

“Cat’s Cradling” the 3D Genome by the Act of LncRNA Transcription

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<http://dx.doi.org/10.1016/j.molcel.2016.05.011>

There is growing evidence that transcription and nuclear organization are tightly linked. Yet, whether transcription of thousands of long noncoding RNAs (lncRNAs) could play a role in this packaging process remains elusive. Although some lncRNAs have been found to have clear roles in nuclear architecture (e.g., *FIRRE*, *NEAT1*, *XIST*, and others), the vast majority remain poorly understood. In this Perspective, we highlight how the act of transcription can affect nuclear architecture. We synthesize several recent findings into a proposed model where the transcription of lncRNAs can serve as guide-posts for shaping genome organization. This model is similar to the game “cat’s cradle,” where the shape of a string is successively changed by opening up new sites for finger placement. Analogously, transcription of lncRNAs could serve as “grip holds” for nuclear proteins to pull the genome into new positions. This model could explain general lncRNA properties such as low abundance and tissue specificity. Overall, we propose a general framework for how the act of lncRNA transcription could play a role in organizing the 3D genome.

Introduction

Since the elucidation of the one-dimensional sequence of the human genome, enormous effort has been placed toward understanding the 2D elements encoded therein (protein-coding genes, enhancers, noncoding RNA genes, and so forth) as well as how these elements interact in three dimensions. It is remarkable to think that two meters of DNA are packaged into a tiny nuclear capsule of 2–10 μm in diameter. Moreover, this process needs to be uniquely regulated in different cell types.

The numerous DNA packaging conformations adopted during lineage commitment have been found to correlate with transcriptional changes (Dixon et al., 2015; Peric-Hupkes et al., 2010). Interestingly, both transcriptional output and nuclear architecture can be used as footprints to classify cell types (Lukk et al., 2010; Melé et al., 2015; Snyder et al., 2016), and links between transcriptional changes and nuclear repositioning have been reported at many levels (reviewed in Gorkin et al., 2014; Hübner et al., 2013).

In light of the tight relationship between transcription and nuclear architecture, an important question that arises is whether transcription plays an active role in organizing the nucleus. One of the most dramatic examples of how noncoding RNA transcription can organize nuclear substructures is the nucleolus. The nucleolus is the largest sub-nuclear compartment, and its function is to produce ribosomal subunits. It is organized based on the recruitment of greater than 400 ribosomal RNA genes, distributed across multiple chromosomes, in order to activate their transcription (Hernandez-Verdun, 2006). Interestingly, inhibition of rRNA transcription is followed by nucleolus disassembly (Oakes et al., 1993), and more importantly, expression of rRNA genes inserted into new chromosomal locations is sufficient to generate nucleolus-like structures (Karpen et al., 1988). Therefore, rRNA transcription is a fundamental process for nucleolus organization.

In the past decade, the number of annotated lncRNA loci detected across diverse cell states has increased dramatically (Table 1). Whereas some lncRNAs, such as *XIST* (Cerase et al., 2015), *FIRRE* (Hacisuleyman et al., 2014), and *NEAT1* (Clemson et al., 2009), play a role in organizing nuclear architecture (reviewed in Caudron-Herger and Rippe, 2012; Quinodoz and Guttman, 2014; Rinn and Guttman, 2014), the function of the vast majority of lncRNAs remains unknown. The possibility that each lncRNA performs a unique role in the cell is still open. However, this notion is unlikely due to the observations that most lncRNAs are expressed at very low abundance (even less than one copy in 1,000 cells (Hangauer et al., 2013) and exhibit low conservation on a primary sequence level. Yet, they all share one very powerful property: the act of being transcribed.

Here, we discuss how the act of transcription, with a specific focus on lncRNA loci, could be a common and pervasive contributor to nuclear architecture, irrespective of the final RNA product. We would also like to avoid attributing function in the classic sense of known lncRNA and mRNA genes, but rather discuss a potential global functionality of transcribing lncRNA loci. Overall, we aim to synthesize a hypothesis of how thousands of transcribed, but low-abundance, transcripts could contribute to higher-order genome architecture.

Correlation between Transcription and Nuclear Architecture: Cause or Consequence?

