1. **Modifications of ncRNAs**

**Small RNAs are modified with N-glycans and displayed on the surface of living cells.**

Flynn, R.A.; Pedram, K.; Malaker, S.A.; Batista, P.J.; Smith, B.A.H.; Johnson, A.G.; George, B.M.; Majzoub, K.; Villalta, P.W.; Carette, J.E.; et al.

Cell 2021, 184, 3109–3124.

Abstract

Glycans modify lipids and proteins to mediate inter- and intramolecular interactions across all domains of life. RNA is not thought to be a major target of glycosylation. Here, we challenge this view with evidence that mammals use RNA as a third scaffold for glycosylation. Using a battery of chemical and biochemical approaches, we found that conserved small noncoding RNAs bear sialylated glycans. These “glycoRNAs” were present in multiple cell types and mammalian species, in cultured cells, and in vivo. GlycoRNA assembly depends on canonical N-glycan biosynthetic machinery and results in structures enriched in sialic acid and fucose. Analysis of living cells revealed that the majority of glycoRNAs were present on the cell surface and can interact with anti-dsRNA antibodies and members of the Siglec receptor family. Collectively, these findings suggest the existence of a direct interface between RNA biology and glycobiology, and an expanded role for RNA in extracellular biology.

1. **RNA processing by Dicer – new functions**

**An isoform of Dicer protects mammalian stem cells against multiple RNA viruses.**

Poirier, E.Z.; Buck, M.D.; Chakravarty, P.; Carvalho, J.; Frederico, B.; Cardoso, A.; Healy, L.; Ulferts, R.; Beale, R.; Reis e Sousa,C.

Science 2021, 373, 231–236.

Abstract

In mammals, early resistance to viruses relies on interferons, which protect differentiated cells but not stem cells from viral replication. Many other organisms rely instead on RNA interference (RNAi) mediated by a specialized Dicer protein that cleaves viral double-stranded RNA. Whether RNAi also contributes to mammalian antiviral immunity remains controversial. We identified an isoform of Dicer, named antiviral Dicer (aviD), that protects tissue stem cells from RNA viruses-including Zika virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-by dicing viral double-stranded RNA to orchestrate antiviral RNAi. Our work sheds light on the molecular regulation of antiviral RNAi in mammalian innate immunity, in which different cell-intrinsic antiviral pathways can be tailored to the differentiation status of cells.

1. **Scaffolding action of lncRNAs:**

**Functional Domains of NEAT1 Architectural lncRNA Induce Paraspeckle Assembly through Phase Separation.**

Tomohiro Yamazaki , Sylvie Souquere , Takeshi Chujo, Simon Kobelke , Yee Seng Chong , Archa H Fox , Charles S Bond , Shinichi Nakagawa , Gerard Pierron , Tetsuro Hirose

Mol Cell. 2018

Summary

A class of long noncoding RNAs (lncRNAs) has architectural functions in nuclear body construction; however, specific RNA domains dictating their architectural functions remain uninvestigated. Here, we identified the domains of the architectural NEAT1 lncRNA that construct paraspeckles. Systematic deletion of NEAT1 portions using CRISPR/Cas9 in haploid cells revealed modular domains of NEAT1 important for RNA stability, isoform switching, and paraspeckle assembly. The middle domain, containing functionally redundant subdomains, was responsible for paraspeckle assembly. Artificial tethering of the NONO protein to a NEAT1\_2 mutant lacking the functional subdomains rescued paraspeckle assembly, and this required the NOPS dimerization domain of NONO. Paraspeckles exhibit phase-separated properties including susceptibility to 1,6-hexanediol treatment. RNA fragments of the NEAT1\_2 subdomains preferentially bound NONO/SFPQ, leading to phase-separated aggregates in vitro. Thus, we demonstrate that the enrichment of NONO dimers on the redundant NEAT1\_2 subdomains initiates construction of phase-separated paraspeckles, providing mechanistic insights into lncRNA-based nuclear body formation.

1. **Regulation of DNA Damage**

**Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-response factors**

Fabio Pessina, Fabio Giavazzi, Yandong Yin, Ubaldo Gioia, Valerio Vitelli, Alessandro Galbiati, Sara Barozzi, Massimiliano Garre, Amanda Oldani, Andrew Flaus, Roberto Cerbino, Dario Parazzoli, Eli Rothenberg, Fabrizio d'Adda di Fagagna

Nat Cell Biol. 2019 Oct;21(10):1286-1299. doi: 10.1038/s41556-019-0392-4. Epub 2019 Sep 30.

