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The long reach of non-coding RNAs

Elena Sotillo and **Andrei Thomas-Tikhonenko***
Division of Cancer Pathobiology, Department of Pathology & Laboratory Medicine, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA

Abstract

Transcription of genomic loci containing protein-coding genes often yields not only cognate mRNAs but also assorted non-coding RNAs (ncRNAs), which typically map in the vicinity of transcription start sites. A new study demonstrates that far from being random byproducts of gene expression, many long ncRNAs (lncRNAs) are synthesized in a coordinate fashion and control important cellular processes, such as survival in the face of DNA damage.

In 1990 Shirley Tilghman's lab made a puzzling discovery: the very abundant RNA encoded by the H19 gene was transcribed by RNA polymerase II, then spliced and polyadenylated, but unlike canonical messenger RNAs (mRNA) never associated with the translational machinery[1]. It was later confirmed that the H19 RNA is a fully functional molecule and plays the key role in the imprinting of its own locus[2]. These studies not only ushered in the long non-coding RNA (lncRNA) era but also set off a prolonged debate whether lncRNAs act locally (in *cis*) or globally (in *trans*). There is plenty of evidence in support of various *cis* modes of action. Up to 70% of protein coding transcripts are thought to be transcribed in both sense and antisense directions[3] and the X-chromosome-encoded Xist RNA “coats” and silences its own chromosome [4]. However, a new study from Howard Chang's laboratory appearing in this issue of Nature Genetics presents evidence for *trans* functions for lncRNAs[5].

Transcribe locally, act globally

Hung and coauthors used ultrahigh-resolution microarray technology to identify more than 200 lncRNAs that are encoded in close proximity to 56 cell cycle-controlling genes (cyclins, cyclin-dependent kinases (cdks), cdk inhibitors, etc)[5]. Predictably, during cell cycle progression, self-renewal, and neoplastic transformation, levels of the cell-cycle-related mRNAs fluctuated - but so did levels of lncRNAs encoded in their vicinity.

When these fluctuating lncRNAs were grouped based on expression patterns, colocalized lncRNAs usually ended up in the same clusters, suggesting that adjacent ncRNAs are regulated in concert. In principle, they could act locally, for instance by regulating the nearby mRNA levels. However, the authors found that the expression of lncRNA clusters did not correlate either positively or negatively with expression of the nearest mRNAs. This finding led the authors to reject the idea that most of lncRNAs function in *cis* and challenged them to identify an alternative mode of action. They focused in particular on a lncRNA that is induced by p53, a master regulator of diverse cellular processes ranging from senescence to apoptosis.

*Corresponding author: 4056 Colket Translational Research Building, 3501 Civic Center Blvd, Philadelphia, PA 19104–4399. Tel.: 267 426 9699; fax: 267 426 8125; andreit@mail.med.upenn.edu.

Trans-fixed by p53

An emerging concept in the RNA field is that lncRNAs can function through binding to and altering the activity of transcription factors [6] and the broader the function of the transcription factor, the longer the reach of the interacting lncRNA. For example, the maternally expressed gene-3 (*MEG3*) was found to increase p53 levels and activity via direct interaction with p53 or indirectly by inhibiting the dedicated ubiquitin ligase MDM2 [7,8]). Also, a locus encoding the long intergenic non-coding (linc) RNA lincRNA-p21 was discovered 15 kilobases upstream of the *CDKN1A* gene. Notably, it clearly impinges on the p53 pathway[9]. Its transcription (along with that of *CDKN1A*) is induced after exposure to DNA damaging agents such as doxorubicin[9], the effects of which are mostly mediated by p53. Once activated, lincRNA-p21 binds to the heterogenous nuclear ribonucleoprotein K (hnRPN-K) known to interact with repressive transcriptional complexes, and in doing so assists p53 in inhibiting gene expression[9]. Thus, p53 both regulates and is regulated by the *CDKN1A* locus. Now it turns out that this feedback loop has another kink.

