Cytotechnology **32:** 109–123, 2000.
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Advances in animal cell recombinant protein production: GS-NS0 expression system

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Received 2 September 1999; accepted 2 September 1999

Key words: gene amplification, glutamine synthetase, methionine sulphoximine, NS0, productivity, recombinant protein

Abstract

The production of recombinant proteins using mammalian cell expression systems is of growing importance within biotechnology, largely due to the ability of specific mammalian cells to carry out post-translational modifications of the correct fidelity. The Glutamine Synthetase-NS0 system is now one such industrially important expression system. Glutamine synthetase catalyses the formation of glutamine from glutamate and ammonia. NS0 cells contain extremely low levels of endogenous glutamine synthetase activity, therefore exogenous glutamine synthetase can be used efficiently as a selectable marker to identify successful transfectants in the absence of glutamine in the media. In addition, the inclusion of methionine sulphoximine, an inhibitor of glutamine synthetase activity, enables further selection of those clones producing relatively high levels of transfected glutamine synthetase and hence any heterologous gene which is coupled to it. The glutamine synthetase system technology has been used for research and development purposes during this decade and its importance is clearly demonstrated now that two therapeutic products produced using this system have reached the market place.

Abbreviations: cAMP, cyclic adenosine monophosphate; CHO, Chinese Hamster Ovary; DHFR, dihydrofolate reductase; ECACC, European Collection of Animal Cell Cultures; FEM, Fortified Eagles Media; GS, glutamine synthetase; hCMV, human cytomegalovirus; MOPC21, mineral oil induced plasmacytoma accession number 21; MSX, methionine sulphoximine; SV40, simian virus 40.

Introduction

The drive to produce greater quantities of recombinant proteins of the correct fidelity for therapeutic use has led to major advances in heterologous expression systems. Largely due to the absolute requirement for correct post-translational modifications and processing, mammalian cells are commonly used (Page, 1988). However, the efficiency with which DNA is taken up into mammalian cells varies between different cell types (Kucherlapati and Skoultchi, 1984) and a method of selecting for cells which have taken up and stably express the DNA of interest is beneficial. A range of biochemical selectable markers, some of which also allow amplification of the exogenous DNA in response to a toxic drug, have been developed. The industrial production of many commercially valuable proteins focuses largely on two such markers, dihydrofolate reductase (DHFR) (Page and Sydenham, 1991) and glutamine synthetase (GS) (Cockett et al., 1990). DHFR is a non-dominant marker and hence is best used in cells which lack any endogenous DHFR. For most cell lines this generally means producing DHFR minus mutants such as the $DHFR^-$ Chinese Hamster Ovary (CHO) cell line, CHO-DUK (Urlaub and Chasin, 1980). GS can act as a dom-

inant selectable marker and hence can theoretically be used in cells which contain an active endogenous GS gene (Bebbington and Hentschel, 1987), as well as cells which are endogenous GS minus. It is most commonly used in CHO cells and NS0 (Non-secreting) myeloma cells. Using the GS system only a single round of amplification, taking typically around 3 months, is often sufficient to result in efficient levels of recombinant protein expression (Bebbington et al., 1992) and in a lot of cases amplification is not necessary at all. However, multiple rounds of amplification are needed with the DHFR system to achieve similar levels of expression and this can increase development time up to around 6 months (Dorai and Moore, 1987). In addition, the GS system usually requires fewer copies of the selectable marker and recombinant protein to obtain efficient levels of production than the DHFR system does. Typically between 4–10 copies are found per cell for the GS system after amplification (Bebbington et al., 1992; Brown et al., 1992) compared to several hundred copies for the DHFR system (Kaufman et al., 1985; Kingston et al., 1994). Also, accumulation of final product has been reported to be around five times more efficient for the GS system compared to the DHFR system (Hodgson, 1993). Due to these reasons, and production levels obtained, the GS expression system is today generally regarded as a high yielding system for the rapid production of recombinant proteins.

The GS system has been used extensively in CHO cells (Table 1). However the myeloma NS0 cells are often favoured as, unlike CHO cells, these cells contain such low levels of GS that phenotypically they are GS minus (Bebbington et al., 1992). Hence, as will be described later, this means they require a lower level of toxic drug to allow selection of cells expressing high levels of exogenous gene. The efficiency of production in NS0 cells is thought to be due to the fact that NS0 cells were originally derived from immunoglobulin-producing tumour cells and are well equipped for producing and secreting proteins. Another advantage of using myeloma cells is that they are suspension cells that can be adapted to grow in serum-free, protein-free culture to high biomass in fermenters which is particularly beneficial in industry (Broad et al., 1991). Finally it is also worth noting the genomic plasticity of CHO cells, which may lead to instability of production, as has been noted for the CHO-DHFR system (Pallavicini et al., 1990).

