

P038 Identification and analysis of post-transcriptional limitations during recombinant protein production in mammalian cells

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Production of recombinant proteins (rP) by *in vitro* cultured cell systems, although greatly enhanced in recent years, remains limited. Vector/promoter systems that yield high mRNA levels have been developed, suggesting that many of the molecular and cellular processes that constrain rP production are post-transcriptional. Our hypothesis is that translational and post-translational mechanisms limit the quantity and quality of rP production. In order to address this hypothesis, we have begun to investigate post-transcriptional limitations on rP production, by determining flux through the entire gene expression pathway. We have initially undertaken this using Chinese hamster ovary (CHO) cells with varying expression levels of a model rP, firefly luciferase, to study translation and folding in the cytoplasm. Using these data we have begun to compare and identify the factors limiting rP production in high, medium and low rP producing cell lines to determine if it is the same cellular factors that mediate these limitations in cells with differing capacities for rP production. Here we present data showing an initial limitation on production levels at the transcriptional level, however in the highest producing cell line post-translational mechanisms affecting both protein turnover and protein folding become severely limiting. Ultimately identification of post-transcriptional limitations on rP production may allow for the rational design of improved expression systems.