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Cytotopic localization by long noncoding RNAs

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Abstract

Cells are highly organized structures. In addition to membrane delimited organelles, proteins and RNAs can organize themselves into specific domains. Some examples include stress granules and subnuclear bodies. This level of organization is essential for the correct execution of multiple processes in the cell, ranging from cell signaling to assembly of structures such as the ribosomes. Here we will review evidence that noncoding RNAs play a critical role in the establishment and regulation of these domains. The unique abilities of RNA to mark the genome in a gene-specific and condition-specific manner and to serve as tethers nominate them as ideal molecular address codes.

The cell nucleus is home to a large variety of nuclear bodies, local accumulations of RNAs and proteins, where several nuclear processes are executed. It has been proposed that formation of nuclear bodies follows a stochastic self-organization assembly model, where highly mobile molecules diffuse throughout the nucleus searching for stable interactions. Stable interactions between molecules result in a steady-state residency in these bodies and, therefore, the formation of a nuclear domain. Several of these nuclear bodies form at sites of transcription, suggesting a role for RNA in the biogenesis of these domains (reviewed in [1]). Long noncoding RNAs (lncRNAs) — defined as transcripts greater than 200 nucleotides that do not code for proteins — are extensively transcribed and are particularly well suited for serving as localization elements.

lncRNAs as seeds to form nuclear bodies

A well studied example of these nuclear domains is the paraspeckle [2], a nuclear body implicated in the retention of hyper edited RNAs [3–5]. The lncRNA NEAT1 is one of the defining components of this domain. NEAT1 is necessary for paraspeckle formation, as depletion of NEAT1 leads to the disruption of paraspeckles [6–9], and tethering of NEAT1 to chromatin is sufficient to induce paraspeckle formation [10[•],11[•]]. Tethering of protein components of the paraspeckle does not lead to the successful formation of a complete paraspeckle [10[•]], suggesting that NEAT1 is the initial nucleating factor functioning as the paraspeckle seed. Unlike the protein components, which show a high rate of dissociation, NEAT1 has a long residency time in the structures. Paraspeckles form close to the site of transcription of NEAT1 and the act of transcription itself is required for paraspeckle formation [10[•]]. These observations suggest a model in which the lncRNA functions as a nucleator for the components that form the paraspeckle (see Figure 1a). Interestingly, although paraspeckles have been observed in almost all cell types studied in culture, human ESC being the only known exception, in living animals they are found only in a specific subpopulation of cells highly expressing the long isoform of NEAT1. The authors of this study have suggested that paraspeckles might be formed in response to changes in the cell environment, changes mimicked by cell culture. This observation suggests that cells use the

transcription of NEAT1 to form a nuclear domain in response to a yet unknown environmental signal [12].

