Biochemical Engineering Journal 81 (2013) 97–107

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular Article

Developing a cyclin blueprint as a tool for mapping the cell cycle in GS-NS0

D.G. García Münzer[a], M. Kostoglou[b], M.C. Georgiadis[c], E.N. Pistikopoulos[a], A. Mantalaris[a,*]

[a] Biological Systems Engineering Laboratory, Centre for Process Systems Engineering, Department of Chemical Engineering, Imperial College London, London SW7 2AZ, UK
[b] Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[c] Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form 3 October 2013
Accepted 8 October 2013
Available online 17 October 2013

Keywords:
Cell cycle
Cyclin
Hybridoma cultures
Growth kinetics
Monoclonal antibodies
Modeling
Bioprocess monitoring

ABSTRACT

The cell cycle is at the center of growth, productivity, and death of mammalian cell cultures. There exists a need to identify and quantify major landmarks in the cell cycle of industrially relevant mammalian cell lines and its association with productivity; central for designing productivity optimization strategies. Herein, we studied the expression of three cyclins, under both perturbed and unperturbed growth, by flow cytometry in batch cultures of GS-NS0. The perturbed systems involved two different DNA synthesis inhibitors, thymidine and dimethyl sulfoxide (DMSO). This approach enables the establishment of characteristic cyclin profiles, timings, and thresholds. In particular, two G_1 class cyclins (D1 and E1), and one G_2 cyclin (B1) were investigated. Cyclin B1 showed a clear cell cycle phase-specific expression increasing during G_2 phase where it was approximately 40% higher when compared to G_1 phase. Similarly, cyclin E1 showed a clear pattern being expressed approximately 10% higher in G_1 compared to G_2 phase and decreased through S phase. Cyclin D1 expression was fairly invariable throughout the cell cycle phases. The observed patterns provide a blueprint of the cell line's cell cycle, which can be used for the development of biologically accurate and experimentally validated distributed cell cycle models.
© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The development of high value bio-pharmaceuticals (biologics), such as monoclonal antibodies (MAb), has grown rapidly [1]. Biologics production uses mammalian cell factories due to their ability to perform vital post-translation modifications. Mammalian cell factories are complex production systems – productivity and product quality are controlled by a large number of coordinated metabolic reactions influenced by culture parameters. The cell cycle is at the center of cellular growth and death as well as productivity, which vary during the different cell cycle phases. It is divided in sequentially distinctive phases: G_1 (first gap), S (synthesis), G_2 (second gap), and M (mitosis). The events and needs of the cells are different in each phase. G_1 is characterized by increased physiological activity due to cell growth and protein synthesis. When conditions (internally or externally) are not favorable for DNA synthesis, the cells enter a resting quiescent state known as G_0 [2]. Cells can remain in this state for a variable period of time before resuming proliferation or undergoing programmed cell death (apoptosis). The S phase represents DNA replication followed by the G_2 phase, where preparation for cell division occurs. During mitosis, duplicate DNA separation and cytokinesis are completed resulting in the birth of two daughters cells. The length of the phases is variable but estimates of the average times for industrially relevant mammalian cell lines have been reported [3,4].

Transition between cell cycle phases is highly regulated [5]. Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), are involved [6]. Cyclins are regulatory units and have no catalytic activity, while CDKs are catalytic subunits and are inactive in the absence of their cyclin partner with which they form a heterodimer. The heterodimer coordinates entry into the next phase, achieved by in- and/or activation of target proteins, such as the enzymes required for DNA replication in the S phase. The presence of CDKs remains in constant excess throughout [7], while cyclin concentration varies in a cyclical fashion (in response to molecular signals or checkpoints). Progression between the G_1/S checkpoint has been reported to be regulated by two cyclins, cyclins D and E [8]. Cyclin D1 is expressed early in G_1, associates with CDK4 and CDK6, and is responsible for the phosphorylation of the retinoblastoma protein (pRb), which is essential for progression to the S phase. Cyclins D expression are reported to remain invariable or at low level in normal proliferating cells [9,10]. In

* Corresponding author. Tel.: +44 0207 594 5601; fax: +44 207 594 5638.
E-mail address: a.mantalaris@ic.ac.uk (A. Mantalaris).

1369-703X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bej.2013.10.008

addition, cyclins D are reported to be regulated by extracellular signals [11], accumulating as a response to extracellular growth factors and declining rapidly in their absence. Therefore, cyclins D provides a link between the external environment and the periodically expressed cyclins E, A and B. Cyclin E1 is expressed in mid-G_1 and associates to CDK2. The cyclin E1–CDK2 complex is bounded with other promoters, such as p33, phosphorylates histone H1 and other proteins in late G_1 and early S phase. Cyclin E1 expression is reported to peak at the G_1/S transition and to degrade gradually through S phase progression [9]. Cyclins D and E together are responsible for carrying the cell through the restriction point between the G_1/S phases. Similarly, cyclin B1 is reported to regulate the cell's entrance into mitosis [12]. Cyclin B1 is expressed in late S phase, accumulates to a maximum between the G_2/M transition and rapidly degrades in late M phase. Cyclin B1 forms a heterodimeric complex with $p34^{cdc2}$ and activates the cdc2 kinase, triggering entrance to mitosis. Cyclin expression patterns are, in general, reproducible in normal cells of different lineages; alas, tumor cell lines display different expression patterns [9].

