Constricting restricted transcription: the (actively?) shrinking web

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It is now quite generally accepted that the mammalian genome is, in essence, littered with functionally critical regulatory elements that can be located anywhere in relationship to the gene that they control: internally, 5' or 3', sometimes even more than a megabase away (Lettice et al. 2002). Furthermore, seemingly straightforward activities of a gene can be controlled by multiple spatially or temporally distinct enhancers, even for eliciting, in a single developing tissue, functions that never could have been identified as distinct by conventional methods (Khandekar et al. 2004). The discovery and initial exploration of the activity of globin gene enhancers has served as a well-documented paradigm for how such activities are mechanistically interpreted. Following discovery of the delocalized β -type globin gene enhancers (that later became known as the locus control region, the LCR), decades of experiment and speculation followed (Choi and Engel 1988; Enver et al. 1990; Tuan et al. 1992; Wijgerde et al. 1995; Martin et al. 1996; Bulger and Groudine 1999) that addressed the mechanisms underlying enhancer/LCR function. The mechanism of long-range enhancer function was at least partially resolved in 2002, thereby ending (at least one aspect of) this ancient debate. Using novel techniques capable of measuring the spatial proximity of distal genomic elements it was shown that the LCR and actively transcribed globin genes were in close physical proximity inside erythroid cells, looping out the sequences lying between the LCR and globin gene promoter (Carter et al. 2002; Tolhuis et al. 2002). The results strongly implied that a direct functional interaction between the LCR and globin gene was necessary for high-level transcription. A report in this issue of *Genes & Development* by Ragoczy et al. (2006) now shows that the LCR directs the nuclear relocation of the globin locus during erythroid differentiation and directs its colocalization to specific nuclear sub-components, providing further insight into the mechanisms of long-range transcriptional regulation and LCR function.

Inside the nucleus

The fact that nuclear compartmentalization exists has been known since the dawn of light microscopy, but the less appreciated possibility that subnuclear organization might functionally contribute to the control over the transmission of genetic information can equally no longer be in doubt. While it has long been appreciated that the nucleus has a visible, dynamic substructure and can be remarkably heterogeneous with regard to the appearance of chromatin structures from different environmental conditions, differentiation states, or different cell types of origin, what has often been missing from experiments that address nuclear structure is a clear indication of whether this organization is the cause or consequence of gene function.

In recent years we have glimpsed the first indications that point to the prospect that in at least some cases genes migrate to specific subnuclear compartments to execute transcription, while in other cases it appears that compartmentalization is more likely to be a consequence of gene activity. What is becoming clear is that many molecular events and mechanisms are intimately integrated into a highly choreographed pathway that lead to the efficient and heritable activation or repression of a given gene. Identification of the major players and understanding their roles in this three-dimensional pavane remains a major challenge in deciphering the cryptic code that controls our genome as well as in understanding its complex translation into function.

Inside vs. way inside

The dynamic repositioning of genes within the nucleus during development and differentiation is nonrandom, and often correlates with gene expression status. Gene colocalization with constitutive heterochromatin or the nuclear periphery is usually associated with silencing (Brown et al. 1997, 1999; Andrusis et al. 1998), whereas a more gene-internal nuclear localization has been generally associated with actual or potential transcriptional activity (Francastel et al. 1999; Kosak et al. 2002). The relationship of a gene to its chromosome territory is also

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Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.1438106>.

highly correlated with expression. Active genes have been seen to extend considerable distances outside their chromosome territories (Cremer and Cremer 2001), whereas inactive genes, in general, tend to remain within or at the surface of their territory (Mahy et al. 2002a,b; Chambeyron and Bickmore 2004). Although only a limited number of cases have been examined in sufficient detail, it is tempting to speculate from these few examples that these subnuclear locations dictate the activity state or epigenetic signature of the genes that enter them. Indeed, a common notion with some support is (for example) that the nuclear periphery is a functionally repressive compartment, actively altering the epigenetic state of genes that are addressed to that compartment to heritably silence gene expression (for review, see Hetzer et al. 2005). However, there are also counterexamples to contradict this generalization. For example, the *IFN γ* gene is located in the extreme nuclear periphery in immature, naive T helper cells, and remains peripheral regardless of whether those cells differentiate along the Th2 pathway (in which case the *IFN γ* locus remains silent) or resolve to a Th1 fate wherein high levels of *IFN γ* are expressed (Hewitt et al. 2004).

Ragoczy et al. (2006) now clearly demonstrate that the nuclear periphery is not necessarily repressive; they show that the mouse β -globin locus is largely positioned in the nuclear periphery in erythroid progenitor cells prior to the most abundant phase of globin gene transcriptional activation. β -Globin transcription begins while the locus is still clearly in the periphery, and only later migrates to a more interior location as erythroid cells differentiate. So, even though a prominent role may exist for silencing compartments in the nuclear periphery, it is clear in both cases cited here that activation can occur there as well. So why does the globin locus move to a more internal location if transcription can occur at the periphery?

