Nucleic Acids Research, Vol. 20, No. 5 997–1003

LCR/MEL: A versatile system for high-level expression of heterologous proteins in erythroid cells

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Received January 6, 1992; Revised and Accepted February 5, 1992

ABSTRACT

We have used the human globin locus control region (LCR) to assemble an expression system capable of high-level, integration position-independent expression of heterologous genes and cDNAs in murine erythroleukaemia (MEL) cells. The cDNAs are inserted between the human β-globin promoter and the second intron of the human β-globin gene, and this expression cassette is then placed downstream of the LCR and transfected into MEL cells. The cDNAs are expressed at levels similar to those of the murine β-globin in the induced MEL cells. Heterologous genomic sequences can also be expressed at similar levels when linked to to the LCR and β-globin promoter. In addition we demonstrate that, after induction of differentiation, MEL cells are capable of secreting heterologous proteins over a prolonged time period, making this system suitable for use in continuous production systems such as hollow fibre bioreactors. The utility of the LCR/MEL cell system is demonstrated by the expression of growth hormone at high levels (>100mg/l) 7 days after induction. Since the expression levels seen do not depend upon gene amplification and are independent of the integration position of the expression cassette, it is possible to obtain clones with stable high-level expression within 3–4 weeks after transfection.

INTRODUCTION

A number of systems have been described that reproducibly direct high-level expression of recombinant proteins from prokaryotic (1), lower eukaryotic (2) and insect cells (3,4). Reproducible high-level expression from animal cells, however, tends to require considerable time, effort and often good fortune. A range of problems are encountered in trying to achieve consistently high overall expression levels. These problems are further compounded when large quantities of recombinant protein are required since very large-scale fermentation of higher eukaryotic cells is not, as yet, possible.

The main factors contributing to the problems encountered in mammalian expression systems are the large variability of expression levels due to so-called 'position effects', in which the DNA surrounding the site of insertion of transfected DNA has a major effect on expression levels from transfected genes (5) and the use of cell lines (eg CHO) which do not normally express one major protein product. In addition these cells should secrete heterologous protein products efficiently and be grown cheaply.

Compensating for position effects has been a major challenge for high-level expression in animal cells and has been approached in the past by development of amplification protocols based on a number of selectable marker genes (6), by development of homologous recombination or viral infection protocols designed to target the incoming DNA to more 'active' regions of the animal cell genome (7), or by using vectors which contain multiple copies of the transcription unit (8). In general, these methods involve considerable manipulation to compensate for position effects and are often only of limited value. We have overcome the problem of position effects by using the globin locus control region (LCR) sequences (9) as an integral part of vectors designed to direct high-level expression of heterologous proteins in erythroid cells. The globin LCR has been shown to confer integration site independent expression on stably transfected genes which are linked in *cis* (10, 11). The LCR is defined by four erythroid specific DNase sensitive sites *in vivo* (9,12,13). Experiments *in vitro* have shown that they are regions containing a high density of non-histone nuclear protein binding sites, including those for the erythroid-specific transcription factors GATA1 and NF-E2 (14,15,16).

Since the globin LCR is erythroid specific, we investigated murine erythroleukaemia (MEL) cells (17) as an expression system. These cells can be induced to undergo terminal differentiation *in vitro* which is accompanied by high levels of expression of the globin proteins (up to 25% of total cellular protein; ref 18). Moreover, MEL cells can be adapted to low serum requirements resulting in a substantial reduction in the cost of large cultures. However the secretory capacity of these cells was questionable because of the complete change in the cell membrane upon erythroid differentiation.

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We describe here a system to rapidly express and secrete a range of biologically active proteins at high levels in MEL cells. In addition, we have increased the overall expression levels using the human β-globin promoter and parts of the β-globin gene which provide mRNA processing and maturation signals and give stability to the final mRNA in the induced cells. The system does not rely upon gene amplification or the use of high copy-numbers for expression. The LCR/MEL system therefore enables rapid and easy identification of high expressing clones, and represents a genetically stable system for expression of a range of heterologous protein types.

MATERIALS AND METHODS

Construction of the cDNA 'expression cassette' vectors

Three 'expression cassette' (EC) vectors were used in this study (Figure 1). The first EC vector, pEC1, has only the β-globin promoter (19) whereas the other two vectors, pEC2 and pEC3, contain the 3′ half of the human β-globin gene, including the second intron, untranslated region and polyadenylation signals. Vector pEC3 differs from pEC2 only in that it has a longer stretch of 3′ flanking sequences.

