Epi-CHO, an Episomal Expression System for Recombinant Protein Production in CHO Cells

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Abstract: This study describes the development of a transient expression system for CHO cells based on autonomous replication and retention of transfected plasmid DNA. A transient expression system that allows extrachromosomal amplification of plasmids permits more plasmid copies to persist in the transfected cell throughout the production phase leading to a significant increase in transgene expression. The expression system, named *Epi*-CHO comprises (1) a CHO-K1 cell line stably transfected with the Polyomavirus (Py) large T (LT) antigen gene (*PyLT*) and (2) a DNA expression vector, pPyEBV encoding the Py origin (PyOri) for autonomous plasmid amplification and encoding Epstein-Barr Virus (EBV) nuclear antigen-1 (EBNA-1) and *Ori*P for plasmid retention. The CHO-K1 cell line expressing PyLT, named CHO-T was adapted to suspension growth in serum-free media to facilitate large-scale transient transfection and recombinant gene expression. Enhanced green fluorescent protein (EGFP) and human growth hormone (hGH) were used as reporter proteins to demonstrate transgene expression and productivity. Transfection of suspension-growing CHO-T cells with the vector pPyEBV encoding hGH resulted in a final concentration of 75 mg L^{-1} of hGH in culture supernatants 11 days following transfection. © 2005 Wiley Periodicals, Inc.
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INTRODUCTION

There is a small repertoire of mammalian cell lines used for the manufacture of biopharmaceuticals with heavy reliance on stably transfected CHO cells. A number of factors contribute to successful commercial production of biopharmaceuticals in CHO cells such as growth in suspension and serum-free media. Recently, the high throughput screening in the drug discovery process has intensified the need for a rapid technique to produce milligram amounts of recombinant protein. To accomplish this need, transient gene expression technology has attracted much interest over the traditional stable expression technology. As an alternative to stable cell line generation, large-scale transient expression of recombinant proteins in mammalian cells have been used to generate sufficient amounts of recombinant protein for early product analysis and high throughput screenings (Durocher et al., 2002; Girard et al., 2002; Meissner et al., 2001). Such large-scale transient expression employs the transformed human embryonic kidney (HEK293) cells engineered to express the Epstein-Barr Virus (EBV) nuclear antigen-1 (EBNA-1). The combination of *Ori*P and EBNA-1 contributes to elevated levels of transgene expression following transient transfection (Pham et al., 2003).

Transient protein production levels of 18–20 mg L^{-1} have been reported in HEK293-EBNA-1 cells using an *Ori*P-bearing vector (Durocher et al., 2002; Pham et al., 2003). In contrast, the yield of transient recombinant protein produced in CHO is significantly lower, 5–8 mg L^{-1} (Derouazi et al., 2004; Schlaeger and Christensen, 1999; Schlaeger et al., 2003; Tait et al., 2004). Since CHO is the most commonly used cell line in commercial production of biopharmaceutical products (Andersen and Krummen, 2002; Chu and Robinson, 2001; Werner et al., 1998) the availability of a high-level transient expression system for CHO would be useful since this would allow early product development to be done in the same parental cell line as in a final bioprocess using a stably derived recombinant clone. To improve the level of transient protein expression in CHO, this study focused on the development of an efficient transient expression system based on episomal replication and plasmid retention in these cells. To establish plasmid episomal replication in CHO cells the Py virus based replication system was chosen due to its simple requirements: only two exogenous elements namely Py origin of replication (PyOri) and PyLT antigen are required to launch episomal replication in CHO cells (Heffernan and Dennis, 1990). A combination of Py elements for plasmid replication and EBV elements (EBNA-1 and *Ori*P) for plasmid maintenance and segregation (Lupton and Levine, 1985; Polvino-Bodnar and

Schaffer, 1992; Yates et al., 1984) was used to develop the transient expression system in CHO cells. The level of transient recombinant protein expression was evaluated using EGFP to measure protein expression on a per cell basis and hGH to measure the overall transient protein yield. The CHO transient expression system named *Epi*-CHO consists of the expression vector pPyEBV capable of episomal replication in the suspension-growing, serum-free adapted cell line CHO-T (CHO cells stably expressing Py LT).

MATERIALS AND METHODS

Cells and Media

CHO-K1 (ATCC CCL 61) cells were cultured in DMEM/F12 (JRH Biosciences Lenexa, KS) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA). CHO-K1 cells were adapted to growth in suspension and cultured in serum-free Ex-Cell 302 media (JRH Biosciences, Lenexa, KS). These cells are referred to as CHO in this study.

