

# lncRNA in worms – Time to meet the neighbors

Soumasree De<sup>1</sup>, Liron Levin<sup>2</sup> and Barak Rotblat<sup>1</sup>

## Abstract

Long noncoding RNA or lncRNA have been in the spotlight in recent years, due to their abundance in the human genome and the crucial regulatory roles they play in diverse biological processes. The mode of action by which lncRNA regulate biological processes is not fully understood however, in many cases it was found that they function by regulating the expression of neighboring genes in *cis*. To date, lncRNA have received little attention from the *Caenorhabditis elegans* research community. In particular, lncRNA functions in *cis* have not been thoroughly investigated. Here we review the known functions of lncRNA in mammals and *C. elegans*. To promote lncRNA studies in *C. elegans*, we provide a catalog of lncRNA neighbor genes in *C. elegans*, their human homologs and the biological categories they belong to. We propose that *C. elegans* could be a powerful model for studying these enigmatic non-protein coding genes.

## Addresses

<sup>1</sup> Ben Gurion University of the Negev, Israel

<sup>2</sup> Bioinformatics Core Facility, National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Corresponding author: Rotblat, Barak ([rotblat@bgu.ac.il](mailto:rotblat@bgu.ac.il))

**Current Opinion in Systems Biology** 2019, 13:10–15

This review comes from a themed issue on **Systems biology of model organisms**

Edited by Denis Dupuy and Baris Tursun

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 23 August 2018

<https://doi.org/10.1016/j.coisb.2018.08.007>

2452-3100/© 2018 Elsevier Ltd. All rights reserved.

## Keywords

Bioinformatics, Evolution, Transcription regulation, Synteny.

## Introduction

LncRNA are a large and diverse class of transcripts ranging from 200 to 100,000 bp in length which do not code for proteins. LncRNA have been identified in plants, invertebrates and mammals [1]. Most lncRNA gene structure resembles protein coding mRNAs with regards to promotor CpG islands, RNA Poly II transcription, a multiexonic structure and, in some cases the transcript is decorated with a 3' poly A tail and a 5' m7GTP cap [2,3]. Although particular transcripts depicted as lncRNA were found to encode short open reading frames [4–6], in most cases there is no evidence that they do code for peptides [7]. LncRNA are

classified by their location relative to the nearby protein coding genes, as long intervening non coding RNA (the genomic loci does not overlap the exons of protein coding genes), antisense lncRNAs (the loci present in the opposite strand of protein coding genes) and intergenic lncRNAs (the loci present between two protein coding genes) [8]. Genome based studies have revealed several functional roles for lncRNA in chromatin structure regulation, gene transcription, development and disease [9].

## Evolution of lncRNA may have promoted complexity

lncRNAs have evolved from either partial or total duplication and divergence of consequent sequences [10,11]. They are linked to a wide range of developmental processes and are found in a diverse range of species, from sponges to primates [10]. A recent systemic investigation in demosponge has identified 2935 lncRNAs, most of them overlapping with protein coding genes [12]. Similarly, a large-scale evolutionary study of lncRNA repertoires and expression patterns in 11 tetrapod species has identified almost 11,000 primate-specific lncRNAs, some of which originated more than 300 million years ago [13].

An interesting possibility regarding lncRNA functions is that by fine tuning gene expression, lncRNA facilitated the increase in complexity found in the late stages of mammalian evolution and in humans in particular [14]. This notion is supported by the finding that there are a significant number of lncRNA associated with human complex traits and, importantly, these lncRNA are suspected of regulating the expression of neighboring protein-coding genes with significant enrichment for chromatin regulators [15]. From a systems biology point of view, one of the major challenges of the lncRNA field is to infer lncRNA function from their sequence [16]. Of note, recent efforts promoted our understanding of the information embedded in lncRNA sequences enabling prediction of their localization [17], protein binding [18] and binding to small RNA [19].

## Mammalian lncRNA function to regulate chromatin

The functions of lncRNA in chromatin regulation have been the focus of lncRNA research from the inception of the field [20,21] with *XIST*, a lncRNA which promotes X inactivation maintenance by recruiting chromatin modifier proteins to the X chromosome [22,23], serving as a classic example for such lncRNA functions in *cis* [10].