Hung et al show that between the protein-coding *CDKN1A* gene and lincRNA-p21 there is a gene for yet another lncRNA, which they named *PANDA*[5] *(P21 Associated ncRNA DNA damage Activated)*. PANDA is one of 12 lncRNAs that displayed expression changes in response to p53 activation via DNA damage. Its close proximity to *CDKN1A* (only 5 kilobases upstream on the antisense strand) suggested that perhaps it could regulate *CDKN1A* expression. However, upon DNA damage PANDA was induced appreciably earlier than *CDKN1A* mRNA . Also, knockdown of PANDA had no effect on p21 expression. Rather, knockdown of PANDA selectively enhanced induction of p53-regulated pro-apoptotic genes such as *FAS* and *APAF1*. These p53 targets are distinguished from cell cycle-related p53 targets by the presence of binding sites for the transcription factor NF-Y[10].

NF-Y connection

NF-Y is a heterotrimeric complex composed of NF-YA, NF-YB and NF-YC, of which NF-YA is an important regulatory subunit[11]. There is a close and complex relationship between NF-Y and p53. On the one hand, p53 seems to rely on NFY to repress transcription of many of its target genes[12]. On the other hand, NF-Y has been shown to function as a trans-activator of a subset of p53 targets, such as *FAS*, that lack a TATA box in their promoter region and require binding of both NF-Y and p53[10].

Hung and collaborators hypothesized that PANDA might function through sequestration of NF-YA away from NF-Y/p53 co-regulated promoters. Using RNA chromatography and chromatin immunoprecipitation, they showed that PANDA indeed binds to NF-YA and its knockdown increases the presence of NF-YA at promoter regions of p53-dependent pro-apoptotic target genes [5] (Figure 1). This could lead to increased cell death in response to DNA damage. Consistent with this idea, reducing PANDA levels with siRNA resulted in increased rates of apoptosis[5]. Whether pro-survival effects of PANDA during DNA damage response are mediated through “eviction” of NF-YA and ensuing repression of FAS remains to be determined. At the very least, it is consistent with the prevailing view that NF-Y transcription factors help orchestrate p53-dependent responses to DNA damage[13]. Thus, while PANDA might be little more that “junk” RNA for the purpose of p53-dependent cell cycle arrest, it plays a key role in protecting the stressed cell from apoptotic death.

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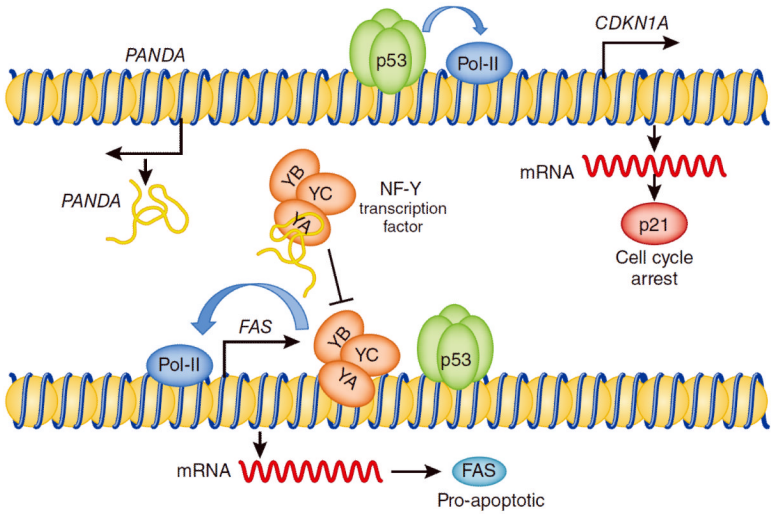

Figure 1.

Model of pro-survival effects of PANDA lncRNA during the DNA damage response. Upon DNA damage, p53 activates expression of target genes such as *CDKN1A* which encodes the cell cycle regulator p21. p53 also activates expression of PANDA lncRNA encoded upstream of the *CDKN1A*..PANDA physically interacts with the NF-YA subunit of the NF-Y transcription factor and prevents it from cooperating with p53 on the promoters of p53-dependent pro-apoptotic targets, such as *FAS*.