Development of CHO-T Cell Line

CHO-K1 cells were transfected with an expression vector encoding PyLT (ATCC). Cells were cultured in the presence of the antibiotic G418 (400 $\mu g\,mL^{-1}$, Invitrogen, Carlsbad) for 2 weeks. The neomycin gene encoded on the vector confers resistance to G418 and is an indication of stable integration of plasmid DNA into the host cell chromosomes. Stable clones were isolated by limiting dilution at 0.5 cells per well and verified for their ability to support DNA replication of the PyOri-containing plasmid and expression of PyLT was confirmed by immunofluorescence staining (data not shown). One CHO-T clone designated clone P1-C11 was adapted to growth in single cell suspension in protein-free Ex-Cell302 (JRH Biosciences).

Transfection

Cationic lipid-mediated transfections were performed using the reagent LipofectAMINE 2000 (Invitrogen, Rockville, MD). Briefly, cells in mid-log phase were washed once with phosphate buffered saline (PBS) and resuspended at 10^6 cells mL^{-1} in Opti-MEM media (Life Technologies, Rockville, MD). Lipofectamine 2000® (10 μL) and DNA (5 μg) were mixed in Opti-MEM media (400 μL) before being added to cells (1.6 mL) in a 6-well plate. Six hours after addition of the Lipofectamine/DNA complex, an equal volume of serum-free media was added. Transfections were scaled proportionately as required.

Isolation of Extrachromosomal DNA and Replication Assay

Extrachromosomal DNA was isolated using a modified Hirt extraction method (Hirt, 1967) at 24, 48, and 72 h post transfection. Hirt-extracted DNA was digested with *Spe*I and *Dpn*I (New England Biolabs, Beverly, MA) and fragments were separated on a 0.8% agarose gel for 1 h. Southern hybridization was carried out according to the method of Ausubel et al. (1997) and probed using ^{32}P-labelled EGFP (BD Bioscience Clontech, Palo Alto, CA). The membrane was exposed to autoradiography film and DNA quantified using a Biorad-525 densitometer.

ELISA

Human growth hormone was detected using an hGH ELISA detection kit (Roche Diagnostics GmbH, Mannheim, Germany).

Vectors

pBasic: The vector pCEP-4 (Invitrogen, Carlsbad) named pEBV in this study was digested with *Cla*I to remove sequences encoding EBNA-1 and *Ori*P.

pPyOri: A 548 bp fragment containing the Polyomavirus origin of DNA replication (PyOri) was amplified by PCR from pPyA3-1 (ATCC) using forward and reverse primers ($^{5'}$actacatcgatcagtctccctcgatgaggtctacta$^{3'}$) and ($^{5'}$tactcatcga-tctacgtatccatgatggtggtgagg$^{3'}$), respectively, with *Cla*I ends.

pPyOriLT: Polyomavirus Large T antigen and the Py origin were isolated from pPyLT-1 (ATCC) and pPyA3-1 (ATCC), respectively, and cloned into pNK (Bailey et al., 1999), encoding EGFP reporter.

pPyEBV: the PyOri-containing *Cla*I fragment was ligated into the *Cla*I-linearised pEBV vector.

pEBV-d2EGFP, pPyOri-d2EGFP, pPyEBV-d2EGFP, and pBasic-d2EGFP: DNA encoding the destabilized EGFP protein, d2EGFP was digested from pCMV-d2EGFP (BD Bioscience Clontech) using *Kpn*I and *Not*I and ligated into pBasic, pPyOri, pEBV, and pPyEBV.

pEBV-hGH, pPyOri-hGH, pPyEBV-hGH, and pBasic-hGH: DNA encoding hGH was digested from pCBhGH (Bailey et al., 2002) and ligated into *Kpn*I site of pBasic, pPyOri, pEBV, and pPyEBV.

Flow Cytometry Analysis

All data presented were collected on a Cytomation MoFlo cytometer (Cytomation, Fort Collins, CO), equipped with Summit 3.0 software and an argon-ion laser operating at 200 mW and tuned to 488 nm in light regulation mode. Forward angle and side-scatter light gating were used to identify viable populations whilst doublets were excluded using forward angle and pulse-width scatter gating. A 525-nm short pass dichroic mirror was used to separate EGFP fluorescence. EGFP emission (whose emission maxima occurs at 508 nm) was detected on FL4 using a 510/23-band pass filter. PMT voltages were adjusted to ensure auto-fluorescence associated with untransfected controls described a Gaussian distribution within the first log-decade. Analysis was maintained at an event rate not exceeding 600 cells per second and a total of 20,000 events were acquired per sample.