Other lncRNA have been shown to play a role in regulation of chromatin states in *trans* by binding chromatin regulating proteins and targeting them to particular regions of the genome [24–28]. *HOXAIR*, yet another well studied lncRNA, was shown to be expressed from the human and mouse *HOXC* cluster and promote silencing of genes within the *HOXD* cluster by recruiting and targeting chromatin modifying enzymes to the *HOXD* locus, thus functioning in *trans* [29,30]. Recent analysis of the *HOXAIR* sequence, chromatin state and expression pattern revealed that it may function both in *trans* and *cis* to regulate gene expression within the HOX clusters [31]. These findings have raised the attractive hypothesis that lncRNA target chromatin regulator proteins to specific regions of the genome to regulate chromatin structure and thus regulate gene expression. Whether these lncRNA chromatin regulatory functions in *trans* are mediated by specific sequences within the lncRNA is still debated. One major chromatin regulating protein, PRC2, was shown to bind promiscuously to RNA [32,33] supporting an alternative model where the genomic location of the lncRNA, rather than the specific sequence, is the major determinant of its functions [33].

### **lncRNA regulate neighboring genes in *cis***

Progress has been made in better understanding the functions of lncRNA as regulators of adjacent genes in *cis* [34]. *Upperhand* is a lncRNA gene neighbor of the transcription factor *Hand2* coding gene which is essential for heart development. To test if *Upperhand* regulates *Hand2* in *cis*, Anderson et al. used TALEN to introduce either a transcription terminator or a fluorescent protein coding sequence into the second exon of the *Upperhand* gene and found that transcription termination, but not sequence swapping, eventuated in reduced *Hand2* expression and defective heart development in mice [35], supporting the model where the lncRNA functions in a sequence independent manner to activate an adjacent gene in *cis*. Similarly, this principal was demonstrated by using CRISPR to insert transcription termination sequences into mouse lncRNA and protein coding genes and analyzing the expression of the adjacent genes [36]. In both cases, inhibiting the transcription of a gene often resulted in reduced expression of the neighboring genes.

To functionally interrogate the role of 10,000 lncRNA in melanoma tumor cells drug resistance, Joung et al. used CRISPR activation and a pooled screen by which they identified 11 lncRNA promoting resistance [34]. In this study, most identified functional lncRNA promoted drug resistance by regulating one, or more, adjacent genes. Similarly, Bester et al. performed a CRISPR activation screen to identify both protein-coding and lncRNA genes promoting drug resistance in acute myeloid leukemia tumor cells and found that the lncRNA *GAS6-AS2* promotes resistance by activating

the adjacent *GAS6* protein coding gene [37]. Interestingly, in a guilt-by-association analysis, lncRNA protecting tumor cells from chemotherapy were enriched for fatty acid metabolism and oxidative phosphorylation categories, suggesting that lncRNA are also involved in regulation of metabolism related genes [37]. Indeed, an earlier study showed that lncRNA transcription negatively regulates the adjacent *DHFR* gene activity [38].

How lncRNA promote or inhibit the expression of neighboring genes is still not completely clear [39]. Using computational and experimental analysis, Lou et al. investigated the functions of divergent lncRNA-protein coding gene pairs (as illustrated in the graphical abstract) which represent 20% of human lncRNA [14], and found that these lncRNA regulate the expression of adjacent genes, by a mechanism which includes binding of the lncRNA to the borders of active chromosomal regions and to chromatin regulating proteins [39].

### **lncRNA in *Caenorhabditis elegans***

Despite the fact that many major discoveries in RNA biology have come from studying the small worm *Caenorhabditis elegans* (*C. elegans*) [40,41] less is known about lncRNA in this model organism. Nam et al. identified 170 lncRNAs in *C. elegans* [42], of which 25% have sequence conservation with endogenous micro RNA, and theoretically, could serve as templates for these small RNA species. The other lncRNA were more conserved and exhibited transcription patterns implicating them in developmental process and differentiation of worms. These lncRNA transcriptional patterns are linked to male identity, sperm formation, and interaction with sperm-specific mRNAs [42].

A recent study attempted to infer biological function of lncRNA in *C. elegans* by following their spatiotemporal expression pattern [43]. Using a GFP knock-in approach, the spatial expression patterns of 68 lncRNAs were examined in 18 tissue categories throughout eight developmental stages. This study found that lncRNA and miRNA promoters are less active at the embryonic stage, as compared with the promoters of transcription factors (TFs), but become comparable to TFs after embryogenesis. Finally, the expression pattern of this lncRNA gene set is similar to that of miRNAs and TFs in mature animals [43].