Construction of the hGH cDNA expression vectors

The hGH cDNA was isolated as a *Hin*dIII to *Sma*I fragment from plasmid pGH, which contains the hGH cDNA (a gift of Dr L. Hall, Bristol University) preceded by a short eukaryotic translation initiator sequence (20) and a *Hin*dIII site for cloning into the EC vectors (AAGCTTGCCACCATG). The fragment was cloned into the multiple cloning site (MCS) of pEC2 and pEC3 to give plasmids pEC2/GHc and pEC3/GHc respectively. The final expression vectors were generated by subcloning the expression cassettes of the relevant plasmids (as *Cla*I to *Asp*718 fragments) into the human globin LCR microlocus plasmid pGSE1417 (ref 10), to generate the plasmids pLCR/EC2/GHc and pLCR/EC3/GHc. These plasmids were used for transfection of MEL cells as described.

Construction of the hGH genomic expression vector

The hGH gene was isolated as a *Hin*dIII-*Eco*RI fragment containing the entire coding and polyadenylation regions from the plasmid pφGH (ref 21), and cloned downstream of the human β-globin promoter in the pEC1. The expression cassette was then subcloned downstream of the LCR in plasmid pGSE1417, as described for the hGH cDNA above and the resulting plasmid, pLCR/EC1/GHg, was transfected into MEL cells as described.

Tissue culture and cell transfections

MEL-C88 cells (22) were cultured in DMEM supplemented with 10% foetal calf serum and 2mM glutamine. MEL-11A21 (a generous gift from M. Oishi, Institute of Applied Microbiology, University of Tokyo), a cell line adapted for growth and induction at low serum concentrations, were cultured in αMEM supplemented with 1% foetal calf serum and 2mM glutamine. Prior to transfection, 100μg of the appropriate expression construct was cut at the unique *Pvu*I site, in the ampicillin resistance gene of the plasmid backbone. Transfection into the cell lines MEL-C88 and MEL-11A21 was performed by either electroporation (23), calcium phosphate precipitation (24), or by lipofection (25) as described in the text.

Directly after transfection, cells were diluted in culture medium to 10^4 and 10^5 cells per ml and 1ml aliquots transferred to each well of a 24 well plate. G418 was added to a concentration of 1mg/ml 24 hr after the transfection to select for stable transfectants. Individual clones were picked or pooled to generate populations seven to ten days after the addition of selective medium.

For expression studies, cells were maintained in exponential growth for a period of at least four days and then DMSO was added to a final concentration of 2% (v/v) to induce differentiation, and hence expression. Samples were taken at various times post induction for mRNA and protein analyses.

RNA analysis

Approximately 4×10^7 cells were washed twice with phosphate buffered saline then resuspended in 3ml of ice cold NTE (0.1M NaCl,10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0) containing 2% Nonidet P-40 (BDH Chemicals Ltd., Poole, England). The cell suspensions were immediately added to an equal volume of phenol/chloroform (1:1 v/v), mixed thoroughly and centrifuged at 9,000 rpm for 10 min. The aqueous layer was transferred to a fresh tube containing a further 3ml of phenol/chloroform, and the samples mixed and centrifuged as above. The final aqueous layer was made 0.3M with sodium acetate pH 5.5 and two volumes of absolute alcohol were added. After precipitation at −70°C the RNA was centrifuged, washed with 70% ethanol and dissolved in distilled water.

RNA was separated by electrophoresis in agarose gels containing 2.2M formaldehyde (26), then transferred to a nylon membrane (Hybond-N, Amersham) in 20×SSC. After transfer, RNA was covalently cross-linked to the membrane by UV

