Increased production of recombinant hIGFBP-1 in PEG induced autofusion of Chinese hamster ovary (CHO) cells

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Abstract

A recombinant Chinese hamster ovary (CHO) cell clone, S1, stably expressing human insulin-like growth factor binding protein-1 (hIGFBP-1), was treated with polyethylene glycol (PEG), resulting in cell fusion, in order to further enhance the protein expression by increasing the gene copy number and/or the amount of organelles important to the protein expression/-secretion. Both the fused cell line, Peg1, and its mother cell line, S1, were adapted to serum-free growth in suspension and were characterised with respect to growth and productivity. Peg1 was easier to adapt to the serum-free suspension conditions and had a higher viability during the adaptation period than S1. Furthermore, Peg1 showed a stable productivity of hIGFBP-1 that was twice as high as that for S1 under both adherent and suspension conditions. A considerable difference in the specific productivity (up to 3–4 times) was noticed during the growth phase. PEG fusion experiments have earlier been studied in our laboratory with CHO cells producing recombinant factor VIII and our results correlates very well with the results obtained with the factor VIII producing cells. Surprisingly, it was possible to obtain high producing recombinant cell lines, which were stable for more than 4 months.

Introduction

Large scale production of recombinant proteins in mammalian cells requires high expression levels for a successful process. A common approach to obtain high expression is to increase the gene copy number e.g. by gene amplification (dihydrofolate reductase, glutamine synthetase, etc.) (Sharma and Schimke, 1994). The cell productivity increases at higher gene copy number but it does not increase linearly (Gu et al., 1992; Dyring et al., 1997). The cells seem to reach a limit at which they cannot make use of any extra gene copies. However, when using the dihydrofolate reductase (DHFR) gene amplification system, increased expression can be achieved in cells cultured over a very long period of time in MTX containing medium. This does not increase the gene copy number. A likely explanation is, however, that the expression and/or secretion rate increases (Dyring et al., 1997).

Fusion of two different cell lines is a well-known method to achieve a new cell line with characteristics from both parent cells. Somatic cell fusion is also used to enhance specific properties of one of the parent cells. The most common use is in the establishment of hybridoma cells for the production of monoclonal antibodies. Cell fusion as a method of enhancing protein production has been described by Chenciner et al., 1990. They produced classical hybrids between a liver cell and a transfected Vero cell. The stable hybrid produced an increased amount of hepatitis B surface antigen. A number of different cell fusion protocols are applicable; the use of polyethylene glycol (PEG), protoplast or Sendai virus has been described by Kennett, 1979, electrofusion by Borraeack and Hagen, 1989, and by Chang et al., 1992. These authors describe the use of classical fusion of two different cells, as a means of obtaining an increased production of a particular product. Stoicheva and Hui, 1994, have described the fusion of CHO cells by the use of an exponen-

tially decaying electric pulse in the presence of PEG, Dextran or Ficoll. They studied the effect of different concentrations and molecular weights of the agents on cell aggregation. PEG causes cell shrinking and aggregation, and after removal of the PEG, cell fusion takes place (Robinson et al., 1979; Krahling, 1981; Hui et al., 1985). A direct interaction of PEG with membrane lipids is assumed to cause a decrease of the membrane fluidity (Ohno et al., 1981). The assembly of cell membranes, stabilised by water, is disturbed by strong interaction with the polymer molecules having a strong hydration power, resulting in membrane fusion (Nakajima and Ikada, 1994).

Human IGFBP-1 is a 26 kDa IGF binding protein with a total of 234 amino acids (Luthman et al., 1993). It acts as an acute modulator of the metabolic activities of IGF. To understand the regulation of IGF bioactivity at the cellular level, it is necessary to study the IGFBPs. In this paper we present a study on the self-fusion of cells from a CHO line with cells from the same line leading to an increased production of recombinant hIGFBP-1.

Materials and methods

Origin of the S1 mother cell population

A CHO cell clone, S1, stably producing human IGFBP-1 for more than four months was used for these experiments: DHFR-deficient CHO cells (DG44 N.Y., a generous gift from D.L. Chasin) were co-transfected with an IGFBP-1 expression vector and a dhfr-vector. For gene amplification, selective medium + 0.1 μg TPA l^{-1} (TPA = phorbol 12-myristate 13-acetate) was added to the cells with stepwise increasing concentrations of MTX up to 100 nm MTX. The tumour promoter, TPA, is shown to enhance the frequency of MTX resistance and DHFR gene amplification (Varshavsky, 1981). Colonies were picked and the one used in this paper was named S1.