The GS technology was developed by scientists at Celltech and Lonza Biologics (formally known as Celltech Biologics). The technology, in particular the GS-NS0 system, is most commonly used within the biotechnology industry and recently therapeutic products produced using the GS-NS0 system have been licensed for the market place. This paper aims to review the GS-NS0 expression system by consideration of the history and nutritional requirement of the cell line, the principles of the GS system which make it so successful and its use for the production of proteins for both research and therapeutic purposes.

NS0 cell history

The discovery by Potter and Boyce in 1962 that injection of mineral oil into the intraperitoneal region of BALB/c mice induced plasma-cell neoplasms was the starting point for the development of the NS0 cell line (Potter and Boyce, 1962). The histology of the development of these plasmacytomas was later detailed in 1964 (Potter and MacCardle, 1964). The sequence of events which led to the development of this cell line are represented in Figure 1.

Tumours were induced within inbred BALB/c female mice by the Animal Production Unit of the National Institute of Health, U.S.A. One such tumour, MOPC21 (mineral oil induced plasmacytoma, accession number 21), was found to secrete IgG_1 (Potter et al., 1965) and Horibata and Harris then went on to use this tumour to establish a continuous tissue culture line (Horibata and Harris, 1970). Initially, the MOPC21 tumour cells were cultured in Fortified Eagles Media (FEM) supplemented with 20% horse serum which resulted in a population with a doubling time of 7 days. Subsequently, the cells were maintained as ascites in mice before re-culture in FEM media supplemented with 10% horse serum. After a few weeks of culture, the cell doubling time was decreased to 16 hours and during a subsequent 3 years period of stationary culture the cells maintained this doubling time (Horibata and Harris, 1970). This heterogeneous population of cells, which grew in suspension, were called P3K cells. The P3K cells, which were shown to synthesize and secrete IgG_1 (Horibata and Harris, 1970), were then cloned and gave rise to P3-X27, a clone that also secreted IgG_1 (Ramasamy et al., 1974). Subsequent re-cloning of P3-X27 gave rise to two cell lines, 289–16 and P3-X63, which were developed separately (Ramasamy et al., 1974).

The 289–16 cell line did not secrete IgG_1, synthesizing the light chain but no heavy chain. At this

Table 1. Examples of Recombinant Protein Expression Using the GS System in NS0 and CHO cells

Products	References	
	GS-NS0	GS-CHO
Antibodies (murine and humanised)	Field et al., 1991; Bebbington et al., 1992; Brown et al., 1992; Hassell et al., 1992; Birch et al., 1993; Sims et al., 1993; Bibila et al., 1994a, b; Birch and Froud 1994; Peakman et al., 1994; Robinson et al., 1994a, b, c; Yoon and Konstantinov, 1994; Yu Ip et al., 1994; Ellis et al., 1995; Lifely et al., 1995; King et al., 1995; Pearce et al., 1995; Robinson et al., 1995; Stephens et al., 1995; Young et al., 1995; DiStefano et al., 1996; Downham et al., 1996; Keen and Hale, 1996; Konstantinov, 1996; Anon, 1997; Duncan et al., 1997; Johnson et al., 1997; Pilson et al., 1997; Ray et al., 1997; Reimann et al., 1997; Zhou et al., 1997; Lonza Press Release, 1998; Paterson et al., 1998.	Bebbington, 1991; Owens et al., 1991; Brown et al., 1992; Hassell et al., 1992; Birch et al., 1993; King et al., 1993; Burton et al., 1994; Ortlepp et al., 1995.
Protease and Inhibitors	Murphy et al., 1991a, b, 1992a, b; Gofton et al., 1992; O'Shea et al., 1992; Nguyen et al., 1993; Willenbrock et al., 1993; Apte et al., 1995; Knäuper et al., 1996; Baker et al., 1997.	Field et al., 1989; Cockett et al., 1990; Jenkins and Hovey, 1993a, b; Hovey et al., 1994a, b.
Cytokine, Hormones and Growth Factors	Hirayama et al., 1994; Rossmann et al., 1996; Zhou et al., 1996; Cannon-Carlson et al., 1998.	McKnight and Classon, 1992;, McInnes et al., 1993; Castro et al., 1995; Morrison et al., 1995; Kim et al., 1997; Orlinick et al., 1997; Salas et al., 1997.
Cell Surface Markers and Receptors	Robinson et al., 1992; Flesher et al., 1995; Tan et al., 1995; Rhode et al., 1996; Hamilton et al., 1997; Shi et al., 1997.	Moore et al., 1989; Williams et al., 1989; Davis et al., 1990; Froud et al., 1991; Classon et al., 1992; Gastinel et al., 1992; Gjörloff et al., 1992; Gloor et al., 1992; Harfst et al., 1992a, b, c; Harfst and Johnstone 1992; McCall et al., 1992; Trowbridge et al., 1992; Ashford et al., 1993; Davis et al., 1993; Feany et al., 1993; Gutman et al., 1993; Kemble et al., 1993; Quilliam et al., 1993; Van der Merwe et al., 1993; Brown and Barclay, 1994; Crouch et al 1994; Fahnestock et al., 1994; Lange et al., 1994; Skonier et al., 1994; Berg et al., 1995; Cosgrove et al., 1995; Fahnestock et al., 1995; Guerini et al., 1995; Siemers et al., 1997; Haumont et al., 1996; McAlister et al., 1998a, b; Pu et al., 1998; Stanley and Hogg 1998.
Intracellular Proteins	Murray et al., 1996.	Cockett et al., 1991; Laubach et al., 1996.
Other		Blochberger et al., 1997.

