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Review

Polyethyleneimine-based transient gene expression processes for suspension-adapted HEK-293E and CHO-DG44 cells

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ABSTRACT

Transient gene expression (TGE) from mammalian cells is an increasingly important tool for the rapid production of recombinant proteins for research applications in biochemistry, structural biology, and biomedicine. Here we review methods for the transfection of human embryo kidney (HEK-293) and Chinese hamster ovary (CHO) cells in suspension culture using the cationic polymer polyethylenimine (PEI) for gene delivery.

Contents

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Introduction

There is a growing interest in the rapid production of recombinant proteins in cultured animal cells for applications in medicine and fundamental research [1–6]. Currently, the two major approaches to rapid protein production are non-viral transient gene expression (TGE)[1] using mammalian cells [7–11] and infection of insect cells with a baculovirus expression vector [12,13]. Cost-effective protein production is achievable by both methods when performed with suspension-adapted cells cultivated in simple and scalable systems.

One of the major differences between the two expression systems is the manner in which the gene of interest (GOI) is delivered to the production host. Construction of the recombinant baculovirus begins with the cloning of the GOI into a transfer vector that allows recombination-mediated transfer of the GOI into the baculovirus genome while it is maintained as an episomal DNA element (bacmid) in *Escherichia coli* [12,13]. After recovery of the recombinant bacmid DNA from *E. coli*, it is transfected into insect cells, resulting in a productive viral infection. The baculovirus stock is then used for the large-scale infection of insect cells to produce the recombinant protein of interest. Unfortunately, the baculovirus infection of insect cells is cytolytic, limiting the production phase to a relatively short period of 2–5 days. In contrast, TGE requires the cloning of the GOI into a mammalian expression vector. After amplification of the plasmid in *E. coli*, it can to be transfected into cells. A protein production phase of up to 2 weeks is possible depending on the protein and the culture conditions. In optimized cultures, volumetric yields up to 1 g/L have been reached [14]. A major drawback of this method, however, is the amount of plasmid DNA, typically 1 mg or more, required per liter of transfection. By comparison, the generation of the recombinant baculovirus vector is time-consuming, but its continued propagation is relatively simple and inexpensive.

Both mammalian and insect cells support correct protein folding, multi-protein complex formation, and post-translational modifications. However, there is a major difference between the two animal cell hosts with regard to *N*-linked glycosylation. Insect cells mainly synthesize oligomannosidic and paucimannosidic glycans with low levels of galactose and sialic acid [13,15]. In contrast, mammalian cells synthesize complex glycans containing mannose, *N*-acetylglucosamine, galactose, and sialic acid [16,17]. For applications in structural biology, it is often beneficial to produce glycoproteins with homogenous oligomannosidic glycans that can be efficiently removed by glycosidases [18–20]. For therapeutic proteins, on the other hand, it is preferable to have complex glycans to enhance both the half-life and functionality of the protein *in vivo* [21–23].

This article focuses on TGE methods with the two major mammalian production hosts, human embryo kidney 293 (HEK-293) and Chinese hamster ovary (CHO) cells, using polyethyleneimine (PEI) for DNA delivery. A non-exhaustive list of examples of proteins which have been produced in these cells is provided in Table 1. Since 2004, over 20 structures have been resolved using proteins produced transiently in HEK-293 cells. In addition, the method has been successfully applied to the production of virus vectors for the purpose of gene delivery (Table 1). Despite these successes, there may be perceptions that TGE is unaffordable for many academic labs and that the cultivation of mammalian cells in suspension is technically difficult. Fortunately, the production yields from transiently transfected mammalian cells have improved considerably in the last decade, and innovative cost-effective, non-instrumented cultivation systems for suspension-adapted mammalian cells have been developed [24–26]. These technical improvements have dramatically reduced protein production costs.

Our objective is to present a practical overview of the key components, methods, and limitations of PEI-based TGE production processes in HEK-293 and CHO cells based on our extensive experience in the Protein Expression Core Facility and the Laboratory of Cellular Biotechnology at the École Polytechnique Fédérale de Lausanne. The point is to provide access to the technology so that new users may better understand the critical steps in order to develop protocols to suit their own needs. We are not providing a comprehensive review of transient transfection methods with animal cells. Other recent reviews can be consulted for a broader perspective of TGE [2,10,11]. In addition, we and others have published step-by-step protocols on TGE using mammalian cells as host [27–33].

Cells

To achieve an economic transient production process, two properties of the host cell are essential. They must be able to grow to a high density (>5 × 10^6 cells/mL) in single-cell suspension culture, and they must be efficiently transfected (DNA uptake in more than 50% of cells) with a low-cost DNA delivery vehicle. The ability to grow in the absence of serum is also highly desirable if the protein product is secreted. Although the TGE methods described here can be adapted to many other mammalian cell lines, most of them do not meet these criteria. Consequently, most efforts to develop TGE systems have focused on HEK-293 and CHO cells as hosts.

HEK-293 cells

HEK-293 cells (American Type Culture Collection, Molsheim, France) were generated from embryonic human kidney tissue by stable transfection with sheared human adenovirus DNA, resulting in cells overexpressing the adenovirus E1A and E1B genes [34]. The cells were initially used to propagate adenovirus mutants deficient in these genes. Due to their ease of cultivation and transfection, they eventually gained popularity as a TGE host. Subsequently, HEK-293T (American Type Culture Collection) and HEK-293E cells, resulting from stable transfection of the parental line with the simian virus 40 (SV40) large T antigen (LT) gene and the Epstein–Barr Virus nuclear antigen 1 (EBNA1) gene, respectively, were generated [35,36]. Both EBNA1 and SV40 LT function in viral DNA synthesis by binding to the cognate viral origin of DNA replication (ori) to recruit the cellular DNA replication machinery. These two cell lines were developed with the expectation that they would support the episomal replication and maintenance of a transfected plasmid DNA bearing the appropriate viral ori [37]. For the TGE system described here, HEK-293E cells were used. Interestingly, the highest protein yields in these cells were achieved under conditions of growth arrest following transfection with plasmids that did not bear the EBV ori [14]. However, there may be other TGE conditions in which episomal replication of the plasmid has a positive effect on recombinant protein yield [38–40].

More recently, HEK-293 subclones, adapted to suspension growth in commercial serum-free media, were made available. HEK-293F™ and Expi293F™ cells (Life Technologies Europe, Zug, Switzerland) were selected for high-density suspension growth in FreeStyle293™ and Expi293™ media (Life Technologies), respectively. A suspension-adapted cell line lacking

[1] *Abbreviations used:* TGE, transient gene expression; CHO, Chinese hamster ovary; PEI, polymer polyethylenimine; GOI, gene of interest; HEK-293, human embryo kidney 293; EBNA1, Epstein–Barr virus nuclear antigen 1; DHFR, dihydrofolate reductase; HT, hypoxanthine and thymidine; Py, polyomavirus; OSRs, orbitally shaken bioreactors; k_La, oxygen mass transfer coefficient; DO, dissolved oxygen; hCMV, human cytomegalovirus; mIE, major immediate early; mCMV, mouse CMV; UTR, untranslated region; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element; CaPi, calcium phosphate; PDI, polydispersity index.

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