Korean J. Chem. Eng., **27**(4), 1042-1048 (2010)
DOI: 10.1007/s11814-010-0278-4

INVITED REVIEW PAPER

Cellular engineering for the high-level production of recombinant proteins in mammalian cell systems

Ju Hyun Park, Hee Ho Park, and Tai Hyun Park†

School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Korea
(*Received 20 May 2010 • accepted 9 June 2010*)

Abstract−The market for protein-drugs has steadily increased due their increased use as alternatives to traditional small molecule drugs. While some therapeutic proteins have been produced in microbial systems, mammalian cell systems such as Chinese hamster ovary (CHO) cells are widely used as the host cell system. To increase the efficiency of producing therapeutic proteins, many researchers have attempted to solve the critical problems that occur in mammalian cell systems. As a result, several serum-free media and advanced culture methods have been developed, and protein productivity has increased considerably through the development of efficient selection methods. However, the prevalence of apoptosis during mammalian cell culture still remains a significant problem. Based on the understanding of apoptotic mechanisms and related proteins, anti-apoptotic engineering has steadily progressed. In this study, we review the strategies that have been developed for high-level production of recombinant proteins in the CHO cell system via a selection of clones, target-gene amplification, optimization of culture systems and an inhibition of apoptosis through genetic modification.

Key words: Mammalian Cell, Chinese Hamster Ovary Cell, Therapeutic Proteins, Apoptosis, Recombinant Proteins, Productivity

INTRODUCTION

During the last 30 years, many recombinant proteins have been produced and released in the therapeutic protein market. In addition, the size of this market is growing at a steady rate every year. Most recombinant proteins were first produced in microbial systems; however, the host cell system for the production of therapeutic proteins has recently shifted from microbial systems to mammalian cell systems. This is due to the limitations of microbial expression systems, such as the formation of an inclusion body and the difficulties associated with the purification steps. However, the most important reason for this shift is that post-translational modification (PTM) is impossible to attain or incomplete in microbial systems. In particular, glycosylation among various PTMs is critical in that it has effects on *in vivo* protein stability. In microbial systems, the glycosylation process is not performed completely. For this reason, many mammalian cell systems have been used as a host for the production of therapeutic proteins [1]. There are various mammalian host cells that can be utilized for this purpose, including the human embryonic kidney (HEK-293), the baby hamster kidney (BHK), and mouse myelomas (NS0) cells [2]. Among these cells, the Chinese hamster ovary (CHO) cell line is most widely used to produce therapeutic proteins. This review article describes the various methods that have been developed for the production of recombinant proteins using the mammalian cell systems including the CHO cell system.

1. CHO Cell Systems for the Production of Therapeutic Proteins

Many therapeutic proteins are produced using the CHO cell system because the recombinant proteins produced from CHO cells have a glycan structure similar to that of the original human protein (Fig. 1). In the early stages of protein production using CHO cells, the productivity was quite low compared with microbial systems. However, even though the basic concept of protein production has not changed, productivity has increased dramatically in the past two decades. For example, in monoclonal antibody production, the maximum cell density, operation time, and specific productivity have increased by approximately 5 times, 3 times, and 9 times, respec-

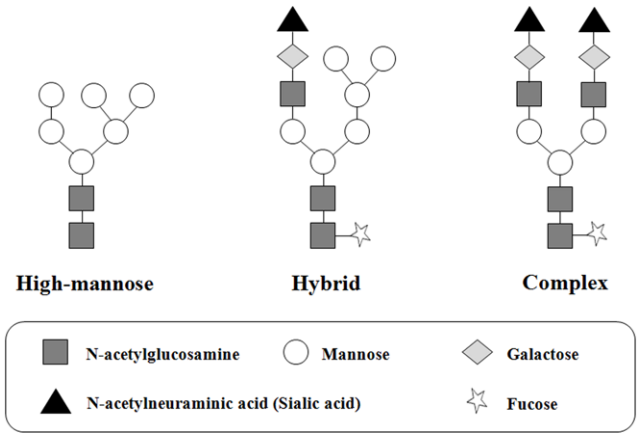

Fig. 1. N-glycan structures of glycoproteins. Although the high-mannose type is a major glycan structure of glycoproteins produced from yeast systems, the complex and hybrid type are the major glycan structures produced from the CHO cell systems. Since the major glycan types of human glycoproteins consist of complex and hybrid types, the glycoproteins produced from CHO cell systems show a higher *in vivo* biological activity in the human body.