Correlation between spatial organization of the genome and transcription has been observed at many different levels (Cohen et al., 2000; Gorkin et al., 2014; Hübner et al., 2013). For example, at the highest organizational level, chromatin can be divided into two large-scale compartments: euchromatin and heterochromatin. Euchromatin has an uncondensed conformation and is transcriptionally active, gene rich, and located in

Table 1. Number of LncRNA Genes or Intergenic LncRNAs Cataloged in Human and Mouse

No. LncRNAs (Intergenic)	Species	Publication	Database and Version
6,736	human	Jia et al., 2010	
8,195	human	Cabili et al., 2011	
15,941 (7,674)	human	Derrien et al., 2012; Harrow et al., 2012	gencode v.24
53,864	human	Hangauer et al., 2013	
58,648	human	Iyer et al., 2015	
90,062	human	Zhao et al., 2016	noncode2016
34,030 ^a	mouse	Maeda et al., 2006	fantom3
(3,289)	mouse	Khalil et al., 2009	
9,072 (3,579)	mouse	Mudge et al., 2013	gencode v.8
79,940	mouse	Zhao et al., 2016	noncode2016

Intergenic lncRNAs are lncRNAs that do not overlap protein-coding gene loci.

^aNumber refers to transcripts rather than genes.

the nuclear interior. In contrast, heterochromatin is highly condensed, gene poor, and located at the nuclear periphery, close to the nuclear lamina (Figure 1). At a more local level, chromosomal looping allows enhancers to interact with promoters of distal genes and regulate their transcription. In fact, there are many examples where changes in chromatin conformation triggering looping can affect transcriptional output (Deng et al., 2012; Rao et al., 2014; also reviewed in Li et al., 2006).

Chromatin conformation capture (3C; Dekker et al., 2002) and the following advances (4C, 5C, and Hi-C, reviewed in Barutcu et al., 2016; de Laat and Dekker, 2012; de Wit and de Laat, 2012) have shown that chromosomes are subdivided into topological associating domains (TADs). For a thorough review on TADs organization and mechanism of formation, see Dixon et al. (2016). TADs are ~1 Mb linear DNA segments that form independent folding units in 3D space. Interactions between sequences within a TAD occur more often than interactions between sequences in neighboring TADs (Dixon et al., 2012; Lieberman-Aiden et al., 2009). TAD boundaries are conserved across cell types and across species, highlighting the functional importance of these chromosomal sub-domains (Dixon et al., 2015, 2012). Interestingly, different TADs have coherent epigenetic states and can be classified as transcriptionally active or inactive (or assigned to compartments named “A” and “B,” respectively). Importantly, although the existence of a TAD is generally conserved, its state—active or inactive—varies across cell types, suggesting that organization of all TADs in transcriptionally active or inactive states plays an important role in defining cell fate (Dixon et al., 2015).

Consistent with these observations, disruption of TAD boundaries can affect transcriptional regulatory programs and ultimately lead to disease. For example, disruption of TAD boundaries on the inactive X chromosome induces new and detrimental long-range interactions (Nora et al., 2012). Furthermore, limb malformations in humans have been shown to originate from a similar event. In this case, disruption of a TAD boundary facilitates interaction between enhancers and pro-

moters normally residing in different TADs, ultimately resulting in transcriptional misregulation and developmental defects (Lupiáñez et al., 2015).

Despite the clear relationship between transcriptional activity and nuclear organization, whether one is the consequence of the other remains unknown (Hübner et al., 2013). One possibility is that changes in nuclear organization could influence and regulate transcription. If this were the case, repositioning of certain DNA segments to new regions of the nucleus could regulate transcription of the genes encoded in such DNA segment. However, it is also possible that transcription itself could reorganize the nucleus. This is an intriguing idea, considering that many regions of the genome are transcribed but have unknown function. Could the transcription of noncoding regions, such as lncRNAs, actively direct the formation of specific nuclear conformations? Below, we present some examples in the literature supporting one of these two models.

