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The role of p21^{cip1} in adaptation of CHO cells to suspension and protein-free culture

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Abstract

The up-regulation of cyclin-dependent kinase inhibitor p21 has been shown to enhance productivity of monoclonal antibodies and has been linked to various regulatory processes. To identify the potential role of p21 in adaptation to suspension and protein-free cultures, we studied the survival and growth of anchorage- and serum-dependent CHO cell lines that differed only in the period of p21-induced arrest. p21 overexpression led to rapid adaptation of cells to suspension and protein-free cultures. The period taken to achieve adaptation was correlated with the time the cells were arrested after transfer from the monolayer and serum-fed culture. Interestingly, cell aggregation associated with protein-free suspension culture was reduced in p21 culture in response to the loss of cellular adherence. The processes of adaptation to suspension and arrest did not decrease monoclonal antibody productivity. In contrast, following adaptation to protein-free growth media, an overall increase in specific productivity was observed. The ability of cells to survive in protein-free suspension cultures was due to the requirement of G1 cells to growth factors and to their relatively high resistance to the hydrodynamic forces. This improved process has the advantage of reducing the duration of critical path activity for developing CHO commercial cell lines from 72 to 36 days.

Keywords: p21^{cip1}; Cell cycle; Proliferation; Anchorage-dependent; Apoptosis; Protein free

1. Introduction

Animal cell cultures have been widely used to produce therapeutic protein products due to the compatibility of post-translational product modification with the therapeutic target; however, the need for increased yields and the requirement to eliminate animal-derived elements are driving industry forward in the search for suitable methodologies that can sustain high-yielding cell lines and speed up the development and production process.

Anchorage-dependent cells must be adapted to grow in suspension cultures due to the scale-up limitation associated with monolayer cultures. Cells which grow in suspension are readily scaled up to production level bioreactors, which are designed to reduce hydrodynamic forces and heterogeneity (Al-Rubeai et al., 1995; Ludwig and Andreas, 1992; Keane and Julian, 2003). Anchorage-dependent cells can be made to be sufficiently robust in the bioreactor suspension environment by a time-consuming adaptation process that includes the addition of protective agents, such as Pluoronic F68 to the media (Kilburn and Webb, 1968; Al-Rubeai et al., 1992). While the effects in suspension culture of hydrodynamic forces and the sensitivity of mammalian cells to hydrodynamic stress have been fully examined, and while the mechanism is well understood (see for example, Hu and Wang, 1986; Peterson et al., 1988; Handa-Corrigan et al., 1989; Oh et al., 1989; Croughan et al., 1989; Jordan et al., 1994; Kunas and Papoutsakis, 1990a,b; van der Pol et al., 1992), the adaptation process has remained essentially unchanged.

Interestingly, it would appear that the susceptibility of any given cell to the forces exerted by the bioreactor environment are increased by, for example, decreased serum concentration (Kunas and Papoutsakis, 1990a,b; McQueen and Bailey, 1989), culture medium viscosity (Croughan et al., 1989), and increased

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cell size (Al-Rubeai et al., 1995). The role of cell size in determining cellular growth, productivity and response to the bioreactor environment may have not been fully appreciated, but cell size regulation is shared with the cell cycle, which, in effect, determines the metabolic potential of a cell and, indeed, its response to the environment.

The ever-increasing need for improved biological safety is pushing industries forward in the development of new serum- and protein-free media formulations. Cell lines will have to be adapted to grow and produce the required product in a defined chemical environment. There have been several approaches for the adaptation of cell lines to the required environments (Kovar and Franek, 1984; Radford et al., 1991; Keen and Steward, 1995; Sinacore et al., 1999; Ozturk et al., 2003) either by a gradual weaning of cultures via the reduction of serum over a set time period or by direct withdrawal of serum. In both cases, the process of adaptation can be extremely long and costly, depending on the cell line and medium selected. Therefore, a process that can speed up the rate of adaptation would provide a valuable tool for both industrial and research applications.

