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# Locus Control Region Mediated Regulation of Adult β-Globin Gene Expression

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## ABSTRACT

Many genes residing in gene clusters and expressed in a differentiation or developmental-stage specific manner are regulated by locus control regions (LCRs). These complex genetic regulatory elements are often composed of several DNAse I hypersensitive sites (HS sites) that function together to regulate the expression of several *cis*-linked genes. Particularly well characterized is the LCR associated with the β-globin gene locus. The β-globin LCR consists of five HS sites that are located upstream of the β-like globin genes. Recent data demonstrate that the LCR is required for the association of the β-globin gene locus with transcription foci or factories. The observation that RNA polymerase II associates with the LCR in erythroid progenitor or hematopoietic stem cells which do not express the globin genes suggests that the LCR is always in an accessible chromatin configuration during differentiation of erythroid cells. We propose that erythroid specific factors together with ubiquitous proteins mediate a change in chromatin configuration that juxtaposes the globin genes and the LCR. The proximity then facilitates the transfer of activities from the LCR to the globin genes. In this article we will discuss recent observations regarding β-globin locus activation with a particular emphasis on LCR mediated activation of adult β-globin gene expression. J. Cell. Biochem. 105: 9–16, 2008. 

**KEY WORDS:** GLOBIN; LOCUS CONTROL REGION; GENE REGULATION; TRANSCRIPTION; TRANSCRIPTION FACTOR

The five human β-like globin genes reside on chromosome 11 and are expressed at high levels exclusively in erythroid cells [Stamatoyannopoulos, 2005]. The order of the genes reflects the sequence of their expression during development. The ε-globin gene is located at the 5′ end of the locus and expressed in the embryonic yolk sac during the first 6 weeks of gestation. With a shift in hematopoiesis to the fetal liver the ε-globin gene is turned off and the two γ-globin genes are activated. Around birth the bone marrow becomes the major site of hematopoiesis, which is accompanied by repression of γ-globin gene expression and up-regulation of the adult δ- and β-globin genes. The δ-globin gene is expressed at low levels due to a mutation in the TATA-box like sequence. High-level expression of the β-like globin genes is mediated by a locus control region (LCR) located far upstream of the ε-globin gene [Grosveld, 1999]. The β-globin LCR, like other LCRs, is a complex element composed of several DNAse I hypersensitive (HS) sites, each 200–400 bp in size and separated from each other by about 2–4 kbp of DNA.

The elements primarily responsible for regulating stage-specific expression of the β-globin genes are located proximal to the genes [Epner et al., 1998]. In the presence of the LCR, however, the relative order of the genes with respect to the LCR is another important factor for correct developmental stage specific expression [Tanimoto et al., 1999]. The LCR could act by a tracking, looping, or reeling mechanism [Choi and Engel, 1988; Tuan et al., 1992; Chakalova et al., 2005]. There is evidence supporting each of these models, and, as we will discuss later, tracking and looping could both be involved in mechanisms leading to high level globin gene expression.

## *trans*-ACTING FACTORS

There are many proteins and *cis*-acting DNA elements involved in the regulation of β-globin gene expression. These proteins function as DNA binding transcription factors, as co-regulators modulating

Abbreviations used: Pol II, RNA polymerase II; LCR, locus control region; USF, upstream stimulatory factor; EKLF, erythroid krupple like factor; TFII-I, transcription factor II-I; NF-E2, nuclear factor erythroid 2; MARE, maf recognition element; Sp1, stimulating protein 1; BP1, Beta protein 1.

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chromatin structure and/or recruiting transcription complexes, and as architectural proteins that change the conformation and perhaps nuclear localization of the globin locus during differentiation and development. We limit our discussion to the putative role of transcription factors involved in locus control region mediated activation of adult-β-globin gene transcription. These factors are the hematopoietic specific proteins GATA-1, EKLF (and related proteins), NF-E2 (and related proteins), and Tal1, as well as the ubiquitously expressed transcription factors Sp1 and USF. Figure 1 summarizes proteins and co-factors interacting with LCR element HS2 and with the adult β-globin gene promoter.