Abstract

Damage-induced long non-coding RNAs (dilncRNA) synthesized at DNA double-strand breaks (DSBs) by RNA polymerase II are necessary for DNA-damage-response (DDR) focus formation. We demonstrate that induction of DSBs results in the assembly of functional promoters that include a complete RNA polymerase II preinitiation complex, MED1 and CDK9. Absence or inactivation of these factors causes a reduction in DDR foci both in vivo and in an in vitro system that reconstitutes DDR events on nucleosomes. We also show that dilncRNAs drive molecular crowding of DDR proteins, such as 53BP1, into foci that exhibit liquid-liquid phase-separation condensate properties. We propose that the assembly of DSB-induced transcriptional promoters drives RNA synthesis, which stimulates phase separation of DDR factors in the shape of foci.

Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks

**Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks**

Flavia Michelini, Sethuramasundaram Pitchiaya, Valerio Vitelli, Sheetal Sharma, Ubaldo Gioia, Fabio Pessina, Matteo Cabrini, Yejun Wang, Ilaria Capozzo, Fabio Iannelli, Valentina Matti, Sofia Francia, G V Shivashankar, Nils G Walter, Fabrizio d'Adda di Fagagna

Nat Cell Biol 2017

Abstract

The DNA damage response (DDR) preserves genomic integrity. Small non-coding RNAs termed DDRNAs are generated at DNA double-strand breaks (DSBs) and are critical for DDR activation. Here we show that active DDRNAs specifically localize to their damaged homologous genomic sites in a transcription-dependent manner. Following DNA damage, RNA polymerase II (RNAPII) binds to the MRE11-RAD50-NBS1 complex, is recruited to DSBs and synthesizes damage-induced long non-coding RNAs (dilncRNAs) from and towards DNA ends. DilncRNAs act both as DDRNA precursors and by recruiting DDRNAs through RNA-RNA pairing. Together, dilncRNAs and DDRNAs fuel DDR focus formation and associate with 53BP1. Accordingly, inhibition of RNAPII prevents DDRNA recruitment, DDR activation and DNA repair. Antisense oligonucleotides matching dilncRNAs and DDRNAs impair site-specific DDR focus formation and DNA repair. We propose that DDR signalling sites, in addition to sharing a common pool of proteins, individually host a unique set of site-specific RNAs necessary for DDR activation.

RNA: and atypical DNA structures

1. **Production and processing of lnRNAs**

**Distinctive patterns of transcription and RNA processing for human lincRNAs.**

Schlackow, M. et al. Mol. Cell 65, 25–38 (2017).

Abstract

Numerous long intervening noncoding RNAs (lincRNAs) are generated from the mammalian genome by RNA polymerase II (Pol II) transcription. Although multiple functions have been ascribed to lincRNAs, their synthesis and turnover remain poorly characterized. Here, we define systematic differences in transcription and RNA processing between protein-coding and lincRNA genes in human HeLa cells. This is based on a range of nascent transcriptomic approaches applied to different nuclear fractions, including mammalian native elongating transcript sequencing (mNET-seq). Notably, mNET-seq patterns specific for different Pol II CTD phosphorylation states reveal weak co-transcriptional splicing and poly(A) signal-independent Pol II termination of lincRNAs as compared to pre-mRNAs. In addition, lincRNAs are mostly restricted to chromatin, since they are rapidly degraded by the RNA exosome. We also show that a lincRNA-specific co-transcriptional RNA cleavage mechanism acts to induce premature termination. In effect, functional lincRNAs must escape from this targeted nuclear surveillance process.

Yin, Y. et al. U1 snRNP regulates chromatin retention of noncoding RNAs. Nature 580, 147–150 (2020).

**Deregulated expression of mammalian lncRNA through loss of SPT6 induces R-loop formation, replication stress, and cellular senescence.**

Nojima, T. et al. Mol. Cell 72, 970–984.e7 (2018).

Abstract

Extensive tracts of the mammalian genome that lack protein-coding function are still transcribed into long noncoding RNA. While these lncRNAs are generally short lived, length restricted, and non-polyadenylated, how their expression is distinguished from protein-coding genes remains enigmatic. Surprisingly, depletion of the ubiquitous Pol-II-associated transcription elongation factor SPT6 promotes a redistribution of H3K36me3 histone marks from active protein coding to lncRNA genes, which correlates with increased lncRNA transcription. SPT6 knockdown also impairs the recruitment of the Integrator complex to chromatin, which results in a transcriptional termination defect for lncRNA genes. This leads to the formation of extended, polyadenylated lncRNAs that are both chromatin restricted and form increased levels of RNA:DNA hybrid (R-loops) that are associated with DNA damage. Additionally, these deregulated lncRNAs overlap with DNA replication origins leading to localized DNA replication stress and a cellular senescence phenotype. Overall, our results underline the importance of restricting lncRNA expression.