stage the cell line was renamed NSI/1 (Cowan et al., 1974) and clones from this cell line, which were resistant to 8-azaguanine, were isolated, one of which also expressed only intracellular κ light chains. This cell line, called P3/NSI/1-Ag4-1 (Köhler et al., 1976), was cloned once more to generate a subline which did not secrete or synthesize heavy or light chains of Ig and hence this murine myeloma cell line was named NS0/1 (Non-Secreting) cells (Galfrè and Milstein, 1981). Table 2 summarizes the immunoglobulin

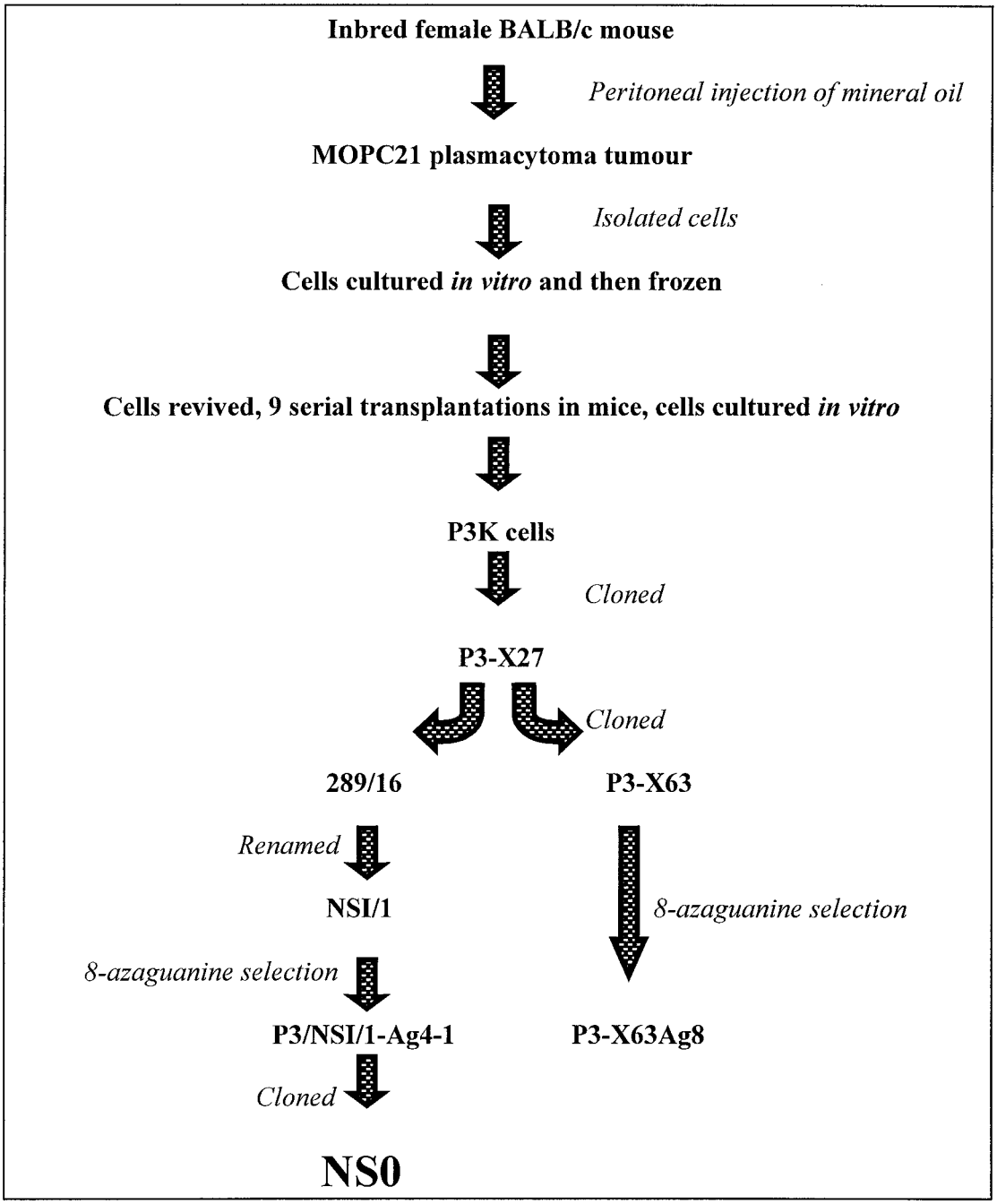

Figure 1. Historical development of the NSO cell line.