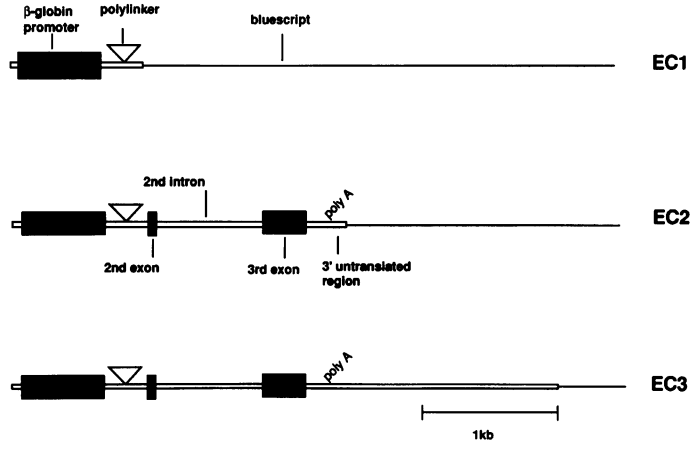


Figure 1. MEL cell expression cassette (EC) vectors. pEC1 is based on pBluescript, with a new polylinker inserted between the *Cla* I and *Sst* I sites. The new polylinker comprises of *Cla* I, *Hin*d III, *Eco*R I, *Sma* I, *Xba* I, *Not* I, *Xho* I and *Bam*H I, *Pst* I, *Asp* 718, *Cla* I and *Sst* I The human β-globin promoter (−420 to +32), was cloned between the 5 prime *Cla* I and *Hin*d III sites of the new polylinker. pEC2 is a modified version of pEC1 containing, in addition, a region of the β-globin gene (2037−3209) which encodes part of the β-globin 2nd exon, the 2nd intron, 3rd exon, poly-A recognition site and approximately 600bp of 3′ flanking DNA, cloned between the *Bam*H I and *Pst* I sites of the polylinker. pEC3 is identical to pEC2 other than it contains a longer region of β-globin 3′ flanking DNA than pEC2 (i.e. up to the natural *Xba* I sight at base 4845), and different polylinker cloning sites between the *Hin*d III and *Bam*H I sites of the pEC1 polylinker.; *Hin*d III, *Eco*R I,*Xho* I, *Bgl* II, *Sal* I , *Not* I and *Bam*H I.

irradiation and prehybridized and hybridized as described (27) using ^{32} P labelled probes.

Growth hormone assays

Samples of cell suspensions were centrifuged at 2,000 rpm for 5 minutes and the supernatant was transferred to a fresh tube to be used directly or diluted prior to assay. Cell pellets were lysed by being resuspended in water and sonicated on ice for 10 seconds. HGH assays were carried out as described by Selden (21).

RESULTS

Expression of human growth hormone cDNA

The accumulation of high levels of β-globin mRNA in MEL cells has been shown to require all four DNase hypersensitive sites of the globin LCR (28). In addition, the minimal promoter elements within 100bp upstream of the transcriptional start (19), and the second large intron (28) must also be present as part of the β-globin gene. Based on these observations we constructed the universal expression cassettes illustrated in Fig. 1. With vector pEC1, the genomic sequences of choice are linked directly to the β-globin promoter. The vectors pEC2 and pEC3, are designed to receive a cDNA copy of the desired gene between the β-globin promoter and the second intron and these vectors also include the β-globin polyadenylation signals. This later expression cassette is used when the natural gene is unavailable or too large to incorporate into the LCR expression vector. Furthermore, the inclusion of the 3′ β-globin sequences as part of the resulting hybrid mRNA, may confer stability in erythroid cells.

Human growth hormone (hGH) was selected as a model protein for expression due to the availability of a simple quantitative assay for this protein (21). The human β-globin promoter was used to drive the expression of the hGH gene (see next section) or the hGH cDNA linked to the human β-globin splice and polyadenylation sequences.

We considered that the position of the cDNA sequences in the final chimaeric (expressed) mRNA would be important for overall expression levels. In order to test this, the hGH cDNA was inserted at various positions within the human β-globin gene. Although hGH expression was easily detectable with all constructs, the mRNA and protein levels were highest with the cDNA inserted as in pEC3/GHc (data not shown).

The expression construct pLCR/EC3/GHc was introduced into MEL-C88 cells by electroporation. Pooled populations and single cell clones were established and cultured as described in Materials and Methods. Following induction, there was a large (>20 fold) increase in the production of hGH mRNA and protein, to levels roughly equivalent to those of the endogenous murine β-major globin. Figure 2 shows the induced level of the chimaeric hGH/β-globin mRNA from the pEC3 expression cassette (panel a), and the corresponding levels of hGH detectable in the supernatants of transfected cells after 4 days of induction (panel c). Clone b did not induce as well as the other samples and this is shown by the reduced level of globin mRNA. Less than 2% of the total hGH activity was detectable in lysed cell extracts (data not shown), indicating that the recombinant hGH produced in MEL-C88 cells was being secreted efficiently.

