 mg ml^{-1} G418 and the appropriate MTX concentration. Cells were routinely passaged twice a week at ratios ranging between 1:3 and 1:6 according to the cell density, so that a fresh monolayer could form before the next passage. Cells were cultured in small T-flasks (NUNC, Denmark) at 37°C in an incubator with controlled CO_2 atmosphere (7% in air).

2.2. In-situ hybridisation

Non-confluent CHO cells were incubated for 4–6 h in the presence of 0.2 μg ml^{-1} Colchizin (Sigma). Mitotic cells were harvested by rigorous shaking and centrifuged at 190 × g. The cells were re-suspended in 5 ml of a 75-mM KCl solution and incubated for 15 min at 37°C. Then 1 ml methanol:acetic acid (3:1) was added, cells were washed twice and finally re-suspended in 1–2 ml of the fixative. Droplets of this suspension were dropped onto ethanol cleaned microscope slides from a height of 20–25 cm. After drying the slides were stored under nitrogen atmosphere at − 20°C.

To reduce background staining the slides were incubated at 37°C with 100 μg ml^{-1} RNase A (Boehringer) in 2 × SSC, rinsed three times with 2 × SSC, dehydrated with a graded series of ethanol, incubated for 10 min at 37°C with 0.02% Pepsin in 10 mM HCl, twice with PBS def. and finally with 50 mM $MgCl_2$ in PBS def. Slides were fixed with 1% formaldehyde in 50 mM $MgCl_2$ in PBS def. and dehydrated with ethanol. The probe stock solution was prepared by co-precipitation of 1 μg of labelled DNA with 50 μg salmon sperm DNA (Boehringer) and 50 μg yeast RNA (Boehringer) with 70% ethanol. The pellet was re-dissolved in 100 μl of a solution containing 50% formamide, 2 × SSC, 50 mM Na-phosphate, pH 7.0. This concentrate can be stored at − 20°C. The probes used were: (a) heavy chain (IgG_1) DNA, digoxigenin labelled, (b) 2F5-light chain DNA, biotin labelled, and (c) dhfr DNA, fluoresceine labelled. They were synthesised by PCR

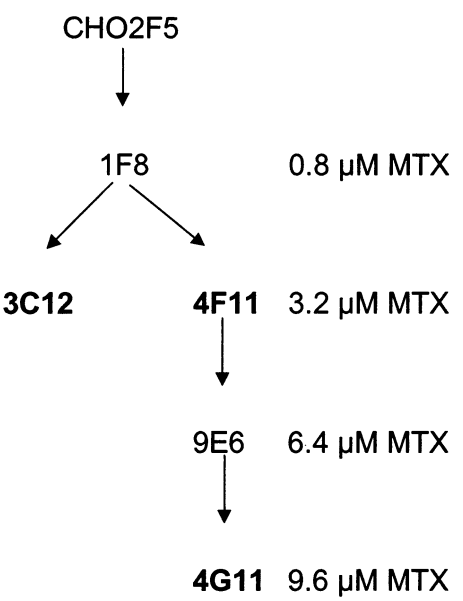

Fig. 1. Genealogy of human antibody producing CHO cell lines and respective MTX concentrations.

using pre-labelled nucleotides (Boehringer). For hybridisation 2 μl of the respective probe stock was diluted with 3 μl formamide solution and 5 μl of 20% dextransulfate in formamide solution to give a final concentration of 2 ng μl^{-1} probe DNA. A total of 10 μl of this hybridisation solution was used per microscope slide and a cover glass fixed with Fixogum (Marabu). Probe and chromosomes were denatured for 4 min at 80°C and then incubated overnight at 37°C in a humid chamber. Slides were then washed three times with 50% formamide in 2 × SSC for 5 min at 45°C, then five times for 2 min with 2 × SSC.

2.2.1. Detection of heavy chain, digoxigenin labelled

Slides were incubated for 30 min at 37°C in TNB buffer (0.1 M Tris–HCl, 0.15 M NaCl, 0.5% blocking reagent (Boehringer), pH 7.5), rinsed with TNT buffer (0.1 M Tris–HCl, 0.15 M NaCl, 0.05% Tween® 20, pH 7.5) and incubated for 30 min at 37°C with mouse-anti-digoxigenin antibody 1:250 (Boehringer) in TNB buffer. Slides were washed three times with TNT buffer and incubated for 30 min at 37°C with goat-anti-mouse-IgG antibody (Sigma), fluorescein labelled 1:64 in TNB buffer. Slides were washed three times in TNT buffer and then incubated with rabbit-anti-goat-IgG-FITC conjugate (Sigma) 1:64 in TNB buffer. Finally slides were rinsed three times for 5 min with TNT buffer and dehydrated with a graded series of ethanol. Air-dried slides were embedded in Citifluor and analysed on a confocal microscope (Biorad).