†To whom correspondence should be addressed.
E-mail: thpark@snu.ac.kr

tively, in 2004 compared with that in 1986 [2]. As a result of these advances, final product titers have reached approximately 5 g/L, whereas the titers were merely 50 mg/L in 1986 [2,3]. In addition, further increases in production could be obtained through a stronger understanding of metabolism, gene expression, and cell growth and death in the CHO cell system.

2. Methods for Gene Delivery into the Host Cell

The most common method for introducing the gene of interest is transfection and electroporation. In these methods, gene integration usually occurs randomly and many transgenes are integrated into the inactive heterochromatin (a non-transcriptional region in the chromosome) because 90% of the chromosomes in mammalian cells consist of inactive heterochromatin and 10% consist of active euchromatin (transcriptional region). Some strategies have been developed to overcome the disadvantages of random integration. Flanking transgenes with *cis*-regulatory elements reduces the effect of heterochromatin and stabilizes gene expression. The *cis*-regulatory elements include scaffold and matrix attachment regions, ubiquitous chromatin opening elements and conserved antirepressors or enhancers [4]. Recently, a new method that utilized enzymes, such as bacteriophage P1 Cre recombinase or yeast Flp recombinase, to integrate the gene of interest into a specific site in the host cell genome has been adopted and broadly used [5]. Through these processes, the target genes can be introduced into the highly active euchromatin, which leads to an increase in recombinant protein production in mammalian cells.

3. Selection of High-productive Clones

The most popular genes used for the selection of high-productive clones are dihydrofolate reductase (DHFR) and glutamine synthetase (GS) [6,7]. In the case of *dhfr*, cells that do not possess these genes cannot survive in the absence of thymidine and hypoxantine [8]. DG44 (CHO cell line without the *dhfr* gene in the entire homologous chromosomes) or DUKX (CHO cell line without the *dhfr* gene in just one of the two homologous chromosomes) are used as the host cell line. Moreover, the gene of interest can be amplified with the *dhfr* genes by increasing the concentration of methotrexate (MTX), an inhibitor of DHFR, in the medium [8,9]. Following this selection, the cells that survive are selected as a single clone and then, the growth and productivity of each individual clone is analyzed. Finally, the clone that shows the appropriate growth and productivity characteristics is chosen. It has been reported that the specific productivity can be increased significantly by the gene amplification using MTX [10]. Furthermore, many advanced methods have been recently developed for the efficient selection of high-producing clones among the thousands of clones [11]. For example, there is a new technology where cells expressing a target protein fused to a fluorescent protein can be selectively collected using flow cytometry.

4. Development of Suspension Culture Systems

The adherent cell culture is the dominant culture method used in the early stages of protein production by mammalian cells. The most common technique for mass production of proteins using adherent cells is the roller bottle culture. After cultivation and when the cells are confluent, the decanted supernatant is harvested and the target recombinant protein is purified from this supernatant. Although it has been reported that productivity can reach as high as 50-200 mg/L using this approach, this method has some limitations in regards to mass production, which is mainly due to the low ratio of cell/operation volume [2]. Therefore, the suspension culture system is more widely used than the adherent culture system because a much higher cell/volume ratio can be obtained in the suspension culture system. However, the adaptation of the adherent cells to the suspension culture requires a number of special techniques and media formulation, which were not available in the 1980s. Today, there are various commercially available media for adaptation to suspension culture [12,13]. Recently, different types of media have been developed that contain components such as Pluronic F-68, which prevents cell aggregation, for adaptation to suspension culture. Despite these advancements in media preparation, cells must still be cultured for several passages in order to attain complete adaptation. It takes at least 6-7 passages and each passage consists of 3-4 days of subcultivation with 2×10^5 cells/ml of the initial seeding concentration [2].

5. Development of Serum-free Medium

For the laboratory scale suspension culture system, spinner flasks are generally used; thus, a transition step to a larger scale bioreactor is imperative for mass production of recombinant proteins. For industrial scale mass production of recombinant proteins, the development of serum-free medium is vital as well as a proper suspension culture system. Animal serum has been used as a supplement for the growth of animal cells. This type of serum commonly contains several hundred components, including many essential nutrients and growth factors. However, there are numerous unknown components in animal serum, and some components such as viruses may be harmful to humans. Most recombinant proteins produced from animal cells are secreted, and the harmful components may not be completely eliminated during the purification process. This has led to a reduction in the use of animal originated-serum or components in the cell culture medium for the production of biopharmaceutics. In the earlier stages of development, researchers had an interest in reducing the concentration of serum in the medium and now many complete serum-free media have been developed. Furthermore, more advanced media, called "chemically defined media," are being developed. The chemically defined serum-free media are basal media containing only biochemically defined low molecular weight additives such as peptides or nutrients.

