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Gene Expression in Mammalian Cells and its Applications

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ABSTRACT

The production of proteins in appropriate quantity and quality is an essential requirement of the present time. There appears to be a progressive increase in the application of mammalian cells for proteins production. Expression systems utilizing mammalian cells for recombinant proteins are able to introduce proper protein folding, post-translational modifications, and product assembly, which are important for complete biological activity. This review article is totally based on literature survey. In this article much emphasis has been done on the mammalian expression system. The author focused on different mammalian cell lines that express the gene. The different vector systems that transfer the gene into mammalian cells like plasmid based expression vectors, adenovirus vectors, vaccinia vectors, retroviral vector and baculovirus as vectors were explored. The processes for the transfer of gene into mammalian cells were also reviewed. Application and limitations of mammalian expression system were also focused. The purpose of research in writing this article is to create awareness in researchers, starting their career in gene expression related to mammalian cells. The principal result and major conclusion of this article is to make available the molecular technologies, expression system and applications of gene expression in mammalian cell lines.

Introduction

Gene is defined as a discrete unit of genetic information which is required for the production of a polypeptide. It includes the coding sequence, the promoter and terminator, and introns. Expression can be described as a transcription and translation of a gene. A particular host is required to express a particular gene. Today, there are wide selections of expression systems available for large-scale recombinant protein production. These expression systems include *E. coli*, baculovirus-mediated insect cell expression, yeast, and several mammalian based systems. Each has its own respective advantages in relation to cost, ease of use, and their post-translational modification profiles. Factors to be considered while considering an expression system for gene expression are listed in Table 1.

Table 1. Factors for selecting an expression system.

S.No	Factors effecting gene expression
1	Facilities of the laboratory and local expertise.
2	Type of protein to be expressed.
3	Whether the protein is toxic?
4	Whether carbohydrate or other modification required?
5	Reqiurement of large yield of protein.
6	How to purify protein?
7	Production costs.
8	Regulatory considerations.
9	Safety considerations.
10	Purpose for which it is required.

This author reported earlier in his articles about gene transfer technology.[1-3] In this article the author reviewed the mammalian expression system. The introduction of large-scale transient transfection has enabled the use of mammalian cells more attractive in terms of speed and ease of use, particularly for cell surface and secreted glycoproteins. The features of the transient expression systems have been listed in the Table 2.

Table 2. Key features for most of transient expression systems

S.No	Important features of transient expression systems
1	Extremely short time-frame for the generation of product (days)
2	Applicability to a wide range of host cell lines
3	Intrinsic genetic stability and consistency due to extremely short time-frame between generation of vector and product recovered.
4	Suitability to multiple processing, allowing study of many genes or mutants at the same time.
5	Simplicity, in particular in the construction of expression vectors.

As compared to insect cells, glycosylation in mammalian cells results in attachment of large and complex glycans to the ex-pressed proteins which generally interfere with crystallization. However, strategies are available that make the glycosylation to

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be modified. This has led to the successful crystallization of glycoproteins produced in mammalian cells.[4] Reports were also made regarding the protein expression in mammalian cells.[5]

This article is totally based on literature survey. The author considered the modern as well as traditional method of literature survey. The search engine and data bases utilized were Science direct, PubMed and Google. The literatures cited in this article are not limited to a particular region but is considered more or less from the entire globe. The author tried to more on the recent research related to the topic. Literatures for the past five years including the year 2012 and valuable literatures of the past were also considered in making this manuscript.

The main objective of this work is to review the different mammalian cell lines that are used for transfection that finally leads to gene expression. The author reviewed the vectors used in transfection in mammalian cell lines. The author also described the various method of gene transfer in mammalian cells. Much stress has been given on the application of gene expression in mammalian cell line. The aim of this article is to create awareness in young researchers whose aim is to express genes in mammalian cell lines to get the desired protein. This article will be beneficial for readers who are initially starting their carrier in biotechnology through animal tissue culture and gene expression.

Requirements for mammalian expression system

The expression level of a gene largely depends upon how efficiently it is transcribed. Transcription of any gene takes place when the RNA polymerase complex interacts with the promoter sequences moves along the gene from a 5^{l} to 3^{l} direction. This produces an RNA transcript and finally dissociating from the gene at the transcription signal, freezing the transcript for eventual translation. The gene expression in mammalian cells needs a suitable cell line and the appropriate vectors that should act as a vehicle to transport the gene of required interest into the required cell lines.