RESULTS

Episomal Replication in CHO Cells

Replication of episomal expression vectors requires the following three elements: (1) a viral origin of DNA replication, (2) the associated viral transacting protein to initiate DNA replication, and (3) permissive host-specific proteins. The expression vector pPyOriLT (Fig. 1A) was transfected into CHO cells to demonstrate episomal replication. Accordingly, pPyOriLT contains PyOri and encodes PyLT, the two viral elements necessary and sufficient to initiate plasmid DNA replication in the presence of permissive CHO cellular factors. Plasmid DNA replication was monitored over 3 days. Low molecular weight DNA was purified from transfected cells as described in ''Materials and Methods'' (Fig. 1B). Plasmid replication was detected by resistance to cleavage by *Dpn*I, which cleaves only when its recognition site is methylated. DNA purified from a dam+ strain of *E. coli* (lanes 1–4) is a substrate for *Dpn*I (lanes 5–10) whereas plasmid DNA which has undergone one or more rounds of

DNA replication in mammalian cells is resistant to *Dpn*I cleavage (lanes 11–20). As shown in Figure 1C, the total amount of non replicated DNA (*Dpn*I-sensitive) drops rapidly from 7000 copies per cell following transfection to under 1000 copies per cell on day 3. Replicated DNA (*Dpn*I-insensitive DNA) reaches maximum levels 2 days post transfection and declines rapidly afterwards. These results indicate that although CHO cells support the episomal replication of pPyOri-containing plasmid in the presence of PyLT, the rapid decline in available template DNA for high-level transient gene expression limits the timeframe for high-level recombinant protein production to 3–4 days post transfection.

Recombinant Gene Expression Vectors Containing EBV and Py Sequences

One objective in this study was to extend the time of transient gene expression in transfected CHO cells. To this end, we designed episomal vectors capable of replicating in CHO cells that will allow for the persistence of molecules over
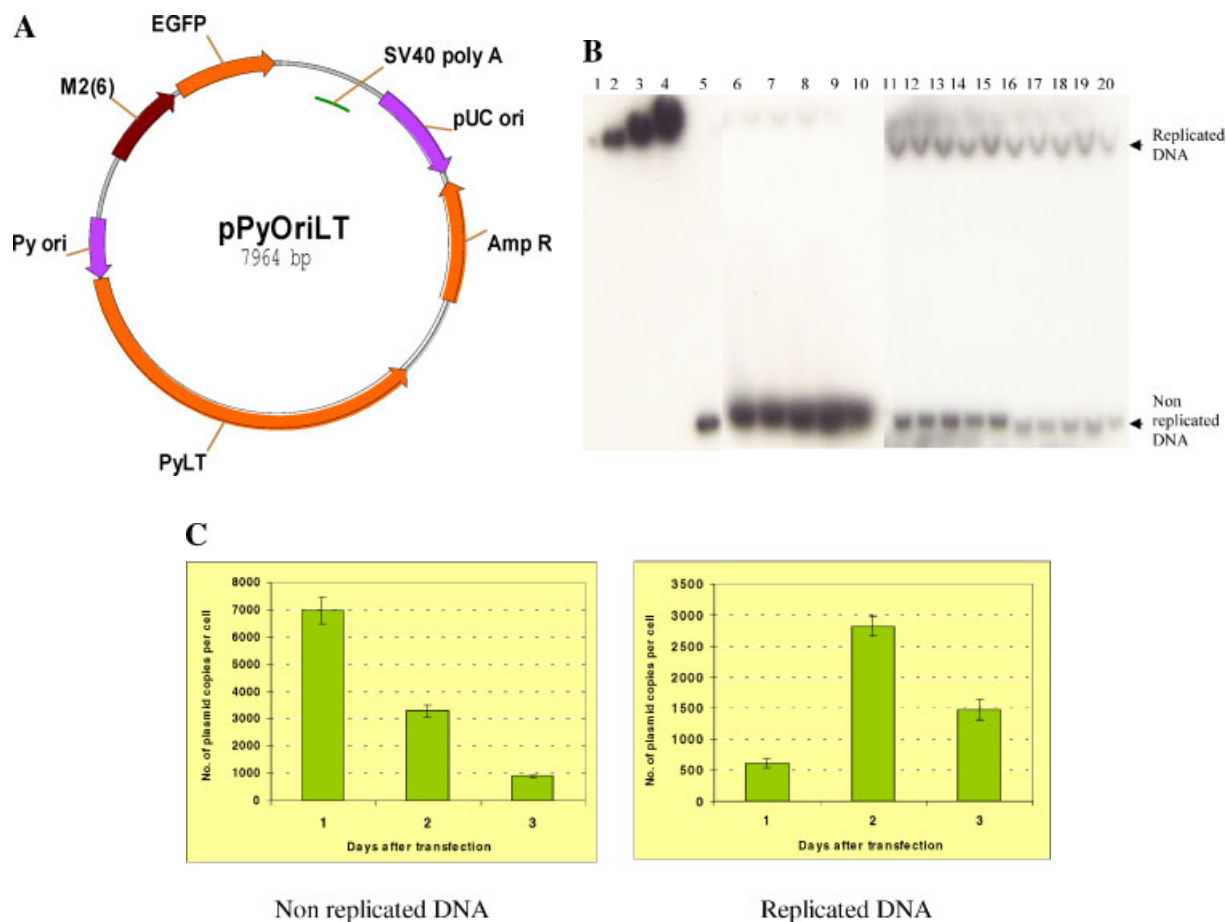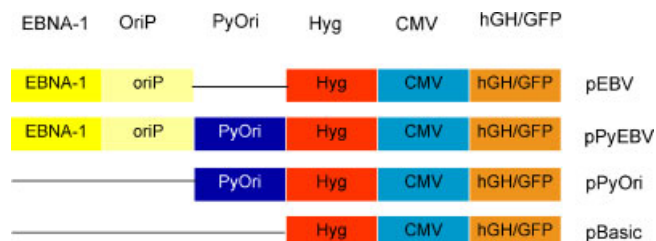

