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Review

FISH-eyed and genome-wide views on the spatial organisation of gene expression

Marieke Simonis, Wouter de Laat *

Department of Cell Biology, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE, Rotterdam, The Netherlands

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ABSTRACT

Eukaryotic cells store their genome inside a nucleus, a dedicated organelle shielded by a double lipid membrane. Pores in these membranes allow the exchange of molecules between the nucleus and cytoplasm. Inside the mammalian cell nucleus, roughly 2 m of DNA, divided over several tens of chromosomes is packed. In addition, protein and RNA molecules functioning in DNA-metabolic processes such as transcription, replication, repair and the processing of RNA fill the nuclear space. While many of the nuclear proteins freely diffuse and display a more or less homogeneous distribution across the nuclear interior, some appear to preferentially cluster and form foci or bodies. A non-random structure is also observed for DNA: increasing evidence shows that selected parts of the genome preferentially contact each other, sometimes even at specific sites in the nucleus. Currently a lot of research is dedicated to understanding the functional significance of nuclear architecture, in particular with respect to the regulation of gene expression. Here we will evaluate evidence implying that the folding of DNA is important for transcriptional control in mammals and we will discuss novel high-throughput techniques expected to further boost our knowledge on nuclear organisation.

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Expression of genes, in particular of tissue-specific genes, is often controlled by regulatory DNA elements like enhancers and silencers that are located away from the gene promoter. In mammals, these DNA elements may be up to 1 megabase apart from the gene [1]. A gene locus is defined as the chromosomal region that carries the gene and its regulatory DNA elements. When evaluating the functional relevance of chromosome folding we consider it important to distinguish between DNA contacts formed within and between gene loci.

1. DNA interactions within gene loci

Within gene loci, it is clear that DNA loops are formed which are functionally meaningful for transcription regulation (Fig. 1). Evidence for the *in vivo* existence of such local chromatin loops was first obtained in the mouse *β-globin* locus. It relied on the development of two novel techniques, RNA-TRAP and Chromosome Conformation Capture (3C) technology (Fig. 2). Both techniques independently showed that the *β-globin* locus control region (LCR), crucial for high *β-globin* gene expression [2–4], contacts active *β-globin* genes by looping out the intervening chromatin fibre *in vivo* [5,6]. 3C technology, in particular, has since become a widely used tool for exploring the functional relevance of DNA interactions. 3C (chromosome conformation capture) is a biochemical method that involves the capture of *in vivo* interacting DNA fragments via formaldehyde crosslinking and ligation. Quantitative PCR across ligation junctions with primers selected for specific DNA fragments subsequently gives a measure for their steady-state interaction frequency in the cell population [7].

Using 3C, it was demonstrated that LCR-gene loops are formed specifically in erythroid cells that express the *β-globin* genes [6]. It was found that during development, the LCR switches its contacts between different *β-globin* genes in relation to their switch in expression. Contacts are only established late during erythroid differentiation when the *β-globin* genes are fully expressed [8] and rely on the transcription factors EKLF and GATA-1, which both are required for high levels of *β-globin* gene expression [9,10]. Collectively, the data show that the LCR increases the transcription rate of the *β-globin* genes by physically contacting the genes. Similar contacts between enhancer-like DNA elements and genes have since been demonstrated by 3C technology in the T helper type 2 cytokine locus [11], the α-*globin* locus [12,13], the *Kit* locus [14] and many other gene loci.

Local chromatin loops not only serve to promote transcription but appear to also function in gene silencing. Silencing DNA elements have been shown to form chromatin loops in an imprinted gene cluster carrying the *Dlx5* and *Dlx6* genes [15] and to contact gene promoters in the *Kit* locus [14]. CTCF, an insulator protein which can block enhancer activity when bound in between an enhancer and promoter, also forms chromatin loops in gene loci. At the imprinted *Igf2-H19* locus, CTCF-mediated loops formed on the maternal allele shield the *Igf2* gene, causing shared enhancers to exclusively act on the maternal *H19* gene [16,17]. In the *β-globin* locus, CTCF mediates

* Corresponding author. Tel.: +31 10 4087164; fax: +31 10 4089468.
E-mail address: w.delaat@erasmusmc.nl (W. de Laat).