2.2.2. Detection of light chain, biotin labelled

The same procedure as described for heavy chain detection was applied using the following conjugates and dilutions: streptavidin-Cy2 (BDS) 1:100; rabbit-anti-streptavidine-biotin labelled (Rockland) 1:500; and streptavidin-Cy2 1:100.

2.2.3. Detection of DHFR, fluorescein labelled

The same procedure as described for heavy chain detection was applied using the following conjugates and dilutions: mouse-anti-fluoresceine antibody 1:250 (Boehringer); goat-anti-mouse-IgG-FITC 1:64; and rabbit-anti-goat-IgG-FITC 1:64.

2.2.4. Calculation of hybridisation index

From each preparation several metaphases were selected that had the marker chromosome M1 well spread and isolated from other chromosomes to allow image analysis. The length of the short arm of chromosome M1 and of the fluorescent region was determined using the COMOS confocal microscope software (Biorad). The hybridisation index was calculated by dividing the length of the fluorescent signal with the length of the short arm of this chromosome to normalise for varying condensation of chromosome preparations.

2.3. Preparation of total RNA and Northern blots

Total mRNA was isolated and Northern blots prepared as described in Borth et al. (1999).

2.4. Immunofluorescence and flow cytometry

Immunofluorescent analysis and flow cytometry were performed as described in Borth et al. (1999). Antibodies used were mouse-anti-human-kappa-chain antibody conjugated to Quantum Red (1:64; Sigma) and goat-anti-human-gamma-chain antibody conjugated to FITC (1:64; Sigma). Cells were analysed on a Becton Dickinson FACS Vantage equipped with a 5-W argon laser tuned to 488 nm. Cells were excited with 250-mW laser power and the fluorescence emissions measured with a 530/30 BP filter and a 660/20BP filter, respectively. Non-producing CHO (*dhfr*) cells were used as negative controls. Their fluorescence was set to lie between 0 and 10 on the logarithmic scale for both parameters. Compensation of spectral overlap between Fl1 and Fl3 was adjusted using cells stained only with goat-anti-human-gamma-chain-FITC.

2.5. ELISA

Antibody titres in culture supernatants were determined by ELISA. Plates were pre-coated with goat-anti-human-gamma chain antibody and the bound human antibodies quantified in a 2^8 dilution series with a goat-anti-human-gamma-chain antibody-HRPO conjugate. For the determination of light chain goat-anti-human-