PEG fusion of S1 cells

Cells were seeded in 225 cm^2 tissue culture flasks at a concentration of 0.2×10^6 cells ml^{-1} in 60 ml growth medium (DMEM/F12 (1:1) + 5% BCS (Hyclone, USA)). The medium was replaced every second day with fresh medium. When the cell culture reached around 90% confluence, the cells were detached from the surface by trypsinisation and washed twice with PBS

in a sterile plastic tube. The fusion protocol developed by Adamson and Spira, 1994, was followed. Polyethylene glycol (PEG₁₅₀₀, Sigma, USA), 1 ml, was added dropwise to the pellet during 1 min. The tube was left for 1 min before it was gently rolled back and forth for 3 min. Slowly, the pellet was resuspended in 60 ml fresh growth medium and seeded in a 225 cm^2 tissue culture flask. The cell population was named Peg1. The following day, the procedure was repeated with Peg1 giving Peg2 and repeated again the next day with Peg2 giving Peg3. As a negative control, 1 ml PEG was added to a sub-confluent cell culture in a 6 ml tissue culture flask and was removed after 3 min. Growth medium (6 ml) was then added to the tissue culture flask.

Morphology, size and growth of the cells

The morphology of the cells in the tissue culture flasks was studied in a phase contrast microscope (Olympus LH50A, Japan) provided with a Polaroid camera (Olympus OM-2, Japan) with Polaroid 667 films (Polaroid, UK). In order to determine the cell size, cells were cultured in tissue culture flasks to around 70% confluence. They were detached from the surface by trypsinisation, washed twice with PBS and resuspended in PBS to a concentration of 10^4 cells ml^{-1} . The cell sample of about 10 000 cells was measured on the cell analyser system, CASY 1 (Schärfe System, Germany). The number and viability of the cells in suspension were determined by trypan-blue exclusion. The growth rate was determined as mean $[(t_2 - t_1)^{-1} \times \ln(C_{V,2} \times C_{V,1}^{-1})]$, where C_V was the concentration of viable cells and t was the time interval between time 2 and time 1.

Adaptation of the cells to serum-free suspension growth

S1 and Peg1 were seeded in 225 cm^2 tissue culture flasks at a concentration of 0.2×10^6 cells ml^{-1} in 60 ml growth medium. The medium was replaced every second day with fresh medium. When the cell culture reached around 90% confluence, the cells were detached from the surface by trypsinisation and transferred to 50 ml defined serum-free medium, named XDYC4 medium (Table 1), with 8 g l^{-1} casein peptone in 250 ml shake flasks (Corning, UK) at a concentration of around 0.5×10^6 cells ml^{-1} . The shake flasks were then placed on a rotating table (40 rpm) in an incubator with 5% CO_2 and 95% humidity. Sam-

Table 1. The composition of the defined serum-free medium, XDYC4, used for suspension cultivations of S1 and Peg1. DMEM, Hams F12 and glutamine was from Gibco, Scotland; HSA, FeCitrate and ascorbic acid from Pharmacia, Sweden; synperonic from Serva, Germany; glucose and hepes from Sigma, USA; Insulin from Novo Nordisk, Denmark and aprotinin from Pentapharm, Switzerland

XDYC4 medium
DMEM/Hams F12 (1:1)
0.4% HSA
0.5 g l ⁻¹ synperonic
a total of 4.5 g l ⁻¹ D-glucose
a total of 8 mM L-glutamine
3.6 g l ⁻¹ hepes
0.6 mM FeCitrate/0.24 mM
Ascorbic acid
10 mg l ⁻¹ Insulin
75 000 KIU l ⁻¹ Aprotinin

ples were taken every day and the cell number was determined. Samples were frozen for later hIGFBP-1 analysis. The medium was fully replaced every second day. After four days, the cells began to grow as single cells and the medium was changed to XDYC4 medium with 4 g l⁻¹ casein peptone for eight days and then changed to XDYC4 medium without casein peptone. Later on, the cells were transferred to 250 ml spinner flasks in 50 ml XDYC4 medium and cultured herein.