Four days post induction was chosen as an end-point for measurement of mRNA and protein levels since the induction of differentiation by DMSO is essentially complete after two days (29). We have also observed that the accumulation of heterologous mRNAs and proteins in cells cultured in flasks peaks at 4 days post induction. The average accumulation of hGH at this timepoint, from populations or single cell clones (transfected by electroporation with pLCR/EC3/GHc), was found to be 15mg/l, although individual clones expressed as much as 33mg/l (Fig. 2c). This clonal variation is due to differences in transfected gene copy number (see Fig. 2 legend and Discussion).

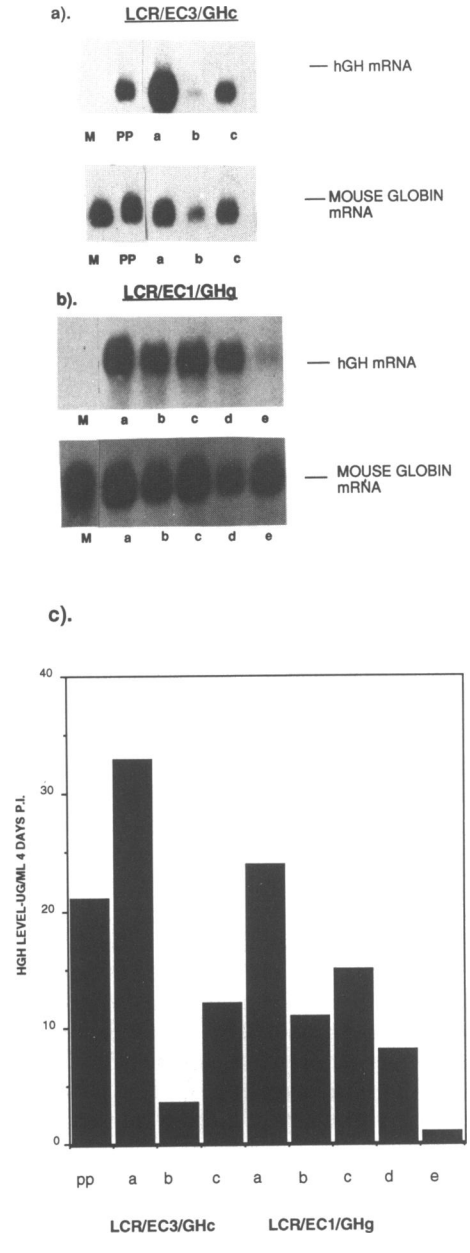


Figure 2. a) Northern blot analysis of RNA from induced MEL-C88 cells (M), MEL-C88 cells transfected with pLCR/EC3/GHc pooled populations (PP) and 3 single cell clones; a(4), b(1), c(2). The numbers in parentheses refer to the transfected gene copy number. Clone b did not induce as well as the other samples and this is shown by the reduced level of globin mRNA. **b)** Northern blot analysis of RNA from induced MEL-C88 cells (M), MEL-C88 cells transfected with pLCR/EC1/GHg single cell clones (a,b,c,d and e). **c)** Results of hGH assay performed on supernatants of induced MEL-C88 cells (M), MEL-C88 cells transfected with pLCR/EC3/GHc pooled populations (PP) and single cell clones (a,b and c) and MEL-C88 cells transfected with pLCR/EC1/GHg single cell clones (a,b,c,d and e).