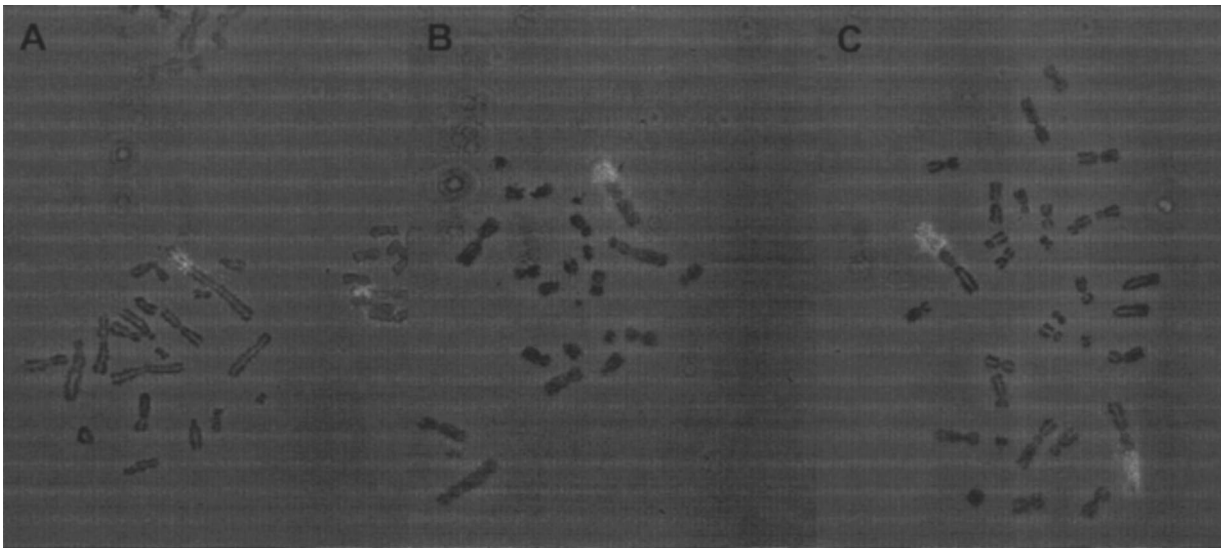

Fig. 2. In-situ hybridisation of chromosomes of subclones CHO2F5/3C12 (A), CHO2F5/4F11 (B) and CHO2F5/4G11 (C) with a probe to the heavy chain gene. The fluorescent signal binds to marker chromosome M1. This localisation of the fluorescent signal was the same for all probes (dhfr and light chain not shown).

kappa-chain antibodies were used accordingly. Reagent grade human IgG (Sigma) was used as standard. The results, though determined using either kappa or gamma chain specific antibodies, are given as whole IgG, so that the differences in molecular weight between light and heavy chain do not come into account. The specific production rate during routine culture was calculated by dividing the amount of antibody per T-flask by the number of cells and the days in culture.

3. Results

3.1. Subclone comparison

To characterise the changes that occur during selection of high producing subclones we have analysed three subclones with specific production rates ranging from 0.3 to 10 pg cell^{-1} day^{-1}. Fig. 2 shows in-situ hybridisation of a heavy chain probe to chromosome spreads of CHO2F5 subclones. The fluorescent signal is localised to the long arm of a marker chromosome M1 in all cases. The lower producing subclones have the specific signal at the same site on M1, but at shorter length. Several metaphase spreads for each probe (heavy chain, light chain, dhfr) were analysed by image analysis using Biorad COMOS software. The lengths of the short arm of M1 and of the fluorescent signal were determined. The hybridisation index given in Table 1 is defined as the length of the fluorescent signal divided by the length of the short arm of M1, to give a value of the relative increase in specific signal normalised for the varying degree of chromosome condensation during preparation. The length of the total chromosome could not be used for this purpose as it did increase with gene amplification. The in-situ hybridisation with the light chain probe and the dhfr probe bound to the same region and at comparable length to the heavy chain sequence (not shown). Results are summarised in Table 1.

Total RNA was isolated from the three subclones in mid-exponential phase and transferred onto a nylon membrane after electrophoresis. This Northern blot was analysed after hybridisation with the heavy and light chain genes using multicolour detection (Fig. 3, lanes 1–3). The bands marked H1 and H2 are heavy chain specific mRNA detected green, while the bands marked

L1 and L2 were detected in red and represent the respective light chain specific bands. The Northern blot was analysed densitometrically using the Phoretix 1D software. The volumes measured for the main band and smaller band were summed for both the heavy and the light chain. The resulting values are presented in Table 2. As the absolute densitometric values depend on the development time of the staining, the mRNA values for the light and heavy chain cannot be quantitatively compared, as they were stained separately. However, for the same probe these values are comparable as equal amounts of total cellular RNA (6 μg/lane) were used for each lane. An increase in both heavy and light chain mRNA can be seen as gene amplification and specific production rates of the subclones increase.

In Fig. 4 the intracellular light and heavy chain content of cells at the end of exponential phase are presented. The high-producing subclone CHO2F5/4G11 is the most uniform, while earlier subclones show different sub-populations with varying light and heavy chain content.

3.1.1. Stability of gene expression in the high producer CHO2F5/4G11

To study their long-term stability CHO2F5/4G11 cells were routinely passaged 1:4 for the first 20 passages, 1:5 for passages 20–90 and 1:6 for the remaining passages twice a week for 160 passages (1.5 years) with and without the addition of G418 and MTX. All three genes were stably present at the same site and length of chromosome M1 up to the 107th passage, when cells were cultivated in the presence of G418 and MTX (Table 1). Some variations are due to the limitations of the method, as only a small number of metaphases can be processed and chromosomes do not always lie straight. Surprisingly, in spite of the continuous presence of the light chain genes, the expression of light chain mRNA, as well as the intracellular content and the secretion of light

Table 1
Hybridisation index of CHO2F5 clones as calculated by dividing the length of the fluorescent sequence by the length of the short arm of chromosome M1[a]

