
Sousa, H. R., Maranhão, A. Q. & Brígido, M. M.
Department of Cell Biology, University of Brasilia. 70910-900, Brazil, Brasília/DF

Introduction: Recombinant antibodies are now a reality in therapeutics. But its production is still a challenging issue. Our group have been developing expression vectors for heterologous expression of recombinant antibodies based on the CMV promoter. We had previously shown that a Z-DNA forming region upstream the promotor/enhancer enhances luciferase expression in a reporter vector. The aim of this study was to explore Z-DNA forming regions, and directly test the role of a Z-DNA stabilizing antibody on a modified CMV promoter (zCMV-IA). Material and Methods: Initially, the GFP gene was fused with a recombinant antibody anti-CD3 and the fusion cassette was introduced in both pCO and pCOΔ600. Then we introduced the Z-DNA forming sequences (Z1, Z3 and Z4) and control sequences (Z2 and Z5) upstream of the CMV-IA promoter. CHO cells were transfection using Lipofectamine LTX 1:1, DNA and LTX reagent. To provide a the trans acting anti-Z-DNA antibody, we cotransfect cells with the vector pMACZ22NLS, that produces a scFv of the anti-Z-DNA mAb Z22 fused to a nuclear localization signal. Flow cytometry was used to follow GFP expression. Results and Discussion: 10 expression vectors were constructed: 5 pCO constructs (pZ1, pZ2, pZ3, pZ4 and pZ5) and 5 pCOΔ600 constructs (ΔZ1, ΔZ2, ΔZ3, ΔZ4 and ΔZ5); The constructions with Z-DNA forming sequences showed an improvement of expression of the reporter gene when compared to the control sequences. Moreover the co-transfection with the anti-Z-DNA vector enhance the MFI of gated GFP⁺ cells by 25%. Conclusion: The Z-DNA forming sequences improve heterologous gene expression and the presence of anti-Z-DNA in trans was shown to enhance the Z-DNA effect probably due to a stabilization effect.

Word Keys: CMV promoter. Biopharmaceuticals. Z-DNA. Supported by FINEP / MCT and FUB