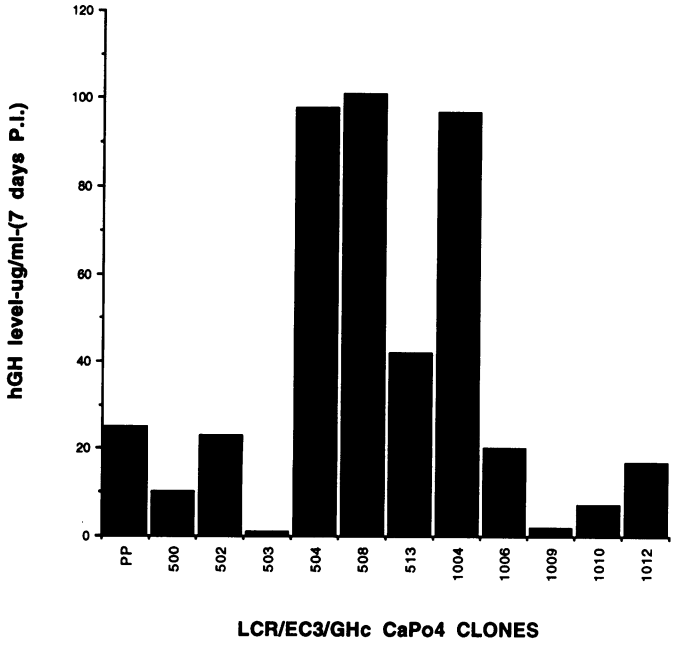


Figure 3. Results of hGH assay performed on supernatants from induced MEL-C88 cells transfected by calcium phosphate precipitation with pLCR/EC3/GHc pooled populations (PP) and single cell clones (500, 502, 503, 504, 508, 513, 1004, 1006, 1009, 1010 and 1012).

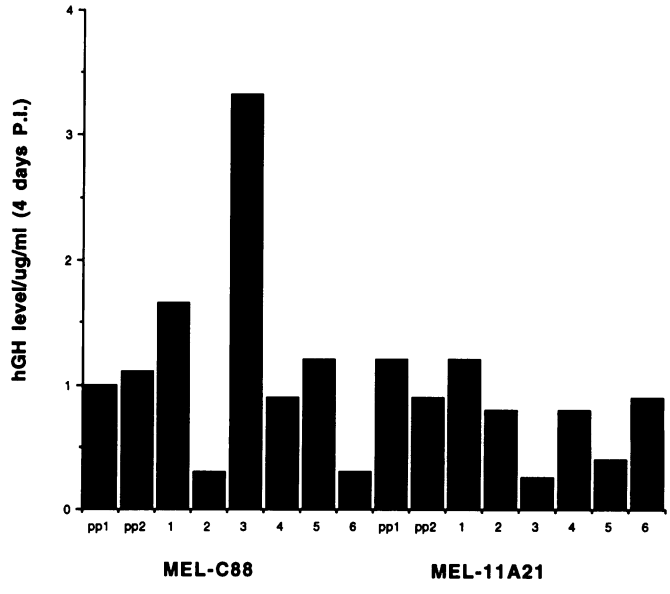


Figure 4. Results of hGH assay performed on supernatants from induced MEL-C88 cells and MEL-11A21 cells transfected by with pLCR/EC1/GHc pooled populations (PP1 and PP2) and single cell clones (1,2,3,4,5 and 6).

Expression of the human growth hormone gene

The vector pEC3 was designed to allow the efficient expression of a range of cDNAs in the MEL cell system. It is also desirable, in certain cases, to express genomic sequences. Again we used hGH as a test protein. The hGH genomic sequences (including the native polyadenylation sequences) from plasmid pϕGH (21), were placed downstream of the human β-globin promoter in plasmid pEC1 and linked to the LCR to generate pLCR/EC1/GHg. The final expression vector was transfected into MEL-C88 cells as described and single cell clones and pooled populations were isolated.

The RNA analysis of a number of transfectants is shown in Figure 2b. As with the hGH cDNA in the pEC3 vector, the level of the hGH mRNA from the genomic construct is as high as that of the murine β-major globin. The overall expression levels of hGH protein are similar in the case of the hGH cDNA in vector pEC3 and the hGH gene in vector pEC1 (Fig. 2c). In both cases, levels of 15mg/l accumulated hGH were attained in pooled populations.

Selection of high-expressing clones

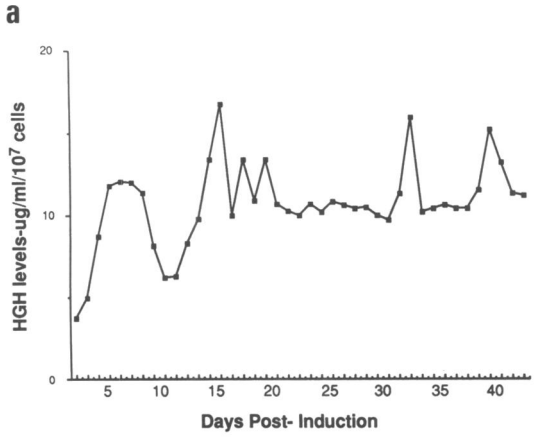
Traditional mammalian expression experiments rely heavily on clone-selection as a means of compensating for position effects and sometimes many clones must be screened to find one with reasonable levels of expression for use directly or as the basis of amplification experiments. In the LCR/MEL cell system, all stable clones which contain a non-rearranged expression unit after transfection (the majority) express at high level. Since the LCR sequences drive expression in a position independent, gene copy-number dependent manner, the overall expression levels can be increased by using transfection methods which result in increased numbers of copies in the original transfected clones.