Clone	Heavy chain	Light chain	Dhfr
3C12, 9th passage	0.44	0.59	0.42
cv	0.38	0.28	0.55
n	6	6	8
4F11, 9th passage	0.75	0.76	0.69
cv	0.17	0.23	0.26
n	8	7	5
4G11, 9th passage	1.08	1.05	0.90
cv	0.11	0.07	0.11
n	10	7	7
4G11, 53rd passage	1.02	0.93	0.82
cv	0.18	0.16	0.12
n	8	10	8
4G11, 106th passage	0.95	1.07	1.00
cv	0.13	0.11	0.10
n	6	6	11
4G11, 103rd passage without G418 or MTX	0.25	0.25	0.25
cv	0.51	0.23	0.27
n	9	9	10

[a] Chromosomes were hybridised with the sequences for light and heavy chain and for dihydrofolate reductase. A total of 5–11 mitoses that had chromosome M1 well spread and separated from other chromosomes were selected for each analysis; cv, coefficient of variation.

Table 2
Chromosome hybridisation index, mRNA content, intracellular antibody content and secretion rate of CHO2F5 clones

Clone (passage)	Hybridisation index			mRNA[a]		Intracellular content[b]		Secretion rate (pg cell^{-1} day^{-1})[c]	
	Heavy chain	Light chain	DHFR	Heavy chain	Light chain	Heavy chain	Light chain	Heavy chain	Light chain
3C12									
(1–9)	0.44	0.59	0.42	82	168	17	28	0.33	1.4
4F11									
(1–9)	0.75	0.76	0.69	135	203	49	31	1.45	3.4
4G11									
(1–9)	1.08	1.05	0.90	180	327	95	57	9.8	16.2
(25)						159	54	8.2	9.0
(37)								8.2	
(47–53)	1.02	0.93	0.82	178	177			6.6	6.6
(71)						254	24		
(85)								6.9	
(97)				143	89			4.3	4.1
(106)	0.95	1.07	1.0			390	23		
(133)								4.1	3.2
(150–153)				111	61			3.5	2.5
(160)						420	15	3.0	1.3
4G11 without MTX or G418									
(103)	0.25	0.25	0.25					0.17	0.1
(143)				47	56			0.11	0.04
(152)						86	3.9	0.12	0.03

[a] mRNA values are relative values expressed as the sum of the grey scale volume of the respective mRNA specific bands in Fig. 4
[b] Intracellular content is the mean channel number of histograms shown in Figs. 5 and 6 and also a relative unit.
[c] The secretion rate, though measured for light and heavy chain specifically, is calculated as whole antibody molecules, to correct for differences in molecular weight.

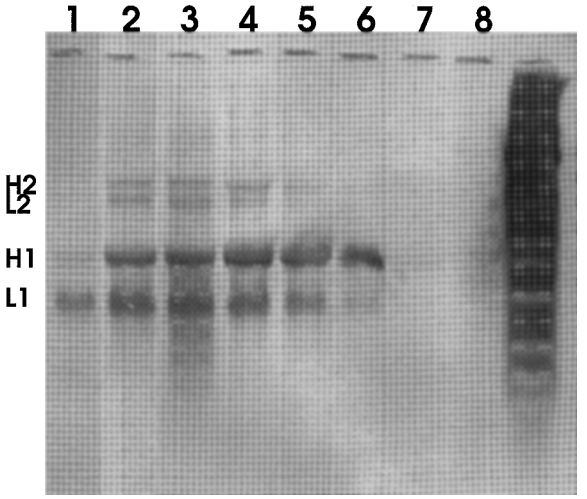

Fig. 3. Northern blot of total cellular RNA isolated from CHO2F5 clones. H1 and H2 are heavy chain specific RNA stained green, L1 and L2 are light chain specific stained red. Lane 1: CHO2F5/3C12; lane 2: CHO2F5/4F11; lane 3: CHO2F5/4G11, two passages old; lane 4: CHO2F5/4G11, 47 passages old; lane 5: CHO2F5/4G11, 97 passages old; lane 6: CHO2F5/4G11, 150 passages old; lane 7: CHO2F5/4G11, 143 passages without G418 or MTX; lane 8: CHO (DHFR −) negative control.

chain molecules did decrease significantly over this time period (Figs. 3, 5 and 6). After 97 passages 80% of the heavy chain mRNA was still expressed, while the light chain mRNA dropped to 27% of its initial value (Table 2). Flow cytometric analysis reveals the appearance of a sub-population with a lower light chain content. This sub-population accumulated heavy chain intracellularly, as the heavy chain apparently could not be secreted without light chain. It is described by region R3 in Fig. 5 and makes up 88% of the total population after 167 passages. The specific secretion rate decreased during the observed period according to the intracellular light chain content (Fig. 6). As seen from the increasing passage ratio necessary during routine culture the growth rate increased with time.