production from cell lines generated during the development of the NS0 cell line and, where relevant, catalogue numbers from the European Collection of Animal Cell Cultures (ECACC).

Myeloma cells are known to be efficient fusion partners for the production of hybrids (Köhler and Milstein, 1975, 1976). As a consequence, NS0 cells have been used for a number of years as fusion partners for the production of hybridoma cells producing monoclonal antibodies (Cazzola et al., 1992; Cloeck-

Table 2. Cell lines generated during the development of the NS0 cell line

Cell line	Description of Ig Production	ECACC number (where appropriate)
MOPC21	Secretes IgG_1	-
P3K	Secretes IgG_1	-
P3-X27	Secretes IgG_1	-
P3-X63	Secretes IgG_1	-
P3-X63Ag8	Secretes IgG_1	85011401
289/16 (NSI/1)	Non-secreting, synthesises light κ chain	-
P3/NSI/1-Ag4-1	Non-secreting, synthesises light κ chain	85011427
NS0	Non-secreting, non-synthesising	85110503

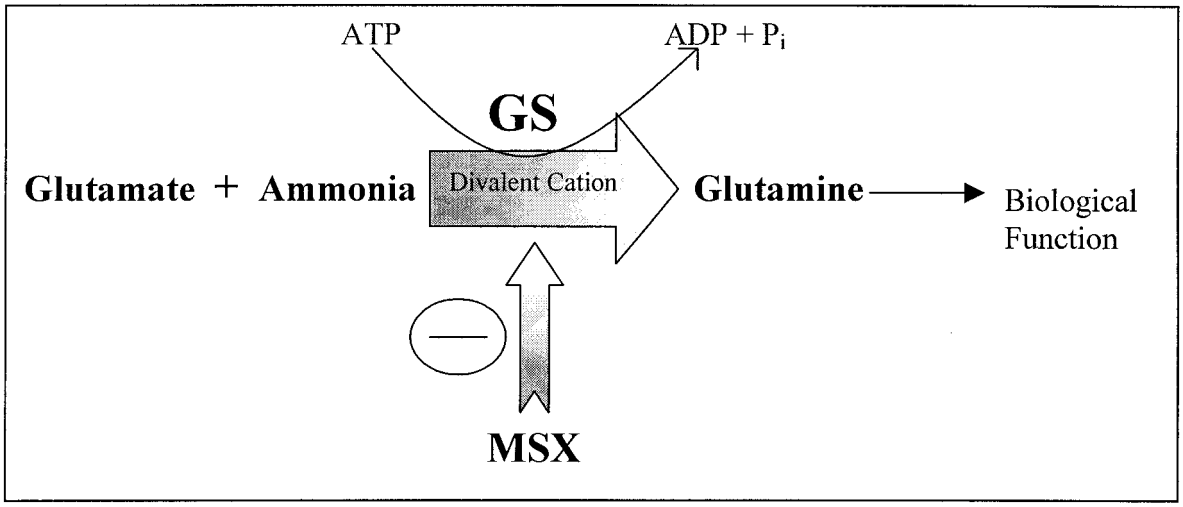

Figure 2. Production of glutamine by glutamine synthetase.

aert et al., 1992; Lyaku et al., 1992; Richards et al., 1992; Zumla et al., 1992; Cloeckaert et al., 1993; Thole and Jakob, 1993; Porro et al., 1994; Green et al., 1995; Ghebrehiwet et al., 1996). However, NS0 cells are now an important host cell for the engineered production of recombinant proteins in their own right, particularly in combination with the GS selection system.

Glutamine and glutamine synthetase (GS): their importance to cell metabolism

The cellular metabolism and function of glutamine is of crucial importance to mammalian cells. It is a non-essential amino acid and hence is synthesised by a large variety of cells and its role within cells is considerable. For example, it is significant in nitrogen metabolism as it provides a source of nitrogen for many biosynthetic pathways. Glutamine is important in protein synthesis, purine and pyrimidine biosynthesis, ammonia formation, the biosynthesis of amino acids, amino sugars and certain cofactors and also for the degradation of amino acids, as well as certain special processes such as phenylacetyl-glutamine formation (Meister, 1974, 1980). In addition, the storage and transport of glutamate and ammonia, as well as the removal of ammonia, all revolve around this amino acid. It is also important to stress that the carbon chain of glutamine can serve as a significant energy source for cells (Reitzer et al., 1979; Zielke et al., 1980).

