Cell

Leading Edge
Perspective

The 3D Genome as Moderator of Chromosomal Communication

Job Dekker[1,*] and Leonid Mirny[2,*]
[1]Howard Hughes Medical Institute, Program in Systems Biology, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 368 Plantation Street, Worcester, MA, 01605-0103, USA
[2]Institute for Medical Engineering and Science and Department of Physics, Massachusetts Institute of Technology, 77 Massachusetts Avenue, E25-526C, Cambridge, MA 02139, USA
*Correspondence: job.dekker@umassmed.edu (J.D.), leonid@mit.edu (L.M.)
http://dx.doi.org/10.1016/j.cell.2016.02.007

Proper expression of genes requires communication with their regulatory elements that can be located elsewhere along the chromosome. The physics of chromatin fibers imposes a range of constraints on such communication. The molecular and biophysical mechanisms by which chromosomal communication is established, or prevented, have become a topic of intense study, and important roles for the spatial organization of chromosomes are being discovered. Here we present a view of the interphase 3D genome characterized by extensive physical compartmentalization and insulation on the one hand and facilitated long-range interactions on the other. We propose the existence of topological machines dedicated to set up and to exploit a 3D genome organization to both promote and censor communication along and between chromosomes.

Chromosomal Communication

Communication involves transfer of information from one party to another. This can be achieved in at least two mechanistically distinct ways. First, the parties directly interact, e.g., two or more people directly speaking to each other. Second, information can be transmitted from one location to another via media or intermediates and it is then received by the appropriate partner(s) at their respective locations. For the first mechanism, the two parties need to be physically close, for the second, there needs to be a means to send, transport and receive information from one place to another. Do similar mechanisms operate inside the cell nucleus where genes are regulated by communicating with regulatory elements that can be located elsewhere in the genome? Here we explore the idea that the spatial organization of a genome, and its physical properties, could constitute an effective mechanical communication device.

Genes do not work as single, isolated units. Their expression is modulated by regulatory elements that can be located from as little as a kb up to as much as several Mb away, although the precise distance distribution between genes and their regulatory elements is still poorly known (Bickmore, 2013; Bulger and Groudine, 1999; Carter et al., 2002; Gibcus and Dekker, 2013; Kleinjan and van Heyningen, 2005; Li et al., 2012; Sanyal et al., 2012; Schwarzer and Spitz, 2014; Tolhuis et al., 2002; West and Fraser, 2005). Since in a given cell thousands of genes are expressed throughout the genome, there is a corresponding abundance of long-range communication between genes and regulatory elements occurring at any moment in each cell nucleus. Over the last decade much has been learned about how this is achieved, revealing critical roles for the spatial organization of chromosomes.

Microscopy-based technologies, such as fluorescence in situ hybridization (FISH) and live cell imaging, and increasingly high resolution chromosome conformation capture (3C)-based methods (Bickmore, 2013; Dekker et al., 2013; Dekker et al., 2002; Fraser et al., 2015; Hsieh et al., 2015; Kalhor et al., 2012; Lieberman-Aiden et al., 2009; Rao et al., 2014; Shachar et al., 2015; Tang et al., 2015) have been instrumental in determining how chromosomes are folded at different length scales (kb up to Gb), and this in turn is starting to provide answers to some long-standing questions related to gene regulation and other chromatin-templated processes. One mechanism by which distal regulatory elements can control genes located far away in the genome is through long-range physical interactions (Figure 1). For instance, enhancer and insulator elements often engage in physical contacts with their target promoters (Carter et al., 2002; Li et al., 2012; Sanyal et al., 2012; Tolhuis et al., 2002), pointing to direct molecular association as a means for long-range communication.

Although such physical associations appear to account for a significant fraction of long-range gene regulatory events, not all chromosomal communications involve direct contacts between the corresponding loci (Figure 1). An example is the case of X chromosome inactivation in female mammals. In this case, the Xist RNA is expressed from one X chromosome only and this RNA spreads along the length of the entire chromosome resulting in gene repression through the Xist-dependent recruitment of a set of silencing complexes (Chu et al., 2015; Galupa and Heard, 2015; Gendrel and Heard, 2014; Jeon et al., 2012; Wutz et al., 2002). Here, communication along the inactivated X chromosome occurs not by direct physical interactions, but by *cis*-spreading of a signal, a non-coding RNA, that delivers silencing proteins to most of the genes linked in *cis* to the Xist gene. X chromosome inactivation also requires initial inter-chromosomal communication to ensure that only one X chromosome expresses Xist. Though Xist loci of the two X chromosomes do