Differential expression of lncRNAs modulates the composition of nuclear bodies

The nucleolus, the nuclear domain where pre-rRNAs are processed, forms around nucleolar organizing regions after each cell division. Nucleoli break down at the onset of mitosis, and reassembly starts during telophase, in a process that requires RNA polymerase I transcription and the recruitment and activation of the pre-rRNA processing machinery (reviewed in [13]). Only 30% of the protein components of the nucleoli have functions related to rRNA maturation. Interestingly, the composition of the nucleolus undergoes dramatic reorganization in response to different types of cellular stress [14–17] (reviewed in [18]), suggesting that the nucleolus, in addition to being the site of rRNA maturation, plays a role in the cellular response to stress. One of the models for nuclear body formation postulates that proteins diffuse freely throughout the nucleus until they are anchored to a nuclear body by an interacting partner, which is a structural component of the nuclear domain. During cellular stress the changes observed in the protein composition of the nucleolus suggest that signaling pathways might lead to changes at the level of the structural components of the nucleolus, leading to changes in the protein composition of this nuclear domain. Acidification of the extracellular milieu leads to the retention of VHL, DNMT1 and POLD1; heat shock stress leads to the retention of HSP70 and inhibition of transcription leads to the retention of MDM2 at the nucleolus. Under the stress conditions that lead to the accumulation of VHL, DNMT1 and POLD1 at the nucleolus, these proteins interact with a region of the large intergenic spacer (IGS) 28 kb downstream of the rRNA transcriptional start site. Similarly, under heat shock stress the protein Hsp70 interacts with regions of the IGS 16 and 22 kb downstream of the rRNA TSS and inhibition of transcription leads to the interaction MDM2 with a region of the IGS 20 kb downstream of the rRNA TSS. In other words, as a response to different types of stress, different proteins will interact with different regions of the rDNA repeat [19**]. Intriguingly, noncoding RNAs are expressed from these regions in response to the cellular stress that leads to the retention of the sequestered proteins at those loci (see Figure 1b). The importance of these RNAs was demonstrated by the inability of the nucleolus to retain the ‘sequestered’ proteins when the relevant IGS RNA was knocked down. In this experimental setting, these proteins were no longer retained at the nucleolus, and became highly mobile. These data suggest that the IGS is capable of promoting the sequestration of proteins under cellular stress through the transcription of multiple lncRNA transcripts that respond to specific stimuli. Expression of each identified transcript is independent from each other, that is, heat shock leads to expression of heat shock-specific IGS RNAs but not of RNAs from IGS positions that respond to other types of cellular stress. Their function is also independent, as inhibition of a specific IGSRNA does not affect the ability of another IGSRNA to sequester its target proteins in response to the specific cellular stress that induces its expression [19**]. Above we have discussed how cells use an RNA molecule as an anchor to form a particular domain in the nucleus. This example demonstrates that cells can modulate the composition of a nuclear domain through the expression of specific lncRNAs in response to different environmental cues.

lncRNAs regulate chromosomal structures under developmental control

Cells can use noncoding RNAs to retain proteins at specific loci as part of developmental programs as well. In *S. pombe*, one of the mechanisms that regulate entry into meiosis is the selective elimination of meiosis-specific transcripts. To prevent the expression of meiotic genes during vegetative growth, the RNA binding protein Mmi1 binds to the determinant of selective removal DSR region of meiotic mRNAs and promotes their degradation [20].

During vegetative growth Mmi1 is localized in multiple nuclear foci. A recently study has shown that in addition to promoting meiotic mRNA degradation through the recruitment of the exosome, recruitment of Mmi1 to nascent mRNA containing the DSR motif also promotes the formation of facultative heterochromatin island at meiotic loci. The formation of these facultative heterochromatin islands is developmentally regulated [21**]. Upon entry in meiosis, when Mmi1 function is deleterious, Mmi1 is sequestered to a structure known as the Mei2 dot, where Mmi1 function is inhibited [20].

The Mei2 dot forms at the *sme2* locus, which expresses two lncRNAs, *meiRNA-S* and *meiRNA-L*, necessary for the formation of the Mei2 dot [22–25]. Recently *meiRNA* has been identified as a target of Mmi1, suggesting that it can compete for Mmi1 binding with meiotic targets. *S. pombe* can therefore use the transcription of noncoding RNAs to shut down the selective elimination of meiosis specific transcripts mechanism, and therefore switch developmental gene expression program [26] (see Figure 1c). It is not yet clear how formation of Mei2 dot formation and *meiRNA* decoy function together. These studies combined show how *S. pombe* utilizes nonprotein coding information in RNA transcripts to ensure that the meiotic gene expression program is silenced during vegetative growth. The DSR sequence of meiotic mRNAs promotes their silencing while the noncoding RNAs at the *sme2* locus allow for expression of the meiotic gene expression program.

As part of meiosis it is necessary that homologous chromosomes recognize each other and become physically linked. The formation of the bivalent structure allows exchange of genetic material between two homologous chromosomes. The mechanism that allows the two homologous chromosomes to recognize each other remains largely unknown, but a recent study in *S. pombe* suggests that in addition to allowing expression of meiotic genes, the noncoding RNAs of the *sme2* locus also play a part in homologous chromosome recognition. In *S. pombe*, the *sme2* locus, where the Mei2 dot forms, displays strong, recombination independent pairing. Deletion of the *sme2* locus leads to loss of robust pairing, while transposition of the *sme2* locus to other chromosomal sites leads to pairing in those ectopic sites. The robust pairing observed at these loci is dependent on the expression of a meiosis-specific noncoding RNA expressed from the *sme2* locus. Although the two RNAs encoded by the *sme2* locus accumulate at the site of transcription only *meiRNA-L* is required for robust pairing. In addition it has been demonstrated that expression of *sme2* transcripts is required from both chromosomes [27**]. This study suggests that RNA-containing complexes can act as site-specific identifiers in the chromosome. Altogether these findings suggest that a multifunctional domain, with important roles during meiosis, forms around the *sme2* locus.

