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High level expression of proteins using sequences from the ferritin heavy chain gene locus

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Abstract

An expression vector has been generated using a gene highly expressed under conditions found in a typical fed-batch bioreactor process. The ferritin heavy chain (HC) gene exhibits higher levels of expression in the late stages of a fed-batch bioreactor than in the early stages. This property was considered advantageous for an expression vector, since the maximal cell density would coincide with maximal expression. The rat ferritin HC genomic region was isolated and converted into an expression vector where large segments of 5′ and 3′ flanking regions were included in an attempt to recreate the same high level of expression in stably transfected cells. Expression from the resulting ferritin HC vector was compared to vectors containing the commonly used strong promoters, CMV IE, and SV40 early promoter/enhancer, in the generation of stable transfectants. The ferritin HC vector was able to generate cell lines with significantly higher expression levels than those under the control of the viral promoters.

Keywords: Gene expression; Ferritin heavy chain; Chinese hamster ovary cell; Stationary phase

1. Introduction

Expression vectors intended for high levels of expression in stably transfected cell lines typically contain strong promoters and efficient polyadenylation signals. Unfortunately, cell lines derived from expression vectors containing only promoters and polyadenylation sequences tend to exhibit wide variations in expression levels due to integration site effects that often lead to repression of transcription (Wilson et al., 1990). For this reason a variety of other types of sequences may be added to enhance performance. Some have overcome integration site repression by including sequences such as matrix attachment regions, CpG islands, anti-repressor elements, or chromatin opening elements

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(Girod et al., 2005; Kalos and Fournier, 1995; Kim et al., 2004, 2005; Kwaks et al., 2003, 2005; Aldrich et al., 2003; Williams et al., 2005). Another successful approach has been through the inclusion of 5′ and 3′ flanking regions of the highly expressed housekeeping gene, EF-1alpha, in an attempt to recreate the highly expressed gene locus around the inserted gene (Running Deer and Allison, 2004). Inclusion of these types of sequences has been shown to increase the frequency of high levels of expression in stably transfected cell lines.

The choice of promoter is usually based on the ability of a promoter to generate high levels of transcript in a selected cell type. If a promoter is only evaluated in exponentially growing cells and not cells in stationary phase, then an important part of the production potential in a batch or fed-batch process may be overlooked. High-level production of recombinant proteins at a commercial scale requires a vector system that supports a high level of expression throughout the production process. The stationary phase of a typical fed-batch process will constitute more than half of the integral cell area, which is a product of the viable cell density and elapsed time. If the activity of the promoter is reduced during stationary phase, then less product may be produced during the time when the maximal number of viable cells are present.

In this paper a housekeeping gene, the ferritin heavy chain (HC), has been identified that is highly expressed throughout exponential and stationary phases of cell growth. Ferritin HC is part of an iron storage protein complex that plays a role in redox homeostasis of the cell and is highly expressed in many cell types. The levels of ferritin in the cell are predominantly controlled at the translational level through the iron regulatory element (IRE) in the 5′ UTR of ferritin mRNA. In addition the gene is also regulated at the transcriptional level by iron, hormones, cytokines, second messengers, differentiation, and oxidative stress (Thomson et al., 1999). Two regions have been shown to contribute to ferrtin HC expression; the proximal promoter region which is within 160 bp of the transcriptional initiation site and the distal enhancer located 4–5 kb 3′ of the initiation site (Beaumont et al., 1994; Bevilacqua et al., 1992; Tsuji et al., 1995).

The expression vector described in this paper is an attempt to take into account three aspects for optimal vector performance. (1) A strong promoter, (2) large flanking regions in order to confer open chromatin and (3) maintenance of promoter strength into stationary phase. The model selected for this work was a serum free fed-batch process developed for a Chinese hamster ovary (CHO) cell line.

2. Materials and methods

2.1. Cells and media

Chinese hamster ovary (CHO) DG44 cells (Urlaub et al., 1986) and CHO cell line transfectants were cultured at 36 °C with 5% CO_2 in either serum free media or Dulbecco's modified Eagle media with 10% fetal calf serum and without nucleosides. DG71712 cells, expressing an LFA3-Fc fusion protein, were grown in the bioreactor in serum free media with a pH setpoint of 7.3, a dissolved O_2 setpoint of 50% and temperature of 36 °C. Cells were fed on days 2 and 6 with insulin, bactotryptone, and asparagine.

2.2. RNA preparation, cDNA libraries

Total RNA was prepared from days 3, 5, 7, and 10 of the bioreactor as well as from a spinner culture of the DG44 host during exponential growth at 36 and 28 °C. The day 5 RNA was used to generate an oligo-dT primed cDNA library. In addition, a CHO-K1 cDNA library was purchased from Invitrogen. The day 5 RNA was also used to generate an oligo-dT primed cDNA that was random primer labeled with biotin-16-dUTP for use as a probe in screening the cDNA libraries.

Colonies from the cDNA libraries were picked and grown overnight for spotting onto Genescreen Plus (NEN) membranes for hybridization. Membranes were then placed on sheets of 3 mm paper soaked with 1.5 M NaCl and 0.5 N NaOH to lyse the cells and denature the DNA. After 5 min the membrane was neutralized in 1.5 M NaCl and 0.5 M Tris–HCl pH 8.0. Neutralized membranes were washed overnight with agitation in 2× SSC with 0.1% SDS to remove bacterial debris. Washed membranes were immersed in 1 M NH_4OAc, 1× SSC and dried. The membranes were then UV crosslinked and treated with proteinase K, quenched with PMSF, and rinsed with 2× SSC. Membranes were placed in sealed bags with a prehybridization solution (6× SSC, 5× Denhardt's and 0.5% SDS) at