The enzymatic synthesis of glutamine is reversible (Levintow and Meister, 1954) and requires hydrolysis of ATP (Speck, 1947, 1949; Elliott, 1948, 1951). The reaction is catalyzed by the enzyme GS (Figure 2),

which is a universal housekeeping enzyme that consists of between 8–12 sub-units. These can range from 42,000–50,000 Da. each depending on the source (Meister, 1980; Pu and Young, 1989); however, there is a high degree of sequence homology between mammalian GS forms (Meister, 1974).

The mechanism of the GS-catalyzed formation of glutamine has been suggested to occur via a γ-glutamyl phosphate enzyme-bound intermediate (Todhunter and Purich, 1975; Midelfort and Rose, 1976), with glutamate being phosphorylated before reacting with ammonia to form glutamine. The ammonia group which reacts with glutamate is provided by asparagine. The full mechanism of action of GS is detailed by Meister (Meister, 1974).

Glucocorticosteriods, insulin and cAMP are among the regulatory signals that are known to affect GS levels (Crook et al., 1978; Miller et al., 1978). Also the specific activity of GS in certain cell lines is inversely proportional to the level of glutamine present with glutamine regulating glutamine synthetase at the post-transcripitional level (Feng et al., 1990).

The importance of glutamine to cell survival (Butler and Jenkins, 1989) and the very low levels of endogenous GS expression within NS0 cells (Bebbington et al., 1992) means these cells have an absolute requirement for exogenous glutamine. This feature has facilitated the use of GS as a biochemical selectable marker.

The principles of the GS-NS0 system

The GS-NS0 system relies on the fact that cells containing very low levels of endogenous GS, when grown in glutamine-free media, require either an exogenous source of glutamine or exogenous GS in order to survive. This is due to the extremely low frequency of generation of natural glutamine-independent cell variants, for example in the case of NS0 cells less than 1 in 10^7 (Bebbington et al., 1992). The incorporation of a GS gene in a plasmid vector containing the gene of a heterologous protein allows selection of cells, in glutamine-free media, that have taken up the plasmid during transfection and are stably expressing the GS gene and hence the heterologous protein. The construction of the plasmid vector can have a great influence on productivity. The GS coding sequence is usually under the control of a weak promoter (e.g. SV40), however the heterologous protein coding sequence(s) is often under the control of a powerful hCMV promoter (Brown et al., 1992; Keen and Hale, 1996) (Figure 3). This theoretically ensures that successful transfectants, surviving in glutamine-free media using the weakly-transcribed GS gene, should produce reasonable levels of heterologous protein due to the powerful hCMV promoter. To minimise promoter occlusion (Kadesch and Berg, 1986; Proudfoot, 1986) the GS sequence can be placed upstream of the recombinant gene (Bebbington et al., 1992).

Generally, the number of plasmid vectors taken up by a cell is low, typically only 1 copy per cell (Brown et al., 1992). However, the GS gene, as well as allowing selection of cells that have taken up the vectors, also allows selection of clones which have high GS expression levels. These high levels may be a result of relatively few copies of the transfected GS gene integrating into particularly active sites within the host cell DNA or of several copies of the GS gene at less active sites. Selection of high GS expressing cells may also be achieved using Methionine Sulphoximine (MSX), which is a specific inhibitor of GS activity (Brown et al., 1992). For the GS-NS0 system the copy number usually increases from 1 to 4–10 copies per cell (Bebbington et al., 1992; Brown et al., 1992). In addition, increase in GS gene copy number as a result of gene amplification caused by increased levels of MSX has been noted (Stark and Wahl, 1984; Bebbington and Hentschel, 1987; Simonsen and McGrogan, 1994). Any gene that is co-transfected with this GS-amplifiable marker, such as a recombinant heterologous protein, will be amplified simultaneously. This leads to an increase in the production level of the heterologous protein due to an increase in its mRNA levels and this in turn is caused by amplification of the copy number of its gene.