6. Apoptosis in the Mammalian Cell-producing Biopharmaceuticals

Apoptosis is genetically programmed cell death, which is distinguishable from passive cell death, necrosis [14,15]. Apoptosis occurs in most animal tissues to remove damaged or abnormal cells such as virus-infected cells. Although the apoptotic process is highly complex and involves the activation of many signal transductions resulting from apoptosis-promoting signals, two dominant apoptotic mechanisms have been identified: the mitochondria-mediated pathway [16,17] and the surface receptor-mediated pathway [18]. Moreover, many recent reports have shown that endoplasmic reticulum (ER)-mediated apoptosis constitutes a third main pathway of apoptosis [19]. A schematic diagram of these apoptotic pathways is presented in Fig. 2. As shown, many components participate in the regulation of apoptosis, and some inhibit the cascade process while others promote it. Caspases are the most representative pro-apoptotic protein family. During apoptosis, these proteins are activated and then cleaved by other caspases. The initiator caspases such as caspase-8,-9, and

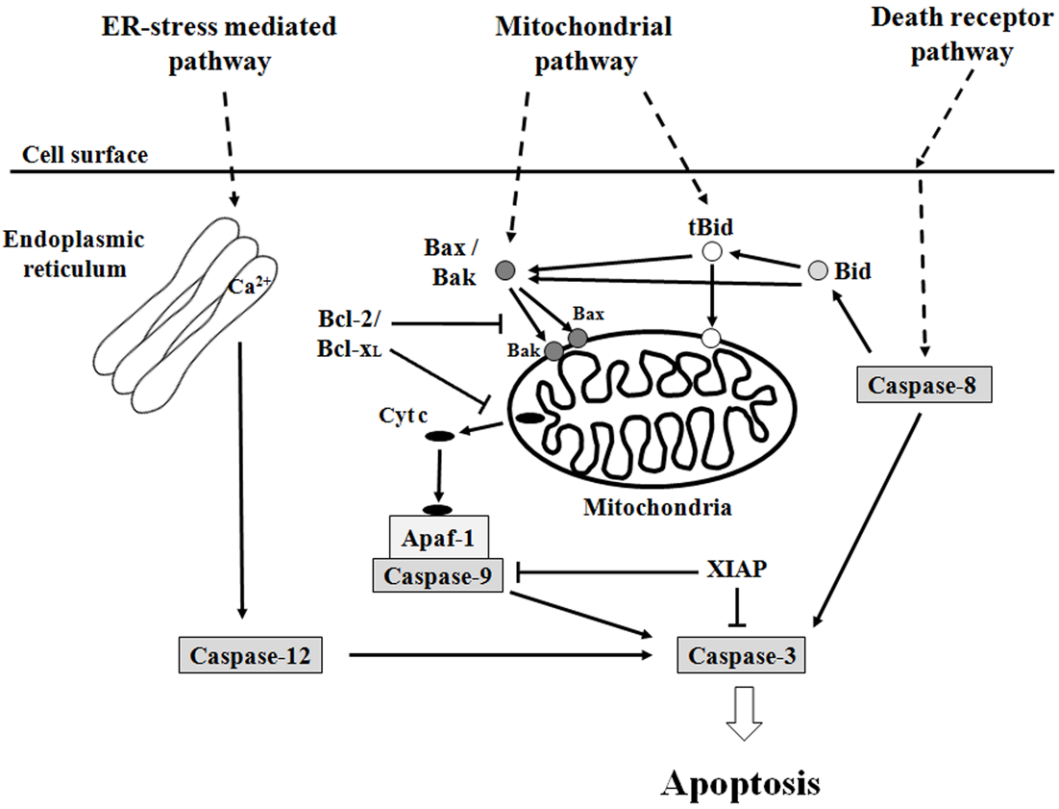

Fig. 2. Molecular pathway of apoptosis. This scheme shows the three main pathways of apoptosis: mitochondrial pathway, death receptor pathway, and ER-stress mediated pathway.

-12 activate the downstream effector caspases such as caspase-3,-6 and -7. Finally, the activation of these effector caspases leads to the execution of apoptosis [20]. The other prominent apoptosis-relating proteins are known as the "Bcl-2 family," which consists of pro- and anti-apoptotic members [21,22].