Location Change and Then Transcription

Initial observations that highly transcribed genes tend to co-localize (Fraser and Bickmore, 2007; Jackson et al., 1993; Osborne et al., 2007, 2004) seemed to suggest that gene activation required DNA repositioning to transcriptionally active regions, termed transcription factories (Osborne et al., 2004). Indeed, later studies showed that genes regulated by similar transcription factors can co-localize in 3D space when they are transcribed (de Wit et al., 2013; Schoenfelder et al., 2010). These observations are consistent with a model—but do not prove it—in which specific chromosomal regions move and transcription is activated as a consequence of their new location (Figure 2A). Consistent with this model, some studies found that artificially relocating specific DNA regions close to the nuclear lamina could turn off or alter transcription (Finlan et al., 2008; Reddy et al., 2008). However, other studies did not observe transcription downregulation by repositioning different DNA regions to the lamina (Kumaran and Spector, 2008).

Transcription and Then Location Change

In some cases, transcription may need to take place before nuclear repositioning occurs (Figure 2B). Zink et al. (2004) studied the localization of *CFTR* in several human cell lines, under both transcriptional activation and repression. In its inactive state, the *CFTR* locus was near the nuclear lamina, and when active, it was closer to the nuclear interior. More importantly, transcriptional activation affected nuclear positioning of the locus, but not vice versa (Zink et al., 2004). Another example of transcription preceding relocation occurs during erythroid maturation, wherein the B-globin gene is activated and subsequently relocated from the nuclear periphery to the nuclear interior (Ragoczy et al., 2006). Importantly, transcription of the B-globin gene starts when the gene is still at the nuclear periphery, suggesting that transcriptional activation of the locus facilitates its relocalization, and not the other way around.

It is also known that transcription of lncRNAs can initiate nuclear reorganization. For example, the transcription of *NEAT1* can induce paraspeckle formation, which are nuclear bodies enriched in proteins that promote nuclear retention of certain mRNAs (Bond and Fox, 2009). The presence of *NEAT1* is sufficient to form the paraspeckle (Mao et al., 2011), and transcription of *NEAT1* is necessary to maintain it (Clemson et al., 2009). Thus,

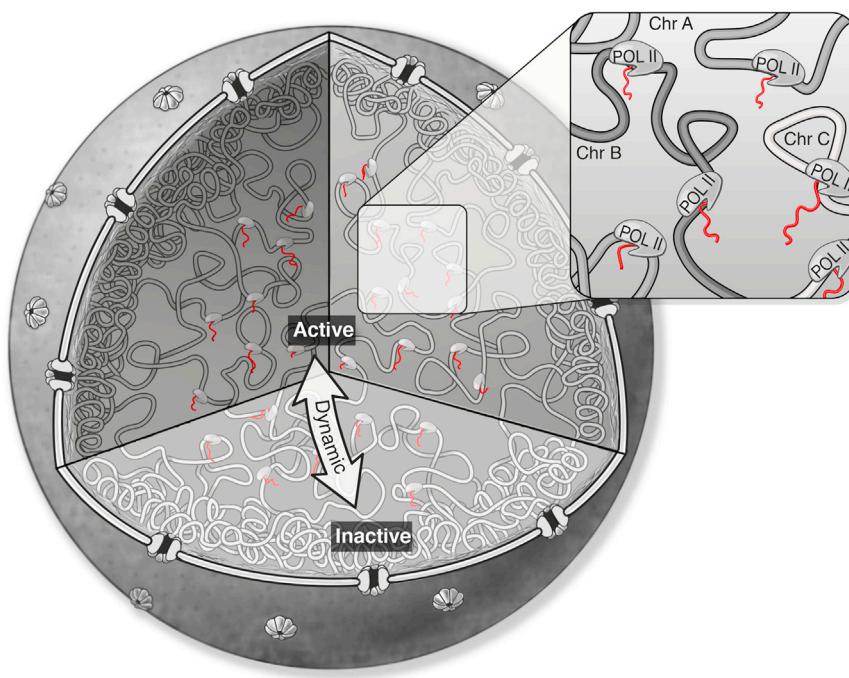


Figure 1. Organization of the Nucleus in Transcriptionally Active and Inactive Compartments

Transcriptionally active DNA tends to be closer to the nuclear center, whereas transcriptionally silenced DNA is more often localized in the nuclear periphery.

transcription of the *NEAT1* RNA is sufficient to generate and maintain the nuclear domain where it localizes.