BIAcore analysis

Real-time biospecific interaction analysis (BIA) has been used to quantify the amount of hIGFBP-1 produced by the cells as described in Dyring et al. (1997). The BIAcoreTM system from Pharmacia Biosensor AB was used. IGF-I (100 µg ml⁻¹, Pharmacia, Sweden) was immobilised on Sensor Chip CM5 which was then blocked with 1 M Ethanolamine Hydrochloride. Standards of hIGFBP-1 (Pharmacia, Sweden) and undiluted samples were injected. Mab anti-hIGFBP-1 (100 µg ml⁻¹, 6303 Medix Biochemica, Finland) was injected after each sample and the sensor chip was prepared for a new sample by washing with HCl.

Southern blot analysis

DNA was extracted from exponentially growing cells by using the Qiagen Cell Culture DNA kit (Qiagen, USA). The procedure in the Qiagen genomic DNA handbook was followed. The method described in Dyring et al. (1997) was followed. The expression vector was cleaved with *Hind*III and *Bam*HI according to the manufacturer and electrofored on a low melting point gel. The 740 bp hIGFBP-1 fragment was isolated, transferred to an Eppendorf tube and labelled with Amersham kit (Amersham, USA). The extracted DNA (1 µg) was cleaved as above and a dilution series of the expression vector was made. A 1% horizontal agarose gel containing EtBr was loaded with Molecular Weight Marker VI (MW VI), different amounts of the expression vector and 1 µg DNA extracted from the cells. The gel was electrofored for 16 h at 30 V. The protein bands were transferred to a gene screen filter (Du Pont, Boston, USA) by the use of a vacuum blotter. The filter was air dried before hybridisation was carried out in hybridisation buffer supplemented with 200 µl ssDNA. The filter was transferred to a plastic film and exposed to X-ray film (DuPont Gronex 4, Du Pont, France) 48 h at -70 °C.

Immunoblotting

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) followed by immunoblotting was used to identify hIGFBP-1. Conditioned medium was mixed with 2× sample buffer (0.125 M Tris-HCl pH 6.8, 0.2% glycerol, 0.005% bromophenol blue, 4% (w/v) SDS, 5% beta-mercaptoethanol) and loaded onto a vertical Novex 12% gel (Novex, San Diego, USA) and electrofored 100 V in running buffer (3 g l⁻¹ Trisma-base, 14.35 g l⁻¹ glycine and 10% SDS). The protein gel was electrophoretically transferred onto Hybond-ECL nitro-cellulose filter (Amersham, UK) at 135 mA using a semi-dry electroblotter (Ancos, Denmark) and 5.8 g l⁻¹ Trisma-base, 4.3 g l⁻¹ glycine, 0.38 g l⁻¹ SDS and 200 ml l⁻¹ MetOH as transfer buffer. The filter was blocked in PBS-T (PBS + 0.1% Tween-20) + 5% milk powder (Semper, Sweden) and the filter was rinsed by washing several times with PBS-T. The filter was then incubated with Mab anti-hIGFBP-1 (6303) in PBS-T and washed as above. The filter was then incubated with peroxidase-conjugated donkey-anti-mouse (715-035-151, Jackson ImmunoResearch, Pennsylvania, USA) in PBS-T and washed as above. The filter was then incubated with

Figure 1. Different numbers of Peg2 cells fuse shortly after the PEG treatment of the cells. Bar = 100 μm .

ECL Western blotting detection Reagents 1 and 2 (Amersham, UK) and exposed to ECL film (Amersham, UK).

For PAGE, running buffer (10.8 g l^{-1} Trisma base, 5.5 g l^{-1} boric acid and 0.74 g l^{-1} EDTA), 2x sample buffer (0.4% running buffer, 20% glycerol and 0.1% bromophenol blue), and transfer buffer (3.94 g l^{-1} Trisma base and 14.4 g l^{-1} glycine) was used. The gels used was vertical Novex 16% gels.