7. Process Development for the Inhibition of Apoptosis

Although various CHO cell lines expressing recombinant proteins show tremendous cell growth and protein productivity in advanced serum-free media, cell death and a reduction in productivity due to serum deprivation are still major problems. Cell death derived from serum deprivation is usually a form of apoptosis. Furthermore, transcription-promoting chemicals, such as sodium butyrate, are sometimes added to the CHO cell culture to improve productivity; however, these chemicals induce apoptosis at high concentrations. Therefore, many biotechnologists have tried controlling cellular apoptosis by regulating apoptosis-related components in cells to reduce cell death and improve recombinant protein production [23,24].

Optimization of the culture process is necessary to prevent cellular apoptosis. For this purpose, an appropriate culture mode should be selected. In addition, medium optimization is also necessary. In the batch culture process, nutrients become exhausted and the pH in the medium decreases as the culture time progresses due to the production of acidic wastes, such as lactate and glutamate, which are produced from the cells during the culture process. These changes lead to cellular apoptosis. Therefore, bioreactors equipped with a pH controller need to be used and other culture modes, such as fed-batch and perfusion culture mode, are applied rather than normal batch culture. The concentration of nutrients can be controlled in the fed-batch culture, and toxic wastes can be removed in the perfusion culture.

8. Inhibition of Apoptosis Using Additives

Inhibition of apoptosis is an effective method to increase recombinant protein productivity in mammalian cells. Many methods have been developed to inhibit apoptosis (Table 1). Continuous feeding of essential amino acids is a beneficial strategy to inhibit apoptosis induced by the depletion of nutrients [25]. Zanghi et al. reported that suramin, a polysulfated naphthylurea, protects CHO cells from apoptosis during the exponential growth phase [26]. Also, Balcarcel et al. demonstrated that rapamycin reduces hybridoma cell death in addition to arresting the cell in the G1-phase [27]. Other kinds of additives for the inhibition of apoptosis include inhibitors of caspases, which play a central role in apoptosis through the activation of various apoptosis-related intracellular proteins. Tinto et al. found that the addition of caspase inhibitors Ac-DEVD-cho and z-VAD-fmk reduces the apoptosis of hybridoma cells deprived of glutamine [28]. Sauerwald et al. reported that spent medium-induced apoptosis of CHO and HEK-293 cells are delayed in the presence of the caspase-8 inhibitor, z-IETD-fmk and the caspase-9 inhibitor, z-LEHD-fmk [29]. Although cellular viability was highly retained in the presence of these caspase inhibitors, a significant increase in protein productivity was not obtained [30]. This is believed to be due to the activity of the mitochondria. Mitochondria play a central role in the ATP generation and ATP-dependent biosynthetic metabolism. Although caspase activations, downstream events in apoptosis, are inhibited by

Table 1. Various methods for inhibiting apoptosis

Method for inhibition of apoptosis		Cell line	Ref.
Medium supplementation	Suramin	CHO	[26]
	Rapamycin	Hybridoma	[27]
	Glutamine feeding	CHO	[25]
	Caspase inhibitors	CHO, HEK-293	[29]
	Silkworm hemolymph	CHO	[51]
Gene expression	Bcl-2	BHK, CHO, Hybridoma	[32-34]
	Bcl-x_L	CHO	[35]
	XIAP	CHO, HEK-293	[29]
	CrmA	CHO, HEK-293	[29]
	Hsp70	NS0	[36]
	Akt	CHO	[37]
	30Kc6	CHO	[52-55]
Gene knock-out	Caspase-3	CHO	[38]
	Caspase-7	CHO	[39]

caspase inhibitors, the disruption of mitochondrial membrane integrity may already occur by the upstream apoptosis signals. Then, this reduced mitochondrial activity causes the lower production of the target protein.