The irreversible inhibition of GS by MSX is a result of MSX being phosphorylated by GS. It occurs in the presence of ATP and divalent metal ions. The structure of MSX and its phosphorylated form is given in Figure 4. The mechanism of this inhibition has been extensively studied (Ronzio and Meister, 1968; Manning et al., 1969; Ronzio et al., 1969; Rowe et al., 1969; Rowe and Meister, 1973) and MSX is considered to be an inhibitory analog of the intermediate formed in the normal GS catalyzed reaction. During the reaction the sulfoximine nitrogen of MSX occupies the same site in GS as the oxygen of phosphorylated glutamate. Only the L, S-isomer of MSX is phosphorylated on the sulfoximine nitrogen to give MSX phosphate (Manning et al., 1969) and this can then bind to GS irreversibly in the presence of ADP

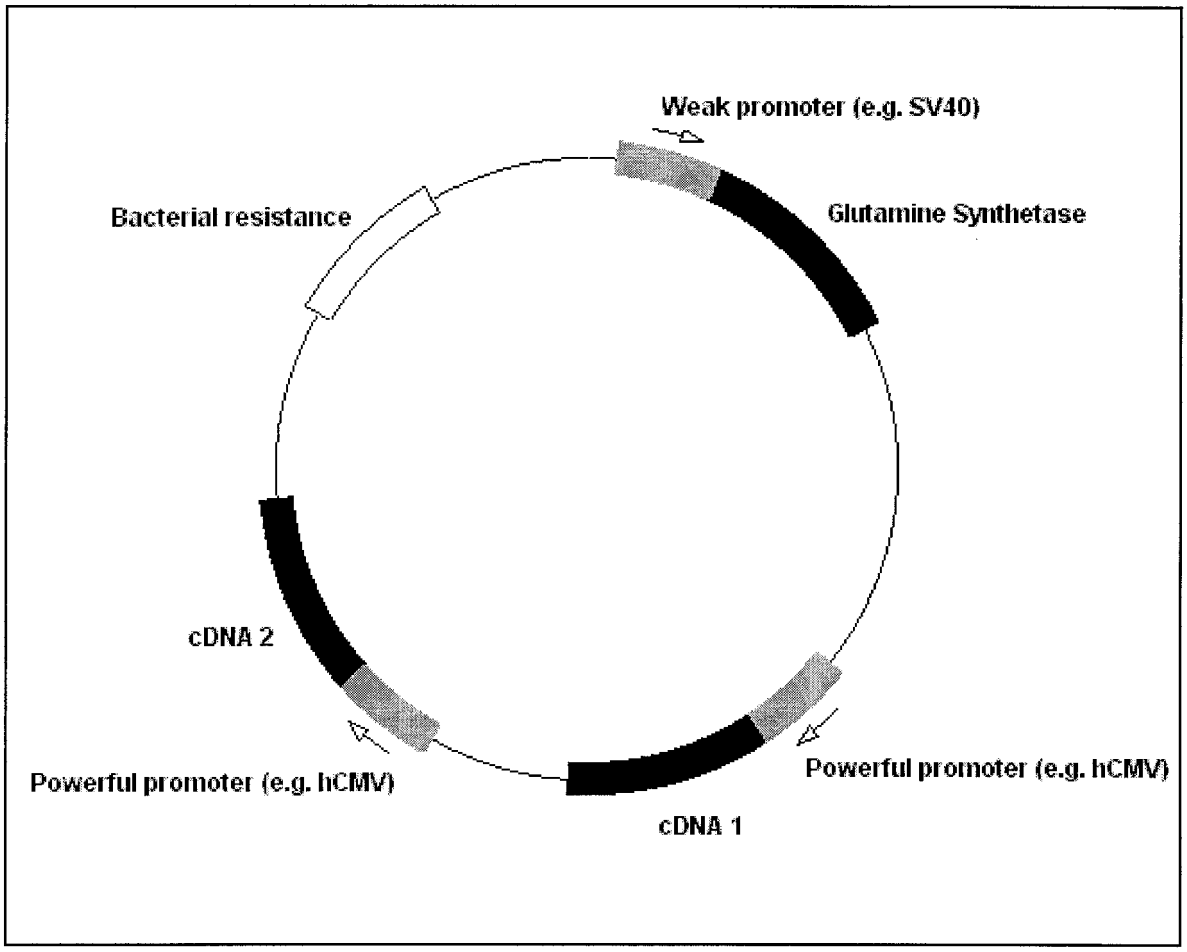

Figure 3. A typical expression vector used with the GS-NSO expression system for the production of multisubunit proteins such as immunoglobulins.