Results

After treatment with PEG, cells were seeded in a tissue culture flask and immediately, the cells began to fuse as shown on Fig. 1. The picture is taken 5 min after the PEG treatment of Peg2 cells. A fusion between different number of cells can be seen. The cells rapidly attached to and spread on the surface of the tissue culture flask. The three obtained fusion cell populations, Peg1-3, appeared as heterogenous populations of non-fused and fused cells, and the fused cells seemed to be derived from different numbers of cells. The untreated S1 cells cultured adherently had a morphology characterised by a fibroblast-like appearance typically for CHO-DG44 cells cultured adherently (Fig. 2a). After the cell fusions, the appearance of the cells changed. Examples are given in Fig. 2b–d. The fused cells were larger than their mother cells, S1, and attached to the surface of the tissue culture flasks, cells with a diameter

Figure 2a. (a) S1 cells before the PEG treatment. The cells were cultured adherent in tissue culture flasks in DMEM/F12 (1:1) + 5% BCS (Hyclone, USA). Appearance of the fused cells: (b) Peg1, (c) Peg2 and (d) Peg3. With each PEG treatment, larger and more apart looking cells were obtained. Bar = 100 μm .

Figure 2b.

Figure 2c.

as big as $200\ \mu\text{m}$ could be seen. The plasma membrane was very diffuse and the cells appeared extremely thin except from a more defined area in the middle of the cell. This area contained a large amount of shining spots in different sizes, which were most probably different organelles.

Determination of the cell size showed, that the mean diameter of the cell population increased with each fusion step (Table 2). It was also seen, that the percentage of small cells with a mean diameter of $10\text{--}15\ \mu\text{m}$ decreased with each fusion step while the percentage of larger cells, $15\text{--}20$ and $20\text{--}25\ \mu\text{m}$, increased. Analysis of the gene copy number by southern blotting one month after the PEG treatment of the cells showed that Peg1 had twice as many copies of the hIGFBP-1 gene as S1, while Peg2 and Peg3 had 1.5 and 1 times as many copies of the gene, respectively, as S1 (Fig. 3).

S1 and Peg1 was adapted to serum-free suspension cultivation and Fig. 4 shows the growth of S1 and Peg1 in 50 ml XDYC4 medium in 250 ml spinner-flasks. The results shown are a mean of three experiments performed simultaneously with each cell population. There seemed to be no difference in cell growth between the two cell populations. Both cell populations had a growth rate of $0.34\ \text{d}^{-1}$ and reached a maximum number of living cells of $2.3 \times 10^6\ \text{cells ml}^{-1}$. The viability of Peg1 was more than 90% during the adaptation to serum-free suspension growth while the

Figure 2d.

viability of S1 was 70–90% most of the adaptation period. Fig. 4 also shows the amount of IGFBP-1 that was produced during 48 h periods consecutively. During the growth phase, more than twice as much IGFBP-1 was produced from Peg1 than from S1 and the amount of IGFBP-1 produced in each 48 h production period was higher throughout the whole experiment. The total amount of hIGFBP-1 which was produced during the 15 days cultivation period was twice as high for Peg1 as for S1 as shown in Fig. 5. According to immunoblotting, there was no apparent difference in the hIGFBP-1 produced from S1 and Peg1 (results not shown).

Table 2. Cell size distribution of S1 and the three PEG-treated cell lines, Peg1-3, determined by the use of CASY 1 in a duplicate experiment. Each measurement was performed on between 10 607 and 12 083 cells. The results were normalised to 10 000 cells. The mean diameter/volume of the cells increased with increasing number of PEG treatments. The number of cells with mean diameter 10–15 μm decreased with increasing number of PEG-treatments while the number of larger cells increased

	S1	Peg1	Peg2	Peg3
Mean diameter (μm) of total cell population	12.4 $\pm$ 0.1	13.0 $\pm$ 0.1	13.3 $\pm$ 0.1	13.8 $\pm$ 0.1
Increase in mean diameter		5%	8%	11%
No. of cells with mean diameter 10–15 μm	7692	7030	6736	6155
Decrease in no. of cells with mean diameter 10–15 μm		9%	12%	20%
No. of cells with mean diameter 15–20 μm	1046	1601	1890	2118
Increase in no. of cells with mean diameter 15–20 μm		52%	81%	102%
No. of cells with mean diameter 20–25 μm	237	322	421	605
Increase in no. of cells with mean diameter 20–25 μm		36%	78%	155%
Mean volume (fl) of total cell population	1175	1380	1518	1729
Increase in mean volume		17%	29%	47%

Figure 3. Southern blotting analysis. Lane 1: Molecular weight marker VI, lane 2: S1, lane 3: Peg1, lane 4: Peg2, lane 5: Peg3 and lane 6: Molecular weight marker VI. For quantification, the bands in the autoradiograms were scanned, quantitated by determining the area under each peak and normalised to the S1 plasmid band.