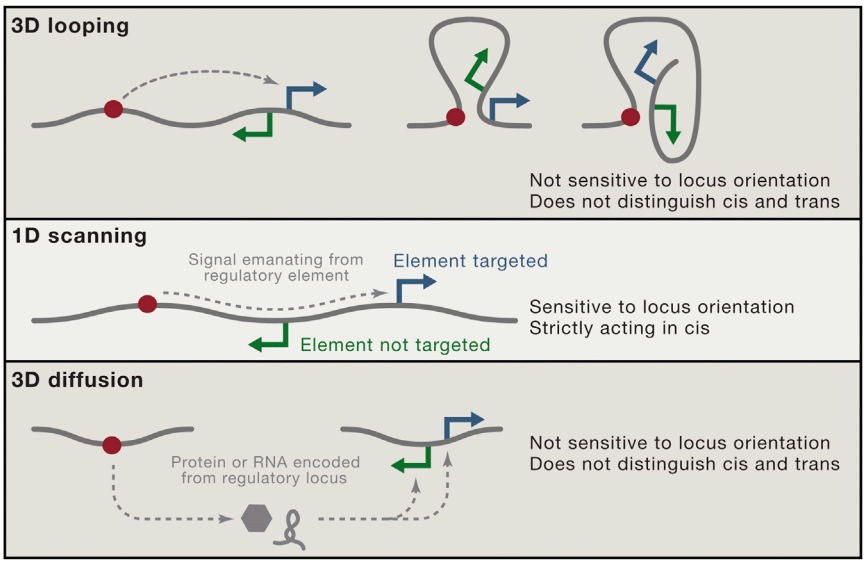

Figure 1. Chromosomal Communication

Top: communication between genomic loci by 3D looping interactions. For large loops, e.g., tens to hundreds of Kb, such interactions are not sensitive to locus orientation. Also, 3D interactions do not readily distinguish interactions with loci located on the same chromosome (in *cis*) or on different chromosomes (in *trans*). Middle: Signals emanating from one locus (e.g., RNA transcribed at that locus, a protein complex recruited at that site) can spread in *cis* along the chromatin fiber till a target locus is reached. This mode of communication can be sensitive to relative orientation of the target locus, and is strictly in *cis*.

Bottom: Communication by 3D diffusion of factors such as RNA or proteins released from one locus till they reach a target loci. This mode of communication is not sensitive to target orientation and cannot distinguish *cis* from trans.

transiently interact (Augui et al., 2007; Masui et al., 2011; Xu et al., 2006), implying physical communication, critical information is transmitted by diffusible proteins such as Rnf12 (Barakat et al., 2014; Galupa and Heard, 2015). The latter mode of communication includes the general and widespread action of transcription factors encoded at one locus but acting throughout the genome. Thus, communication involves direct physical associations, *cis*-spreading of information, and diffusional signals including proteins and RNAs, that can move between chromosomes (Figure 1). In this perspective we do not discuss diffusion-based communication through transcription factors and instead focus on communication through long-range chromatin interactions and spreading of signals in *cis* along chromosomes. In addition, we mostly discuss chromosome organization and long-range communication in mammalian genomes, even though other organisms including bacteria may employ similar mechanisms.

Not all chromosomal communication is for regulating gene expression. An interesting example is intra-chromosomal communication to control somatic recombination in the immunoglobulin loci, such as V(D)J recombination and antibody class switching. During these processes, specific pairs of double-stranded breaks located up to 200 kb apart need to interact to be joined for successful recombination events. Recent studies (Dong et al., 2015; Gostissa et al., 2014) have revealed a surprising orientation bias in the IGH class switch recombination process where genomic orientation is preserved in the vast majority of recombination events prior to any further selection. During the process, recombination occurs between recombination sequences that undergo activation-induced cytidine deaminase (AID)-dependent DNA break formation. Interestingly, re-joining of ends is orientation-specific implying long-range communication between the break sites in a manner that maintains the relative orientation of the sites even when they are separated by hundreds of Kb. Thus, communication between two sites where double-stranded breaks are initiated requires not only direct proximity between them, but also preservation of their genomic orientation, pointing to specific processes to facilitate and or mediate their association in a directional manner (discussed below in more detail).

With the phenomenon of long-range communication well established, and the roles of chromosome structure and dynamics becoming increasingly clear, many new questions arise: first, how are long-range interactions established, i.e., how do distal elements find one another inside the crowded nucleus? Further, what determines specificity of such interactions and what prevents any of the thousands of active regulatory elements in the genome from inappropriately engaging in contacts with any of the thousands of genes? How do signals spread along chromosomes and how can such spreading be contained to a single chromosome (e.g., X chromosome inactivation)? How is robustness and precision achieved so that important communication is efficiently and rapidly established in most or all cells? Answers to these questions start to emerge now that deeper knowledge is obtained about nuclear organization, the structural compartmentalization of chromosomes, the physical and mechanical properties of chromosomes and their dynamics, and the identification of molecular machines that can actively fold chromosomes to orchestrate and guide long-range communication.

Physics of Chromosomal Communication

Chromosomes are long polymers and many of their structural properties, dynamics and cell-to-cell variability in folding can be understood from their polymer nature. In fact, the polymer state of chromosomes has critical consequences for which pairs of loci have an opportunity to interact, the kinetics of their search for each other, and the number of cells in the population in which interactions occur (Figure 2). To illustrate this we will first explore the scenario in which the 3D genome is determined solely by the physical polymer state of chromosomes.

Three physical phenomena are central to our understanding of spatial genomic communication: the short-range character of molecular interactions, the polymeric nature of chromosomes, and the localized dynamics of chromosomal loci (Figure 2). Below we discuss implications of these aspects to genomic communications.