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Review

Communication of genome regulatory elements in a folded chromosome

Sergey V. Razin [a,b,c,d,*], Alexey A. Gavrilov [a,c,d,e], Elena S. Ioudinkova [a,c,d], Olga V. Iarovaia [a,c,d]

[a] Institute of Gene Biology of the Russian Academy of Sciences, 119334 Moscow, Russia
[b] Faculty of Biology, M.V. Lomonosov Moscow State University, 119992 Moscow, Russia
[c] LIA 1066 French-Russian Joint Cancer Research Laboratory, 94805 Villejuif, France
[d] LIA 1066 French-Russian Joint Cancer Research Laboratory, 119334 Moscow, Russia
[e] University of Oslo, Center for Medical Studies in Russia, 119334 Moscow, Russia

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ABSTRACT

The most popular model of gene activation by remote enhancers postulates that the enhancers interact directly with target promoters via the looping of intervening DNA fragments. This interaction is thought to be necessary for the stabilization of the Pol II pre-initiation complex and/or for the transfer of transcription factors and Pol II, which are initially accumulated at the enhancer, to the promoter. The direct interaction of enhancer(s) and promoter(s) is only possible when these elements are located in close proximity within the nuclear space. Here, we discuss the molecular mechanisms for maintaining the close proximity of the remote regulatory elements of the eukaryotic genome. The models of an active chromatin hub (ACH) and an active nuclear compartment are considered, focusing on the role of chromatin folding in juxtaposing remote DNA sequences. The interconnection between the functionally dependent architecture of the interphase chromosome and nuclear compartmentalization is also discussed.

1. Introduction

The importance of the genome spatial organization for the regulation of gene expression has been first demonstrated through the preferential sensitivity of active genes to DNase I [1]. In the last decade, increasing interest in this problem has been triggered by observations that the mutual positioning of genes and regulatory sequences within the nuclear space and their positioning with respect to functional nuclear compartments might be important for transcriptional control. The studies performed using both cytological (fluorescence in situ hybridization (FISH), immunostaining) and biochemical approaches (chromosome conformation capture (3C) and derivative protocols commonly known as C-methods [2]) resulted in an integral view of the eukaryotic cell nucleus, where the domain organization of the genome is highly associated with chromatin folding and the functional compartmentalization of the nuclear space [3,4]. The chromosomal territories separated and perforated by the ramified interchromatin domain constitute the basis for nuclear organization. The territorial organization of interphase chromosomes has been extensively reviewed [5–7] and we will not discuss it here. Promoter–enhancer communication is of particular importance as a possible mechanism for mediating the functionally dependent spatial positioning of genes and regulatory elements. Although there are different models of enhancer-mediated promoter activation, the most popular mechanisms suggest that the physical approach and direct contact between enhancers and promoters is necessary for the enhancer action [8]. Particularly, the active chromatin hub (ACH) model [9] postulates that distant regulatory elements that control the expression of tissue-specific genes assemble into a common ACH complex, to which the promoters of transcribed genes are recruited. The model is supported by evidence obtained using the 3C protocol to study several experimental models, particularly, the domains of beta- and alpha-globin genes of vertebrates [10–15]. Although the 3C protocol [16] and derivative methods [2] demonstrate the interactions between distant genomic elements, the portion of cells in which two particular DNA sequences interact cannot be estimated, as the average interaction profile for a cell population is analyzed in these studies. Thus, it is difficult to determine whether the identified complexes are stable or short-lived. Furthermore, the 3C protocol [16] only illustrates the pairwise interactions of different genomic regions. The existence of complex assemblies of regulatory elements can be inferred based on the existence of a set of pairwise interactions between several

Abbreviations: FISH, fluorescence in situ hybridization; 3C, chromosome conformation capture; ACH, active chromatin hub; LCR, locus control region; HS, DNAse I hypersensitive site; RCH, repressive chromatin hub; S/MAR, scaffold/matrix attachment regions

* Corresponding author at: Institute of Gene Biology of the Russian Academy of Sciences, 119334 Moscow, Russia. Fax: +7 9031233549.
E-mail address: sergey.v.razin@inbox.ru (S.V. Razin).

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genomic regions, but these inferences cannot be accurately proven. Moreover, the alternative possibility that different pairwise interactions exist in different cells present in a population cannot be ruled out based on the results of the 3C experiments.

Although the idea that enhancers and promoters should be juxtaposed in the nuclear space has gained much support, the exact mechanism of this juxtaposition remains unknown. The classical model of ACH [9] suggests that ACHs are stabilized through interactions between proteins bound to different regulatory elements. However, it is equally possible that promoters and distant regulatory elements transiently interact and are held in spatial proximity through the specific folding of a relatively large chromatin domain. In this case, the folded chromatin domain containing the juxtaposed regulatory elements could be considered as a nuclear compartment (an expression hub [17,18]). Notably, the structural basis of nuclear compartmentalization and functionally dependent genome folding remains a matter of debate. The popular hypotheses include, but are not limited to, the following: (i) fully functional interactions, in particular, associations between the transcribed genes in transcription factories [19]; (ii) the existence of an uncharacterized nuclear skeleton or matrix [20–22]; and (iii) the effect of macromolecular crowding [23,24]. Here, we will discuss different mechanisms that might mediate the mutual positioning of regulatory elements in interphase chromosomes.