Discussion

The described results show, that self-fusion with PEG of the stable recombinant cell line, S1, lead to a stable cell population, Peg1, with an increased specific hIGFBP-1 production and with cells that had an altered appearance. It was observed, that the Peg1 population contained cells that were larger than the S1 cells three weeks after the PEG-treatment. This indicates, that at

least a part of the fused cells had survived and that the average cell size had increased.

After months of cultivation, the mean Peg1 cells were still larger than the non-fused S1 cells (data not shown) and were stably producing increased amounts of hIGFBP-1. These results correlate very well with the results obtained by Adamson and Spira, 1994. They found that, by treating stable, recombinant factor VIII producing CHO cells with PEG₄₀₀₀, the mean diameter of the cells as well as the specific productivity increased. The increased size of the cells and the increased specific productivity of recombinant factor VIII were stable for more than five months in cultivation.

Southern blotting showed an increased number of hIGFBP-1 gene copies in Peg1 and Peg2 as a result of the cell fusion. As described by Robinson et al., 1979, and Stoicheva and Hui, 1994, multinucleate cells are formed after polyethylene-induced cell fusion. These cells which have two or more separate nuclei are called heterocaryons. Eventually, a heterocaryon will proceed to mitosis and produce a hybrid cell in which the separate nuclear envelopes have been disassembled, allowing all the chromosomes to be brought together in a single large nucleus (Alberts et al., 1994). However, such hybrid cells tend to be unstable and lose chromosomes at random giving rise to a variety of hybrid cell lines, each of which contains different numbers of chromosomes. A selection favoring cells with the most optimal number of chromosomes (if any) may occur with time. Our results indicate that a state where the cells have more than one set of chromosomes may be of advantage for some cells, since the mean gene copy

Figure 4. Growth and hIGFBP-1 production of S1 ($\square$) and PEG1 ($\blacksquare$) in 50 ml XDYC4 medium in 250 ml spinner flasks. The results shown are a mean of a triplicate experiment performed simultaneously with each cell line. No difference in growth was observed but PEG1 produced increased amounts of IGFBP-1.

number of Peg1 is twice that of S1 after one month of cultivation. Adamson and Spira (1994), however, did not find any increase in gene copy number or chromosome number in the PEG treated factor VIII producing cells.

The increased specific productivity of hIGFBP-1 in Peg1 could either be a direct result of the increased hIGFBP-1 gene copy number and/or a result of an increased number of organelles, in particular ER, mitochondria and even Golgi as described by Adamson and Spira, 1994. They studied the PEG-treated recombinant factor VIII producing cells with Transmission Electron Microscopy (TEM) and detected a larger number of high energetic mitochondria in the PEG cells, which indicated that these cells probably had a larger energy pool than their mother cells. The TEM studies were confirmed by FCM (flow cytometry) analyses of mitochondria in living cells after incubation with Rhodamin 123 and in fixed cells after incubation with antibodies against mitochondrion inner membrane. The PEG cells showed a much stronger fluorescence than the mother cell population. A further

support for this theory was energy level measurement performed by Adamson and Spira, 1994, that showed, that the amount of ATP/cell was higher for the PEG cells. An increased requirement for ATP in the larger, fused cells may lead to an increase in metabolic capacity and production of ATP and thereby an increase in protein expression rate.

The self-fusion also lead to cells which were less sensitive to adaptation to serum-free suspension growth, observed by the higher viability during the adaptation period. Based on the results obtained by Stoicheva and Hui, 1994, it is likely to think, that less than 20% of the S1 cells fused, which means that the fused cells in average produced more than 6 times as much hIGFBP-1 as their mother cells. Sorting out the 20–30% largest cells in the Peg1 population by e.g. FACS could most probably lead to a population of cells with a very high specific production of hIGFBP-1.

Peg2 and -3 cells had less hIGFBP-1 gene copies and a lower specific productivity than Peg1 cells. They were bigger and appeared more abnormal in the microscope than Peg1 cells. A maximum cell size where the

Figure 5. Accumulation of harvested hIGFBP-1 during the 15 days cultivation period in spinner flasks; S1 $\square$, PEG1 $\blacksquare$. The results shown are a mean of a triplicate experiment, see Fig. 4, performed simultaneously with each cell.

cells could survive, may have been reached with the first PEG treatment, so that the fused cells in Peg2 and -3 were too abnormal not to be outgrown by smaller, less abnormal cells. This could explain both why the gene copy number of the Peg3 cell population was close to that of S1 and why the specific productivity was lower in the cultures that had been repeatedly fused.

