How Exactly Does The Locus Control Region Regulate Beta-Globin Genes?

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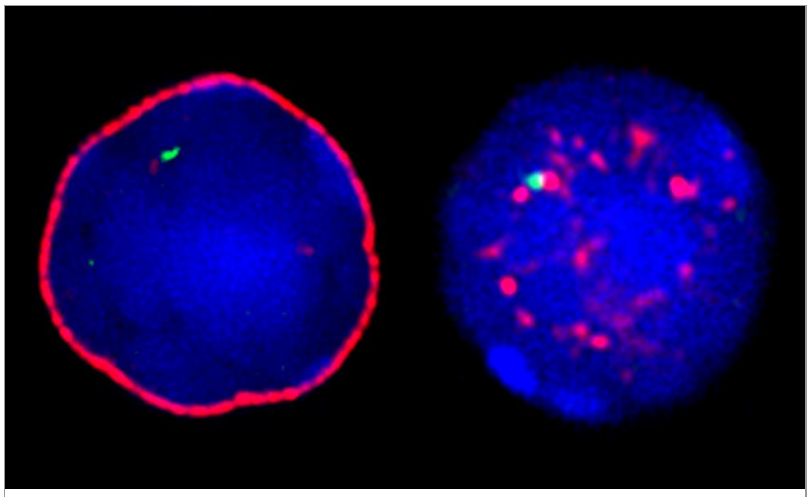
For cell differentiation to unfold normally, certain subsets of genes must be activated, and others silenced, at specific time points in development. *β-globin* gene regulation during red blood cell maturation has become a powerful model for inquiry into how dynamic nuclear organization contributes generally to the control of gene expression during development. The *β-globin* locus consists of five genes encoding components of the oxygen transport protein, hemoglobin, plus an upstream locus control region (LCR) that works to drive high-level expression of these genes. In an earlier study from the laboratory of Dr. Mark Groudine (Basic Sciences Division), Dr. Tobias Ragoczy *et al.* (2006) demonstrated that red blood cell maturation was accompanied by movement of the *β-globin* locus away from the nuclear periphery, and that this correlated with increased *β-globin* gene expression. The research team also showed that transcription factories (TFs), foci of RNA polymerase II complexes, decreased in number and contracted toward the nuclear interior during erythroid development. The LCR was clearly necessary for efficient relocalization of the *β-globin* locus, its association with TFs, and high-levels of transcription, thus raising a new question about this process. What, exactly, was it about the LCR that regulates *β-globin* position and expression?

The LCR consists of a cluster of DNase I hypersensitive sites (HSs). Controversy has surrounded whether certain HSs contribute additively, synergistically or redundantly to LCR-mediated gene activation. To address this, and to investigate the intricacies of how the LCR regulates gene expression, Dr. Bender (affiliate member in the Clinical Research Division), Dr. Groudine and co-authors recently compared differentiation-dependent *β-globin* gene transcription, nuclear positioning of the *β-globin* locus and its association with TFs among a series of mice with targeted HS deletions. Some of their mice lacked a single HS, while others had combinations of two HSs deleted. They show that each HS independently contributes to the activation of expression in an additive manner. By contrast, the authors found that even with two HSs missing, the remaining HSs are sufficient for a nearly normal probability that an individual *β-globin* locus is associated with a TF. Furthermore, the remaining HSs also suffice to activate transcription above baseline. These results are consistent with individual HSs acting in a redundant manner. How then does the control of *β-globin* gene transcription arise? To tackle this question, Bender *et al.* also investigated the regulatory

role of HSs after*β-globin* genes associate with TFs. In contrast to the mild effect that loss of HSs has on the probability of *β-globin* genes being actively transcribed, the authors found that HSs have a strong effect on the amount of *β-globin* RNA generated during each burst of transcription. This seems to occur because loss of HSs impairs the recruitment of factors required for efficient transcription elongation during each burst of expression. The authors' systematic dissection of the LCR defines several steps in *β-globin* gene activation, providing insights that will help scientists understand how enhancers regulate many other genes that are structurally simpler than the *β-globin* locus.

Bender MA, Ragoczy T, Lee J, Byron R, Telling A, Dean A, Groudine M. 2012. The hypersensitive sites of the murine *β-globin* locuscontrol region act independently to affect nuclear localization and transcriptional elongation. *Blood*, published online ahead of print, 29 February 2012, doi:10.1182/blood-2011-09-380485.

Also see: Ragoczy T, Bender MA, Telling A, Byron R, Groudine M. 2006. The locus control region is required for association of the murine β-globin locus with engaged transcription factories during erythroid maturation. *Genes & Dev.* 20:1447-1457.



Images courtesy of Dr. Tobias Ragoczy, staff scientist in the Groudine Lab

During red blood cell maturation, the beta-globin locus (green) moves away from the nuclear periphery and becomes associated with a focus of serine-5 phosphorylated RNA polymerase II, designated a transcription factory. Left and right images are nuclei of erythroid cells from wild type fetal mouse liver. With laser precision, the authors collected these cells at desired developmental stages using resources and expertise in the Hutchinson Center's Flow Cytometry Core Facility. Shown above are nuclei at stage 4 of development, rather late during erythroid maturation. In the case of each nucleus, the beta-globin locus was probed by DNA FISH (green), and the nucleoplasm was counterstained with DAPI (blue). Left: immunostaining of LaminB1 (red) defines the nuclear periphery. Right: immunostaining of phospho-PolI II (red) defines numerous transcription factories, one of which is associated with the beta-globin locus.