In the absence of G418 and MTX 75% of the gene copies were lost after 103 passages (Table 2). Accordingly the mRNA content of cells was reduced to 26% of the heavy chain and 17% of the light chain mRNA (Fig. 3). The intracellular light chain content decreased to the value seen in the negative controls, while some heavy chain was still found in the cells (Fig. 5E)

4. Discussion

The hybridisation index data in Table 1 show that the light and heavy chain genes were co-amplified with the *dhfr* sequence in our CHO clones. Gu et al. (1992) have found a uniform increase in DHFR expression according to the methotrexate concentration used, but an inhomogeneous expression of the co-expressed β-galactosidase sequence. This was presumably due to

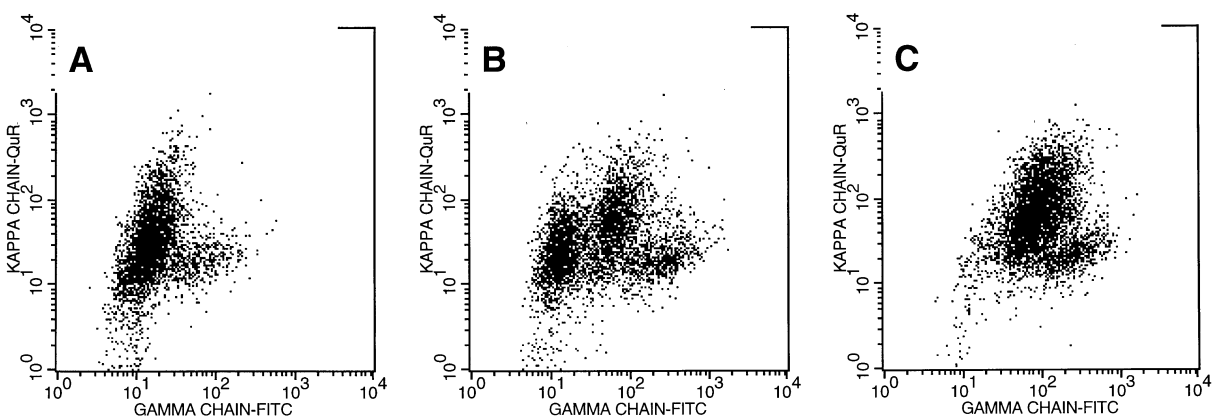

Fig. 4. Immunofluorescent staining of recombinant CHO2F5 clones. Cells were double-stained with goat-anti-gamma chain-FITC and goat-anti-kappa chain-Quantum Red. A: CHO2F5/3C12; B: CHO2F5/4F11; C: CHO2F5/4G11.