Figure 1. (**A**) Restriction map of pPyOriLT consisting of viral sequences PyOri and PyLT, a eukaryotic promoter expressing GFP, and sequences necessary for replication and selection in bacteria. (**B**) Replication assay. CHO cells were transfected with pPyOriLT and grown for 1 day, (**lanes 6–10**) 2 days (**lanes 11–16**) and 3 days (**lanes 17–20**). Five replicate samples are shown for each day. The replication assay was conducted as described in ''Materials and Methods.'' Replicated DNA refers to 7.9 kb *Spe*I digested and *Dpn*I resistant fragment. Non-replicated DNA refers to *Spe*I and *Dpn*I sensitive fragments. Purified pPyOriLT from bacterial cells was used as standard for quantitation purposes (**lanes 1–4**). Plasmid DNA extracted from *E. coli* was digested with *Dpn*I (**lane 5**) as negative control. (**C**) Plasmid copy number per cell either non-replicated DNA (*Dpn*I-sensitive) or replicated DNA (*Dpn*I-resistent) was determined using densitometry. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]
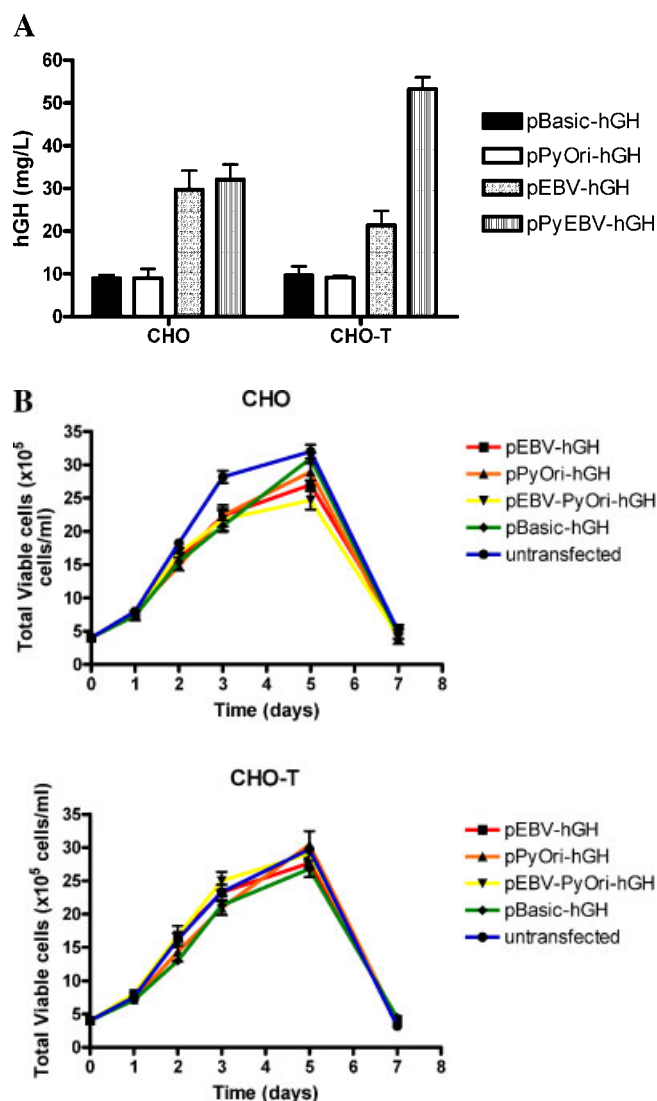
prolonged periods of culture. Epstein-Barr virus elements (EBNA-1 and *Ori*P) were chosen to complement PyOri-vectors. EBV vectors anchor to the nuclear matrix through a high-affinity matrix attachment region containing the *Ori*P sequence (Jankelevich et al., 1992; Mattia et al., 1999). Interaction of *Ori*P with the origin binding protein, EBNA1 is required for EBV vector replication, maintenance, and segregation in primate cells (Lupton and Levine, 1985; Polvino-Bodnar and Schaffer, 1992; Yates et al., 1984). However, EBV vectors are not known to replicate in rodent hosts such as CHO cells due to the non-permissive nature of rodent hosts to EBV infection and DNA replication. Therefore, a hybrid vector, pPyEBV, was constructed to encode both PyOri and EBV elements. For comparison three additional vectors were constructed lacking PyOri or EBV elements or both as depicted in Figure 2. The vector pEBV contains the *cis*-acting sequence *Ori*P and encodes the DNA binding protein EBNA-1. Plasmid pPyOri contains the *cis*-acting sequence, PyOri without EBV sequences. The hybrid vector pPyEBV contains *Ori*P, EBNA1 of EBV and PyOri of Py. Plasmid pBasic lacks both EBV and Py viral elements. The reporter genes *d2EGFP* or *hGH* were cloned into all four plasmid vectors downstream of the CMV promoter. The cell line, CHO-T, a CHO-K1 cell line constitutively expressing PyLT was used for transfection and recombinant gene expression analysis (see "Materials and Methods" for the isolation of the CHO-T cell line).