### GATA-1

GATA factors are small zinc finger proteins that bind to DNA elements containing the GATA motif and interact with different co-regulators including Fog (friend of GATA), CBP, a co-activator with histone acetyl transferase (HAT) activity, and mediator, a large co-activator complex associated with RNA polymerase II [Kim and Bresnick, 2007]. GATA-1 acts early during the specification of erythroid cells and has been shown to increase histone acetylation in the LCR and in globin gene promoters, to participate in the recruitment of RNA polymerase II to the LCR and to the promoters, and together with Fog1 to mediate proximity between the LCR and the adult β-globin gene [Johnson et al., 2002; Vakoc et al., 2005]. GATA-1 does not indiscriminately bind to all GATA sites in accessible chromatin and interaction with specific sites is facilitated by Fog1 [Kim and Bresnick, 2007]. Recently GATA-1 has also been implicated in transcription repression and associates with the NURD co-repressor complex [Rodriguez et al., 2005]. It is likely that the sequence context and perhaps cellular environment determines whether GATA-1 bound to DNA acts as activator or repressor of transcription.

### EKLF

Erythroid krupple like factor (EKLF) is another zinc finger protein that interacts with specific DNA sequences in LCR elements HS2 and

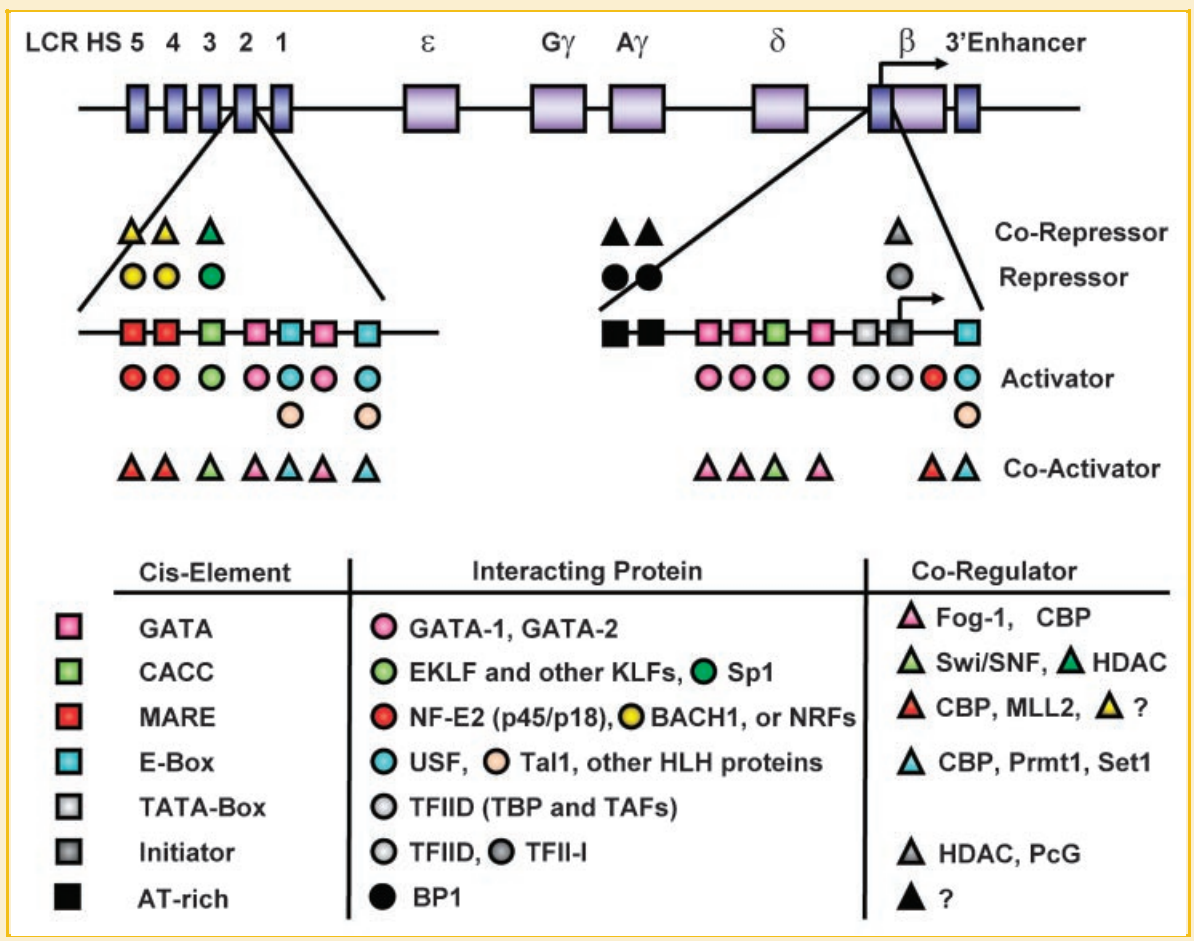


Fig. 1. Summary of proteins and co-regulators interacting with LCR element HS2 and with the adult β-globin gene promoter. Shown on top is the overall organization of the human β-globin gene locus depicting the LCR and the embryonic (ε), fetal (Gγ and Aγ), and adult (δ and β) globin genes. The diagram below illustrates the overall organization of regulatory elements associated with adult β-globin gene regulation. Transcription factor binding sites (boxes) in LCR element HS2 (on the left) and the β-globin promoter (on the right) are bound by transcription factors (circles) that either activate (shown below the binding sites) or repress (shown above the binding sites) transcription through the recruitment of co-regulators (triangles). The binding sequences, interacting proteins, and co-regulators are listed in the table. DNA binding motifs as well as interacting proteins and their known co-regulators are color matched. No co-regulators have been described for Bach1 and Bp1 (indicated by question marks).

HS3 as well as with the β-globin gene promoter [Bieker, 2001]. EKLF deficient mice reveal a reduction in adult β-globin gene expression and in the formation of HS sites associated with the LCR (HS2 and 3) as well as with the β-globin gene promoter. Subsequent work has shown that EKLF recruits nucleosome remodeling activities to the globin gene locus [Armstrong et al., 1998]. EKLF is also required for mediating proximity between the LCR and the adult β-globin gene promoter [Drissen et al., 2004]. Although the main function of EKLF appears to be in activating adult β-globin gene expression it is present in primitive erythroid cells and surprisingly was found to bind the β-globin gene in these cells [Zhou et al., 2006]. EKLF expression increases during development and it was speculated that increased expression leads to the association with positive-co-factors which would then be recruited to the β-globin gene in adult erythroid cells.

