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Sub-physiological culture temperature boosts expression levels of membrane proteins in CHO cells

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Membrane proteins especially G-protein coupled receptors (GPCR) are the targets of a large number of small molecule therapeutics. With the advances in biopharmaceutical therapeutic approach, development of antibodies against the membrane proteins are increasingly attempted. Generation of therapeutic antibodies against these targets is a major challenge due to the limited accessibility of these proteins *in vivo*. Stable clonal cell lines expressing the membrane proteins are used as antigen-presenting cells for antibody development in one of two ways: whole-cell based selection including complexing with B cells, or immunization. They also serve as valuable reagents in characterizing the binding properties of antibodies. Expression levels for multi-span membrane proteins are often low and unstable. Insufficient expression levels of the membrane protein of interest, amid endogenous membrane proteins, is often a constraint for the success of the above applications especially antibody discovery. We employed serum-free methods to generate stable CHO cells expressing membrane proteins, and in the course of cell line development, most clones showed a decline in expression levels during culture scale up, before stabilizing. We reasoned that this is associated with an increase in cell growth rate that accompanies scale up. We found that retarding the cell growth by culturing them at a sub-physiological culture temperature substantially boosted the expression of membrane proteins. CHO cell lines expressing a chemokine receptor (CCR, a GPCR), when cultured at 31 °C, grew at a very low rate; the CCR expression level increased 3.5 fold within a day, and continued to increase for 3 days, reaching a maximum increment of 9 fold compared to the 37 °C counterparts. In a second study, 31 °C-cultures of lead CHO lines expressing a human-, murine- and cyno-type I membrane protein showed 7, 4 and 5 fold increases, respectively, compared to the 37 °C-cells. Thus, culturing at a slightly hypothermic condition serves as a simple method to boost CHO cell expression of membrane proteins just as it does for secreted proteins.