Overall control of the cell cycle, and thus cellular proliferation, is based on the activity of cyclin-dependent kinases (cdks), which are activated by their association with cyclin regulatory subunits via phosphorylation and may, in turn, be inhibited by the alternate binding of cdk inhibitors (ciks), such as p21. The effect of p21 activity in terms of its effect on productivity in cell culture is well documented (Fussenegger et al., 1998; Watanabe et al., 2002; Bi et al., 2004; Ibara et al., 2003); however, it is the ability of p21 to induce arrest that is of particular interest in this study, which examines new roles for p21 in process development.

Examination of the proteins and pathways involved in responding to the stresses caused by chemical or physical means has shown that an inbuilt adaptive response exists which is capable of adjusting the levels and/or activity of the genome-protecting machinery, usually through the synchronization of cell cycle arrest, DNA repair and apoptosis (Hofseth, 2004). By initiating p21 overexpression, cells accumulate in the G1 phase of the cell cycle. This arrest of cell cycle progression may allow additional time for repair of environmentally derived damage before the cell is replicated (Weinert and Hartwell, 1988). We hypothesize that an extended period of cell cycle arrest allows cells to survive in a phase where extracellular growth factors are not needed, and/or the accumulation of extracellular autocrine growth factors to a level that can support proliferation. It may also allow the resulting genome damage, created through the direct withdrawal of serum, to be repaired prior to cell cycle release. Interestingly, recent studies also indicate that p21 expression may also provide some protection against apoptosis during the process of adaptation to genotoxic stresses. A recent study (Roninson, 2002) showed that wild-type p21 has the ability to inhibit apoptosis and stimulate transcription of secreted factors with mitogenic and antiapoptotic activities. In this paper, we examine the effect of p21-induced cell cycle arrest on the ability of an anchorage-dependent CHO cell line to survive and adapt to a suspension and protein-free growth environment whilst maintaining productivity.

2. Materials and methods

2.1. Cell-line maintenance

The recombinant Chinese hamster ovary cell line CHO3B2 was previously created by transfecting the mouse–human chimeric B72.3 IgG4 antibody expressing cell line 22H11, previously obtained from Lonza Biologics, with an inducible copy of $p21^{cip}$ using the LacSwitch system (Bebbington et al., 1992; Bi et al., 2004).

Cells were maintained for several passages prior to experimentation in DMEM F12 minus glutamine supplemented with 10% heat-inactivated dialysed fetal calf serum (dFCS) (PAA, Yeovil, UK), 50 μM methionine sulphoximine (MSX) and 1× GS supplement (GSEM, Sigma, Poole, UK). Cells were cultured in static vented T-flasks with 5% CO_2 at 37 °C. All cultures were passaged every 72 h by washing the cells with warm 1× PBS followed by the addition of nonenzymatic cell dissociation solution (CDS) (Sigma–Aldrich). Cells were then washed with warm growth medium before being centrifuged at 1000 rpm for 5 min and resuspended in fresh growth medium.

2.2. Cell counts

Viable cell number, total cell number, and viability were obtained using the Trypan blue exclusion method via a haemocytometer and phase contrast microscopy to distinguish viable and nonviable cells based on stain uptake.

2.3. Adaptation to suspension culture

Cultures previously grown in static conditions, as described above, were detached from the surface of the culture flask using cell dissociation solution and resuspension in fresh growth medium. The viable cell number and percentage viability was determined before and after the cells were centrifuged at 1000 rpm for 5 min and resuspended in fresh growth medium at a cell density of 4×10^5 cells per ml into a final volume of 100 ml in vented 250 ml Erlenmeyer flasks (Corning). The culture was agitated at a rate of 125 rpm at 37 °C in the presence of 5% CO_2. Adaptation was considered successful following the return to an equal or better growth and production rate to that observed with the original culture.

For each set of experiments, four flasks were set up as described above. We added 5 mM of IPTG to the medium in three of the flasks at time point 0; the other flask was kept as a control (0 mM IPTG). Every 72 h, the arrested nondividing cultures were spun down and resuspended in fresh growth medium. The remaining nonarrested culture was passaged back to 4×10^5 cells/ml. At days 3, 6, and 9, cell cycle arrest was reversed by the removal of IPTG from the culture medium. Each culture was examined daily in triplicate via the Trypan blue exclusion method to determine viable and total cell num-