Cell lines

In the previous decade, protein therapeutics produced from mammalian cells have changed the landscape of human healthcare. The value of protein therapeutics has made the search in the forward direction for more cost-effective and efficient cell lines that are capable of producing high quality protein products. Bioprocesses based on mammalian cell have been applied in the manufacture of viral vaccines, diagnostic and therapeutic proteins in the past. In the production of protein therapeutics, cells are the host for producing proteins. The most widely used host mammalian cells are chinese hamster ovary (CHO) cells and mouse myeloma cells, including NS0 and Sp2/0 cells.[6] Two derivatives of the CHO cell line, CHO-K1 and CHO pro-3, gave rise to the two most commonly used cell lines in bioprocessing today, DUKX-X11 and DG44. These two cell lines were engineered to be deficient in dihydrofolate reductase (DHFR) activity.[7-8]

A number of mammalian cell lines have been utilized for protein expression with the most common being HEK 293 (Human embryonic kidney) and CHO (Chinese hamster ovary). These cell lines can be transfected using polyethyleneimine (PEI) or calcium phosphate. HEK 293 cells exhibit the highest level of PEI-mediated transfection with 50–80% of cells showing green fluorescent protein (GFP) expression,[9] and are now widely used for production of recombinant proteins both by transient transfection as well as by the formation of stable cell lines.

Protein expression in mammalian cells can also be achieved using viral-mediated transduction by such techniques as the BacMam system.[10] This technology utilizes recombinant baculoviruses for simple transduction of mammalian cells, allowing for production of milligram quantities of protein for structural studies.[11] Other cell lines such as COS and Vero (both green African monkey kidney), HeLa (Human cervical cancer), and NS0 (Mouse myeloma) have also been used for structural studies. Some of these cell lines such as NS0 are more difficult to transfect. Transfection can be usually achieved using electroporation, and are only used in stable cell line production. A number of mammalian cell lines have been summarized in Table 3.

Table 3. Mammalian cell lines.

S.No	Mammalian cell lines	References
1	HeLa	12
2	HEK293T	12
3	U2OS	12
4	A549	12
5	HT1080	12
6	CAD,	12
7	P19	12
8	NIH 3T3	12
9	L929	12
10	N2a	12
11	Human embryonic kidney 293 cells	12
12	Recombinant Chinese hemester ovary cell line	13
13	MCF-7	14
14	Y79	15
15	SO-Rb50	15
16	Hep G2	16
17	DUKX-X11	17
18	J558L	18
23	Baby hamster kidney (BHK) cells	18

The main advantages of mammalian cell expression are that the signals for synthesis, processing and secretion of eukaryotic proteins are properly and efficiently

recognized by the mammalian cells. However, it should be noted that there are differences between species.[19]

Vectors

Vectors are autonomously replicating DNA molecules that can be used to carry foreign DNA fragments. It is a vehicle used in gene cloning. DNA of interest is first cloned into an appropriate vector and then by transfection, the gene can be inserted into the host for its expression. For expressing hetrologous genes in mammalian cells, usually vectors derived from mammalian viruses are used. These include viruses such as Simian Viruses 40 (SV40), polyomavirus, herpesvirus and papovirus. Inorder to construct vector the requirement is to select an efficient promoter and also the selection marker. In this article a number of vectors have been discussed by the author.

Plasmid based expression vectors

Expression vector is a vector that allows the transcription and translation of a foreign gene inserted into it. Plasmids are circular DNA molecules that lead an independent existence in the bacterial cells. They are naturally occurring, extra chromosomal DNA fragments that are stably inherited from one generation to another generation in an extra chromosomal state.

In most cases, first attempts to transiently express recombinant proteins were executed with 'standard' expression vectors that contain strong viral promoters, such as SV40 or a

promoter from cytomegalovirus (CMV).[20-21] More recently, at least one non-viral promoter, the elongation factor (EF)-1 promotor, has received strong endorsements because it appears to be as strong or stronger than some viral promoters.[22]

Adenovirus vectors

Adenovirus is medium sized, non-enveloped icosahedral virus. It is composed of a nucleocapsid and a double stranded linear DNA genome that can be used as a cloning vector. The extensive knowledge and data collected over the years on adenovirus transcription regulation favored the engineering of adenovirus vectors modified for heterologous expression.[23-24] For this purpose, the early regions E1 and E3 were deleted. This makes the virus replication incompetent and this function must then be provided in trans by the host cell. An expression cassette was set in place of the deleted E1 region. In the cassette, the recombinant gene was placed under control of an additional (or ectopic) major late promoter or under control of an exogenous promoter, such as cytomegalovirus.[25] Very recently, a new series of vectors, pAdBM5, was constructed[26] with enhancers added to the ectopic major late promoter region. The fact that the virus can be propagated in suspension cell cultures is of considerable advantage for large scale work. Reports were made regarding the recombinant adeno associated viral vectors also.[27]