Migration by remote control?

Ragoczy et al. (2006) address the question of nuclear position versus transcriptional status by charting the course of the β -globin locus during erythroid maturation. After purification of staged progenitors from both wild-type and LCR-deleted mouse strains, precursor cells representing the various stages of erythroid differentiation were monitored by FISH and immunolocalization. Without the LCR, the locus departs the periphery later during differentiation than does the wild-type locus, and never fully traverses the course of the wild-type locus into the interior of the nucleus. These results imply that placement (or timing of repositioning) is important, and suggest that the LCR directly or indirectly affects the nuclear address of the globin locus. But how does it do this, and why should intranuclear position matter?

The LCR was originally defined in transgenic assays as a complex element that was capable of conferring position-independent, copy number-dependent expression to linked transgenes (Grosveld et al. 1987). These properties

led to the suggestion that the LCR was responsible for opening an erythroid-specific chromatin domain as a (possibly critical) initial step in activating the entire locus. However, examination of the endogenous murine locus later showed that the LCR was not required, either for an open chromatin structure or an active histone modification profile across the whole locus (Bender et al. 2000; Schubeler et al. 2001), implying that other sequence elements within or nearby controlled these functions (Gribnau et al. 2000). In those studies, deletion of the LCR did profoundly affect globin gene expression levels, suggesting that the primary function of the LCR is to act as a powerful long-range enhancer. Ragoczy et al. (2006) now show (using RNA FISH) that the drastically diminished expression of the adult β -globin gene in the LCR-deleted mouse strain is due to the fact that only a minority of the globin genes are transcribed at any given time. Furthermore, by using DNA FISH and immunological detection of hyperphosphorylated RNA polymerase II, they find that only a fraction of the LCR-deleted loci colocalize with active RNA polymerase II foci, also known as “transcription factories” (Fig. 1). Previous reports have shown that transcription factory subnuclear foci are sites of nascent gene transcription that are highly enriched in the active, hyperphosphorylated form of RNA polymerase II (Jackson et al. 1993; Wansink et al. 1993; Iborra et al. 1996; Grande et al. 1997), and importantly, each factory potentially contains multiple, actively transcribed genes (Jackson et al. 1998; Osborne et al. 2004). Normally, nearly every globin locus in a population of transcriptionally active erythroid cells is in contact with a transcription factory, and therefore displays a primary transcript RNA FISH signal, implying almost continuous globin gene transcription (Osborne et al. 2004). However, for other genes that are less abundantly transcribed than globin (i.e., everything else in an erythroid cell) only a minority of alleles colocalize with factories, strongly correlating with their less frequent transcription and lower expression. Ragoczy et al. (2006) show that in erythroid cells that lack the LCR, the globin genes now behave like these other more moderately expressed genes: They adopt a configuration in which they are less frequently engaged with active transcription factories and thus less frequently transcribed. One thus reaches the inescapable conclusion that some property conferred by the LCR must be responsible for homing to active factories.

These observations raise central questions regarding the nature of transcription factories, the topology of LCR–gene–nuclear substructure complexes, and the relationships between them. Work conducted more than two decades ago suggested that transcription factories are relatively stable aggregations of RNA polymerase II attached to some sort of nuclear substructure, or skeleton (Jackson and Cook 1985; Jackson et al. 1993). More recent work indicates that individual factory components are continually exchanged (Kimura et al. 2002), suggesting that transcription factories are dynamic self-organizing (Misteli 2001) collectives of transcriptional components that are comparatively fixed in space.

Figure 1. Role of the LCR in nuclear organization of the β -globin genes during erythroid differentiation. A schematic summary of the changes in nuclear position of the murine β -globin genes (red dots) and their relationship to transcription factories (black dots). In wild-type erythroid cells prior to abundant globin expression, the β -globin genes are found in the nuclear periphery and are not associated with transcription factories. Globin gene expression (pink cytoplasm) begins in stage 1 of differentiation when the genes colocalize with transcription factories (Ragoczy et al. 2006). At differentiation stage 4, factory numbers are reduced and redistributed toward the nuclear interior. Globin gene expression is high (red cytoplasm) and globin loci are found predominantly in the nuclear interior in association with transcription factories. (*Bottom panel*) In the LCR knockout erythroid progenitors, β -globin gene association with transcription factories is incomplete or intermittent, resulting in uniform, but diminished, β -globin expression in all erythroid cells. In stage 4 cells of the LCR deletion mutants, a significant number of globin alleles remain in the periphery, and only a small fraction are coincident with transcription factories.