2. ACH model

The ACH model postulates that distant regulatory elements (enhancers) and promoters of target genes are assembled in a common complex [9] (Fig. 1A). This complex is likely stabilized through interactions between proteins bound to the DNA sequences involved in the assembly of ACH. Both transcription factors [25–27] and special "communication" proteins [28–30] were reported to mediate the interaction of distant regulatory elements. Of particular interest is the observation that some DNA elements are indispensable for the assembly of ACH, while the others may or may not be present. In human alpha-globin gene domain, the locus control region (LCR)-like element, known as HS-40 (DNAse I hypersensitive site −40), is essential for the formation of ACH. The removal of this element abolished all other spatial interactions within the domain. Interestingly, the correct spatial organization of the domain was restored when the −40 element (normally located 40 Kb upstream of the alpha-globin gene cluster) was reinserted downstream of the cluster [15]. A service element, which does not possess an enhancer activity but is necessary for the formation of ACH, has been identified in the human *AML* gene [31].

Although the pairwise interactions of the regulatory elements assembled into an ACH have been well documented in different model systems (for a review see [32]), the exact nature of the ACH remains obscure. The isolation of DNA–protein complexes, which could represent ACHs, has not been reported. The resolution of fluorescent microscopy is not high enough to study the fine structure of ACH. A recent study demonstrated that upon differentiation of mouse erythroid cells, the beta-globin locus acquired a more compact configuration, as evidenced from the reduction of the volume occupied by the locus [33]. Furthermore, it was demonstrated that upon induction of globin gene transcription, the shape of a folded chromatin domain occupied by the beta-globin locus became more round [33]. Although these observations are consistent with predictions based on the ACH model, the validity of the expression hub/active chromatin compartment model (see below) cannot be ruled out, as both models suggest that promoters and distant regulatory elements of a gene domain are brought in close vicinity in the nuclear space to ensure transcription activation. The resolution of the approach used in this study only permitted the analysis of the shape and dimensions, but not the internal organization, of the relatively large chromatin domain (128 Kb of DNA, more than 200 nm in each direction).

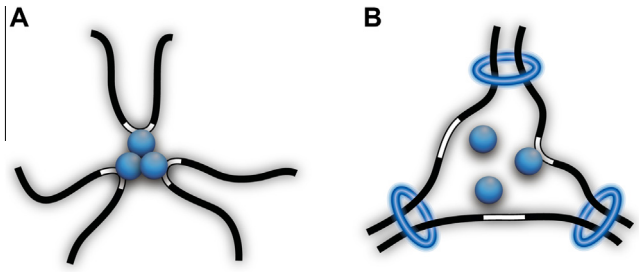

Fig. 1. The ACH models. (A) The chromatin hub as a rigid complex of regulatory elements. (B) The chromatin hub as a nuclear compartment. The black lines represent segments of chromatin fibers with regulatory elements shown as white boxes. The circles symbolize transcription factors and transcription machinery proteins; rings–cohesion complexes.

3. Active nuclear compartment (expression hub)

As discussed in the previous section, the ACH model suggests that enhancers and promoters are held together through the interaction of proteins bound to each of these elements (Fig. 1A). It was assumed that these DNA–protein complexes could be further stabilized via formaldehyde cross-linking and solubilized after DNA cleavage by restriction enzyme(s) and nuclei lysis by SDS [10,16,34]. Recent studies have demonstrated that this assumption, which constitutes the basis of the 3C procedure, might be incorrect [35,36]. It has been reported that the major portion of DNA/chromatin fragments cannot be solubilized from cross-linked nuclei. Consequently, the proximity ligation step in the 3C procedure proceeds in non-lysed nuclei in a chromatin cage rather than in a diluted solution, as originally proposed. Furthermore, the maintenance of the chromatin cage appears essential for the detection of 3C signals [36]. Based on these observations, we have suggested that the higher-order chromatin domain folding constitutes a primary determinant in establishing/securing the proper mutual positions of enhancers and promoters [36] (Fig. 1B). It was previously proposed that transcribing genes and distant regulatory elements should be placed in the same nuclear compartment, an expression hub, where they form different short-lived associations [18]. Our data [36] fit this model perfectly. The disposition of an enhancer (or a block of enhancers) and several promoters in close spatial vicinity secured through the specific folding of the large chromosomal domain (Fig. 1B) facilitates the establishment of short-lived alternating associations between the enhancers and different promoters, resulting in the alternating transcription of different genes, a phenomenon observed in cells expressing globin genes [37]. At first view the results demonstrating the ability of the LCR-like element HS-40 of the human alpha-globin gene locus to ensure the correct spatial positioning of globin genes promoters and enhancers [15] argue against the supposition that the configuration of a chromosomal domain is essential to secure this positioning. Indeed, relocation of a regulatory element to a new genomic position should drastically affect its ability to participate in shaping of the parental chromatin domain due to the perturbation of spatial connections. On the other hand, being inserted in an ectopic position this element still may constitute a platform for the assembly of a chromatin hub. However, in the above-mentioned experiments, the HS-40 was reinserted immediately downstream of the alpha-globin gene cluster [15]. Thus, HS-40 remained within the same chromosomal locality and possibly could support the correct configuration of the chromatin domain harboring alpha-globin genes.

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