Vaccinia vectors

The genome of vaccinia virus is made up of double stranded DNA of nearly 200,000 bp and replicates in the cytoplasm of the host cell.[28] Cells infected with the vaccinia virus produces up to 5000 virus particles per cell, which leads to high levels of recombinant protein expression. The vaccinia system has been efficiently used at very large scale (1000 L) to produce many different kinds of proteins, such as HIV-1 rgp160 by Pasteur-Merieux[29] and human pro-thrombin by Immuno AG.[30]

Retroviral vector

Retroviruses are RNA viruses that replicate via a dsDNA intermediate. The reason for making retrovirus as vector lies in the fact that most retroviruses do not kill the host, but produce progeny virons over an indefinite period of time. Retroviral vectors can therefore be applied to make stably transformed cell lines. The next reason is that viral gene expression is driven by strong promoters, which can be subverted to control the expression of transgenes. The other reason is that some retroviruses, such as amphotropic strains of murine leukaemia virus (MLV), have a broad host range, allowing the transduction of many cell types.

It has been reported that exogenous gene expression system based on the retroviral vector is an alternative method for the generation of stable and high-expressing mammalian cell lines.[31] Several methods utilized for transfection of mature osteoclasts and their precursors using lentiviruses, and adenoviruses have been described.[32] The retroviral-vector-targeted *CD59* gene was constructed and transfected into breast cells (MCF-7).[14] Retroviral system has also been used for the production of recombinant human factor IX.[16]

Baculovirus as vectors

Baculoviruses possess rod-shaped capsids with large double stranded DNA genomes. They productively infect arthropods, particularly insects. Two baculoviruses have been extensively developed as vectors, namely the *Autographa californica multiple nuclear polyhedrosis virus* (AcMNPV) and the *Bombyx morinuclear polyhedrosis virus* (BmNPV). The former is used for protein expression in insect cell lines, particularly those derived from *Spodoptera frugiperda* (e.g. Sf9, Sf21). The latter infects the silkworm for the production of recombinant protein.

Construction and application of a recombinant baculovirus containing a bicistronic expression cassette that can be used for stable protein expression in mammalian cells have been reported.[33] Expression of a secreted protein in mammalian cells using baculovirus particles was also studied.[34]

Process for transfer of gene

The choice of the vector depends on the method used for the introduction of the foreign gene into the mammalian cells and on the control elements utilized

for the efficient mRNA expression and protein synthesis. There are two general methods for the introduction of foreign DNA into mammalian cells. One is mediated by virus infection and the other by direct transfer of DNA into the cells employing chemical liposomes, calcium phosphate, DEAE-dextran and polybrene and physical electroporation and microinjection methods.[19]

Calcium-phosphate, PEI and electroporation have been shown to be useful as vehicles/approaches for large-scale transient gene expression.[35-38] Commercially available products for DNA transfer are usually sold in small quantities and are not designed to be used in reactors or with large cell masses. Calcium phosphate and PEI achieve DNA transfer by forming complexes with DNA under suitable conditions and these complexes are taken up by cells through endocytosis. A number of methods used to transform cell has been also reported by this author also.[1-3]

Application of mammalian expression system

The first approved biologic from a mammalian bioprocess platform was tissue plasminogen activator (tPA), produced in 1987 by Genentech Inc. Today, production of biologics in mammalian cells dominates: of the 58 biopharmaceutical products approved between 2006 and 2010, 32 were produced in mammalian cells, 17 were produced in *E. coli*, four in yeast, three in transgenic animals, and two in insect cultures.[17]

Production of monoclonal antibody

Mammalian cells are currently the main hosts for commercial production of therapeutic proteins, including monoclonal antibody (mAbs).[39-42] African green monkey kidney (COS) cells may be appropriate if the aim is to produce small-scale quantities of mAbs, as for preliminary investigation.[43] Indeed, they have been used for transient expression of active antibodies since 1987. However, they are not the most suitable cells for large-scale processes of production, since they lose the production ability over time. Actually, if the aim is large-scale production, the most currently used cells for this application are CHO cells, which have assumed an increasing importance in laboratory. Guidelines to cell engineering for mAbs production were also reported.[44] Using heterologous promoters, enhancers and amplifiable genetic markers, the yields of antibody and antibody fragments can be increased. High levels well above the levels seen from parental hybridomas of chimeric antibodies and recombinant antibody fragments have been achieved by using low copy number cell lines.[19]