lncRNAs and gene movement in response to changes in gene expression

Above we have discussed how lncRNAs can, once expressed, function as anchors to retain certain proteins in specific domains. A recent study has demonstrated how lncRNAs can control the subnuclear localization of proteins depending on the protein modification status. Polycomb 2 protein (Pc2) has been shown to interact with distinct ncRNAs, depending on its methylation status. Methylated Pc2 interacts with TUG1, leading to the retention of Pc2 and growth control genes in Polycomb bodies (PcGs), where their expression is repressed. Unmethylated Pc2 interacts with MALAT1/NEAT2 and localizes to interchromatin granules (ICGs), where Pc2 bound growth control genes can be expressed [28**]. Although TUG1 and MALAT1/NEAT2 are not required for the formation of Polycomb bodies or ICGs, respectively, their presence in these domains is necessary for the specific retention of Pc2 and growth control gene promoters bound by Pc2 in response to signaling pathways [28**]. These lncRNA thus seem to function as anchors for different forms of Pc2 (see Figure 1d).

A role for lncRNAs in the regulation of cytoplasmic structures

The same principle of RNA-mediated cytotopic function extends also to the cytoplasm, and includes messenger RNAs that moonlight in a noncoding regulatory capacity. VegT mRNA and the noncoding Xlirts are required for the organization of the cytokeratin filaments in *Xenopus laevis*. VegT and Xlirts RNAs are components of the cytokeratin network in the vegetal cortex of stage VI *X. laevis* oocytes and destruction of either one of these transcripts leads to disruption of the cytokeratin cytoskeleton and the release of several RNAs from the vegetal cortex, thus interfering with proper formation of the germinal granules. Inhibition of VegT translation by morpholinos had no impact on the cytokeratin filaments, revealing a noncoding role for the VegT mRNA. Addition of exogenous VegT mRNA can rescue the cytokeratin filaments in oocytes where cytokeratin filaments are disrupted by destruction of endogenous VegT or injection of antibodies targeting components of the cytokeratin filaments, suggesting that VegT mRNA operates at the level of subunit assembly [29]. A recent study demonstrated that VegT mRNA functions by influencing the polymerization status of cytokeratin filaments [30].

In summary, cells use RNA molecules to create and regulate subcellular domains, in response to environmental stimuli or as part of a developmental program. Protein scaffolds are known to play a central role in a wide range of pathways by favoring the interaction between relevant partners (review in [31]). In the nucleus, RNAs are the ideal candidates to play a central role in the organization of nuclear domains. The act of transcription of RNA provides positional identity in a gene-specific and allele-specific fashion without the need of translation, allowing for a fast response to environmental cues. Using RNAs as anchors for subcellular domains allows the cells to quickly form important structures by sequestering proteins already available. Although some of the advantages of RNAs as anchors are more relevant in the nucleus, RNAs are also known to play roles in biology of the cytoplasm. These mechanisms appear to have emerged repeatedly in evolution, suggesting their utility. One limitation of our current knowledge is that the physiologic functions of these subcellular domains are not well known, which should be addressed in future studies.

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Figure 1.

(a) Transcription of the lncRNA NEAT1 leads to the accumulation of protein components at the site of transcription, and the formation of the Paraspeckle. (b) Cellular stress leads to the expression of IGSRNA, resulting in the retention at the nucleolus of proteins that would otherwise diffuse through the nucleoplasm. (c) During vegetative growth, meiotic mRNAs are targeted by for silencing by the protein Mmi1, which promotes RNA degradation and formation of facultative heterochromatic islands. Upon entry in meiosis, Mmi1 is recruited to the Mei2 dot, allowing for the expression of meiotic mRNAs. (d) The Pc2 complex is differentially methylated depending on the nutritional state of the cell. Depending on the state of methylation, Pc2 interacts with different lncRNA, leading to the presence of genes bound by Pc2 in different nuclear domains.