9. Genetic Engineering for the Inhibition of Apoptosis

In recent reports, methods to effectively inhibit apoptosis through genetic engineering have been demonstrated. The expression of a gene coding for an anti-apoptosis factor can modify the cell death response and delay apoptosis in mammalian cell cultures. The X-linked inhibitor of apoptosis protein (XIAP) is an inhibitor of caspase-9, -3, and -7, and overexpression of *xiap* in the cells reduced the activation of capase-9, -3, and -7. It was also reported that the overexpression of *crmA*, a cytokine response modifier that inhibits caspase-8, also inhibits CHO cell death from growth factor withdrawal-induced apoptosis [29,31]. Bcl-2 and Bcl-x_L are prominent anti-apoptotic proteins that inhibit the release of pro-apoptotic proteins into the cytoplasm from the mitochondria. It has been shown that Bcl-2 is an anti-apoptotic protein that protects cells from various apoptotic stimuli, including UV-irradiation, cytotoxic drug, heat and the mis-regulated oncogenes as well as the serum deprivation [32,33]. Many researchers have reported that the overexpression of *bcl-2* and *bcl-x_L* leads not only to the inhibition of apoptosis but also to a significant increase in protein productivity [34,35]. This occurs because Bcl-2 acts upstream of apoptosis, including in the mitochondria. In addition, the overexpression of various heat shock proteins (Hsp) has been demonstrated to reduce the formation of reactive oxygen species (ROS) and to neutralize cell toxicity [36]. It was recently reported that the overexpression of *Akt* (protein kinase B, a serine/threonine kinase), one of the major survival proteins, has the ability to inhibit apoptosis in antibody-producing CHO cells [37]. The inhibition or knock-out of pro-apoptotic genes is also useful for reducing cell death and increasing protein productivity as well as the expression of anti-apoptotic genes. For example, it was shown that the down-regulation of only caspase-3 and both caspase-3 and -7 by small interference RNA (siRNA) inhibits sodium butyrate-induced CHO cells' apoptosis [38,39].

10. Inhibition of Apoptosis Using Proteins and Genes Derived from Silkworm

Researchers have attempted to use insect hemolymph as a substitute for mammalian serum such as fetal bovine serum (FBS) because mammalian serum is very expensive and may contain dangerous components such as viruses that can cause fatal diseases in humans. Silkworm hemolymph is the most well-understood insect hemolymph; thus, there have been several studies that examined

Table 2. Anti-apoptotic properties of silkworm hemolymph and its components. 30 K proteins and SP proteins are the major proteins in silkworm hemolymph. 30 K proteins consist of five similar proteins (30Kc6, 30Kc12, 30Kc19, 30Kc21 and 30Kc23), and SP proteins consist of two proteins (SP 1 and SP 2)

Apoptosis-inhibiting material		Cell line	Apoptosis inducer	Ref.
Silkworm hemolymph (SH)		Sf9	Baculovirus	[40,41]
			Actinomycin D, Camptothecin, Staurosporine	[42]
		HeLa	Vaccinia virus	[43]
30 K protein	30Kc19 purified from SH	Sf9	Baculovirus	[44]
	Recombinant 30Kc6 produced by *E. coli*	Sf9	Baculovirus, Actinomycin D	[45,46]
		HeLa	Staurosporine	
	Expression of *30Kc6*	HEK-293, CHO-K1	Staurosporine	[47]
	Recombinant 30Kc19 produced by *E. coli*	Sf9	Actinomycin D	[48]
SP protein	SP 2 purified from SH	HeLa	Staurosporine, Hydrogen peroxide	[50]

the use of silkworm hemolymph as a medium additive instead of FBS. Our group found that silkworm hemolymph and its components inhibit apoptosis (Table 2). Silkworm hemolymph was shown to inhibit baculovirus-induced insect cell apoptosis as well as replacing FBS [40,41]. Furthermore, it has been found that silkworm hemolymph inhibits apoptosis in insect cells induced by various chemicals such as camptothecin, actinomycin D and staurosporine [42] and apoptosis of the human cell line induced by vaccinia virus [43]. Using size exclusion- and ion exchange-chromatography, silkworm hemolymph was fractionized and the anti-apoptotic activity of each fraction was tested. The fraction that had the highest anti-apoptotic characteristics was identified using N-terminal amino acid sequencing, and the identified protein was shown to have a 95% homology with one of the so-called "30 K proteins" [44]. 30 K proteins are a group of structurally related proteins that are a kind of plasma protein in silkworm hemolymph, which have molecular weights of approximately 30 kDa. The cDNA coding for each 30 K protein was synthesized from mRNA in silkworm fat body cells using RT-PCR [45]. Among the five 30 K proteins, 30Kc6 was used as a representative 30 K protein in order to examine the anti-apoptotic activity of this group of proteins [46]. The *30Kc6* gene was introduced into mammalian cells and expressed. It was shown that apoptosis induced by the cytotoxic chemical, straurosporine, was inhibited by the expression of *30Kc6* [47]. Moreover, recombinant 30Kc19, a typical 30 K protein, also inhibited apoptosis in insect cells [48]. Consequently, it was demonstrated that the 30 K protein originating from silkworm has the ability to inhibit apoptosis in various animal cells [49]. Recently, SP proteins, another major group of proteins in the silkworm hemolymph, were also shown to have an anti-apoptotic effect in mammalian cell systems [50].