Several lncRNAs were also identified in the muscle transcriptome profile of dauer and aging worms [44]. Using tissue-specific RNA-seq, splicing-based RNA tagging (SRT), the authors detected 461 novel RNA transcripts in worm muscles, most of which are predicted to be lncRNA. Using reporter assay, 5 out of 8 tested transcripts were shown to be expressed in muscle. It is

therefore clear that we do not yet know the full repertoire of lncRNA in *C. elegans*.

The *C. elegans* 800 bp lncRNA *rncs-1* is expressed in the hypodermis and intestine of the adult worm. Its double stranded structure facilitates *rncs-1* *in vitro* binding to Dicer, a major component of the RNA interference pathway, and thus *rncs-1* may compete with endogenous double stranded RNA (siRNA or miRNA) involved in gene silencing, for Dicer [45]. This is an early example of a functional lncRNA in *C. elegans*.

Recently, a study revealed ribosomal association of the lncRNA *tts-1* [46]. In *C. elegans*, *daf-2*, *clk-1* or *eat-2* mutations extend the worm lifespan [47]. Depletion of *tts-1* in *daf-2* or *clk-1*, but not in *eat-2*, mutants increased ribosome levels and significantly decreased life span. Thus, *tts-1* is required, at least in some cases, for the life-extension phenotypes of *C. elegans* [46]. Together, these studies demonstrate that lncRNA do function in *trans* in *C. elegans*, however, whether worm lncRNA function in *cis* is still an open question.

### The protein coding neighbors of *C. elegans* lncRNA

To gain some clues regarding the possible *cis* functions of lncRNA in *C. elegans* we asked some basic questions:

1. Do worm lncRNA have neighbor coding genes?
2. Do these protein-coding neighbors have human homologs and do the homologues have a neighbor lncRNA in the human genome?
3. Do the worm protein-coding genes with lncRNA neighbors share common biological functions?

To answer these questions, we performed a bioinformatics analysis (Figure 1; all data available in Table S1–6). We used the 172 genes annotated as lncRNA in the *C. elegans* genome by ENSAMBLE and asked if there is a coding gene within 5000 bp of each lncRNA. Using the

**Table 1**

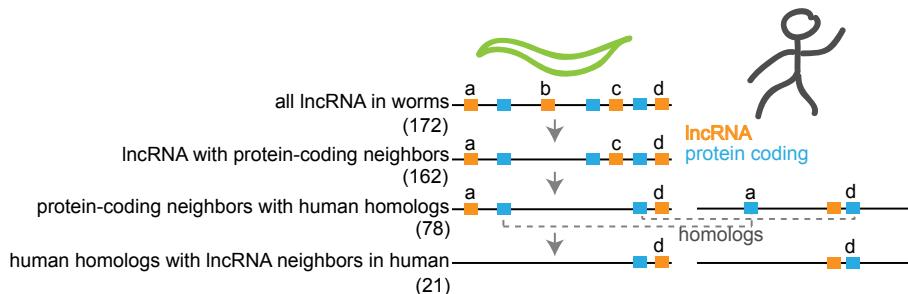
*C. elegans* protein-coding neighbors of lncRNA which have human homologs that are neighbors of lncRNA in the human genome. As detailed in Table S6.

Worm	Human		
	IncRNA	Protein neighbor	Human homolog
LINC-85	SCPL-3	CTDSPL2	ENSG00000179523
LINC-105	TAT-3	ATP10A	ENSG00000259011
LINC-80	FASN-1	AC106795.1	ENSG00000250101
LINC-108	LSM-5	ACSF2	ENSG00000275897
LINC-61	HIS-1	HIST2H3C	ENSG00000272993
LINC-73	UNC-104	THAP4	ENSG00000273113
LINC-4	F13H8.2	HLCS	ENSG00000224790
LINC-96	RHR-1	RHBG	ENSG00000237390
LINC-135	ANT-1.4	SLC25A6	ENSG00000236871
LINC-65	HIL-4	HIST1H1A	ENSG00000272810
LINC-113	CEH-6	POU3F2	ENSG00000283010
LINC-37	CEEH-1	EPHX3	ENSG00000269635
LINC-81	GLY-4	GALNT16	ENSG00000258520
LINC-35	FLCN-1	FLCN	ENSG00000266498
LINC-50	FKH-7	FOXP1	ENSG00000270562
LINC-63	PGK-1	MRC2	ENSG00000277463
LINC-2	PCF-11	PCF11	ENSG00000269939
LINC-58	JPH-1	JPH3	ENSG00000226180
LINC-110	KLP-3	KIFC3	ENSG00000187185
LINC-133	KLP-3	KIFC3	ENSG00000187185