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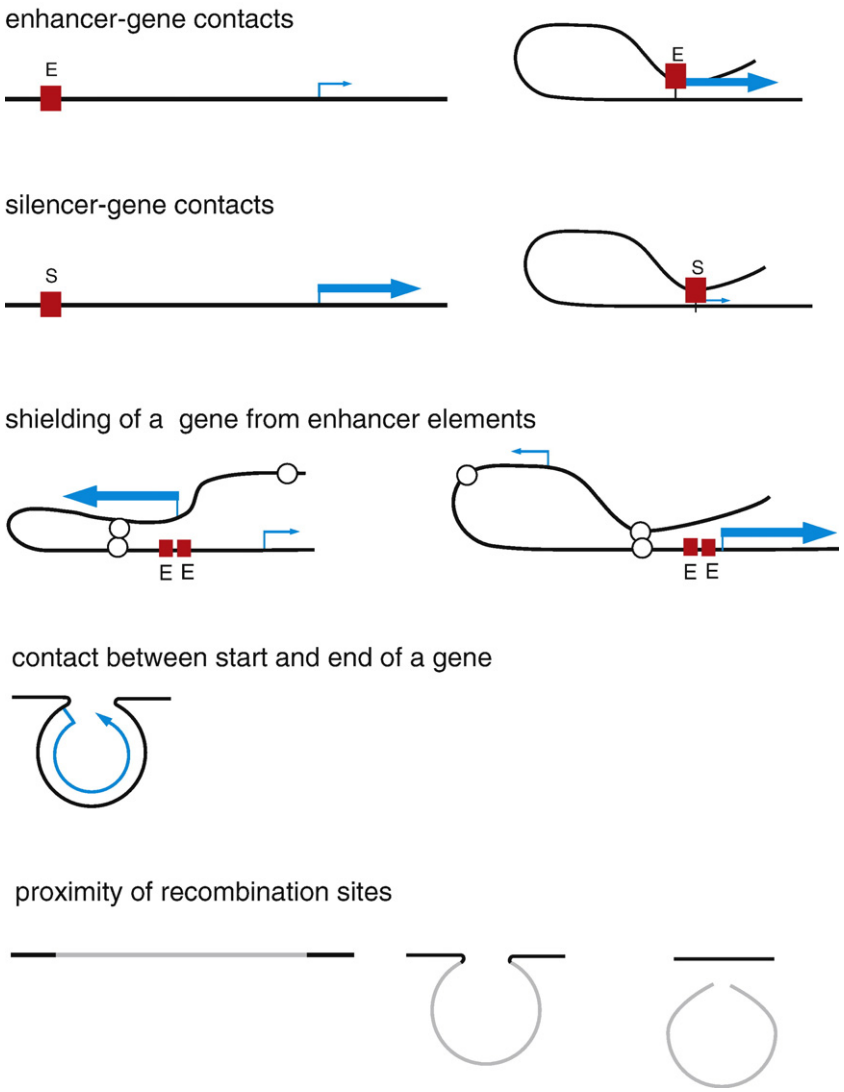

Fig. 1. Overview of mechanisms in which looping of DNA is involved. Looping of enhancers or silencers towards gene promoters can influence transcriptional activity [5,6]. Looped structures can shield a gene from its enhancers, resulting in gene silencing [14,15]. Contacts between a gene's start and end have been reported and are proposed to facilitate recycling of RNA *Pol*II [17]. DNA sequences that are removed during recombination events in immune receptor loci are looped out, while the recombination sites come in close proximity [19,23].

the formation of loops between cognate binding sites flanking the locus. These loops are formed only when the locus is active, but they seem to not influence the expression of the *β-globin* genes [18]. In yeast, loops have been demonstrated between the two ends of actively transcribed genes and a similar observation was recently made for the HIV-1 provirus integrated into human cells [19,20]. It has been suggested that physical proximity between the end and the start of a transcription unit facilitates the recycling of RNA polymerase II (RNAP II), thus stimulating transcription re-initiation. Interestingly, recent live cell imaging studies in flies confirm local recycling of fluorescently tagged RNAP II at highly transcribed heat-shock loci, however apparently without these genes forming loop structures [21]. Future research therefore should uncover whether gene looping is a general phenomenon and how it influences the transcription process.

Apart from transcription, chromatin loops have been implicated in the rearrangement process that joins the various segments of immunoglobulin loci and T cell receptor loci to assemble a functional antigen receptor gene [22–24]. These loci are very large, spanning hundreds of kilobases or even a few megabases of DNA, and fluorescence in situ hybridisation (FISH) could therefore be applied to independently confirm the presence of these chromatin loops in individual cells under the confocal microscope [23,24]. Ideally, loops detected by 3C in much smaller loci should also be validated by FISH, but this is currently impeded by the limited resolution of microscopes. Novel microscopy techniques such as 4pi [25] may enable the visualisation of these smaller chromatin loops in the near future.

2. The significance of local chromatin loops

How would chromatin loops influence processes like transcription and recombination? In the case of recombination it seems clear: to join two DNA segments they need to physically meet. An unanswered question still is how two sites separated on the chromosome physically come together. It may involve a deterministic search process, but we would predict contacts are the consequence of the random collisions between DNA sites that occur as consequence of the flexibility of the chromatin fibre. A productive recombination event then takes place as soon as the appropriate sites juxtapose.

The relevance of chromatin loops for transcription regulation seems more difficult to envisage in molecular terms. Looping brings DNA-binding sites for transcription factors in close proximity to the promoter. It has been proposed that this causes a local accumulation of transcription factors, which will reinforce the expression status of the

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