The Role of LncRNA Transcription in Chromatin Remodeling and Nuclear Architecture

The number of recently annotated lncRNAs has continued to increase, with the latest catalogs containing more than 50,000 lncRNA genes (Table 1). However, the number of functionally characterized lncRNAs is still limited to a few hundred (Amaral et al., 2011; Quek et al., 2015). This gap can be partially explained by the difficulty of identifying RNA function by sequence analysis alone, an approach that is relatively straightforward for proteins (Goff and Rinn, 2015). As a result, the relative importance of the RNA product versus the act of their transcription is largely unknown. However, the fact that transcription and nuclear architecture are tightly correlated opens the possibility that the act of lncRNA transcription contributes to nuclear organization. Indeed, there are many publications reporting that the act of transcription of noncoding RNAs has an effect on chromatin reorganization.

One of the first cases in which the act of intergenic transcription was shown to play a crucial role in chromatin remodeling and nuclear architecture was in *Hox* gene clusters (Schmitt et al., 2005). The *Hox* genes are key developmental transcription factors that regulate body patterning across the anterior-posterior axis. Activators such as the trithorax group (TrxG) and repressors such as the Polycomb group (PcG) proteins coordinate a complex choreography of *Hox* gene expression patterns that define specific body patterning (Schuettengruber et al., 2007). In *Drosophila*, *Hox* gene repression is in part mediated by the binding of PcG proteins to DNA motifs termed PcG response elements (PREs). When the region is turned on, TrxG response

elements (TREs) recruit TrxG proteins to bind and activate transcription of the *Hox* genes. Yet, how this switch from the off state to the active state occurred was a mystery until Schmitt et al. (2005) found that the act of transcription through a PRE element rather than the RNA product could trigger the switch from PRE to TRE. Thus, the act of transcription changes a key developmental gene from a heterochromatic to a euchromatic state.

Other studies involving *Hox* gene regulation in *Drosophila* found that the act of transcription could also induce transcriptional repression through a mechanism named transcriptional interference (Petruk et al., 2006). Transcriptional interference occurs when transcription in one site has a negative impact on transcription in another site in *cis* (Shearwin et al., 2005). In this case, the act of transcription of *Bxd* ncRNAs represses expression of the downstream *Ubx* gene in *cis* (Petruk et al., 2006).

A similar mechanism by which the act of transcription represses expression of a downstream gene occurs in the context of imprinting (Latos et al., 2012). Imprinted genes are expressed based on their parental origin, so that only one copy (maternal or paternal) is selectively expressed, while the other copy is silenced. There are around 100 imprinted genes in mice and humans, and most are located in clusters (Ferguson-Smith, 2011). In three of these clusters, epigenetic silencing is controlled by the expression of specific lncRNAs in *cis*: *Kcnq1ot1* (Mancini-Dinardo et al., 2006), *Gnas* (Williamson et al., 2011), and *Airn* (Sleutels et al., 2002). In the case of *Airn*, the act of transcription, rather than the RNA product itself, triggers epigenetic silencing of the paternal allele (Latos et al., 2012).

Early studies suggested that the presence of the *Airn* RNA transcript is necessary for a long-range looping event that changes the 3D organization of the paternal allele, interfering with *Igf2r* gene promoter and inducing epigenetic silencing (Sleutels et al., 2002). However, a more recent study revealed that only the act of transcription across the *Airn* locus was necessary to induce this event (Latos et al., 2012). Using polyA cassettes to truncate *Airn* transcripts at different lengths, Latus and colleagues found that only *Airn* transcripts that overlap *Igf2r* promoter are able to silence *Igf2r* transcription, indicating that silencing of the paternal *Igf2r* gene is carried out by the act of *Airn* transcription. However, the mechanism by which the other genes in the cluster, such as *Slc22a1* and *Slc22a3*, are silenced is still unknown. Paurer et al. (2012) suggested that the *Airn* locus

