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IDENTIFYING OPPORTUNITIES IN CELL ENGINEERING FOR THE PRODUCTION OF 'DIFFICULT TO EXPRESS' RECOMBINANT PROTEINS

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There is a growing demand for production of recombinant proteins of many structural varieties in mammalian expression systems, either as therapeutics or for protein characterisation. However, certain recombinant proteins are "difficult to express" in mammalian expression systems requiring extensive cell line and process optimisation which, as a result, can have significant consequences for drug development processes. The Tissue Inhibitors of Metalloproteinase (TIMP) protein family, TIMP-2, -3 and -4, are naturally secreted proteins that share significant structural homology (~50% identity and ~70% similarity in amino acid sequence), but show profound differences in secretion in mammalian expression systems. Computational sequence analysis of the TIMPs shows areas of significant amino acid difference mainly locating to flexible loop regions. This study has investigated the molecular mechanisms that selectively restrict expression of recombinant proteins of extensive sequence similarity. The loci of the molecular steps that limit successful expression have been defined by quantitative real-time polymerase chain reaction, proteomic analyses, cellular fractionation and immunofluorescence microscopy. All three TIMPs were readily detectable at mRNA and protein level within the cell but only TIMP-2 was secreted effectively into the culture medium. Analysis of protein localisation showed intracellular protein for all three TIMPs, mainly co-localised in the organellar and cytoskeleton fractions. In addition, immunofluorescence microscopy showed all three TIMPs to be detectable within the endoplasmic reticulum. TIMP-3, which was not secreted, was detected within the cell in both expected glycosylated and non-glycosylated forms. Treatment of intracellular TIMP-3 with glycosidases suggests the presence of an immature high mannose glycoform. Knockout of the TIMP-3 glycan site did not result in secretion. These data suggest that the post-translational processing of poorly expressed TIMPs limits transit through the secretory pathway. To overcome this challenge, cell engineering of limiting secretory pathway components could enhance production of these "difficult to express" recombinant proteins.