### NF-E2

NF-E2 is a heterodimer composed of a small (p18, small maf) ubiquitously expressed subunit and a large (p45) hematopoietic subunit that both contain leucine zipper and DNA binding domains and interact with maf-recognition elements [MAREs, Kim and Bresnick, 2007]. Elucidating the role of NF-E2 in erythroid cells has been a challenge due to the presence of related proteins that appear to function in a redundant manner [Kim and Bresnick, 2007]. Mice deficient for p45 do not exhibit a significant erythroid phenotype or reduction in β-globin gene expression. Recent studies by Kooren et al. [2007] have shown that a decrease in p45 activity is accompanied by an increase in occupancy of β-globin locus associated NF-E2 sites by the NF-E2 related protein NRF2. The same group demonstrated that NF-E2 is not required for conformational changes that reduce the distance between the LCR and β-globin gene promoter. NF-E2 has been shown to interact with other proteins, including components of the TFII-D complex and protein complexes with chromatin modifying activities [Demers et al., 2007; Kim and Bresnick, 2007]. It appears that in erythroid progenitor cells MARE sites in the LCR are occupied by Bach1, an Nf-E2 related protein that functions as a repressor [Ogawa et al., 2001]. Heme induced differentiation leads to the dissociation of Bach1 and the subsequent association of the activator NF-E2.

### TAL1/SCL

Tal1/SCL is a hematopoietic specific helix-loop-helix protein that heterodimerizes with ubiquitously expressed E12/E47 proteins and interacts with E-box sequences [Kim and Bresnick, 2007]. Tal1 has an essential role during early stages of erythroid specification, but the function of Tal1 in more differentiated and adult erythroid cells is far less understood. Tal1 and its heterodimeric partner interact with a variety of proteins and can recruit both co-activators and/or co-repressors to target regulatory elements. It also forms a complex with NL1/Ldb1 and GATA-1 and this complex interacts with a modular *cis*-element composed of GATA and E-box sequences [Kim and Bresnick, 2007], although it is not clear whether individual sites are able to recruit the entire complex in vivo. Tal1 has been shown to interact in vitro with an E-box located in LCR HS2 [Elnitski et al., 1997]. More recently, Dean and colleagues have shown that Tal1 can be crosslinked to the LCR as well as to the β-globin gene promoter and furthermore that the Tal1 associated protein NL1/Ldb1 is required for conformational changes that bring the LCR and the adult β-globin gene into close proximity [Song et al., 2007].