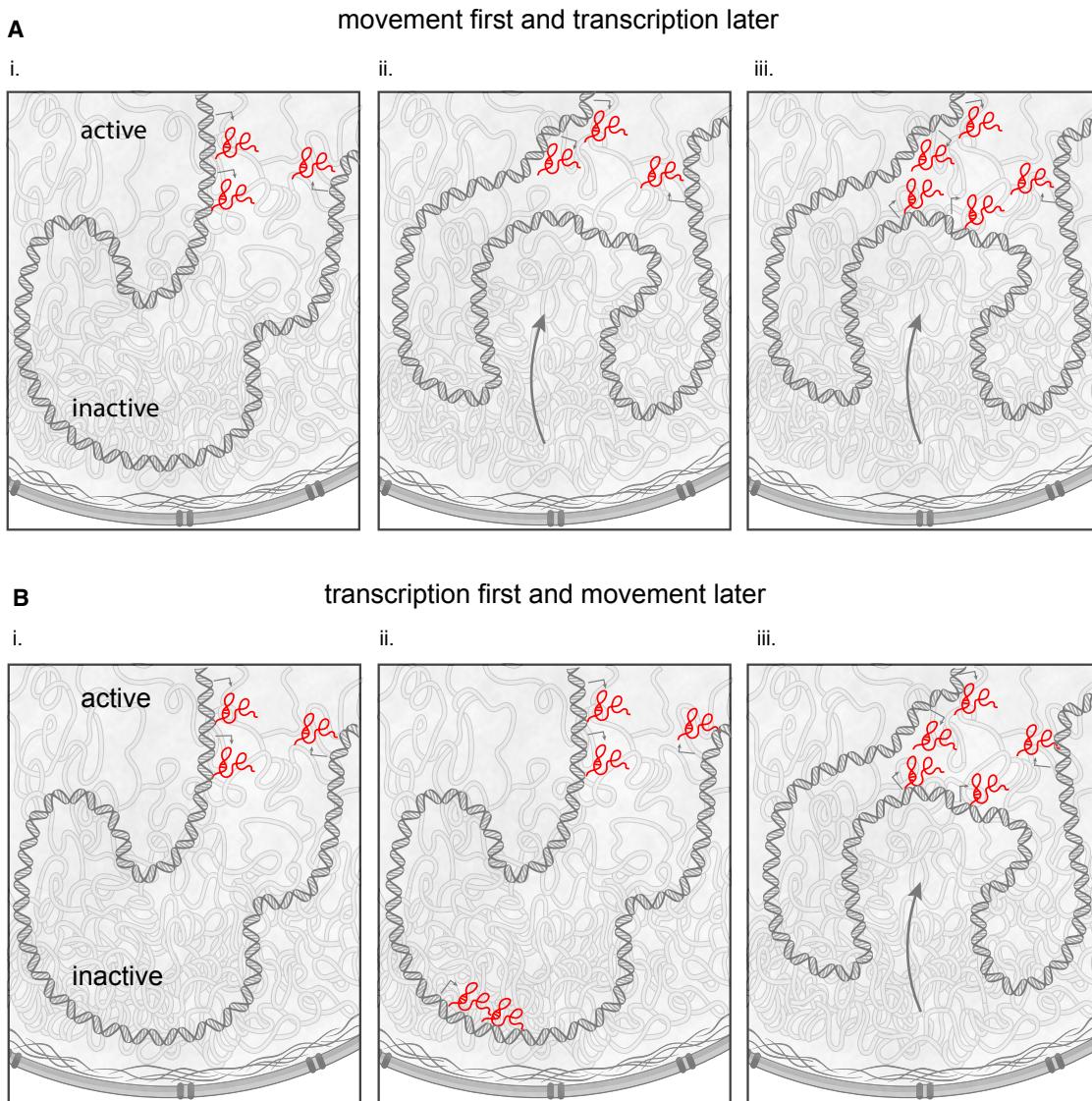


Figure 2. Two Possible Models Explaining the Relationship between Nuclear Organization and Transcription

(A) DNA repositioning allows transcriptional activation. Initial state with active chromatin in the inner nucleus and inactive chromatin in the periphery (i). DNA repositioning from the periphery to the inner nucleus (ii) will trigger transcription activation (iii).

