TO SUMMARIZE MY STUDY . . . . . .

The production of therapeutic proteins at very large scale is a prerequisite for a Biotechnology – based Pharmaceutical Industry, for being able to fulfill the requirements of producing novel drug Entities in order to meet the ever – increasing demand of the market.

Prokaryotic systems for the production of a high amount of therapeutic proteins have been developed and are now available for use. But during the evolution of biotechnology processes, it has been noticed that these systems, generally lacking the folding, post transcriptional & post translational modifications are not good enough for production of fully active proteins. As the bioactivity is directly related with these modifications; especially the post translational modifications, the focus of molecular cell biologists has shifted to using Mammalian expression systems. The biggest advantage of using Mammalian cells is that they are the closest to human cells , and thus possess most of the machinery for active protein processing.

CHO cells and a few other cell lines have been extensively studied and used for recombinant protein production. This is due to the ease in handling these cells as compared to other cells. Also the amount of literature available has accumulated to address most of the problems related with these systems. Still a lot of study has to be done in order to understand the biology of these cells.

Also, there are many Expression systems used in experiments where the production of recombinant protein is the ultimate aim; as was the case in the present study. In such cases, it is appropriate for the transgene expression to be maximized.

Although different expression systems vary in terms of their total potential yield, in terms of their vector design and methodology ; the following considerations should be applied when high – level expression is required :

1.) The use of a strong and constitutive promoter to drive transgene expression
2.) The inclusion of an Intron
3.) The inclusion of a PolyAdenylation signal
4.) Optimization of the transgene sequence for translational efficiency
5.) Subcloning into the expression vector, other additional Elements, that help to enhance gene expression.

The 3 elements that we have used in this study to subclone into the expression vector are :

a.) A Tripartite Leader Sequence
b.) A Virus – Associated element
c.) An Intron sequence.

All of these elements work in synergy in order to enhance gene expression of the recombinant gene cloned into the vector.

A lot of study has gone into obtaining high levels of Transgene expression , not only at the Molecular cloning level, but also at the Bioreactor level. A lot of processes are yet to be optimized in order to obtain high – level expression.
However, through this study, we were successful in establishing that an intron sequence, when incorporated into the expression vector, enhances expression levels to a considerable extent.

Not to undermine the other assembly elements, we were pleasantly surprised to find that an expression vector consisting of all of the above – mentioned elements showed a 4 –fold higher level of expression than the vector containing no element.

Currently, the Genomics Lab of Zydus Research Centre, is starting afresh in order to construct an expression vector which is able to achieve the highest possible level of gene expression, by cloning the above mentioned elements into an expression system that drives the expression of the transgene product of **Enbrel**, or **Etanercept**. Enbrel is a TNF (Tumor Necrosis Factor) – Blocking drug, which has been classified as a Biological Response Modifier. **Enbrel** is widely used by patients suffering from Rheumatoid Arthritis, juvenile Rheumatoid arthritis, Ankylosing spondylitis, and Psoriatic arthritis. Etanercept / Enbrel is a synthetic protein that binds to TNFα and acts like a sponge to remove most of the TNFα molecules from the joints and blood, where they cause inflammation.

Every day, lots of new chemical entities are being discovered as therapeutics to treat dysfunctions of the human body; and a lot of research is responsible for their reaching to the common man. However, these drugs and chemicals being expensive, it is much easier to express them by cloning their naturally occurring cDNA sequence into a cloning vector, and transfecting this vector into a mammalian system to obtain a fully

functional and biologically active product. Hence, the need for high – expression – level vectors has arisen, and studies are going on to achieve such vectors.

The present study thus has been carried out by me, along with my guides, keeping in mind the need for such vectors, which enable very high level of the recombinant gene expression, which are normally not achievable.

- Pardon The errors