### USF

USF is a ubiquitously expressed dimeric helix–loop–helix leucine zipper protein normally composed of the highly related proteins USF1 and USF2. USF interacts with E-box elements located in LCR HS2 and in the β-globin gene promoter [Elnitski et al., 1997; Crusselle-Davis et al., 2006]. Inhibition of USF activity in erythroid cells led to a reduction in adult β-globin gene expression and a decrease in the association of Pol II with LCR HS2 and the β-globin gene promoter [Crusselle-Davis et al., 2006].

USF proteins are ubiquitously expressed but appear to primarily regulate genes that are expressed in a differentiation and tissue-specific manner [Corre and Galibert, 2005]. A recent genome wide interaction study in hepatocytes using ChIP followed by hybridization to genomic arrays revealed that USF proteins occupy genes in close proximity to transcription start sites and that the interaction correlates with increased levels of histone H3 acetylation [Rada-Iglesias et al., 2008]. In this respect, it is interesting to note that USF has been shown to interact with the histone acetyltransferase CBP/p300 [Crusselle-Davis et al., 2007]. USF also interacts with large co-regulator complexes including the histone methyltransferases Prmt1 and Set1 [Huang et al., 2007]. This suggests that USF at least in part regulates chromatin accessibility which may facilitate the assembly of transcription complexes at the LCR and at the adult β-globin gene promoter.

### PROTEINS INVOLVED IN REPRESSING ADULT β-GLOBIN GENE EXPRESSION

Sp1 is a ubiquitously expressed zinc-finger transcription factor that binds to GC rich elements in enhancer and promoter regions. There are several Sp1 related proteins that can act as activators or repressors of transcription. Sp1 binds to LCR element HS2 and represses LCR function possibly through the recruitment of co-repressors like HDAC [Feng and Kan, 2005]. Sp1 is replaced by the activator fetal krupple like factor (FKLF) upon induction of globin gene transcription.

β-Globin protein 1 (BP1) is a homeobox-transcription factor that binds two AT-rich sequences located upstream of the adult β-globin gene promoter and represses its transcription [Mpollo et al., 2006]. TFII-I has also been shown to repress transcription by interacting with the adult β-globin initiator and recruiting HDACs and perhaps polycomb group (PcG) complexes [Crusselle-Davis et al., 2007]. Bp1 and TFII-I could be involved in mediating silencing of the adult β-globin gene in primitive erythroid cells, in undifferentiated erythroid cells, or in non-erythroid cells.

## *cis*-REGULATORY ELEMENTS

The main regulatory elements that mediate tissue-specific regulation of the adult β-globin gene are located proximal to the gene, including the 5′ promoter, downstream promoter elements, an intronic enhancer, and a 3′enhancer [Stamatoyannopoulos, 2005]. These elements likely mediate tissue- and developmental

stage-specific expression. The LCR is required for high-level expression. As illustrated in Figure 1, many proteins that bind to LCR core elements, for example HS2, also contact the β-globin gene promoter.

### THE β-GLOBIN PROMOTER

The β-globin promoter consists of basal promoter elements and regulatory sequences [Stamatoyannopoulos, 2005]. The basal promoter contains a TATA-like sequence and an initiator, which interact with components of the TFII-D complex. In addition there are two E-box motifs located downstream from the transcription start site. The upstream promoter region contains important elements for tissue- and stage-specific regulation, including multiple binding sites for GATA-1and EKLF (or Sp1). A binding site for Bp1 is located further upstream and is involved in repression of β-globin gene expression. There is a partial Ap1/MARE-like binding site in the downstream promoter region, which has been shown to bind NF-E2 with low affinity [Leach et al., 2003].