Prolonged and Enhanced Recombinant Protein Expression

Both CHO-T and CHO cells were transfected with each of the four expression vectors encoding the human growth hormone gene (*hGH*) and grown in separate cultures. Culture protein yields were assayed on day 7 post transfection and are shown in Figure 3A. Vectors that are incapable of replication in CHO-T cells (pBasic-hGH, pEBV-hGH and all vectors in CHO cells) gave final protein yields from approximately 10–30 mg L^{-1}. Cultures transfected with the vector pBasic-hGH had similar product concentrations in both CHO and CHO-T cells (10 mg L^{-1}). CHO-T cells transfected with the replication competent vector pPyEBV-hGH had a final product

Figure 3. Batch cultures of CHO and CHO-T cells showing (**A**) protein yield and (**B**) growth kinetics. Cells were transfected with the indicated expression vectors. Cells were transfected and seeded at a concentration of 3×10^5 cells mL^{-1} and cultured for 7 days. A viable cell count was performed daily and culture supernatant was assayed for productivity on day 7 using hGH ELISA (Roche Diagnostics GmbH). $n = 3 \pm$ SD. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

concentration in excess of 50 mg L^{-1} indicating the requirement for plasmid replication as a necessary determinant towards greater yields of recombinant product. Although the plasmid pPyOri-hGH is capable of replication in CHO-T cells, these cultures resulted in comparatively low final product yield (10 mg L^{-1}). These data indicate that the difference in product concentration is due to more than simply plasmid replication alone. Cultures transfected with expression vector pEBV-hGH gave intermediate product levels (20–30 mg L^{-1}) in both CHO and CHO-T. In the absence of plasmid replication in these cultures, enhanced expression could be due to the interaction of EBNA-1 and *Ori*P and/or the enhancing properties of EBNA-1 (Langle-Rouault et al., 1998). Plasmid retention and segregation,

Figure 2. Schematic diagram of expression vectors used in this study showing relevant sequences containing PyOri and/or EBV sequences, *Ori*P and *EBNA-1* gene as described in "Materials and Methods." Plasmid pBasic lacks both EBV and Py viral elements. The reporter genes *d2EGFP* or *hGH* were cloned in to all four plasmids downstream of the CMV promoter. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

a property of the interaction of EBNA-1 and OriP (Lupton and Levine, 1985) together with plasmid replication would result in more DNA template available for gene expression resulting in greater product yield in a transient expression system. Growth kinetics and viable cell densities of both transfected CHO and CHO-T cell cultures were similar (Fig. 3B) indicating that episomal replication does not adversely affect growth of CHO-T cells.

Retention and segregation capability of the replication-competent expression vector, pPyEBV is illustrated in Figure 4A. CHO and CHO-T cells were transfected with individual plasmid constructs encoding the marker protein EGFP. Two days post transfection, the populations of cells were incubated in the presence of selection. Transient expression of EGFP was monitored by flow cytometry in cell cultures over a period of 15 days. Results are presented as the percentage of EGFP-fluorescing cells in transfected