(B) Transcription activation promotes DNA repositioning. Transcription activation in a previously inactive region (i) triggers DNA repositioning to the inner nucleus (ii), closer to a transcriptionally active environment (iii).

can loop and end up in physical proximity to the other genes in the IGF2R cluster. In their model, the act of *Aimn* transcription could trigger epigenetic silencing by abrogating the binding of transcription activators to other genes in that region.

Another crucial process where the act of transcription underlies the establishment and maintenance of higher-order chromatin architecture occurs during the process of X-inactivation in females. In mammals, inactivation of one of the X chromosomes is a developmentally regulated process that intrinsically depends on the lncRNA *Xist*. Specifically, *Xist* is initially transcribed from one of the X chromosomes and it spreads in *cis*, ultimately triggering X chromosome inactivation (Augui et al., 2011). Several studies have shown that the act of transcription

of *Tsix*, a lncRNA antisense to *Xist*, can induce chromatin changes on a local scale, ultimately regulating *Xist* expression (Navarro et al., 2009, 2006, 2005; Ohhata et al., 2008; Sado et al., 2005). Consistent with the role of transcription in maintaining higher-order chromatin architecture, a different study using lymphoblastoid cell lines showed that transcription is enough to maintain chromatin in an open state. Specifically, this study showed that transcription on the active X chromosome promotes an open chromatin environment and that under transcription inhibition, chromatin is condensed to a heterochromatic state. Thus, the act of transcription is enough to maintain chromatin in an open state on the active X chromosome (Naughton et al., 2010).

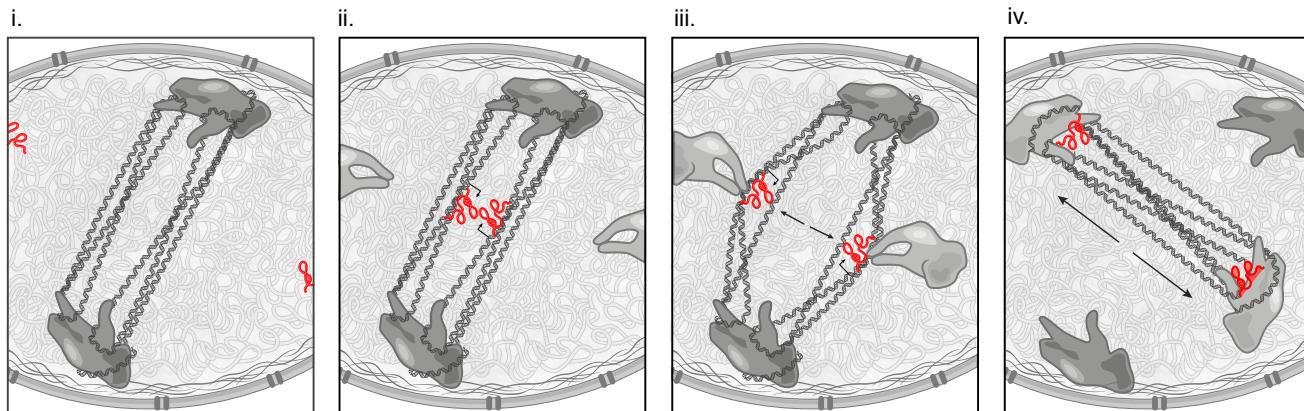


Figure 3. The Cat's Cradle Model

In a specific cell state, DNA is folded in a specific 3D conformation (i). Then, transcription of lncRNAs in specific DNA regions (ii) marks the spot for specific proteins to bind (iii) and pull the DNA to a new conformation (iv). Similarly, at the beginning of the cat's cradle game, the string is folded in a specific conformation (i). Then, another person's fingers grip to specific string locations (ii) and pull (iii) to form a new string conformation (iv). It is important to notice that this representation is a cartoon and that the size of proteins/hands and DNA/string has been magnified for simplicity.