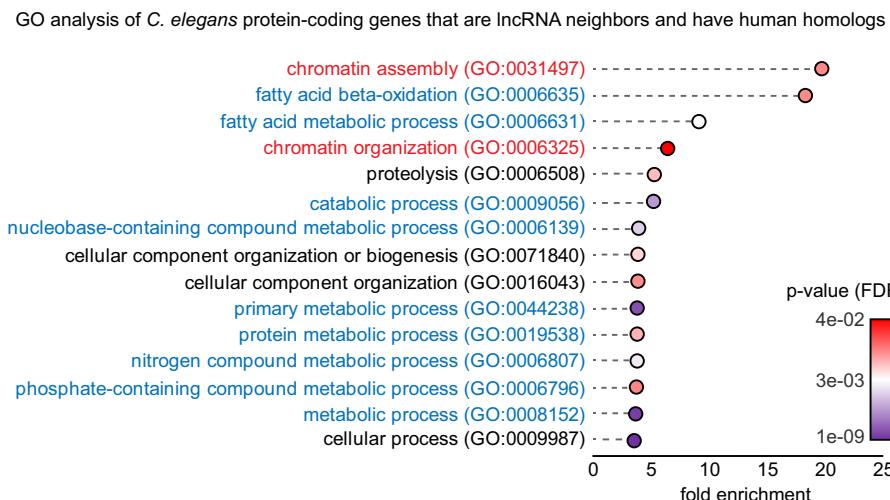
5000 bp cutoff (as in Ref. [14]), we found that the vast majority (162; 94.2%) of worm lncRNA have at least one neighbor protein-coding gene (Table S2; 64% are in the divergent orientation [14]). Out of these 162 worm protein-coding genes with lncRNA neighbors, 78 (48.1%) have human homologs (Table S4) where 21 (26.9%) of these human protein-coding genes have a lncRNA neighbor in the human genome as well (Table 1 and Table S6). Interestingly, two of these lncRNA, LINC-35 and LINC-2 were found to be specifically expressed in muscle [44].

We performed gene ontology (GO) analysis using the list of the protein-coding genes which are neighbors of the

**Figure 1**



**Analysis of *C. elegans* lncRNA, their protein-coding neighbors and their human homologs.** First we listed the protein-coding genes which are lncRNA neighbors in worms (Table S2). Next, we listed those which have human homologs (Table S4). Finally, we list the human homologs with lncRNA neighbors in the human genome (Table S6). We used OrthoList [49] (<http://www.greenwaldlab.org/ortholist/>) for worm to human orthologs information, we considered only genes that were identified as orthologs in at least two out of the four orthology prediction programs reported in OrthoList.

**Figure 2**

**GO analysis of protein-coding neighbors of lncRNA in *C. elegans* which have a human homolog.** Gene ontology (GO) analysis was performed using the Gene Ontology Consortium website (<http://www.geneontology.org/>) and PANTHER [50] (<http://pantherdb.org/>). Fold enrichment and p value using false discovery rate (FDR) are depicted. Red = chromatin related categories; Blue = metabolism related categories. Genes listed in Table S4 and results of analysis in Table S5.

worm lncRNA and found that they are enriched for metabolic and catabolic processes (Table S3). We also performed a GO analysis on the list of worm protein-coding genes with lncRNA neighbors who have human homologs and found enrichment for more categories including metabolic categories and chromatin related processes (Figure 2 and Table S5). Finally, we interrogated the list of human homologs which have a lncRNA neighbor in the human genome (Table 1 and Table S6) and found that this group of genes was not enriched for specific biological categories.