cultures over time. CHO-T population transfected with pPyEBV-EGFP underwent an increase in the percentage of EGFP-expressing cells from approximately 25%–60% 15 days after transfection. In contrast, the same cells and CHO cells (Fig. 4B) transfected with either pEBV-, pPyOri- or pBasic-EGFP revealed no discernible increase over time and only 10%–15% of these populations express the transgene after this period. These results suggest that the number of CHO-T cells that harbor the pPyEBV-EGFP vector increased in the transfected pool due to both plasmid replication (PyLT initiates DNA replication by binding to the PyOri sequence) and plasmid maintenance and segregation (interaction of EBNA-1 and OriP) (Lupton and Levine, 1985). Transfected populations with replication incompetent vectors in CHO-T cells and all vectors in CHO cells showed a decrease in transgene expression over time. Although pPyOri- has been shown to replicate in CHO-T cells, the percentage of fluorescent cells also decreased over the same period. Lack of both replication and plasmid retention leads to loss of plasmid DNA through cell division, degradation, and/or random chromosomal integration.
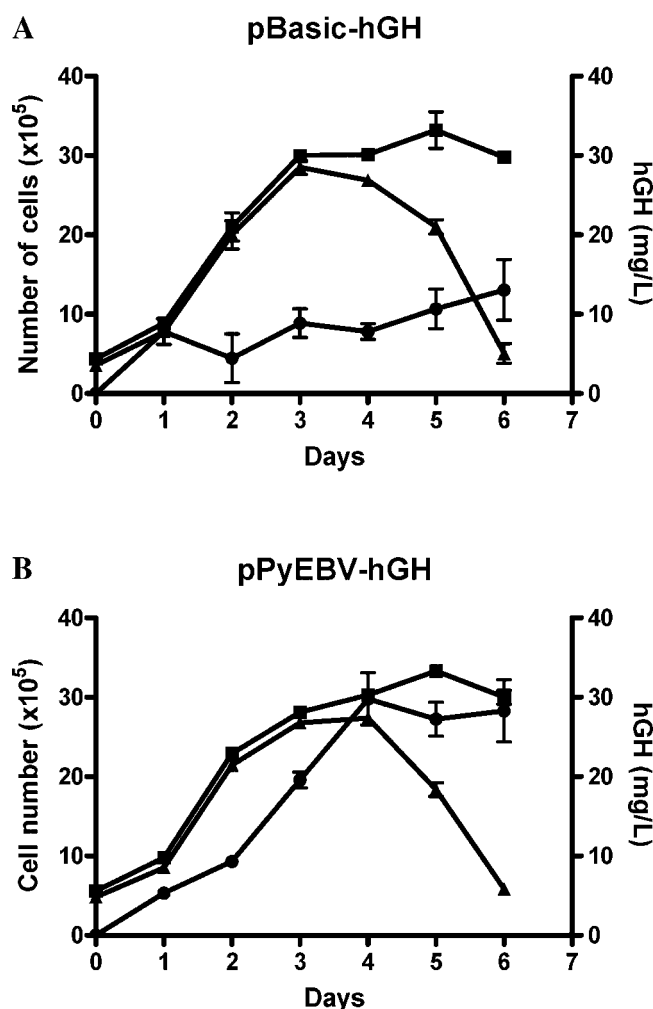
Scaled Transient Production of Recombinant Protein in Epi-CHO

The Epi-CHO expression system comprising the pPyEBV expression vector in CHO-T cells was tested for scaled transient expression of the secreted protein, hGH. Cells were transfected with either the replication-competent expression vector pPyEBV-hGH or the replication incompetent vector pBasic-hGH. Cells were seeded at a concentration of 5×10^5 cells mL^{-1} and cultured in spinner culture flasks in a total volume of 100 mL. As shown in Figure 5, both cultures reached similar maximum viable cell numbers with viability remaining above 90% of total cell numbers up to 4 days post inoculation followed by a rapid decline in viability presumably due to nutrient depletion. Product yields reached 10 and 30 mg L^{-1} in pBasic-hGH (A) and pPyEBV-hGH (B) transfectants, respectively. The increased yield in productivity in Epi-CHO (pPyEBV-hGH in CHO-T cells) results from plasmid replication and segregation. In a subsequent semi-continuous batch culture, a media replacement strategy (50% every 48 h) was used to improve culture viability. Figure 6 shows the growth profile and productivity obtained from a transient transfection in Epi-CHO with media replenishment following inoculation. The feeding strategy employed was to encourage continued cell division. Cell division may have arrested from day 6 onward (as shown by large errors in total and viable cell counts). The decline in productivity at this stage may be a result of a halt in cell division and hence plasmid replication. A continuous feeding strategy would presumably allow for continued cell division and hence plasmid replication.