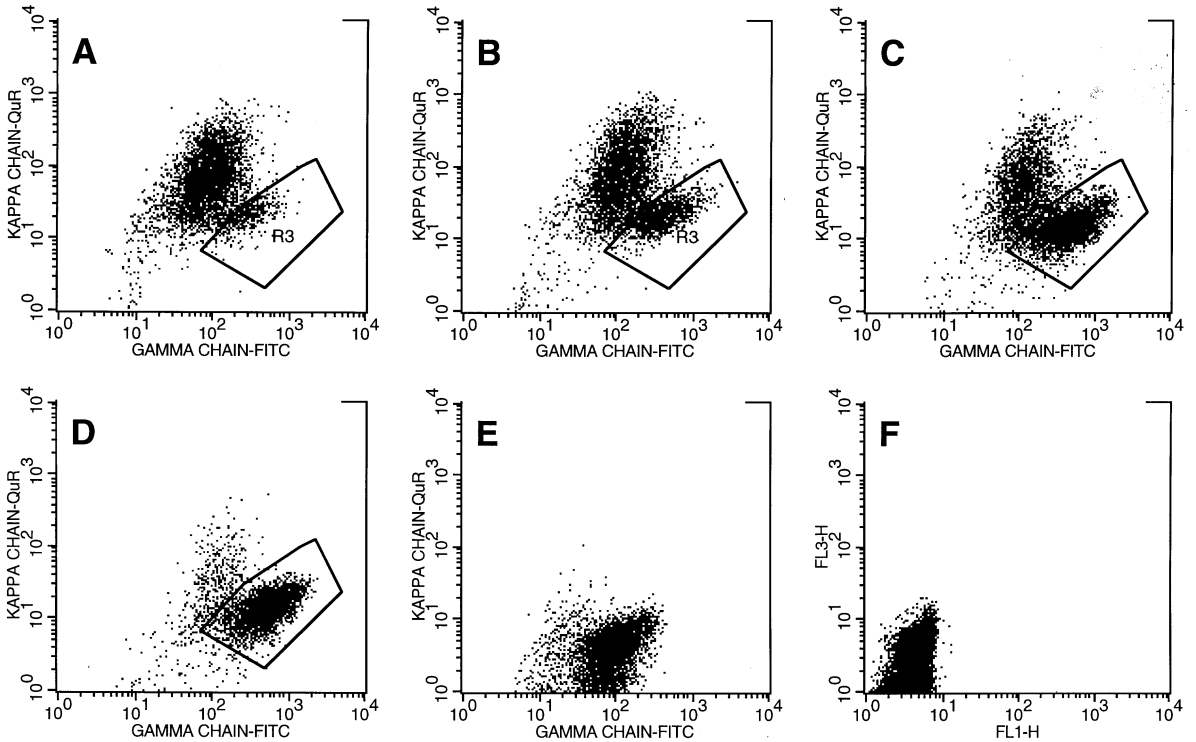

Fig. 5. Immunofluorescent staining of intracellular content of heavy and light chain molecules in CHO2F5/4G11 cells with culture age. Numbers in brackets are the percent of cells that fall within the region depicted in the graphs. A: nine passages old (9%); B: 25 passages old (25%), C: 71 passages old (60%), D: 167 passages old (88%); E: 152 passages cultured without MTX or G418; F: CHO *dhfr* −, negative control.

heterogeneous co-amplification of the genes. However, in our case apparently those cells were selected during subcloning that had co-amplified the product genes together with the DHFR sequence and thus had increased production rates. Pallavicini et al. (1990) have described a high genetic heterogeneity in the CHO clones they analysed by in-situ hybridisation, which they found to be caused by the presence of MTX. We did not find such a heterogeneity in our subclones. In none of the metaphases observed did we see a specific fluorescent signal at any other site but on chromosome M1. This may be due to the fact that the cell lines we used were derived by subcloning and were already at a higher level of amplification. During selection the heterogeneity of cell populations actually decreases. This can be seen by the reduced coefficient of variation for the hybridisation index as well as in the intracellular staining of light and heavy chain (Table 1, Fig. 4).

Due to the amplification of the gene copy number the production of specific mRNA for both the light and heavy chain increases during development of higher producing subclones (Fig. 3). The mRNA values correlate reasonably well to the hybridisation index. Pendse et al. (1992) also found a linear correlation between mRNA and specific secretion rates of hepatitis B surface antigen. They described the steps required for production of a recombinant product as transcription, translation, glycosylation and folding and secretion. However, this will hold true only for a monomeric protein, such as hepatitis B surface antigen. In the case of antibodies the additional step of chain assembly needs to be added. Lenny and Green (1991) already found that IgM heavy chain transfected into COS cells alone is not secreted. Fouser et al. (1992) also found a higher overall secretion rate when expression of kappa

chain was improved by using native intron sequences. Mocikat et al. (1993) showed that the secretion rate of heavy chain did not correlate to the heavy chain mRNA when no light chain genes were co-transfected into CHO cells. After co-transfection of light chain, however they found such a correlation between heavy chain mRNA and secretion rate. In our study, as well, the assembly step appears to be of significant importance as the overall secretion rate of both light and heavy chain correlates better to light chain mRNA concentrations than to heavy chain mRNA (Fig. 7). This would argue for a higher assembly rate at high light chain availability. Together with the accumulation of intracellular heavy chain when light chain is lacking, as seen in the aging studies discussed below, this emphasises that post-translational processing is one of the major limiting steps in cellular production (Kaufman et al., 1988; Pendse et al., 1992; Gebert and Gray, 1994; Schröder and Friedl, 1997).

