A SYSTEMS BIOLOGY APPROACH FOR UNDERSTANDING OSMOTIC STRESS IN ANTIBODY-PRODUCING CELL LINES

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ABSTRACT

Growing demand for monoclonal antibodies (mAbs) presents a challenge to the biotechnology industry. Meeting these demands will require improvements in overall viability, proliferation and specific productivity of antibody-producing cell lines. Osmotic stress, when applied to these cultures, has been shown to improve specific productivity. In this work we investigate several aspects of the cellular response to osmotic stress. Well known signaling molecules are probed by western blotting and it is determined that industrially relevant antibody producing Chinese hamster ovary (CHO) cells exhibit constitutive activation of signaling species. It is also demonstrated that these signaling molecules are further activated under hyperosmotic conditions. Osmotic response element (ORE) binding proteins are shown by electrophoretic mobility shift assay to be present in antibody-producing CHO cells. These proteins are shown to increase their ORE binding behavior when these cells experience an increase in extracellular osmolarity. We show that transfecting CHO cells with active and inactive forms of the tonicity element binding protein (TonEBP), an osmotically responsive transcription factor, affects the growth and proliferation of those cells. Hyperosmotic stress is shown to have a dose dependent effect on the regulatory control of cell volume. Also, it is shown that populations of CHO cells under hyperosmotic stress contain a distinct subpopulation with larger cell diameters than those in the bulk of the population. Additionally, we demonstrate the generation of artificial biochemical reaction networks by using a genetic algorithm. We show that networks with specific stochastic behaviors can be generated by the appropriate tuning of the genetic algorithm. These techniques show good potential to be able to integrate broad sets of experimental data into functional models.