Overall, there are many other examples where the act of transcription, rather than the RNA product, is relevant for chromatin reorganization and downstream transcriptional regulation (Abarategui and Krangel, 2007; Gribnau et al., 2000; Han et al., 2008; Lefevre et al., 2008; Martens et al., 2004; Uhler et al., 2007). Given the large number of yet-uncharacterized transcriptional events that occur within mammalian cells, in the following section we propose a model by which the act of transcription, and/or possibly the RNA product, could play a global role in regulating nuclear architecture.

"Cat's Cradling" the 3D Genome by LncRNA

Transcription

A global relationship exists between nuclear architecture and transcription. Interestingly, there are tens of thousands of actively transcribed loci with no known function—such as most lncRNAs—or for which the mechanism of action is unclear, such as enhancer RNAs. Here, we attempt to integrate the vast majority of this non-coding transcription into a model wherein the act of lncRNA transcription itself is capable of organizing chromatin architecture.

One could think of this model—in which the thousands of lncRNA loci facilitate organization of subnuclear compartments—as a molecular game of cat's cradle. The cat's cradle game is based on two or more players making specific structures with a string. One player starts with a specific string conformation and then the next player will pull the strings at specific locations and generate a new string conformation (https://en.wikipedia.org/wiki/Cat%27s_cradle) (Figure 3). In this model, the first string conformation would be analogous to the way DNA is folded in one cell state. Then, during cell fate transitions, transcriptional activation of cell-type-specific lncRNAs could produce new "grip holds" with which proteins pull and change the 3D organization of the genome into a new state (Figure 3). Therefore, transcription of lncRNAs would mark the spot for nuclear proteins such as lamins or nuclear organizing hnRNP proteins to pull the DNA so that, by changing the transcriptional

landscape (activating lncRNAs), both the nuclear organization and the cell state can change.

Our model implies that, for many lncRNAs, what is functionally relevant may be the act of transcription rather than the RNA molecule itself. This would be consistent with the observed low abundance and high tissue specificity for many lncRNAs (Cabilio et al., 2011; Derrien et al., 2012; Molyneaux et al., 2015). LncRNAs would not need to accumulate to high abundance since the process of transcription would change the epigenetic state of a given locus and facilitate new interactions with nuclear organization protein complexes. Moreover, since the biologically relevant step occurs early in the lncRNA's "life cycle," there would be little or no consequence to rapidly degrading the final transcript.

With the advent of all the related chromatin conformation capture technologies (Barutcu et al., 2016; de Laat and Dekker, 2012; de Wit and de Laat, 2012), it is now feasible to quantitatively assess how chromosomal interactions could potentially be affected if lncRNA transcription were disrupted. In fact, it is now possible to both disrupt or activate transcription in thousands of specific genomic loci, such as lncRNAs, using CRISPR/Cas9 technology (Gilbert et al., 2014) and assess downstream effects in nuclear organization using Hi-C (Lieberman-Aiden et al., 2009). Similarly, we could visualize chromosomal interactions in live cells after transcriptional activation or repression by labeling specific genomic regions using CRISPR/Cas9 (Chen et al., 2013; Ma et al., 2015; Shechner et al., 2015). Finally, a key point to test our hypothesis would be to disentangle whether any observed effects on nuclear architecture are related to changes in the act of transcription rather than the transcribed lncRNA (Grote and Herrmann, 2015). To address this, approaches such as adding a polyA tail or knocking down the lncRNA transcript have been successful in unraveling the two possibilities (Latos et al., 2012; Lee et al., 2016; Paralkar et al., 2016).

After the sequencing of the human genome, one of the biggest surprises has been that thousands of RNAs of unknown function are actively transcribed. It is now becoming clear that such

transcriptional events correlate with the 3D organization of the genome. Our model anticipates that the thousands of transcriptional events that simultaneously occur in each cell may play a role in organizing nuclear architecture. We predict that lncRNAs are key players in the process. Future research needs to decode the interplay between the act of transcription and nuclear architecture. We anticipate that this will be crucial to understand how cell identity is defined and how disruption of such interactions can lead to disease.

ACKNOWLEDGMENTS

We thank the following members of the lab of J.L.R. for thorough comments on this Perspective: Kaia Mattioli, Abbie Groff, Philipp Maass, David R. Kelley, Rasim Barutcu, and especially David M. Shechner.

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