Although a number of studies have dealt with cyclin regulation in various human cell lines [6,13,14], few have addressed this for industrially relevant cell lines. Studies on industrially relevant cell lines have mainly focused on the underlying mechanisms of regulatory pathways [8,15]. Nonetheless, cell productivity has been reported to be cell cycle, cell line, and promoter dependent [16,17]. Therefore, knowledge of the cell cycle-associated production profiles can aid the development of optimization strategies for improving productivity [18]. However, an appropriate metric is required to quantify the relation between productivity and cell population dynamics [19]. Despite several studies have focused on the enhancement of productivity by considering DNA/mRNA distributions or metabolic profiling [20–22], no information on cyclin profiles has been considered. Capturing the heterogeneity of the cell population facilitates accounting for individual characteristics, such as cell cycle associated production profiles. Two main challenges limit the use of segregation in bioprocesses: (1) experimental validation and (2) computational tractability. Experimental techniques, such as flow cytometry, provide quantitative cellular information [23]. In particular, bivariate analysis of cyclin expression alongside DNA content can prove a suitable biomarker for cell cycle transition; while mathematical models can provide a systematic means to study such complex systems [24]. The use of a systematic modeling framework has been proven to improve the reliability of predictive models [25,26]. Previous studies [27,28] have helped to address numerical difficulties of including segregation and have highlighted its potential use as an optimization tool. Others have attempted to capture mammalian cell complexity by coupling a population balance equation (PBE) to a single cell model [29]. A major shortcoming of segregated models is the use of mass or age [30,31] as the distributed variable for the cell cycle transition in mammalian cells. More elaborated models [32,33] have introduced additional biological detail (e.g. protein transcription), but have failed to provide experimental validation. Consequently, inclusion of segregation still remains a challenge. In order to rigorously capture cell cycle heterogeneity, utilization of biomarkers is required.

To the best of our knowledge, cyclin phase-dependent expression profiles and thresholds for industrially relevant cell lines are currently lacking. Herein, we have studied the timing of expression of three cyclins (D1, E1 and B1) under both perturbed and unperturbed growth for the GS-NS0 cell line by flow cytometry. The perturbed cultures involved arresting the cells using two different DNA synthesis inhibitors, thymidine and DMSO. A cyclin blueprint was generated that maps the GS-NS0 cell cycle as well as elucidates the effect of different factors on the cell behavior. This information is of interest in an industrial context, particularly in developing productivity optimization strategies.

2. Materials and methods

2.1. Cell culture

GS-NS0 cells (kindly provided by Lonza; passage 5) with expression of a chimaeric B72.3 IgG4 antibody were cultured in triplicate in 1 L Erlenmeyer flasks (Corning) with 200 mL working volume. Batch cultures over 4 days were carried out in a NU-5500E (Triple Red) humidified incubator set at 37 °C and 5% CO_2. Mixing was accomplished using a Stuart SSL1 orbital shaker (Bibby Scientific) at 125 rpm. The culture medium consisted of glutamine free X2, Non Essential Amino Acids (Sigma Aldrich, cat no. M7145) X1, MEM-Amino Acids (Gibco, cat no. 11130-036) X2, GS-Supplement (SAFC, cat no. 58672C) X2, Penicillin/Streptomycin (Gibco, cat no. 15070-063) X1, 4.5 g/L MSX (Sigma–Aldrich, cat no. M5379) and 10% (v/v) fetal bovine serum (Gibco, Lot 07F7511F).

2.2. Cell cycle arrest

By using different chemical agents we aimed to arrest and synchronize a cohort or fraction of the cell population. Synchronization refers to the fact that the cells were aligned to a particular property, which in this case is the DNA content. After releasing the cells from the arrest, the moving population was tracked using the aligned DNA content as a criterion until it was no longer possible to distinguish that population.

Several chemical agents have been utilized to synchronize cells at different phases [34,35]. DMSO is a frequently used chemical agent in bioprocessing that has proved to be efficient and reversible for G_1 arrest [36–38]. In addition, this agent is reported to act directly on the cell cycle regulation machinery [15,37]. Moreover, it has been reported that DMSO can lead to improved MAb production in different cell lines [39–41]. Thymidine also inhibits the cell cycle, however in an indirect manner. The thymidine action mechanism involves the non-selective inhibition of potassium ion channels proteins [42] while arresting the cells in two phases, G_1 and S, providing a different arrest mechanism [43]. Thymidine has also been used for the study of the cell cycle and productivity in hybridomas [16,44–47]. Herein, the above mentioned arresting agents together with appropriate controls were utilized to develop the cyclin blueprint of the GS-NS0 cell line. Cell cycle arrest using thymidine (final concentration of 2 mM in culture medium; Sigma Aldrich) consisted of a double block. Thymidine was added to the culture medium of cells in mid-exponential growth for 12 h, then the cells were washed twice with phosphate buffered saline (PBS; Gibco) and re-suspended in the culture medium and incubated for another 12 h at which time thymidine was added to the medium for a second time (second block) for 12 h. The cells were then washed twice and re-suspended in culture medium and cultured for 4 days. A control group, which included all the centrifugation and washing steps without exposure to thymidine, was established. Sampling was performed at the beginning and ends of each blocking. After the arrest the culture was sampled every 2 h for the first 10 h and every 24 h afterwards.

Cell cycle arrest using DMSO (final concentration 0.45%, v/v in culture medium) consisted of a single block. DMSO was added to the culture medium of cells in mid-exponential growth for 36 h, and then the cells were washed twice with PBS and re-suspended in the culture medium and cultured for 4 days. A control group, which included all the centrifugation and washing steps without exposure to DMSO, was established. Sampling was performed at 0, 24 and 36 h during the arrest phase and after the arrest every 2 h between 12 and 24 h, and every 24 h afterwards.

Download English Version:

https://daneshyari.com/en/article/3223

Download Persian Version:

https://daneshyari.com/article/3223

Daneshyari.com