Production of urokinase

Urokinase is a serine protease that activates plasminogen into plasmin, which in turn degrades fibrin clots. Hence urokinase finds its value as an important anti-thromboembolic drug. The need for urokinase production has increased significantly in recent years, and current production levels have not kept the same pace. Mammalian cells provide the post-translational modifications; therefore mammalian cell lines are nowadays preferred for production of recombinant urokinase. CHO cells are suitable hosts for recombinant urokinase production because they grow exceptionally well inside the bioreactor.[45]

Production of follicle stimulating hormone

Follicle-stimulating hormone (FSH) is produced by the gonadotropic cells of the anterior pituitary. FSH play its role for follicular growth and development in females. It is essential for males for the pubertal initiation and maintenance of quantitatively normal adult spermatogenesis. Besides their therapeutic application in the treatment of infertility, recombinant gonadotrophins have been used for studying structure and functions of glycoprotein hormones. They are free from contaminating hormones, and their structure and function can be studied extensively because of their availability in large excess. Many groups have prepared and described the actions of human recombinant (rec) FSH produced by transfected CHO cells.[46-50] Recombinant rat FSH production by CHO cells, purification and functional characterization was also reported.[51] Cloning and expression of cynomolgus monkey (Macaca fascicularis) gonadotropins luteinizing hormone and FSH were also described.[52]

Other applications of mammalian expression system

Novel mammalian cell lines expressing reporter genes for the detection of environmental chemicals activating endogenous aryl hydrocarbon receptors or estrogen receptors were reported.[53] Hemophilia B is a genetic disease of the coagulation system that affects one in 30,000 males worldwide. Recombinant human Factor IX (rhFIX) has been used for hemophilia B treatment. Stable and high-level production of recombinant Factor IX in human hepatic cell line was reported.[16]

Limitation of mammalian expression system

As it is known that mammalian expression system provides protein which is functional due to glycosylation. In other words protein obtained through gene expression in mammalian cells always remains biological active. In spite of various benefits the mammalian expression has its limitation. As author has good expierence in working in this system. According to this author this system is highly expensive. The complicated technology, and potential contamination with animal viruses of mammalian cell expression have been bottlenecks for its use in large-scale industrial production, this system is often utilized to express many heterologous proteins including viral structural protein and bioactive peptide for specific functional analysis.[54]

Improvement of mammalian expression system

Improvement of mammalian expression can be achieved by proper designing of vector. It includes using strong promoter, proper signal peptide, selected introns and product gene codon optimization.[55] Moreover the use of transcription control regions is also important.[56] Common approach used in generating cell lines for the production of therapeutic proteins relies on gene amplification induced by a selective marker such as DHFR[57] or glutamine synthetase (GS).[58-59] To achieve high levels of gene expression, vectors usually have strong promoters such as cytomegalovirus (CMV) promoter to drive high level messenger RNA transcription.[56] Codon optimization for the target cell type, GC/AT ratio balancing, and signal sequence optimization have been shown to accelerate mRNA processing and improve secretion.[55] Besides, gene-targeting technology, chromatin opening elements and attachment regions have been incorporated into vector optimization to improve final product production.[60]

Concluding remarks

Expression systems for recombinant proteins using mammalian cells are able to introduce proper protein folding, post-translational modifications, which are often essential for full biological activity. The main conclusion is that the author has summarized the latest advancements in the field of mammalian expression system which has its importance in animal biotechnology. The author explained about the cell lines, vectors and the transfection methodology used in mammalian expression system which are highly important for those researchers who are starting their career in animal biotechnology. In last two decades, mammalian cell protein expression has become the dominant recombinant protein production system for clinical applications, producing more than half of the biopharmaceutical products in the market and several hundreds of candidates in clinical development. Significant progress in developing and engineering new cell lines, introducing novel genetic mechanisms in expression, gene silencing, and gene targeting have been achieved. An optimal expression system can be selected only if the productivity, bioactivity, purpose, and physicochemical characteristics of the interest protein are taken into consideration, together with the cost, convenience and safety of the system itself. It is the present need to discover more mammalian cell lines and vector system and technologies to express the gene to get more efficient proteins having high biological activities.

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Conflict of Interest

As this author is first and corresponding author so there is no competing interests.

Abbreviations

CHO: Chinese hamster ovary; DHFR: Dihydrofolate reductase; HEK: Hhuman embryonic kidney; PEI: Polyethyleneimine; GFP: Green fluorescent protein; SV40: Simian viruses 40; CMV: Cytomegalovirus; MLV: Murine leukaemia virus; AcMNPV: Autographa californica multiple nuclear polyhedrosis virus; BmNPV: Bombyx morinuclear polyhedrosis virus.

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آموزش نرم‌افزار Word برای پژوهشگران

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