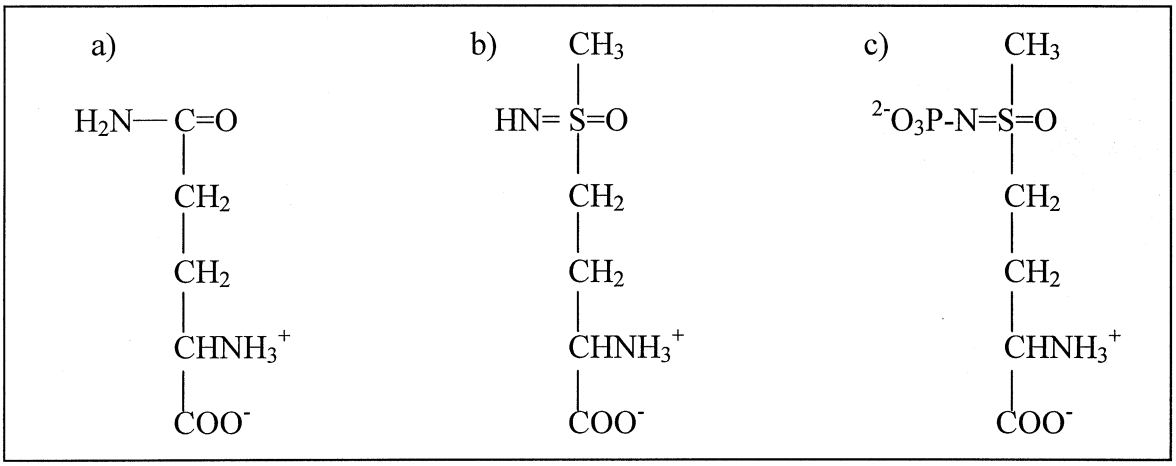

Figure 4. Structure of a) glutamine, b) MSX, c) MSX Phosphate.

and hence block subsequent GS activity.

Due to negligible endogenous GS activity in NS0 cells, concentrations of 10–100μM MSX are typically used and further selection in higher levels of MSX does not appear to increase productivity or copy number of the recombinant gene (Bebbington et al., 1992; Brown et al., 1992). This level of MSX is low compared to levels required in GS-CHO systems which range between 250–500μM MSX due to endogenous GS present within CHO cells (Cockett et al., 1990; Bebbington et al., 1992).

The GS-NS0 system has been reported to be stable in terms of antibody production in suspension culture for at least 65 generations in the absence of MSX (Bebbington et al., 1992). Removal of MSX, once amplification is achieved, eliminates the need to assay for MSX in the final product and reduces the cost and any possible toxic effects on the cells. However, reports concerning the CHO-DHFR system have suggested that the removal of selective pressure generated by the presence of toxic drugs can lead to instability (Weidle et al., 1988).

Product optimisation: the importance of culture conditions

The nutritional requirements of NS0 cells are important factors relating to optimisation of cell growth and productivity. There is little detailed information available in the literature on specific requirements for GS-NS0 cell lines as this work is largely performed within an industrial context. Such information is therefore a closely guarded secret as the derivation of the best media for productivity and growth is something which can be both time consuming and costly. However, some generalities can be made about the requirement of the cell line as a whole. Due to safety, cost, consistency, efficiency and regulatory approval, industrial companies favour the growth of these cells in serum-free media (Broad et al., 1991). Also, protein-free media is beneficial due largely to purification considerations.

NS0 cells are cholesterol auxotrophs (Keen and Steward, 1995), although reports of some cholesterol-independent variants have been noted (Birch et al., 1994; Keen and Steward, 1995). However, generally for the successful growth of these cells cholesterol has to be included in media. Due to the low solubility of cholesterol in aqueous solution, this is usually achieved in the form of complex protein solutions. It has been reported that addition of phosphatidylcholine: cholesterol vesicles with the carbohydrate carrier cyclodextrin to the media aids growth (Keen and Hale, 1996). However, adverse effects on cell viability have been noted when using cholestrol containing vesicles, for example due to lipid precipitation which can cause an increase in cell death (Keen and Hale, 1996).

The nutritional requirements of wild-type NS0 and NS0 cells transfected with GS-containing vectors have been noted to differ widely (Gould et al., 1992; Robinson et al., 1994a). Wild-type NS0 cells contain extremely low levels of GS activity, a feature which the GS system exploits. Thus, whereas wild-type NS0 cells need to be cultured in media which contains glutamine, NS0 cell lines transfected with GS-containing vectors no longer have this requirement and can survive without exogenous glutamine in the media by utilizing more glutamate and asparagine. GS-NS0 cells produce low levels of lactate and hardly any ammonia when grown in glutamine-free media (DiStefano et al., 1996). Asparagine is thought to be used by these cells as a metabolic source of ammonia, which is combined with glutamate to produce glutamine using the GS catalyzed reaction (Figure 2). The requirement for asparagine has been suggested to be due to the possible defects of alternative ammonia producing pathways within myeloma cells (Bebbington et al., 1992).

Recent characterisation of the nutrient requirements of wild-type and transfected NS0 cells has arisen from analysis of the effects of nutrient deprivation in causing or enhancing death by apoptosis (Mercille and Massie, 1994; Robinson et al., 1994a; DiStefano et al., 1996). The exact feeding strategy and conditions of culture are specific for each cell line (Robinson et al., 1994a), however successful nutrient control has been noted to increase monoclonal antibody productivity substantially in GS-NS0 cell lines sometimes by up to 10-fold (Robinson et al., 1994a). During batch culture of recombinant GS-NS0 cells asparagine, cystine, histidine, isoleucine, methionine, valine and, in particular, glutamate and leucine have been noted to be rapidly depleted (Keen and Hale, 1996). However amino acid consumption rates can vary depending on cellular growth state (Robinson et al., 1994a). Medium osmolarity has also been reported to affect NS0 cell growth and secretion of products, and culture under certain hyperosmotic conditions can potentially increase productivity of the cells (Bibila et al., 1994a; Duncan et al., 1997).