### THE LCR

Evidence from transgenic studies suggests that the LCR HS sites function together to activate globin gene expression [Bungert et al., 1995; Fang et al., 2005]. Individual HS sites of the LCR bind proteins that have the potential to interact with other proteins bound at other LCR HS sites. One well-characterized example is Bach1, which interacts with MARE sites in LCR HS2, 3, and 4 and is able to mediate the interaction of HS sites through a BTB/Poz domain [Yoshida et al., 1999]. Other proteins, for example Sp1, likely contribute to and stabilize HS site interactions [Feng and Kan, 2005]. These interactions may occur early in differentiation and may be responsible for formation of a postulated chromatin hub (CH) which contains all β-globin associated HS sites [Palstra et al., 2003]. Some evidence suggests that transcription complexes are already associated with HS sites in undifferentiated cells [Vieira et al., 2004]. Bach1 and Sp1 are repressors of globin gene transcription and are replaced by activating proteins that bind to the same DNA motifs, NF-E2 and EKLF/FKLF, respectively [Ogawa et al., 2001; Feng and Kan, 2005]. After Bach1 and other proteins are replaced from the LCR, the HS sites may adopt a conformation in which the sites no longer directly interact with each other but remain in close proximity to create a highly accessible but somewhat dynamic and open chromatin environment (Fig. 2). This highly accessible chromatin environment is mediated by the high concentration of erythroid-specific and ubiquitously expressed transcription factors and associated co-regulators that interact with the LCR core HS sites. The switch in LCR conformation from a closed to a more open configuration would then allow mechanisms to take place that bring the genes into close proximity to the LCR.

## MODELS OF LCR MEDIATED STIMULATION OF GLOBIN GENE EXPRESSION

Many models have been proposed over the last 15 years to explain how the globin genes are regulated in a developmental-stage

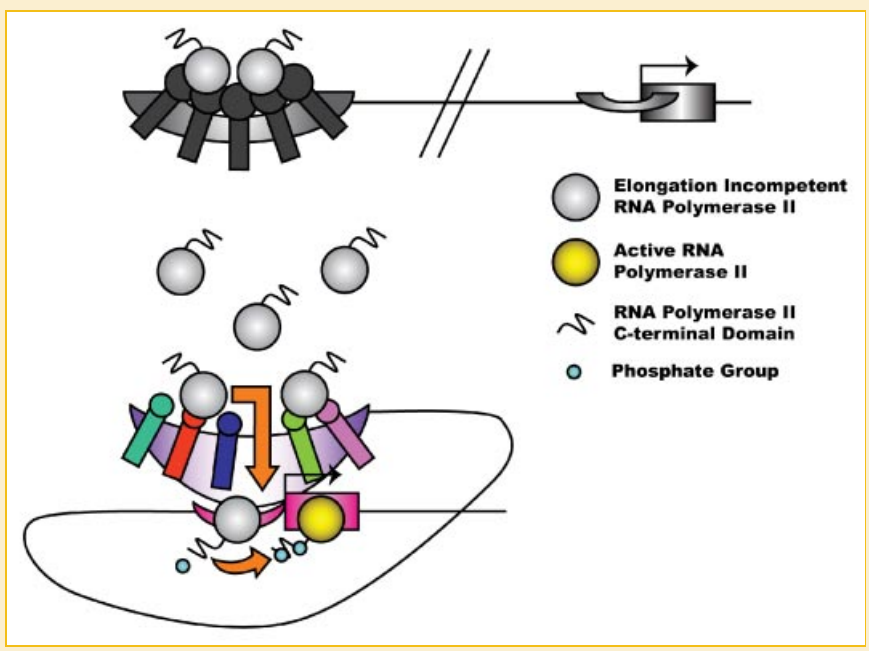


Fig. 2. LCR holocomplex and transfer of transcription complexes to the adult β-globin gene promoter. In progenitor cells the HS sites interact with each other, which is mediated by protein/DNA and protein/protein interactions. This involves, among other proteins, Bach1, Sp1, and USF. Transcription complexes are already recruited to the LCR and may transcribe through the HS sites to maintain accessible domains during differentiation of erythroid cells. In mature erythroid cells expressing the adult β-globin gene LCR HS sites are bound by erythroid-specific and ubiquitously expressed proteins and adopt a more open and dynamic configuration. Elongation incompetent transcription complexes are attracted and loosely associate with proteins or co-regulators bound at the LCR. These transcription complexes are transferred to the β-globin gene promoter, which contains strong basal promoter elements. Once recruited to the promoter, LCR associated activities stabilize the polymerase, protect it from proteasome mediated degradation and/or provide CTD kinase activity. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

specific manner and how the LCR enhances the expression of the globin genes. We restrict our discussion to current models that have gained support over the recent years.