11. Enhancement of Recombinant Protein Productivity by Inhibiting Apoptosis

Based on the results described above, we have attempted to inhibit CHO cell apoptosis and enhance recombinant protein productivity during an industrial CHO cell culture process using silkworm hemolymph, 30 K proteins and its genes (Table 3). In these experiments, we found that silkworm hemolymph increased cell viability when apoptosis was induced by serum deprivation. Moreover, silkworm hemolymph inhibited the release of cytochrome c from the mitochondria to the cytosol, an upstream event in apoptosis. Consequently, caspase activation and DNA fragmentation, which are downstream events, were inhibited. It was also demonstrated that productivity of recombinant proteins was increased by the silkworm hemolymph [51]. In recent studies, the effect of stable expression of 3*0Kc6* on CHO cell growth and recombinant protein production was examined. After introduction of *30Kc6* genes into the erythropoietin-producing CHO cells, several selection steps to eliminate clones that were not able to express the *30Kc6* gene were performed. Finally, one clone that showed stable expression of *30Kc6* was selected and was then compared with the control clone, which did not express *30Kc6*. In these experiments, it was shown that cell growth was promoted and viability was maintained by expressing *30Kc6*. Furthermore, the specific production rate increased significantly as well as the total production of erythropoietin [52]. These results may have occurred because the 30 K protein could act upstream of the apoptosis pathway as was shown through supplementation of silkworm hemolymph containing the 30 K protein. The expression of *30Kc6* showed positive effects on not only the recombinant protein productivity but also the mitochondrial membrane potential and ATP generation. In addition, some case studies that examined the effect of 30Kc6 on cell growth and protein productivity were performed. Park et al. demonstrated that expression of *30Kc6* resulted in anti-apoptotic and productivity-enhancing effects in various commercial serum-free medium systems [53]. Koo et al. tried to apply these effects to suspension culture systems [54]. After several *30Kc6*-expressed CHO cell clones producing interferon-β were selected, cell growth and interferon-β productivity of each clone were analyzed. Compared with the control clone, all of the *30Kc6*-expressing clones showed higher values in cell longevity and interferon-β productivity in the suspension culture when serum-free media were used. Furthermore, the clones with higher levels of *30Kc6* expression showed greater effects when compared with the clones that had lower levels of expression. These results suggest that these beneficial effects are not due to physiological variation among several clones, but rather to the expression of *30Kc6*. Recently, Wang et al. showed that the expression of *30Kc6* inhibited cell death induced by serum deprivation and led to increased monoclonal antibody production in CHO cell systems [55].

CONCLUSIONS

The biopharmaceutical market has continued to grow at a rate of over 20% per year. Although microbial systems were previously used as the host for recombinant protein production, many recent products are being produced from mammalian cells. To enhance productivity in the mammalian cell system, numerous efforts and studies are being conducted in several industries and laboratories. Through advanced gene amplification methods, the *dhfr* gene and MTX selection system, as well as suspension culture techniques, low productivity can be overcome. Moreover, recently developed serum-free media, which leads to increased productivity, has allowed for the possible elimination of animal serum in cultures. The inhibi-

Table 3. Inhibition of apoptosis and enhancement of recombinant protein production using silkworm hemolymph and 30 K proteins

Apoptosis-inhibiting material	Method	Recombinant product	Culture mode	Ref.
Silkworm hemolymph	Medium supplementation	Erythropoietin	Adhesion (2-phase)	[51]
30Kc6	Gene expression	Erythropoietin	Adhesion (2-phase)	[52]
	Gene expression	None (host cell)	Adhesion	[53]
	Gene expression	Interferon-β	Suspension	[54]
	Gene expression	Monoclonal antibody	Adhesion (2-phase)	[55]

tion of apoptosis is another important strategy for enhancing productivity. In addition to the development of culture protocols such as nutrient feeding or the removal of toxic metabolites, the genetic engineering of host cells is being intensively investigated based on the understanding of the mechanism of apoptosis. For example, it has been shown that the expression of *bcl-2*, an anti-apoptotic protein, inhibited CHO cell apoptosis and led to increased recombinant protein production. In this review article, we have summarized the anti-apoptotic activities of proteins and genes derived from silkworm. It was shown that 30 K protein blocks the apoptosis pathway upstream, and thus this protein not only inhibits apoptosis efficiently but also increases protein productivity similar to other anti-apoptotic proteins such as Bcl-2. Through these efforts in the past decade, recombinant protein productivity in the CHO cell system has increased enormously. However, many challenges still remain. For instance, control over protein glycosylation is a very important topic although a higher productivity is still required. It is anticipated that many studies will be conducted to overcome these challenges and will lead to an improvement in therapeutic protein production using CHO cells.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (No. 2009-0081997, 2010-0000825). This work was also supported by the Pioneer Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science, and Technology (MEST) (No. 2009-0082946).

