USE OF A 'MOLECULAR TUG' TO OVERCOME LIMITATIONS IN THE PRODUCTION OF 'DIFFICULT TO EXPRESS' RECOMBINANT PROTEINS

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In recent years there has been an increased drive towards the production of recombinant proteins in large amounts using rapid cell culture processes. Mammalian expression systems such as Chinese Hamster Ovary (CHO) cells have remained the preferred choice for large-scale recombinant protein production (Walsh, 2014). However, in mammalian cells certain recombinant targets can prove to be 'difficult to express' and require extensive upstream process optimisation which can have a negative impact on industrial processes. This study has investigated the molecular mechanisms that are responsible for poor recombinant protein production. Model proteins from the Tissue Inhibitors of Metalloproteinase (TIMP) family, TIMP-2, TIMP-3 and TIMP-4, were subject to detailed study to characterise the molecular mechanisms that limit production of recombinant proteins with high sequence homology (Hussain et al., 2017). TIMP-2, -3 and -4, share significant sequence/structural homology (Douglas et al., 1997, Garcia et al., 2012), but show differences when produced in a transient CHO expression system (Hussain et al., 2017) . A systematic screen of the protein expression pathway showed all three TIMPs were detectable at the mRNA and protein level within the cell but only TIMP-2 was secreted in significant amounts into the culture medium. Analysis of the intracellular protein suggested the post-translational processing of poorly expressed TIMPs was limiting. A protein engineering approach was employed to overcome challenges in the production of these 'difficult to express' TIMP proteins. This approach involved the attachment of a furin-cleavable pro-sequence from a secretory growth factor to recombinant targets. The pro-sequence was predicted to act as a 'molecular tug' to aid transit through the protein expression pathway and/or promote correct post-translational processing. Initially, the furin-cleavable pro-sequence was added to TIMP-3 (non-secreted), which resulted in secretion of TIMP-3, however incomplete processing of the pro-sequence was observed. The protein engineering approach was optimised further and applied in combination with cell engineering (furin overexpression) to TIMP-4 (poorly secreted), which was also successfully detected in significantly higher amounts in the culture medium (Hussain et al., 2017). Together, the described protein engineering approach presents a novel strategy to increase the production of 'difficult-to-express' recombinant targets.

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