The genome of *C. elegans* is ~30 times smaller than the human genome and 82.8% of the *C. elegans* open reading frames have a gene neighbor within the 5000 bp [48] raising the question of what is ‘close’ or ‘gene neighbor’ in *C. elegans*? We argue that if the molecular mechanism by which lncRNA regulate the activity of gene neighbors in *C. elegans* is similar to the human one, then the 5000 bp cutoff would be appropriate however, if such molecular mechanism evolved to match the genome size of the organism than a ‘neighbor’ would be expected to be 30 times closer, a size that will encompass mostly the promoters of the genes [48]. We performed neighbor analysis using the 1500 bp cutoff, of which 52.4% of the *C. elegans* genes have a gene neighbor [48], and found 53 protein coding gene neighbors which have human homologs (Table S7). In accord with our analysis using the 5000 bp cutoff, GO analysis showed enrichment for both metabolic and chromatin related processes (Table S8).

## Conclusion

Whether in *C. elegans* lncRNA regulate neighboring genes in *cis* is not known. The finding that the *C. elegans* protein-coding genes which are neighbors of lncRNA and have human homologs are enriched for chromatin regulators and metabolic genes raises some fundamental questions: are chromatin regulators and metabolic genes more likely to be regulated by neighboring lncRNA? If yes, is this regulation conserved in evolution? Do these types of genes require a more sophisticated regulation as compared with other genes? Is this regulation important in development or stress tolerance? And finally, what might be the molecular mechanism by which lncRNA regulate adjacent genes?

We believe that *C. elegans* may serve as a powerful tool to answer these and other questions regarding the functions and mechanisms of action of lncRNA, and, in particular, unravel their functions in activating neighboring genes in *cis*.

## Conflict of interest statement

Nothing declared.

## Acknowledgments

We thank Anat Ben-Zvi and Maya Bar for critically reading the manuscript and Oded Rechavi for discussion. This research was supported by the Israel Science Foundation (grant No. 221152), the Ministry of Science, Technology & Space of the State of Israel and the German Cancer Research Center (DKFZ), and the Israeli Cancer Association.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.coisb.2018.08.007>.