We constructed vectors to give high level mRNA expression from a single integrated copy to facilitate the rapid analysis of recombinant clones. In order to generate cells expressing higher levels of hGH we transfected the pLCR/EC3/GHc vector into MEL-C88 cells by calcium phosphate precipitation, which we find gives higher average copy numbers than electroporation, and analysed the expression levels of the resultant clones. The results are shown in Figure 3. Whilst it can be seen that the level of expression generated by this method is variable, a screen through twelve clones was sufficient to identify three clones which accumulate hGH at approximately 100mg/L after induction.

Expression of hGH in MEL-11A21 cells

The MEL-11A21 cell line is capable of normal growth and induction in medium supplemented with only 1% foetal calf serum. This is important since it represents a 90% reduction in the added foetal calf serum, which not only makes processing of the recombinant protein much easier but also represents a significant reduction in the cost of protein production using the LCR/MEL cell system. MEL-11A21 cells were transfected by electroporation with the construct pLCR/EC2/GHc, and subsequent induction of transfected clones and populations resulted in hGH expression at levels similar to those seen with the same construct in MEL-C88 cells grown and induced in the presence of 10% foetal calf serum (Figure 4).

The doubling time of MEL-11A21 cells grown in medium supplemented with 1% foetal calf serum is slightly longer than that of MEL-C88 cells grown in medium supplemented with 10% foetal calf serum, and therefore the density of the MEL-11A21 cells at 4 days post induction is, on average, $2-3\times10^6$ cells/ml compared with $5-6\times10^6$ cells/ml for MEL-C88 cells. This probably accounts for the slightly lower levels of hGH seen in the MEL-11A21 cells. Indeed, when corrected for cell number, the hGH expression levels are higher in the MEL-11A21 cells than in the MEL-C88 cells.



b

sample	cell number	hGH level (ug/ml)
clone 2b 4 day uninduced	5.5 x 10^6/ml	6
2b 4 day P.I.	4.6 x 10^6/ml	33
2b long term (2bL) induced log phase.	2-5 x 10^5/ml (24 hrs growth)	1.6
clone 2bL 4 day uninduced	6.1x 10^6/ml	16
clone 2bL 4 days P.I.	5.5 x 10^6/ml	51

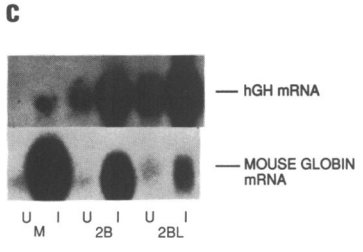


Figure 5. a) Results of hGH assay performed on supernatants of MEL-C88 cells transfected with pLCR/EC3/GHc (single cell clone 2b), induced on day 0, then the culture medium replaced every 24 hrs (maintaining DMSO at 2%). **b)** Results of hGH assay performed on supernatants of clone 2b, which had been induced long-term (42 days) then transferred to medium lacking DMSO, and cultured in log phase growth (lanes 1,2,3,4 and 5).The samples were then left for 4 days either uninduced, in normal growth medium (U), or induced in the presence of 2% DMSO(I). **c)** Northern blot analysis of RNA from MEL-C88 cells, uninduced (MU) and induced (MI), MEL-C88 cells transfected with pLCR/EC3/GHc (single cell clone 2b) uninduced (2bU) and induced (2bI), and from clone 2b after long term induction (42 days) followed by 5 days log-phase growth then left for 4 days uninduced (2bLU), and induced (2bLI).

Long term expression and secretion

To investigate further the ability of MEL-C88 cells to secrete heterologous proteins, cells transfected with the pLCR/EC3/GHc construct were induced to differentiate and the medium then replaced every 24 hours, (maintaining DMSO at 2%). Prior to the media being replaced 1ml samples were taken for hGH analysis. The results (see Figure 5a) show that the induced cells continued to maintain secretion levels similar to those seen 4 days post induction, over a period of 40 days. A further long-term experiment demonstrated that transfected cells were capable of hGH secretion for greater than 80 days after induction (data not shown).

