

## **FERRITIN HEAVY CHAIN LOCALIZES TO THE NUCLEUS AND REPRESSES THE ADULT HUMAN $\beta$ -GLOBIN GENE IN CULTURED CELLS**

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Understanding mechanisms of gene repression is crucial to the goal of targeting specific repressors to specific genes. A repressor thus delivered could be useful in gene regulation therapy for sickle cell disease, cancers and other major diseases. Stage-specific combinations of DNA-binding proteins that interact via DNA looping are thought to mediate gene repression, in developmental and tissue-specific regulation as well as in carcinogenesis. We hypothesize that such interactions mediate repression of the human adult  $\beta$ -globin gene in embryonic erythroid cells. Nuclear Ferritin-H (ferritin heavy chain, FH) has been found to repress EKLF-activated human  $\beta$ -globin-reporter constructs in a transfection assay in CV-1 cells (Broyles et al., *PNAS* **98**: 9145-9150, 31 July 2001). Mutation of the -150 CAGTGC motif inhibits *in vitro* FH binding 20-fold and abolishes repression in the co-transfection assay, linking binding with function. The dissociation constant ( $K_D$ ) for K562 cell nuclear FH is approximately  $10^{-10}$ M, suggesting very tight binding and very effective repression. To further study the repression mechanism, we developed an *in vitro* DNA looping assay to show interaction between the FH (repressor) binding site at -153/-148 of the  $\beta$ -globin promoter, and the silencer-binding protein BP1 sites that map to -302/-294 and -553/-527. Partially pure K562 nuclear extract, enriched in FH and BP1, was reacted with a -610/+20  $\beta$ -globin promoter, yielding a single EMSA band. Cutting the DNA-protein complex with *Sau* 96A at -210/-209 of the  $\beta$ -globin promoter before electrophoresis, gave a complex that also migrated as a single band. Removing the proteins in this single-band complex yielded DNA that migrated as two bands corresponding to the expected restriction fragments, confirming that the cut fragments were kept together by interactions of their bound proteins and indicating looping. EMSA supershift bands with anti-ferritin- or anti-BP1-specific antisera confirmed that the looped complex contained both FH and BP1. The binding of these proteins to the DNA exhibits cooperativity, resulting in a very stable complex. Co-transfections in CV-1 cells and chromatin pull-down (ChIP) assays with K562 cells are being used to confirm these interactions *in vivo*. Our recent data also show that a GFP-FH fusion protein made from a transfected recombinant EGFP-C1 plasmid localizes to the nucleus of cultured CV-1 cells, suggesting FH-gene or FH-protein transfection is potentially applicable to a variety of cell types. Treatment with FH may also ameliorate the effects of excess free iron that occurs in the iron overload of sickle cell disease, in many cancers, in vascular disease, and in certain neurodegenerative diseases.

Poster presented at the IBIS first meeting, NIH, Bethesda, Maryland, USA, spring 2003.