Cumulative protein yield reached a concentration of 75 mg L^{-1} with a calculated maximum specific productivity of 7.8 pg cell^{-1} day^{-1}. Accordingly, plasmid replication, retention, and segregation taken together resulted in an
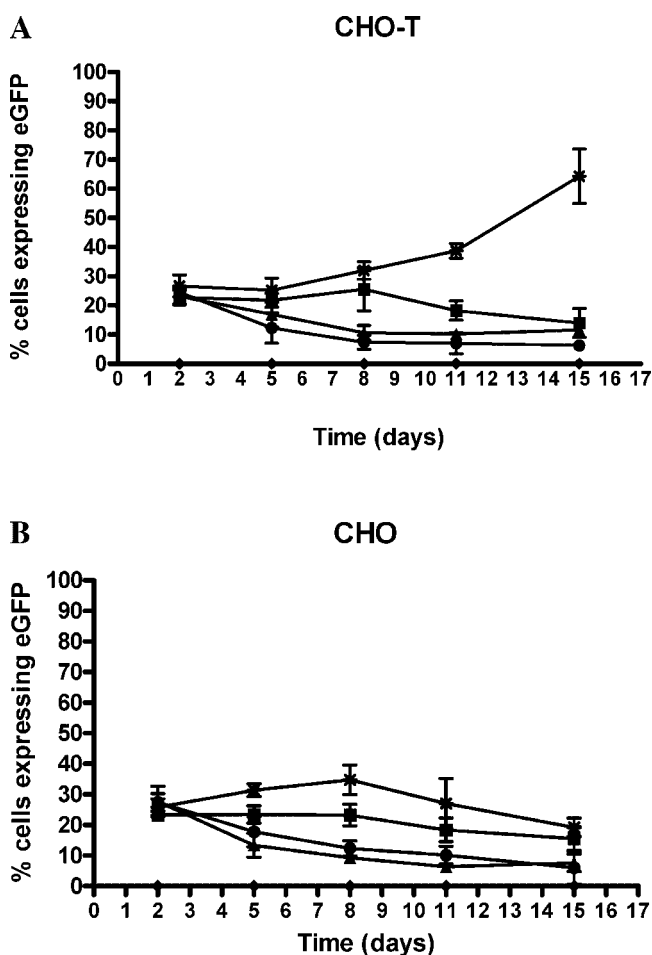
Figure 4. GFP-fluorescing cells within a transfected pool of (**A**) CHO-T and (**B**) CHO cells following transfection. Cells were transfected and seeded into 6-well microtitre plates. Hygromycin (400 μg mL^{-1}) was added to the media on day 2 following transfection. Time indicates days post transfection. Symbols represent the percentage of cells in a transfected pool that have a fluorescence above that of background untransfected cells. Stars, pPyEBV-d2EGFP; squares, pEBV-d2EGFP; triangles, pPyOri-d2EGFP; Circles, pBasic-d2EGFP. $n = 3 \pm$ SD.

A **pBasic-hGH**

B **pPyEBV-hGH**

Figure 5. Batch study of recombinant protein production in CHO-T transfected with (**A**) pBasic- and (**B**) pPyEBV-vectors encoding hGH. Cells were transfected and seeded into 100 mL spinner flasks. Cell counts were determined using a hemocytomer and viability was determined using the trypan blue method. Product concentration was determined using hGH ELISA kit (Roche Diagnostics GmbH). Squares, total cell numbers; triangles, total viable cell numbers; circles, hGH yield.

increase in recombinant gene expression capabilities and enhanced product yield.

DISCUSSION

Transient expression technology is an alternative approach to generate large amounts of recombinant protein in mammalian cells other than the use of stable clones for this purpose. Large-scale transient gene expression has been described for scaling the process and offers the advantage of being extremely rapid (Durocher et al., 2002; Girard et al., 2002; Meissner et al., 2001; Pham et al., 2003; Schlaeger and Christensen, 1999; Schlaeger et al., 2003).

High level of transient protein production has been successfully achieved in suspension adapted transformed human embryonic kidney HEK293(EBNA) host cells, engineered to

A **Semi Continuous Batch culture**

B **Cumulative hGH productivity**
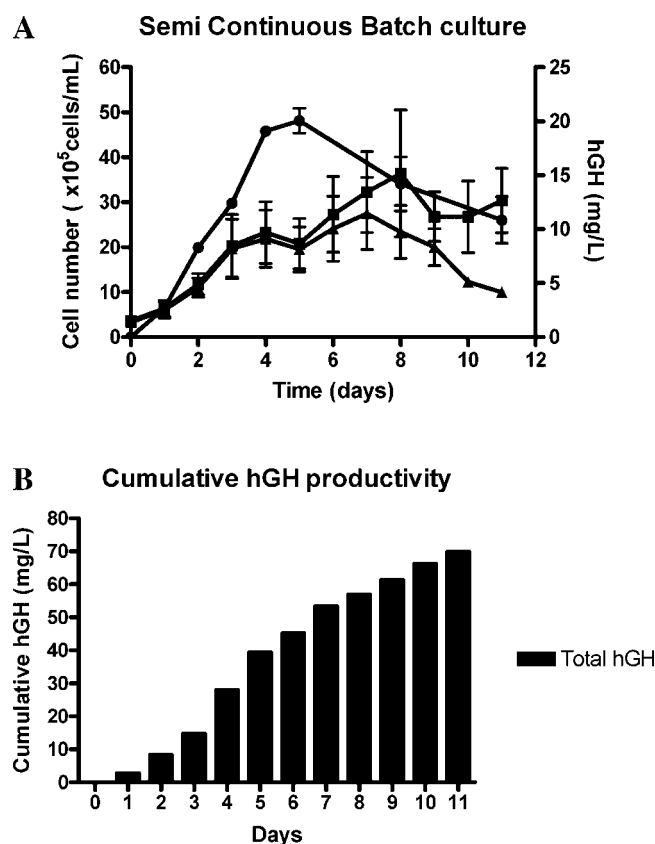
Figure 6. *Epi*-CHO expression system. (**A**) Semi-continuous batch culture with medium replacement of CHO-T cells transfected with pPyEBV encoding hGH. Cells were transfected and seeded into 100 mL spinner flasks. At 48 h intervals, conditioned media was removed and replaced with fresh media. Squares, total cell number; triangles, viable cell number; circles, hGH yield (mg L^{-1}). (**B**) Daily cumulative yield of hGH from batch culture with medium replacement. A maximum specific productivity of 7.8 pg cell^{-1} day^{-1} was achieved.