At the completion of the 40 day induction experiment, the same cells were cultured in log-phase growth in the absence of DMSO and 1ml of supernatant was removed every 24 hours for hGH assay. The cells salvaged from this experiment had a doubling rate of approximately 16 hours (compared to 10 hours for the original clone prior to long term induction) and expressed hGH at $1-2\mu g/ml/5\times10^5$ cells/24 hr before induction (see Figure 5b/c). The log phase cells were then induced and supernatants were assayed for hGH activity after four days. RNA was extracted from the cells and analysed as before.

The hGH levels accumulated in the supernatants was 30ug/ml i.e. 3 fold higher than the level seen in an identical culture grown in the absence of DMSO. RNA analysis (see Figure 5b) shows that the uninduced level of hGH RNA is higher than that seen in the same clone prior to long term induction but still lower than the induced level (either prior to or after long term induction).

Expression of a range of cDNAs

We have expressed a variety of cDNAs in MEL-C88 cells. In all cases the expression levels achieved have compared favourably with expression levels seen in other animal cell expression systems. We have expressed human granulocyte colony stimulating factor (GCSF) at the level of 0.4mg/l in the supernatants of transfected MEL-C88 cells (see Figure 6). In addition, we have achieved levels of >2mg/l using the human synovial phospholipase-A2 cDNA in MEL-C88 cells (C. Gooding *et al.*, manuscript in preparation) which is 100-fold better than the highest previously reported expression levels for this enzyme in amplified CHO cells (30).

DISCUSSION

We describe here a versatile system for high level expression of a range of heterologous proteins using vectors containing the human globin LCR and regions of the human β-globin gene. It has previously been shown that the human globin LCR is capable of directing expression of heterologous genes in MEL cells (11,19) and transgenic mice (Harvey and Grosveld, unpublished). Furthermore, the human β-globin promoter has been shown to direct high-level expression of the human β-globin and heterologous genes in MEL cells when placed adjacent to the

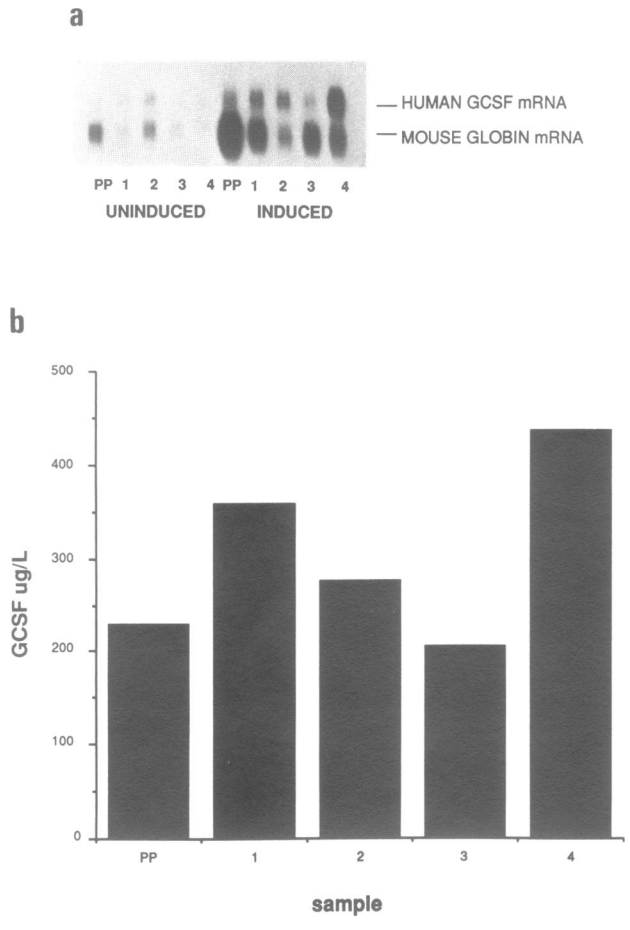


Figure 6. a) Northern blot analysis of RNA from MEL-C88 cells transfected with pLCR/EC2/GCSF pooled population (PP) and single cell clones (1, 2, 3 and 4). **b)** Human GCSF protein levels rom MEL-C88 cells transfected with pLCR/EC2/GCSF pooled population (PP) and single cell clones (1, 2, 3 and 4).

globin LCR sequences (28,19). We have developed this system for high level expression of heterologous proteins from cDNAs and genomic sequences. Since it has been shown that β-globin mRNA is highly stable in induced MEL cells (31), we retained much of the β-globin sequences in the final cDNA expression vectors.

