The long term objectives of this project are to determine the mechanisms by which erythroid Krüppel-like factor (EKLF) contributes specifically to the developmental control of β-globin gene expression and more generally to erythropoiesis *in vivo*. Utilizing an EKLF-dependent erythroblast model, studies of the structural determinants of EKLF function have identified separable chromatin remodeling and transactivation domains. Moreover, these experiments demonstrate that additional sequences outside the previously defined *in vitro* remodeling domain are required for modulation of β-globin promoter structure. In contrast to studies utilizing transient reporter assays, a novel internal activation domain, which is sufficient for induction of endogenous β-globin gene expression to wild type levels was observed. To extend these observations, the first specific aim will assess the ability of the defined domains to modulate local and regional chromatin remodeling, transcription and globin gene switching in the context of an intact animal. This will be accomplished by deriving knock-in mouse strains that express various EKLF domains. Two of these mouse lines will test the hypothesis that an EKLF domain which can mediated chromatin remodeling but lacks transactivation potential, is sufficient to recruit the distal locus control region enhancer to the β-globin promoter in definitive erythroid cells. In complementary experiments, a similarly derived knock-in EKLF mutant encoding the novel transactivation domain but lacking a second previously described amino terminal transactivation region will be tested for its ability to rescue normal erythropoiesis. The determination that additional polypeptide sequences are required for remodeling of the endogenous β-globin promoter has resulted in a working hypothesis that additional as yet unidentified factors are necessary for this process. Studies in the second specific aim focus on the identification and characterization of these factors. Biochemical approaches utilizing reagents already in hand will be exploited to identify the components of this complex. Long-term, the genes identified will be studied by deriving mice in which the corresponding genomic loci are targeted. Together, the studies will provide important insights into the critical functions of EKLF that are essential for erythropoiesis. This fundamental knowledge is likely to expand our understanding of the molecular mechanisms regulating the γ- to β-switch in globin gene expression, potentially identifying therapeutic targets for the treatment of sickle cell disease.