REFERENCES

1. G. Walsh, *Nat. Biotechnol.*, **21**, 865 (2003).
2. F. M. Wurm, *Nat. Biotechnol.*, **22**, 1393 (2004).
3. D. K. Robinson and K. W. Memmert, *Biotechnol. Bioeng.*, **38**, 972 (1991).
4. T. H. J. Kwaks and A. P. Otte, *Trends Biotechnol.*, **24**, 137 (2006).
5. T. J. Wilson and I. Kola, *Methods Mol. Biol.*, **158**, 83 (2001).
6. G. Urlaub and L. A. Chasin, *Proc. Natl. Acad. Sci. USA*, **77**, 4216 (1980).
7. G. Urlaub, E. Kas, A. M. Carothers and L. A. Chasin, *Cell*, **33**, 405 (1983).
8. M. G. Pallavicini, P. S. DeTeresa, C. Rosette, J. W. Gray and F. M. Wurm, *Mol. Cell. Biol.*, **10**, 401 (1990).
9. C. Gandor, C. Leist, A. Fiechter and F. A. M. Asselbergs, *FEBS Lett.*, **377**, 290 (1995).
10. M. Wirth, J. Bode, G. Zettlmeissl and H. Hauser, *Gene*, **73**, 419 (1988).
11. S. M. Browne and M. Al-Rubeai, *Trends Biotechnol.*, **25**, 425 (2007).
12. M. S. Sinacore, D. Drapeau and S. R. Adamson, *Mol. Biotechnol.*, **15**, 249 (2000).
13. M. J. De Jesus, P. Girard, M. Bourgeois, G. Baumgartner, B. Jacko, H, Amstutz and F. M. Wurm, *Biochem. Eng. J.*, **17**, 217 (2004).
14. N. I. Walker, B. V. Harmon, G. C. Gobe and J. F. Kerr, *Methods Achiev. Exp. Pathol.*, **13**, 18 (1988).
15. A. H. Wyllie, R. G. Morris, A. L. Smith and D. Dunlop, *J. Pathol.*, **142**, 66 (1984).
16. S. A. Susin, N. Zamzami and G. Kroemer, *Biochim. Biophys. Acta*, **1366**, 151 (1998).
17. X. Liu, C. N. Kim, J. Yang, R. Jemmerson and X. Wang, *Cell*, **86**, 147 (1996).
18. A. Ashkenazi, *Nat. Rev. Cancer*, **2**, 420 (2002).
19. S. Oyadomari, E. Araki and M. Mori, *Apoptosis*, **7**, 335 (2002).
20. J. M. Adam and S. Cory, *Curr. Opin. Cell Biol.*, **14**, 715 (2002).
21. T. Subramanian and G. Chinnadurai, *J. Cell. Biochem.*, **89**, 1102 (2003).
22. E. H. Y. Cheng, B. Levine, L. H. Boise, C. B. Thomson and J. M. Hardwick, *Nature*, **379**, 554 (1996).
23. A. J. Mastrangelo and M. J. Betenbaugh, *Trends Biotechnol*., **16**, 88 (1998).
24. N. Arden and M. J. Betenbaugh, *Trends Biotechnol.*, **22**, 174 (2004).
25. A. Sanfeliu and G. Stephanopoulos, *Biotechnol. Bioeng.*, **64**, 46 (1999).
26. J. A. Zanghi, W. A. Renner, J. E. Bailey and M. Fussenegger, *Biotechnol. Prog.*, **16**, 319 (2000).
27. R. R. Balcarcel and G. Stephanopoulos, *Biotechnol. Bioeng.*, **76**, 1 (2001).
28. A. Tinto, C. Gabernet, J. Vives, E. Prats, J. J. Cairo, L. Cornudella and F. Godia, *J. Biotechnol.*, **95**, 205 (2002).
29. T. M. Sauerwald, G. A. Oyler and M. J. Betenbaugh, *Biotechnol. Bioeng.*, **81**, 329 (2003).
30. S. L. McKenna and T. G. Cotter, *Biotechnol. Bioeng.*, **67**, 165 (2000).
31. J. Vives, S. Juanola, J. J. Cairo and F. Godia, *Metab. Eng.*, **5**, 124 (2003).
32. J. Vives, S. Juanola, J. J. Cairo, E. Prats, L. Cornudella and F. Godia, *Biotechnol. Prog.*, **19**, 84 (2003).
33. A. J. Mastrangelo, J. M. Hardwick, F. Bex and M. J. Betenbaugh, *Biotechnol. Bioeng.*, **67**, 544 (2000).
34. Y. H. Sung and G. M. Lee, *Biotechnol. Prog.*, **21**, 50 (2005).
35. B. Figueroa, T. M. Sauerwald, G. A. Oyler, J. M. Hardwick and M. J. Betenbaugh, *Metab. Eng.*, **5**, 230 (2003).
36. E. B. Lasunskaia, I. I. Fridlianskaia, Z. A. Darieva, M. S. R. da Silva, M. M. Kanashiro and B. A. Margulis, *Biotechnol. Bioeng.*, **81**, 496 (2003).
37. S. O. Hwang and G. M. Lee, *J. Biotechnol.*, **139**, 89 (2009).
38. N. S. Kim and G. M. Lee, *Biotechnol. Bioeng.*, **78**, 217 (2002).
39. Y. H. Sung, J. S. Lee, S. H. Park, J. Koo and G. M. Lee, *Metab. Eng.*, **9**, 452 (2007).
40. W. J. Rhee, E. J. Kim and T. H. Park, *Biotechnol. Prog.*, **15**, 1028 (1999).
41. W. J. Rhee and T. H. Park, *Biochem. Biophys. Res. Commun*., **271**, 186 (2000).
42. W. J. Rhee, E. J. Kim and T. H. Park, *Biochem. Biophys. Res. Commun*., **295**, 779 (2002).
43. S. S. Choi, W. J. Rhee and T. H. Park, *Biotechnol. Prog*., **18**, 874 (2002).
44. E. J. Kim, W. J. Rhee and T. H. Park, *Biochem. Biophys. Res. Commun.*, **285**, 224 (2001).
45. E. J. Kim, H. J. Park and T. H. Park, *Biochem. Biophys. Res. Commun*., **308**, 523 (2003).
46. H. J. Park, E. J. Kim, T. Y. Koo and T. H. Park, *Enzyme Microb. Technol*., **33**, 466 (2003).
47. E. J. Kim, W. J. Rhee and T. H. Park, *Biotechnol. Prog.*, **20**, 324 (2004).