A range of expression constructs, in which the heterologous cDNA was placed at various positions within the β-globin gene, were made. In the simplest construct, we retained the entire β-globin gene and inserted the cDNA fused at the translational initiation site. This resulted in very low steady-state levels of hybrid mRNA even when linked to the LCR sequences. We believe that these constructs mimic the situation seen with some human β°-thalassaemias, where point mutations which generate translational termination signals in the first exon of the human β-globin gene, lead to drastically reduced levels of steady state mRNA (32). One potential explanation for this is that these mRNAs contain an unusually long 3′ untranslated region which is perhaps more susceptible to degradation.

Further constructs were therefore designed to reduce the length of the 3′ untranslated sequences in the chimaeric mRNAs in the hope of increasing the overall stability of the message. It has been shown that the β-globin large (2nd) intron is essential for high level expression in this system (28) and we therefore included this in the subsequent cDNA expression vectors. In the constructs pEC2 and pEC3, all of the human β-globin gene first exon and the majority of the 2nd exon were replaced by the heterologous cDNA reducing the length of the 3′ untranslated region without removing other regions which may be necessary for the stability of the hybrid transcript.

The steady state level of the hybrid (hGH/β-globin) mRNA in cells transfected with pLCR/EC2/GHc and pLCR/EC3/GHc is similar to that of the native mouse globin mRNA. Comparison of the levels of hGH protein with those produced by these vectors indicates that the pEC3 vector produces significantly more hGH. This may be due to the longer 3′ sequences (downstream of the poly-A addition sequence) providing a better polyadenylation signal in the pEC3 vector resulting in slightly increased steady-state mRNA levels and increased translation (33,34).

We have used a range of techniques to generate transfectants which express hGH at levels in excess of those of mouse globin. In those clones which express the highest level of heterologous mRNA, a reduced level of mouse globin mRNA is observed by Northern blotting, suggesting that a factor involved in transcription or in mRNA stability is limiting and that the high steady state levels of the hybrid mRNA are maintained 'at the expense' of the native mouse globin mRNA (Fig. 5).

We have also shown that MEL-C88 cells are capable of secreting recombinant proteins for extended periods. This was surprising since erythroid differentiation involves a process of extreme diversification and cells may enucleate and rapidly degrade mRNAs other than those of the globins and related proteins. It is therefore reasonable to assume that the 'machinery' necessary for secretion of proteins would also break down. Our results show that induced MEL-C88 cells are capable of secreting proteins for a period of more than 80 days after the differentiation of the culture has begun. It is unlikely that heterologous mRNAs, synthesized in the early stages of differentiation, would remain stable and available for translation for long periods so we assume that de novo synthesis of mRNA is taking place many days after the culture is induced to differentiate. After long term induction, it is likely that a partially induced subpopulation which can still grow in the presence of DMSO (see ref 35 and references therein), accounts for the majority of the secreting cells in the culture. The cells can be removed from the inducing media following long term induction and shown to reinduce when subjected to inducing agents at a later stage.

The LCR/MEL cell expression system described here has significant advantages over previously described mammalian expression systems. The MEL-C88 cells have a very short doubling time (10 to 16 hours) and are not adherent, making them straightforward to culture. In addition, the cells are easy to transfect by a range of standard techniques and the resulting clones (even high copy number clones) are stable. High-level expression is achieved from low copy numbers of the transfected DNA and it is possible to go from transfection to analysis of secreted protein from a recombinant clone in around three weeks. Furthermore, an extra dimension of flexibility is offered by the fact that the level of expression per gene copy can be varied by using either a mutant LCR (28) or β-globin promoter (19). Such control would be invaluable in cases where expression of a potentially toxic product is desired. The system can also serve as a test bed for studying the control of mRNA stability and translation.

The LCR/MEL cell system has all of the utility and advantages of the insect cell/baculovirus system without the disadvantages of needing to prepare virus or control infection and lysis. In addition MEL cells are robust and amenable to growth in large scale fermentors. We imagine that the LCR/MEL cell system will find utility in all areas currently using baculovirus expression systems or mammalian gene amplification systems. The overall speed and productivity of this system is such that, for the first time, it is possible to undertake *in vitro* mutagenesis and structure/function studies in animal cells and expect results in a reasonable timescale.

ACKNOWLEDGEMENTS

We would like to acknowledge the help of Jennifer Scott with some of the experiments described here and thank colleagues at ICI Pharmaceuticals for support during the development of the system.

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