## TRACKING MODEL

A tracking model of LCR function was proposed by Tuan et al. [1992] and is based on the observation that LCR HS2 initiates the formation of long non-coding transcripts. Most of this non-coding transcription proceeds unidirectionally toward the globin genes. According to the tracking model, the LCR recruits transcription complexes that track along the DNA until they reach the globin gene promoters. The model explains the chromatin opening function of the LCR because elongating transcription complexes in eukaryotic cells harbor chromatin modifying activities that either remodel nucleosomes or modify histone tails [Orphanides and Reinberg, 2000]. It also explains the enhancer function because the mechanism of tracking would deliver the polymerase to the genes.

Several recent findings support a Pol II tracking based mechanism for globin activation. Insulators placed between the LCR and the globin genes reduce expression of the β-globin genes [Tanimoto et al., 2003; Zhu et al., 2007]. Furthermore, the Tuan group recently not only demonstrated that an insulator placed between the LCR and the embryonic ε-globin gene represses expression of the downstream gene but also, and perhaps more importantly, that Pol II transcription complexes accumulate at the insulator [Zhu et al., 2007]. The role of intergenic transcription in modulation of chromatin structure is somewhat controversial. Inhibiting transcription elongation by DRB (5,6-dichloro-1-β-D-ribofuranosylbenzamidazole) in erythroid cells has no effect on chromatin modifications downstream of the LCR, suggesting that transcription elongation is not required for chromatin opening [Johnson et al., 2003]. DRB mediated inhibition also fails to prevent conformational changes that bring LCR and globin genes in close proximity [Palstra et al., 2008]. The fact that deletion of the murine endogenous LCR does not affect DNase I sensitivity or increase in histone acetylation in the remainder of the globin gene locus also argues against the hypothesis that LCR initiated intergenic transcription is required to open chromatin structure [Epner et al., 1998].

Haussecker and Proudfoot [2005] found that intergenic transcription does not correlate with open chromatin domains in the globin gene locus and that inhibition of dicer causes an increase in the abundance of intergenic transcripts. These results suggest that intergenic transcription could in fact have a negative role in mediating silencing of globin locus domains that are inactive at specific developmental stages.

## LOOPING MODEL

The looping model proposes that LCR HS sites interact with the globin genes to activate transcription [Choi and Engel, 1988; Engel and Tanimoto, 2000]. The interaction could be mediated by transcription factors and co-factors that interact with the HS sites and the globin gene promoters. In this view the looping model is a contact model, suggesting direct contacts between the regulatory elements and the genes. It is also possible that there are no direct interactions between the LCR and the genes but that these elements instead are brought together in close physical proximity to allow transfer of activities from the LCR to the genes or to allow modifications of promoter bound activities by those recruited to the LCR. The recently applied 3C technology, which assays proximities between genes and regulatory elements, does demonstrate that the LCR and actively transcribed globin genes are in close proximity in the context of an active chromatin hub (ACH) [Palstra et al., 2003]. However, how close these elements come together is not known. A more open and perhaps dynamic configuration appears to be consistent with previous observations showing that γ-globin and β-globin genes rapidly switch their interactions with the LCR [Wijgerde et al., 1995] and also with previously proposed competition models [Choi and Engel, 1988].

The transcription tracking mechanism immediately explains gene activation; but how does looping lead to enhanced expression of the globin genes? LCR mediated gene activation either results in enhanced recruitment of transcription complexes to the globin genes [Levings and Bungert, 2002] and/or in the conversion of transcription initiation complexes to elongation active complexes [Sawado et al., 2003]. This could be achieved by providing activities that are first recruited to the LCR and subsequently transferred to globin genes. For example, transcription complexes could first be recruited to the LCR and looping would mediate the transfer to the globin gene promoters. The ACH would provide a high local concentration of transcription factors that could efficiently capture transcription complexes which would then be recruited to and positioned at the basal promoters of the globin genes to engage in productive transcription (Fig. 2). Alternatively, or additionally, elongation incompetent transcription complexes are recruited to the genes and the LCR provides activities necessary for activation, for example kinases that phosphorylate serine residues at the RNA polymerase II carboxy-terminal domain (CTD), or co-regulator complexes that modify chromatin structure at the globin gene promoters either to enable recruitment of transcription complexes and/or to allow elongation.