## References

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Frith MC, Bailey TL, Kasukawa T, Mignone F, Kummerfeld SK, Madera M, Sunkara S, Furuno M, Bult CJ, Quackenbush J, et al.: **Discrimination of non-protein-coding transcripts from protein-coding mRNA.** *RNA Biol* 2006, **3**:40–48.
  2. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, et al.: **Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals.** *Nature* 2009, **458**:223–227.
  3. Brosnan CA, Voinnet O: **The long and the short of noncoding RNAs.** *Curr Opin Cell Biol* 2009, **21**:416–425.
  4. Anderson DM, Anderson KM, Chang CL, Makarewicz CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R, et al.: **A micropeptide encoded by a putative long noncoding RNA regulates muscle performance.** *Cell* 2015, **160**:595–606.
  5. Cai B, Li Z, Ma M, Wang Z, Han P, Abdalla BA, Nie Q, Zhang X: **LncRNA-Six1 encodes a micropeptide to activate Six1 in Cis and is involved in cell proliferation and muscle growth.** *Front Physiol* 2017, **8**:1–13.
  6. Matsumoto A, Pasut A, Matsumoto M, Yamashita R, Fung J, Monteleone E, Saghatelyan A, Nakayama KI, Clohessy JG, Pandolfi PP: **MTORC1 and muscle regeneration are regulated by the LINC00961-encoded SPAR polypeptide.** *Nature* 2017, **541**:228–232.
  7. Verheggen K, Volders P-J, Mestdagh P, Menschaert G, Van Damme P, Gevaert K, Martens L, Vandesompele J: **Non-coding after all: biases in proteomics data do not explain observed absence of lncRNA translation products.** *J Proteome Res* 2017, **16**:2508–2515, <https://doi.org/10.1021/acs.jproteome.7b00085>.
  8. Rinn JL, Chang HY: **Genome regulation by long noncoding RNAs.** *Annu Rev Biochem* 2012, **81**:145–166.
  9. Gomes AQ, Nolasco S, Soares H: **Non-coding RNAs: multi-tasking molecules in the cell.** *Int J Mol Sci* 2013, **14**:16010–16039.
  10. Ponting CP, Oliver PL, Reik W: **Evolution and functions of long noncoding RNAs.** *Cell* 2009, **136**:629–641.
  11. Ulitsky I, Bartel D: **lincRNAs: genomics, evolution, and mechanisms.** *Cell* 2013, **154**:26–46.
  12. Gaiti F, Fernandez-Valverde SL, Nakanishi N, Calcino AD, Yanai I, Tanurdzic M, Degnan BM: **Dynamic and widespread lncRNA expression in a sponge and the origin of animal complexity.** *Mol Biol Evol* 2015, **32**:2367–2382.
  13. Necsulea A, Soumillon M, Warnefors M, Liechti A, Daish T, Zeller U, Baker JC, Grützner F, Kaessmann H: **The evolution of lncRNA repertoires and expression patterns in tetrapods.** *Nature* 2014, **505**:635–640.
  14. Luo S, Lu JY, Liu L, Yin Y, Chen C, Han X, Wu B, Xu R, Liu W, Yan P, et al.: **Divergent lncRNAs regulate gene expression and lineage differentiation in pluripotent cells.** *Cell Stem Cell* 2016, **18**:637–652.
  15. Tan JY, Smith AAT, Ferreira da Silva M, Matthey-Doret C, Rueedi R, Sönmez R, Ding D, Katalik Z, Bergmann S, Marques AC: **cis-acting complex-trait-associated lncRNA expression correlates with modulation of chromosomal architecture.** *Cell Rep* 2017, **18**:2280–2288.
  16. Toiber D, Leprvier G, Rotblat B: **Long noncoding RNA: noncoding and not coded.** *Cell Death Dis* 2017, **3**:16104.
  17. Lubelsky Y, Ulitsky I: **Sequences enriched in Alu repeats drive nuclear localization of long RNAs in human cells.** *Nature* 2018, **555**:107–111.
  18. Tichon A, Perry RB-T, Stojic L, Ulitsky I: **SAM68 is required for regulation of Pumilio by the NORAD long noncoding RNA.** *Genes Dev* 2018, **32**:70–78.
  19. Yan P, Luo S, Lu JY, Shen X: **Cis- and trans-acting lncRNAs in pluripotency and reprogramming.** *Curr Opin Genet Dev* 2017, **46**:170–178.
  20. Bernstein E, Allis CD: **RNA meets chromatin.** *Genes Dev* 2005, **19**:1635–1655.
  21. Johnson WL, Straight AF: **RNA-mediated regulation of heterochromatin.** *Curr Opin Cell Biol* 2017, **46**:102–109.
  22. Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, Wang H, de la Cruz CC, Otté AP, Panning B, Zhang Y: **Role of histone H3 lysine 27 methylation in X inactivation.** *Science* (80-) 2003, **300**:131–135.
  23. McHugh CA, Chen C-K, Chow A, Surka CF, Tran C, McDonel P, Pandya-Jones A, Blanco M, Burghard C, Moradian A, et al.: **The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3.** *Nature* 2015, **521**:232–236.
  24. Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, et al.: **A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response.** *Cell* 2010, **142**:409–419.
  25. Khalil AM, Rinn JL: **RNA-protein interactions in human health and disease.** *Semin Cell Dev Biol* 2011, **22**:359–365.
  26. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, et al.: **Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression.** *Proc Natl Acad Sci USA* 2009, **106**:11667–11672.
  27. Dimitrova N, Zamudio JR, Jong RM, Soukup D, Resnick R, Sarma K, Ward AJ, Raj A, Lee JT, Sharp PA, et al.: **LncRNA-p21 activates p21 in cis to promote polycomb target gene expression and to enforce the G1/S checkpoint.** *Mol Cell* 2014, **54**:777–790.
  28. Postepska-Igielska A, Giwojna A, Gasri-Plotnitsky L, Schmitt N, Dold A, Ginsberg D, Grummt I: **LncRNA Khps1 regulates expression of the proto-oncogene SPHK1 via triplex-mediated changes in chromatin structure.** *Mol Cell* 2015, **60**:626–636.
  29. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann S a, Goodnough LH, Helms J a, Farnham PJ, Segal E, et al.: **Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs.** *Cell* 2007, **129**:1311–1323.
  30. Gupta R a, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai M-C, Hung T, Argani P, Rinn JL, et al.: **Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis.** *Nature* 2010, **464**:1071–1076.
  31. Nepal C, Hadzhev Y, Pundhir S, Mydel P, Lenhard B, Mueller F, Andersen JB: **HOTAIR ancient sequence suggests regulatory roles both in cis and trans.** *bioRxiv* 2018, **154**:26–46, <https://doi.org/10.1101/250621>.
  32. Davidovich C, Zheng L, Goodrich KJ, Cech TR: **Promiscuous RNA binding by polycomb repressive complex 2.** *Nat Struct Mol Biol* 2013, **20**:1250–1257.
  33. Wang X, Goodrich KJ, Gooding AR, Naeem H, Archer S, Paucek RD, Youmans DT, Cech TR, Davidovich C: **Targeting of polycomb repressive complex 2 to RNA by short repeats of consecutive guanines.** *Mol Cell* 2017, **65**:1056–1067.e5.
  34. Joung J, Engreitz JM, Konermann S, Abudayyeh OO, Verdine VK, Aguet F, Gootenberg JS, Sanjana NE, Wright JB, Fulco CP, et al.: **Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood.** *Nature* 2017, **548**:343–346.
  35. Anderson KM, Anderson DM, McAnally JR, Shelton JM, Bassel-Duby R, Olson EN: **Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development.** *Nature* 2016, **539**:433–436, <https://doi.org/10.1038/nature20128>.
  36. Engreitz AJM, Haines JE, Munson G, Chen J, Elizabeth M, Kane M, McDonel PE, Guttman M, Lander ES: **Neighborhood**