48. W. J. Rhee, E. H. Lee and T. H. Park, *Biotechnol. Bioprocess Eng.*, **14**, 645 (2009).
49. E. J. Kim and T. H. Park, *Biotechnol. Bioprocess Eng.*, **8**, 76 (2003).
50. W. J. Rhee, E. H. Lee, J. H. Park, J. E. Lee and T. H. Park, *Biotechnol. Prog.*, **23**, 1441 (2007).
51. S. S. Choi, W. J. Rhee and T. H. Park, *Biotechnol. Bioeng.*, **91**, 793 (2005).
52. S. S. Choi, W. J. Rhee, E. J. Kim and T. H. Park, *Biotechnol. Bioeng.*, **95**, 459 (2006).
53. J. G. Park, S. S. Choi and T. H. Park, *Process Biochem.*, **42**, 8 (2007).
54. T. Y. Koo, J. H. Park, H. H. Park and T. H. Park, *Process Biochem.*, **44**, 146 (2009).
55. Z. Wang, J. H. Park, H. H. Park, W. Tan and T. H. Park, *Process Biochem.*, **in press**, doi:10.1016/j.procbio.2010.03.029 (2010).

Tai Hyun Park is a Professor in the School of Chemical and Biological Engineering at Seoul National University in Korea. He received his B.S. degree (Seoul National University, Korea), M.S. degree (KAIST, Korea), and Ph.D. degree (Purdue University) all in Chemical Engineering and was a postdoctoral fellow at the University of California at Irvine. He worked for several years at the LG Biotech Research Institute and taught at Sung Kyun Kwan University before joining Seoul National University in 1997. He was a visiting professor at the University of California at Irvine and Cornell University. His research interests include cellular engineering, olfactory and taste biosensor, nanobiotechnology, biohydrogen, and biorefinery.