express EBNA-1. To date, antibody and secreted recombinant proteins are routinely expressed at 10–20 mg L^{-1} in HEK293-EBNA-1 cells (Durocher et al., 2002; Pham et al., 2003) at a scale of up to 100 L (Girard et al., 2002). By comparison, the yield of transient protein production in CHO is lower. Schlaeger et al. (2003) showed that the average SEAP expression in HEK293-EBNA-1 cells was between 20 and 30 mg L^{-1} and approximately 7–8 mg L^{-1} in CHO. For antibody production, approximately 5 mg L^{-1} of IgG has been reported in CHO (Derouazi et al., 2004; Tait et al., 2004). To improve the level of transient protein production in CHO, this study focused on the development of transient expression system that is capable of episomal replication in CHO cells. Plasmid copy number is a key element that affects the level of protein production in transient expression technology. The level of transient recombinant protein production can be elevated when more plasmid copies persist in transfected cells. Replication is required for the maintenance of the extrachromosomal state. Chromosome-based vectors that replicate extrachromosomally in mammalian cells have been described for use in gene therapy (Lipps et al.,

2003; Piechaczek et al., 1999; Schaarschmidt et al., 2004). Another way to accomplish episomal replication of plasmid DNA is to incorporate a host cell specific viral origin of DNA replication into the expression vector. A Py-based vector was chosen to establish episomal replication in CHO, requiring only two exogenous elements namely PyOri and PyLT (Heffernan and Dennis, 1990). Plasmid vectors harboring the EBV origin of DNA replication (*Ori*P), through their interaction with EBNA-1 are capable of autonomous replication and nuclear retention (Langle-Rouault et al., 1998; Sclimenti and Calos, 1998; Van Craenenbroeck et al., 2000; Yates et al., 1985). Although some reports demonstrate that plasmids containing the *Ori*P and EBNA-1 sequences can replicate in some rodent cells (Krysan and Calos, 1993; Mizuguchi et al., 2000) this has not been demonstrated in CHO cells.

Plasmid replication contributes to increased transient gene expression. In this study we have shown that CHO cells support the episomal replication of PyOri-containing plasmid DNA in the presence of PyLT (Fig. 1B). However, plasmid replication reaches a maximum 2 days following transfection and the amount of episomal DNA declines rapidly afterwards (Fig. 1C). This limits the duration of transgene expression to only a few days following transfection. In order to prolong transgene expression in CHO cells, DNA sequences encoding EBNA-1 and *Ori*P from the Epstein-Barr virus were used to complement the PyOri vector. The interaction of EBNA-1 and *Ori*P tethers the plasmid to the nuclear matrix and facilitates segregation of plasmid DNA to daughter cells. Segregation of replicated plasmids occurs during cell division. Plasmid retention and segregation, together with replication results in greater levels of transient gene expression. In this study, the hybrid vector, pPyEBV, containing elements from both Py and EBV results in a significantly greater yield of hGH, than vectors lacking either element (Fig. 3A). Transient gene expression of destabilized EGFP (Fig. 4A) is prolonged in cultures transfected using pPyEBV demonstrating the capability of plasmid retention and segregation.

The CHO-T cell line was developed to facilitate transient gene expression. The cell line was derived from the parental CHO-K1 to constitutively express PyLT. Constitutive expression of PyLT ensures replication of PyOri-containing plasmid DNA in CHO cells. In order for the cell line to be useful as a host for large-scale transient production of recombinant protein, the CHO-T cell line was adapted to growth in suspension in serum-free media. The CHO-T clone P1-C11 used in this study was further developed for high-cell density growth. The expression system named *Epi*-CHO consists of CHO-T cells transfected with the vector pPyEBV. An episomal expression system as efficient as the HEK293(EBNA) system has not been utilized for scaled-up transient production in CHO cells. Different host cell types can confer different post-translational modifications, which may significantly affect the properties of therapeutic recombinant proteins (Haack et al., 1999). The development of a stable cell line is costly and time-consuming. Since CHO cells are extensively used in the biotechnology industry to produce stable cell lines for therapeutic protein production, the *Epi*-CHO expression system offers the advantage of using the same parental cell line for the production of transient material. Significant amounts of protein can be produced quickly at an early stage in product development using *Epi*-CHO before a stable CHO cell line is generated.

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