**Nascent RNA antagonizes the interaction of a set of regulatory proteins with chromatin.**

Skalska, L.; Begley, V.; Beltran, M.; Lukauskas, S.; Khandelwal, G.; Faull, P.; Bhamra, A.; Tavares, M.;Wellman, R.; Tvardovskiy,A.; et al.

Mol.Cell 2021, 81, 2944–2959.e10.

Abstract

A number of regulatory factors are recruited to chromatin by specialized RNAs. Whether RNA has a more general role in regulating the interaction of proteins with chromatin has not been determined. We used proteomics methods to measure the global impact of nascent RNA on chromatin in embryonic stem cells. Surprisingly, we found that nascent RNA primarily antagonized the interaction of chromatin modifiers and transcriptional regulators with chromatin. Transcriptional inhibition and RNA degradation induced recruitment of a set of transcriptional regulators, chromatin modifiers, nucleosome remodelers, and regulators of higher-order structure. RNA directly bound to factors, including BAF, NuRD, EHMT1, and INO80 and inhibited their interaction with nucleosomes. The transcriptional elongation factor P-TEFb directly bound pre-mRNA, and its recruitment to chromatin upon Pol II inhibition was regulated by the 7SK ribonucleoprotein complex. We postulate that by antagonizing the interaction of regulatory proteins with chromatin, nascent RNA links transcriptional output with chromatin composition.

1. **lncRNAs in the cytoplasma**

**Gene architecture and sequence composition underpin selective dependency of nuclear export of long RNAs on NXF1 and the TREX complex.**

Zuckerman, B., Ron, M., Mikl, M., Segal, E. & Ulitsky, I.

Mol. Cell 79, 251–267 (2020).

Abstract

The core components of the nuclear RNA export pathway are thought to be required for export of virtually all polyadenylated RNAs. Here, we depleted different proteins that act in nuclear export in human cells and quantified the transcriptome-wide consequences on RNA localization. Different genes exhibited substantially variable sensitivities, with depletion of NXF1 and TREX components causing some transcripts to become strongly retained in the nucleus while others were not affected. Specifically, NXF1 is preferentially required for export of single- or few-exon transcripts with long exons or high A/U content, whereas depletion of TREX complex components preferentially affects spliced and G/C-rich transcripts. Using massively parallel reporter assays, we identified short sequence elements that render transcripts dependent on NXF1 for their export and identified synergistic effects of splicing and NXF1. These results revise the current model of how nuclear export shapes the distribution of RNA within human cells.

**Cytoplasmic long noncoding RNAs are frequently bound to and degraded at ribosomes in**

**human cells.**

Carlevaro-Fita, J., Rahim, A., Guigo, R., Vardy, L. A. & Johnson, R.

RNA 22, 867–882 (2016).

Abstract

Recent footprinting studies have made the surprising observation that long noncoding RNAs (lncRNAs) physically interact with ribosomes. However, these findings remain controversial, and the overall proportion of cytoplasmic lncRNAs involved is unknown. Here we make a global, absolute estimate of the cytoplasmic and ribosome-associated population of stringently filtered lncRNAs in a human cell line using polysome profiling coupled to spike-in normalized microarray analysis. Fifty-four percent of expressed lncRNAs are detected in the cytoplasm. The majority of these (70%) have >50% of their cytoplasmic copies associated with polysomal fractions. These interactions are lost upon disruption of ribosomes by puromycin. Polysomal lncRNAs are distinguished by a number of 5' mRNA-like features, including capping and 5'UTR length. On the other hand, nonpolysomal "free cytoplasmic" lncRNAs have more conserved promoters and a wider range of expression across cell types. Exons of polysomal lncRNAs are depleted of endogenous retroviral insertions, suggesting a role for repetitive elements in lncRNA localization. Finally, we show that blocking of ribosomal elongation results in stabilization of many associated lncRNAs. Together these findings suggest that the ribosome is the default destination for the majority of cytoplasmic long noncoding RNAs and may play a role in their degradation.

1. **Cis and trans acting roles of lnRNAs in controlling chromosome architecture**

**Topological organization of multichromosomal regions by the long intergenic noncoding RNA Firre.**

Hacisuleyman E, Goff LA, Trapnell C, Williams A, Henao-Mejia J, Sun L, McClanahan P, Hendrickson DG, Sauvageau M, Kelley DR, Morse M, Engreitz J, Lander ES, Guttman M, Lodish HF, Flavell R, Raj A, Rinn JL.Nat Struct Mol Biol. 2014

Abstract

RNA, including long noncoding RNA (lncRNA), is known to be an abundant and important structural component of the nuclear matrix. However, the molecular identities, functional roles and localization dynamics of lncRNAs that influence nuclear architecture remain poorly understood. Here, we describe one lncRNA, Firre, that interacts with the nuclear-matrix factor hnRNPU through a 156-bp repeating sequence and localizes across an ~5-Mb domain on the X chromosome. We further observed Firre localization across five distinct trans-chromosomal loci, which reside in spatial proximity to the Firre genomic locus on the X chromosome. Both genetic deletion of the Firre locus and knockdown of hnRNPU resulted in loss of colocalization of these trans-chromosomal interacting loci. Thus, our data suggest a model in which lncRNAs such as Firre can interface with and modulate nuclear architecture across chromosomes.