## MODEL FOR LCR MEDIATED GLOBIN GENE REGULATION

In the following we will integrate established and more recent data into a model of LCR mediated globin gene regulation (Fig. 3). Proteins binding to individual HS sites mediate the formation of a highly accessible LCR holocomplex. The LCR already adopts an open accessible chromatin configuration in hematopoietic stem cells or in erythroid progenitor cells. This open accessible chromatin conformation is likely mediated by proteins like USF and Sp1 that interact with multiple sites in LCR HS elements. This hypothesis is supported by the observation that transcription complexes can be detected at the LCR but not at the globin genes in embryonic stem cells or in progenitor cells not expressing the globin genes [Vieira et al., 2004]. It is also consistent with findings showing that the LCR is in a poised open configuration in erythroid progenitor cells [Bottardi et al., 2003, 2006]. Transcription through LCR HS sites could aide in maintaining an open chromatin configuration during early differentiation stages [Levings et al., 2006]. Only when stem cells and hematopoietic stem cells commit to other cell lineages will the LCR and the entire globin gene locus be packed into a heterochromatic domain.

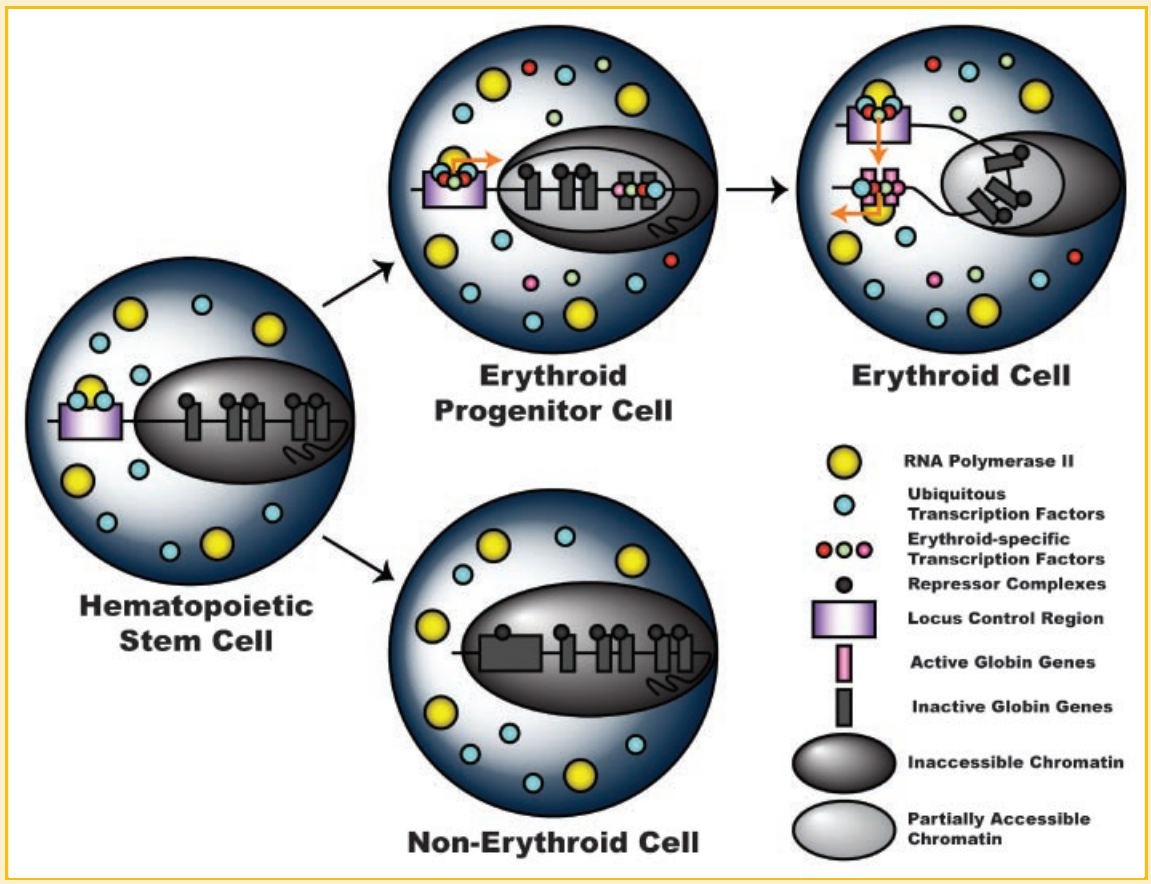


Fig. 3. LCR mediated regulation of β-globin gene expression. The LCR adopts or retains an accessible chromatin conformation in hematopoietic stem cells. As discussed in Figure 2 transcription complexes are recruited and maintain an open configuration at this stage. The rest of the globin locus is in an inaccessible configuration. The entire globin locus is packed into heterochromatin when the cells differentiate into non-erythroid cells. During differentiation of erythroid cells erythroid-specific factors and associated co-regulators open sub-domains in the globin gene locus. These sub-domains unfold and localize to an LCR proximal position. Erythroid-specific and ubiquitously active proteins mediate the transfer of transcription complexes from the LCR to the globin gene promoter, stabilize the transcription complex, and enhance transcription elongation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