- regulation by lncRNA promoters, transcription, and splicing.** *Nature* 2016, <https://doi.org/10.1101/050948>.
37. Bester AC, Lee JD, Chavez A, Lee YR, Nachmani D, Vora S, • Victor J, Sauvageau M, Monteleone E, Rinn JL, et al.: **An integrated genome-wide CRISPRa approach to functionalize lncRNAs in drug resistance.** *Cell* 2018, **173**:649–652.e20.
  38. Martianov I, Ramadass A, Serra Barros A, Chow N, Akoulitchev A: **Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript.** *Nature* 2007, **445**:666–670.
  39. Long Y, Wang X, Youmans DT, Cech TR: **How do lncRNAs regulate transcription?** *Sci Adv* 2017, **3**, eaa02110.
  40. Rine J: **A future of the model organism model.** *Mol Biol Cell* 2014, **25**:549–553.
  41. Wani S, Kuroyanagi H: **An emerging model organism *Caenorhabditis elegans* for alternative pre-mRNA processing in vivo.** *Wiley Interdiscip Rev RNA* 2017, **8**, <https://doi.org/10.1002/wrna.1428>.
  42. Nam J, Bartel DP: **Long noncoding RNAs in *C. elegans*.** • *Genome Res* 2012, **22**:2529–2540, [https://doi.org/10.1101/gr.140475.112.Freely](https://doi.org/10.1101/gr.140475.112).
  43. Liu W, Yu E, Chen S, Ma X, Lu Y, Liu X: **Spatiotemporal expression profiling of long intervening noncoding RNAs in *Caenorhabditis elegans*.** *Sci Rep* 2017, **7**:1–9.
  44. Ma X, Zhan G, Sleumer MC, Chen S, Liu W, Zhang MQ, Liu X: **Analysis of *C. elegans* muscle transcriptome using trans-splicing-based RNA tagging (SRT).** *Nucleic Acids Res* 2016, **44**:e156.
  45. Hellwig S, Bass BL: **A starvation-induced noncoding RNA modulates expression of Dicer-regulated genes.** *Proc Natl Acad Sci USA* 2008, **105**:12897–12902.
  46. Essers PB, Nonnenkens J, Goos YJ, Betist MC, Viester MD, • Mossink B, Lansu N, Korswagen HC, Jelier R, Brenkman AB, et al.: **A long noncoding RNA on the ribosome is required for lifespan extension.** *Cell Rep* 2015, **10**:339–345.
  47. Riera CE, Merkworth C, De Magalhaes Filho CD, Dillin A: **Signaling networks determining life span.** *Annu Rev Biochem* 2016, **85**:35–64.
  48. Dupuy D, Li Q-R, Deplancke B, Boxem M, Hao T, Lamesch P, Sequerra R, Bosak S, Doucette-Stamm L, Hope IA, et al.: **A first version of the *Caenorhabditis elegans* promoterome.** *Genome Res* 2004, **14**:2169–2175.
  49. Shaye DD, Greenwald I: **OrthoList: a compendium of *C. elegans* genes with human orthologs.** *PLoS One* 2011, **6**, e20085.
  50. Mi H, Muruganujan A, Thomas PD: **PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees.** *Nucleic Acids Res* 2012, **41**:D377–D386.