Therefore it is clear that maintaining these cells in a viable productive state for extended periods of time can be achieved to some degree by the optimization of culture conditions and this can often lead to substantial increases in productivity.

Products generated using the GS-NS0 system

As indicated in Table 1, a range of products have now been generated using the GS system, both in NS0 cells (most commonly for recombinant antibodies) and in CHO cells (most commonly for receptors and cell surface markers). Scientists at Celltech and Lonza Biologics (formerly Celltech Biologics) developed the GS expression system in the late 1980s. However, since then it has been licensed to a number of biotechnology companies, for example, Merck, Bayer Corporation, Hoffmann-La Roche, GlaxoWellcome and MedImmune. As a consequence, the majority of publications using the GS system, and in particular the GS-NS0 system, have been performed by or in collaboration with biotechnology companies.

Among the early papers on the GS-NS0 and GS-CHO systems yields of 560 mg/l for a recombinant antibody produced by the GS-NS0 system in fed-batch airlift fermenter (Bebbington et al., 1992), and 180 mg/l for tissue inhibitor of metalloproteinase produced by the GS-CHO system in shake flasks (Cockett et al., 1990) were reported. However, it is important to bear in mind that the method of culture can potentially affect productivity. In addition, care must be taken when comparing production of different antibodies from cells as slight changes to amino acid sequence have been noted to influence secretion rates (Hendershot et al., 1987; Nakaki et al., 1989). In summarising all the published production values it is clear that amplification using MSX results in an increase in production values. For the GS-CHO system in batch culture these range from an average of 70 mg/l for unamplified cell to 270 mg/l for amplified lines (Field et al., 1989, 1991; Brown et al., 1992; Birch et al., 1993). Similar observations have been made for the GS-NS0 system where average expression levels in batch culture of 210 mg/l have been recorded in unamplified lines but amplification resulted in an increase to an average of 510 mg/l (Brown et al., 1992; Birch et al., 1993; Bibila et al., 1994a; Robinson et al., 1994a, b). In contrast to the GS-CHO system there has been considerable optimisation of fed-batch conditions for the GS-NS0 system which has resulted in substantial increases in production. Various fed-batch protocols increase productivity for amplified lines to an average of 1300 mg/l (Bebbington et al., 1992; Brown et al., 1992; Robinson et al., 1994b; Zhou et al., 1997). Fed-batch regimes can also enhance productivity of unamplified GS-NS0 cell lines (from about 210 mg/l to 640 mg/l on average) (Robinson et al., 1994a, b; Zhou et al., 1996). Hence, careful optimisation of culture conditions, especially for the GS-NS0 system can generate greatly enhanced productivity. Values of 2.7 g/l of monoclonal antibody has recently been reported for GS-NS0 cells in fed-batch culture (Zhou et al., 1997). Therefore it is clear that coupling of culture optimisation to the GS-NS0 system provides great promise for future recombinant protein production.

The importance of, and the need for, a review of the GS-NS0 system subject area was prompted by the arrival on the market of two therapeutic products produced using this system. Zenapax® (dacliximab) is a humanised monoclonal antibody for the prophylaxis of acute organ rejection which was marketed in December 1997. This product is produced by Hoffmann-La Roche and represented the first licensing of a human therapeutic produced using the GS system (Anon, 1997; Lonza Press Release, 1998). Since then a second product, Synagis™ (palivizumab) produced by MedImmune has reached the market. This is a humanised monoclonal antibody used in pediatric patients to prevent serious lower respiratory tract disease caused by respiratory syncytial virus and is the first monoclonal antibody licensed for any infectious disease (Lonza Press Release, 1998).

GS-NS0 is a heterologous mammalian expression system that allows rapid high level expression of recombinant proteins using a dominant selection system and is not restricted to use in mutant cell lines. The fact that two products generated using the GS-NS0 system are now on the human therapeutics market highlights the acceptance of this system by regulatory authorities. It is expected that, in the coming years, more announcements will be made of products reaching the market which have been generated from this system and as time goes by the frequency with which these statements are made is expected to increase.

Acknowledgments

We would like to thank the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK and GlaxoWellcome for financial support. We would

also like to thank Justin Newton and Stephen J. Froud for resolving discrepancies in the literature concerning the genealogy of the NS0 cell line.

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