Stability of gene expression in recombinant CHO cells was described by several groups with differing results. Both cases with stable expression and with rapid loss of gene expression in the absence of MTX were reported (Kaufman et al., 1985; Pallavicini et al., 1990; Gu et al., 1992). Gu et al. (1992) reported that their recombinant CHO cell line, producing β-galactosidase, lost expression of dihydrofolate reductase during 76 days when methotrexate was removed. Kaufman et al. (1985) reported that some of their clones producing tPA were stable in the absence of methotrexate for up to 36 cell doublings while some had already lost significant amounts of tPA production after 17 doublings. The cells analysed in this study still had equal amounts of all three exogenous genes after more than a 100 passages in the presence of MTX, as all the metaphases analysed did bear the in-situ hybridised sequences for all three genes at comparable length. However, even though the light and heavy chain genes were under the control of the same promoter and integrated at the same site, it is predominantly the light chain expression which is lost. At present we do not have an explanation for this phenomenon. Due to the lack of light chain the intracellular content in heavy chain actually increases with culture age, as it is retained in the ER. Although analysis of mRNA of antibody producing cell lines is usually done of both the light and heavy chain mRNA (Merten et al., 1994), flow cytometric analyses are usually done for the heavy chain only (Frame and Hu, 1990; Heath et al., 1990; Lee and Palsson, 1990; Lee et al., 1993; Kromenaker and Srienc, 1994; Park and Ryu, 1994; Couture and Heath, 1995). Our results demonstrate the importance of analysing both the heavy

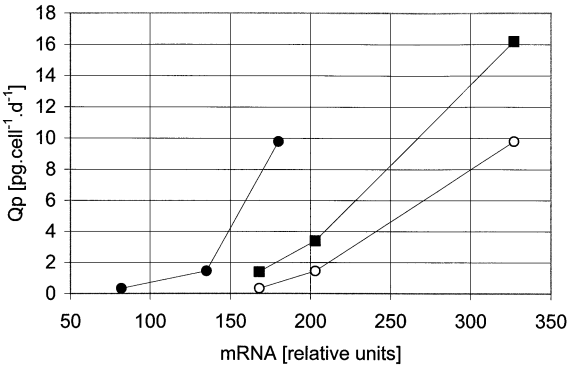

Fig. 7. Specific secretion rate in relation to mRNA content of CHO2F5 clones: ●, specific heavy chain secretion rate versus relative heavy chain mRNA content; ■, specific light chain secretion rate versus relative light chain mRNA content, ○, specific heavy chain secretion rate versus relative light chain mRNA content.

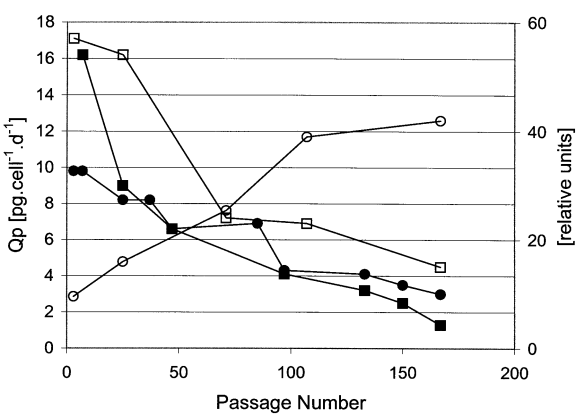

Fig. 6. Specific secretion rates and intracellular content of light and heavy chain of CHO2F5/4G11 cells with culture age. ●, heavy chain secretion rate; ■, light chain secretion rate; ○, intracellular heavy chain content (left axis); □, intracellular light chain content (left axis).

and light chain expression simultaneously when looking at antibody expression by flow cytometry. Analysis of only the heavy chain would have shown an increase in intracellular content and a simultaneous decrease in secretion rate, without giving a possible explanation for this phenomenon.

Comparison of our results with the results obtained for the heterohybridoma cell line (Borth et al., 1999) shows that in both cases assembly of light and heavy chain molecules to a complete antibody is one of the primary rate limiting factors. For the recombinant cell line gene copy number is an additional contributing factor, as was expected. In both cases loss of production rates is not due to gene loss (in the case of the heterohybridoma because no non-producing subclone was found, in the case of the recombinant CHO because genes were stably present). Rather it appears that the rate of transcription or translation was reduced. As to the cause of this reduction our results give no clue. However, as cells with lower production rates usually have higher growth rates, it is not surprising that such cells will increase in number.

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