**Trans- and cis-acting effects of Firre on epigenetic features of the inactive X chromosome**.

Fang H, Bonora G, Lewandowski JP, Thakur J, Filippova GN, Henikoff S, Shendure J, Duan Z, Rinn JL, Deng X, Noble WS, Disteche CM.

Nat Commun. 2020 Nov 27;11(1):6053. doi: 10.1038/s41467-020-19879-3.

Abstract

Firre encodes a lncRNA involved in nuclear organization. Here, we show that Firre RNA expressed from the active X chromosome maintains histone H3K27me3 enrichment on the inactive X chromosome (Xi) in somatic cells. This trans-acting effect involves SUZ12, reflecting interactions between Firre RNA and components of the Polycomb repressive complexes. Without Firre RNA, H3K27me3 decreases on the Xi and the Xi-perinucleolar location is disrupted, possibly due to decreased CTCF binding on the Xi. We also observe widespread gene dysregulation, but not on the Xi. These effects are measurably rescued by ectopic expression of mouse or human Firre/FIRRE transgenes, supporting conserved trans-acting roles. We also find that the compact 3D structure of the Xi partly depends on the Firre locus and its RNA. In common lymphoid progenitors and T-cells Firre exerts a cis-acting effect on maintenance of H3K27me3 in a 26 Mb region around the locus, demonstrating cell type-specific trans- and cis-acting roles of this lncRNA.

1. **lncRNAs sponging miRNAs:**

**A regulated PNUTS mRNA to lncRNA splice switch mediates EMT and tumour progression.**

Simon Grelet, Laura A Link, Breege Howley, Clémence Obellianne, Viswanathan Palanisamy, Vamsi K Gangaraju, J Alan Diehl, Philip H Howe

Nat. Cell Biol. 19, 1105–1115 (2017).

Abstract:

The contribution of lncRNAs to tumor progression and regulatory mechanisms driving their expression are areas of intense investigation. Here, we characterize the binding of heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1) to a nucleic acid structural element located in exon 12 of PNUTS (also known as PPP1R10) pre-RNA that regulates its alternative splicing. HnRNP E1 release from this structural element, following its silencing, nucleo-cytoplasmic translocation or in response to TGFβ, allows alternative splicing and generates a non-coding isoform of PNUTS. Functionally the lncRNA-PNUTS serves as a competitive sponge for miR-205 during epithelial-mesenchymal transition. In mesenchymal breast tumor cells and in breast tumor samples, the expression of lncRNA-PNUTS is elevated and correlates with levels of ZEB mRNAs. Thus, PNUTS is a bifunctional RNA encoding both PNUTS-mRNA and lncRNA-PNUTS each eliciting distinct biological functions. While PNUTS-mRNA is ubiquitously expressed, lncRNA-PNUTS appears to be tightly regulated dependent on hnRNP E1’s status and tumor context.

1. **Gene regulation by lncRNAs**

**Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments**

Fanucchi, S. et al. Nat. Genet. 51, 138–150 (2019).

Abstract

Accumulation of trimethylation of histone H3 at lysine 4 (H3K4me3) on immune-related gene promoters underlies robust transcription during trained immunity. However, the molecular basis for this remains unknown. Here we show three-dimensional chromatin topology enables immune genes to engage in chromosomal contacts with a subset of long noncoding RNAs (lncRNAs) we have defined as immune gene-priming lncRNAs (IPLs). We show that the prototypical IPL, UMLILO, acts in cis to direct the WD repeat-containing protein 5 (WDR5)-mixed lineage leukemia protein 1 (MLL1) complex across the chemokine promoters, facilitating their H3K4me3 epigenetic priming. This mechanism is shared amongst several trained immune genes. Training mediated by β-glucan epigenetically reprograms immune genes by upregulating IPLs in manner dependent on nuclear factor of activated T cells. The murine chemokine topologically associating domain lacks an IPL, and the Cxcl genes are not trained. Strikingly, the insertion of UMLILO into the chemokine topologically associating domain in mouse macrophages resulted in training of Cxcl genes. This provides strong evidence that lncRNA-mediated regulation is central to the establishment of trained immunity.