Human Hemoglobin Switching

GEORGE STAMATOYANNOPOULOS

THE SWITCHES OF HUMAN GLOBIN GENE EXPRESSION, FROM embryonic (ε, ζ) in the yolk sac, to fetal (γ) during intrauterine life, and to adult (β) after birth, provide a model for the study of human gene expression during development. Hemoglobin switching is also of medical interest because reactivation of fetal hemoglobin in patients with sickle cell disease and β thalassemia ameliorates their disease. Past work focused on cell biology and in vivo studies (*1*). Recently, the discovery of the locus control region (LCR) and experiments in transgenic mice have provided insights on the molecular control of globin gene switching.

The LCR, located 6 to 20 kb upstream of the human ε gene (Fig. 1), consists of a series of deoxyribonuclease (DNase) I hypersensitive sites that are specific for the cells of the erythroid lineage and are developmentally stable (*2*). Formation of the hypersensitive sites is a prerequisite for β-globin expression (*3*). LCR increases expression of the β-globin gene and causes expression to be specific to the erythroid lineage and dependent on copy number in transgenic mice; this expression is not influenced by the position of integration of the transgene (*4*). Linkage to β-globin LCR confers high-level expression on heterologous erythroid or housekeeping genes and reprograms the tissue specificity of expression of nonerythroid genes (*4*). Deletions of LCR result in inactivation of the downstream β-globin gene and a phenotype of β thalassemia (*1*). The LCR may be a regulatory element with two functions: it organizes the globin locus into an active chromatin domain and acts as an enhancer of globin gene transcription. How LCR performs these functions and how it interacts with the genes of the globin locus is still unknown. Comparisons of human, goat, and mouse LCRs disclose extensive sequence homologies and conservation of the spatial arrangements of the DNase I hypersensitive sites, suggesting that the overall organization of LCR is important for function (*5*). How are the globin genes developmentally controlled in the presence of such a powerful element? Experiments with transgenic mice suggest at least two mechanisms: an autonomous mode, exemplified by the control of the embryonic gene, and a competitive mode, illustrated by the switch from fetal to adult globin gene expression. Autonomous and competitive mechanisms have also been proposed for hemoglobin switching in the chicken (*6*).

The temporal control of embryonic genes has been studied in transgenic mice by means of constructs in which either the ε or the ζ human genes were linked to sequences of LCR. LCR–human embryonic gene constructs are regulated correctly in transgenic mice in that they are expressed in the primitive cells of the yolk sac and are completely silenced in the cells of the definitive erythropoiesis (*7*). This suggests that the embryonic genes respond to the influence of LCR in primitive erythroid cells, but the genes escape the LCR effect in definitive erythroid cells. Because LCR is active in definitive cells, the silencing of the embryonic genes in the presence of LCR suggests that the developmental regulation of embryonic genes is autonomous and that it is mediated by a negative mechanism that cannot be overcome by LCR. A silencer has been identified in the 5′ flanking region of the ε globin gene (*8*).

The control of the switch from fetal to adult globin appears to be different from the process that turns off expression of the embryonic genes. Normally the murine β-globin gene expresses only in definitive erythroid cells and not in the embryonic cells yolk sac erythropoietic cells. Constructs containing the human β-globin genes without an LCR display proper developmental control: the human β-globin gene is expressed only in definitive erythroid cells and is silent in the yolk sac cells of the transgenic mouse. However, when the human β-globin gene is linked to the LCR, it loses developmental specificity and is expressed in both embryonic and definitive cells (*9*). The human γ gene behaves in transgenic mice like an embryonic gene: it is expressed in the yolk sacs cell, but is silent in definitive cells. However, when the same γ-gene constructs are linked to the LCR, specificity of developmental expression is lost, and the γ gene is expressed in cells of primitive as well as definitive erythropoiesis (*9*). Correct developmental control of genes is restored when the constructs contain the LCR as well as the γ and the β genes in their normal chromosomal arrangement (*9*). Thus, although LCR overrides the developmental control of individual globin genes, it is unable to do so when both genes are present in the same construct. This suggests that the β and γ genes are in competition with each other for interaction with the LCR (Fig. 1). The outcome of this competition may depend on the availability of developmentally regulated (but unidentified) trans-acting factors that alter the ability of the globin genes to interact with the LCR.

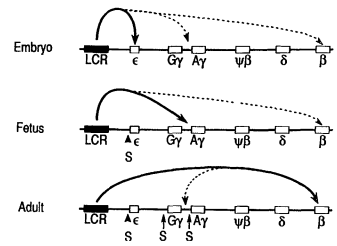

Fig. 1. Human globin gene switching. Solid and broken arrows illustrate the competition between genes for interaction with LCR. S denotes silencing sequences; the arrowheads indicate proven silencers and the thin arrows indicate postulated silencing.

Although a competitive mechanism accounts for the developmental control of the β-globin gene, complete silencing of the γ gene seems to require cis-negative elements. This possibility is supported by the finding that, although the LCR-γ transgene expresses in the adult animal, the amount of fetal globin expression is lower than that in the embryo (*9*).

Delineation of the control of globin gene switching will require several advances. The function of the LCR and its mode of interaction with the globin genes need to be understood. Trans factors interacting with the LCR and the globin genes need to be characterized and cloned. The mechanism that controls the timing of globin gene switching (the developmental clock of switching) must be discovered. The studies of developmental expression of human globin genes in transgenic mice have given us only a glimpse into the complexities of the control of globin gene switching.

Department of Medicine, University of Washington, Seattle, WA 98195.

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