When cells differentiate along the erythroid lineage, erythroid specific and ubiquitously expressed transcription factors like Bach1 and Sp1 first associate with the LCR but keep the LCR in an inactive closed configuration. The proposed formation of a chromatin hub (CH) in which globin locus associated HS sites interact in progenitor cells that do not express the globin genes is consistent with the hypothesis that transcription factors mediate the formation of a poised but inactive LCR holocomplex [Palstra et al., 2003]. At later differentiation stages erythroid-specific transcription factors like GATA-1 and EKLF could invade the globin locus and mediate partial unfolding of the chromatin fiber. This partial unfolding may force the genes out of a packed chromatin environment and the genes could come in close proximity to the LCR, which may be fixed at the surface of compact chromatin. This may or may not involve intergenic transcription.

Increasing evidence suggests that transcription of highly expressed genes takes place in the context of transcription factories [Iborra et al., 1996]. As mentioned previously, Pol II transcription complexes are detectable at the LCR before they interact with the genes, and recent observations demonstrate that the LCR is required for the association of the globin genes with transcription factories [Ragoczy et al., 2006]. This suggests either that the LCR anchors the globin locus at transcription factories or that the LCR nucleates the formation of erythroid-specific transcription factories. Data showing that in the absence of the LCR transcription complexes are recruited to the adult β-globin gene but reveal defects in elongation are not necessarily in conflict with this model [Sawado et al., 2003]. In the presence of the LCR the β-globin gene is expressed at extremely high-levels in erythroid cells. In the absence of the LCR, recruitment of Pol II to the adult β-globin gene is somewhat reduced and its expression levels are likely comparable to that of most cellular genes.

We propose that the LCR provides a highly accessible environment for the efficient recruitment of transcription complexes (Fig. 2), which are transiently associated with LCR bound proteins and efficiently transferred to globin gene promoters containing high affinity binding sites for the basal transcription apparatus [Leach et al., 2001; Levings and Bungert, 2002]. This has also recently been discussed for long-range activation in the α-globin gene locus [Vernimmen et al., 2007]. In addition, the LCR or associated

transcription factories may provide an environment for efficient transcription elongation. The LCR could provide elongation factors or stabilize transcription initiation and/or elongation complexes. Early elongation complexes are unstable and recent work suggests that unproductive transcription complexes are subject to ubiquitination and proteasome mediated degradation [Szutorisz et al., 2006]. Perhaps the LCR or transcription factories provide an environment that excludes ubiquitinating enzymes and/or proteasomes and may thus facilitate conversion from unstable early elongation to stable elongation complexes.

## CONCLUSIONS AND PROSPECT

Regulatory mechanisms in the β-globin gene locus have been intensively studied over the last 30 years. Immense progress has been made in identifying *trans*-acting proteins and *cis*-regulatory sequences that together regulate differentiation and developmental-stage specific expression of the globin gene. It is almost certain that additional erythroid-specific and ubiquitous transcription factors and co-regulators will be discovered that act on the globin gene locus. The mechanism of LCR mediated activation of globin gene expression is still not completely understood, but with progress in imaging and tagging technologies as well as through the continued use of genetically modified systems to analyze the contribution of proteins and DNA regulatory elements, a complete understanding of the LCR may emerge in the near future. The idea that the LCR nucleates the formation of erythroid-specific transcription factories is interesting, but addressing this issue will require novel technologies that could distinguish between LCR nucleation of transcription factories and LCR mediated association with pre-formed factories. Perhaps the use of tagged proteins in combination with ChIP/3C technology will allow identification and analysis of formation of tissue-specific transcription factories. Another unresolved question is the role of intergenic transcription in the globin gene locus. Further studies will have to address the potential role of non-coding transcription in either activating or repressing globin locus sub-domains.

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