Greener pastures, or roll your own?

While current work leaves open the answers to many remaining questions, there are clues that provide some interesting suggestions. Ragoczy et al. (2006) show that RNA polymerase II factories are redistributed during erythroid maturation (Fig. 1). In early erythroid progenitors, factories are greater in number and relatively evenly distributed between the nuclear interior and periphery. In the most mature erythroid progenitor cells, factory numbers are reduced and redistributed toward the nuclear interior with a noticeable decrease in the periphery. It is possible that the globin locus and hundreds of other coexpressed genes are drawn toward the nuclear interior in pursuit of increasingly scarce transcriptional machinery. This type of model (Fig. 2A) implies that the redistribution of the factories plays a decisive role in the reorganization of the nucleus during differentiation (Chakalova et al. 2005). Globin loci that are missing the LCR, and therefore cannot form a stable association with a factory, would be left behind during this migration, interacting only intermittently and unstably with the few factories remaining in the periphery. Conversely, the LCR and the factors that bind to it may confer some sort of directionality that result in a relocation of the locus to the nuclear interior. In this scenario (Fig. 2B), other active genes would also require elements or marks that direct them to interior nuclear locations. Chromatin structure and histone modifications would be an obvious choice owing to the visible segregation of eu- and het-

erchromatin under the light microscope, but chromatin structure and histone modifications are both relatively unperturbed in the LCR mutant locus with the exception of the promoter hypersensitive sites (Schubeler et al. 2001). Interestingly, promoter hypersensitivity and transcription can be segregated at the β -globin promoter by examining differential activities of erythroid kruppel-like factor (EKLF), which has functionally separate BRG1 (chromatin modifying) interaction and transactivation domains (Brown et al. 2002). EKLF-mediated recruitment of BRG1 to the β -globin gene promoter through the BRG1 interaction domain results in hypersensitive site formation but only low-level transcription, while abundant transcription more typical of this gene additionally requires the transactivation domain. EKLF also appears to be involved in the formation of the LCR–gene complex (Drissen et al. 2004). All of these phenomena have been proposed to be required for efficient recruitment of the polymerase complex to the adult β -globin promoter; however, the data could be interpreted equally well as a circular argument in favor of the hypothesis that they increase affinity of the gene for a factory.

Future resolution

With the advent of this and other 21st century investigations that address subnuclear localization and its relevance to control over gene expression come a host of important and unanswered mechanistic questions. First, it is not yet clear whether transcription factories exist as such by themselves, in which case an enhancer may simply increase the affinity for (i.e., frequency of interaction with) factories, or whether factories preferentially nucleate on the enhancers of all expressed loci in a given cell type, in which alternative case the enhancer might function by increasing the ability of a locus to nucleate an active factory. Second, what is the dynamic and which are the molecules that seemingly draw inward the transcriptional net of factories, enhancers, and promoters as an erythroid cell matures? Are the confocal sections that we see through the light microscope reflecting increasing spatial/temporal constraints on chromatin, on the nuclear envelope, or on both (i.e., are active, energy-requiring physical processes within the nucleus, dictating

Figure 2. Possible models to explain the relocation of the β -globin genes in stage 4 erythroid cells. (A) β -Globin and other coexpressed genes migrate to the nuclear interior in pursuit of the increasingly scarce transcriptional machinery, which has been redistributed to the nuclear interior. (B) β -Globin and other coexpressed genes have specific *cis* elements that direct their migration to the nuclear interior where transcription factories form on them and their regulatory elements.

that transcription can only take place in “open” subcompartments) (Verschure et al. 1999; Cremer et al. 2000), or is the three-dimensional factory “net” that we visualize in these images (e.g., Fig. 5 in Ragoczy et al. 2006) simply reflecting the fact that we can see only what we look for, and that the underlying mechanisms are controlled by cryptic molecules whose identity has not yet been revealed? In other words, are there subnuclear machines that actively constrain the transcriptional apparatus to an ever more refined organization as an erythroid cell differentiates, or is the entire process controlled purely by occupancy of the transcriptional apparatus into whatever nuclear space remains that happens not to be occupied by (hetero)chromatin? Resolution of thorny “chicken and egg” issues like these will require not only the discovery of new molecules that play key roles in defining and elaborating the process, but will likely also require the development of novel imaging applications. Given the progress in this emerging discipline over the past 5 years, we should expect answers sooner rather than later to many of these most basic questions about nuclear architecture and its active versus passive roles in directing transcription.

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Genes Dev. 2006, **20**:

Access the most recent version at doi:[10.1101/gad.1438106](https://doi.org/10.1101/gad.1438106)

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