In order to study whether polyethylene glycol (PEG) itself induced higher specific productivity, PEG was added to the cells in such a way that the cells could not fuse (there was no contact between the cells). No increase in specific productivity was observed.

We further investigated whether the higher concentration of IGFBP-1 found in medium from Peg1 was a direct result of a lower protease activity in this cell population. The decrease in the hIGFBP-1 concentration throughout the week of incubation was small and did not differ between the cell populations.

Taken together, these results show that cell fusion by polyethylene glycol treatment induced a stable change in the expression capability of the hIGFBP-1 producing cells. Furthermore, cells which were easier to adapt to serum-free suspension culture and which were less sensitive to mechanical stress, were obtained.

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References

- Adamson L and Spira J (1994) Increasing protein expression by self fusion of producer cells to form stable cell lines providing higher yields of therapeutic protein. Patent No. WO 94/19459.
- Alberts B, Bray D, Lewis J, Raff M, Roberts K and Watson JD (eds) (1994) Molecular biology of the cell, third edition. Garland Publishing, New York.
- Borrbæck CAK and Hagen I (eds) (1989) Electromanipulation in hybridoma technology. Laboratory manual. Macmillan: Stockton Press, New York.
- Chang DC, Chassy BM, Saunders JA and Sowers AE (eds) (1992) Guide to Electroporation and Electrofusion. Academic Press, San Diego.
- Chenciner N, Delpeyrox F, Israel N, Lambert M, Lin A, Strecek RE and Houssais JF (1990) Enhancement of gene expression by somatic hybridization with primary cells: high-level synthesis of the hepatitis B surface antigen in monkey vero cells by fusion with primary hepatocytes. *Bio/Technology* 8: 858–862.
- Dyring C and Mellström K (1997) Recombinant expression of human insulin-like growth factor binding protein-1 (hIGFBP-1) in chinese hamster ovary (CHO) cells. (*Cytotechnology*, in press).
- Gu MB, Kern JA, Todd P and Kompala DS (1992) Effect of amplification of *dhfr* and lac Z genes on growth and beta-galactosidase expression in suspension cultures of recombinant CHO cells. *Cytotechnology* 9: 237–245.
- Hui SW, Isac T, Boni LT and Sen A (1985) Action of polyethylene glycol on the fusion of human erythrocyte membranes. *J. Membrane Biol.* 84: 137–146.
- Kennett RH (1979) in *Cell Culture, Methods in Enzymology LVIII*, Academic Press, San Diego. Jakoby WB and Pastan IH (eds), pp. 345–359.
- Krahling H (1981) Discrimination between two fusogenic properties of aqueous polyethylene glycol solutions. *Z. Naturforsch.* 36: 593–596.
- Luthman H, Söderling-Barros J, Persson B, Engberg C, Stern I, Lake M, Franzén S, Israelsson M, Rådén B, Lindgren B, Hjelmqvist L, Enerbäck S, Carlsson P, Bjursell G, Póvoa G, Hall K and Jörnvall H (1989) Human insulin-like growth-factor-binding protein. Low-molecular-mass form: protein sequence and cDNA cloning. *Eur. J. Biochem.* 180: 259–265.
- Nakajima N and Ikada Y (1994) Fusogenic activity of various water-soluble polymers. *J. Biomater. Sci. Polymer. Edn.* 6(8): 751–759.
- Ohno H, Shimidzu N, Tsuchida E, Sasakawa S and Honda K (1981) Fluorescence polarization study on the increase of membrane fluidity of human erythrocyte ghosts induced by synthetic water-soluble polymers. *Biochim. Biophys. Acta* 649(2): 221–228.
- Robinson JM, Roos DS, Davidson RL and Karnovsky MJ (1979) Membrane alterations and other morphological features associated with polyethylene glycol-induced cell fusion. *J. Cell. Sci.* 40: 63–75.
- Sharma RC and Schimke RT (1994) The propensity for gene amplification: a comparison of protocols, cell lines and selection agents. *Mutat. Res.* 304(2): 243–260.
- Stoicheva NG and Hui SW (1994) Electrically induced fusion of mammalian cells in the presence of polyethylene glycol. *J. Membrane Biol.* 140: 177–182.

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