Evaluation of stable and highly productive gene amplified CHO cell line based on the location of amplified genes

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Abstract
In order to establish an easy and quick construction method for obtaining a stable and highly productive gene-amplified recombinant Chinese Hamster Ovary (CHO) cell line, various kinds of stepwise methotrexate (MTX) selection were carried out. The specific growth and production rates of the cell were compared with each other, and the distribution of the amplified gene location was determined using fluorescence in situ hybridization (FISH). The specific growth and production rates of the cell pool reached the highest levels under the selection condition in which the stepwise increase in the MTX concentration was most gradual; about 82% of amplified genes were observed near the telomeric region. During long-term cultivation without MTX, the percentage of amplified genes near the telomeric region hardly changed, but that of amplified genes at other regions decreased. Based on these results, stable and highly productive cell pools could be easily and quickly constructed and amplified and gradual stepwise increase of the MTX concentration. In addition, the FISH technique was powerful tool to evaluate highly productive and stable gene-amplified cells based on the chromosomal location of the amplified gene.

Abbreviations: CHO, chinese hamster ovary; dhfr, dihydrofolate reductase; hGM-CSF, human granulocyte macrophage colony stimulating factor; FISH, fluorescence in situ hybridization; MTX, methotrexate.

Introduction
The amplified genes are usually located either within expanded chromosomal regions, termed homogeneously staining regions (HSRs), in abnormally banded regions (ABRs), or in extrachromosomal elements termed double minutes (DM) (Qumsiten et al., 1993; Misawa et al., 1987). Some molecular biological mechanism for gene amplification have been proposed, for example unequal sister chromatid exchange, telomeric fusions and bridge-breakage-fusion cycles, centromere recombination, and chromosome breakage-acentric element models (Kaufman, 1993; Mariani et al., 1984; Smith et al., 1990; Stark, 1993; Von Hoff, 1991; Windle et al., 1992). However, the relationship between the selection methods and the gene amplification mechanisms remains open to investigation.

In general, MTX-tolerant cell lines develop resistance against MTX owing to dhfr gene amplification. Other mechanisms of tolerance to MTX have been reported, such as the membrane-permeability mutation (Assaraf and Schimke, 1987), the expression of DHFR, with no- or low sensitivity to MTX (Haber et al., 1981). The appearance of these other tolerant cells might correspond to the increase method of MTX concentration.

Gene amplification techniques using recombinant mammalian cells are frequently employed for the production of glycoproteins (Cockett et al., 1990; Kane...
Figure 1. Construction of dhfr and hGM-CSF gene amplification vector. The pSV2-dhfr/hGM-CSF vector was constructed from pSV2-dhfr and pcD-hGM-CSF. The pSV2-dhfr vector contained dhfr cDNA derived from mouse and the pcD-hGM-CSF vector contained human GM-CSF gene.

et al., 1988; Kaufman et al., 1985; Kaufman, 1993), among which the dihydrofolate reductase (dhfr) gene amplification system in the Chinese Hamster Ovary (CHO) cell lines are the most widely used (Alt et al., 1978; Kaufman, 1993). In regard to the application of a gene amplification system for industrial processes, one of the most important factors is the selection method employed for obtaining highly productive recombinant CHO cell lines that can stably produce the desired recombinant proteins (Kaufman et al., 1982; Pallavicini et al., 1990; Rath et al., 1984; Weidle et al., 1988). However, the selection has so far been carried out only empirically.

Rath et al. (1984) investigated the time required for mouse 3T6 cells to develop resistance to 200 nM methotrexate (MTX) based on three selection protocols, and found that multistep selection was the most effective for rapid emergence of resistance. In single-step (rapid) selection, gene-amplified cell lines were not obtained, whereas in two or greater number of step selections, amplified dhfr genes were obtained. However, in these experiments, stability of the amplified genes in the absence of MTX was not evaluated. Using mammalian cells including CHO cells, Kaufman et al. (1985) and Pallavicini et al. (1990) examined the stability of amplified gene in the presence and absence of MTX (Pallavicini et al., 1990; Weidle et al., 1988), and concluded that the amplified genes were more stable when the cells were cultured with MTX.

In order to obtain a ‘gene-amplified’ cell pools, recombinant cell pools were cultivated in a selection medium containing MTX, which inhibits DHFR. With increase in MTX concentration most of the cells died, but a proportion in which dhfr was amplified, was able to survive in the medium containing MTX. In order to systematically construct highly productive cell pools, an optimal strategy for stepwise selection must be devised. Therefore, we carried out stepwise increase in MTX concentration under five different selection conditions (pattern 1 to 5 in Figure 2a) (Omasa et al., 1996). Therefore, in order to obtain a highly productive and stable cell, systematic evaluation of methods for the selection of a stable and highly productive cell is essential. We previously reported that the specific growth and production rates were affected by stepwise selection patterns (Omasa et al., 1996). In this experiment, we investigated the effect of the pattern of increase of the MTX concentration on the specific