

Dark matter of the genome, a safeguard of genetic integrity

Commentary on Fabrizio d'Adda di Fagagna's paper published on Nature

by Geneviève Almouzni & Antonin Morillon

With the advances of DNA sequencing technologies, the realization that 99% of the human genome is non-protein coding has highlighted the under-appreciated potential that lies within the dark matter of the genome. More strikingly, the recent ENCODE project estimated that the number of expressed non-coding genes, so called pervasive transcripts, is now more prominent than the number of coding genes [1]. Thus, understanding how non-coding transcription can be associated with particular function in vivo is an area that is subject of debate [2] and deserves deeper investigations. In particular it is important to explore how the pervasive transcription that generates thousands of non-coding transcripts can regulate gene expression and have broad impacts on development and disease in many organisms [3]. Long and small non-coding RNAs have no protein-coding potential and do not overlap with characterized classes of non-coding RNAs [4, 5].

In contrast to RNAi, miRNA and piRNA-mediated gene regulation, the novel classes of long and short ncRNA are still poorly defined whether it is for their putative regulatory role(s) or for their systematic catalog in living cells. In addition, if the majority of the ncRNA do not have function yet associated with their expression, gene regulation-mediated by ncRNA remains the most studied activity, leaving as terra incognita the role of the ncRNA on general DNA maintenance and chromosome stability.

A few examples of DNA and chromatin territories modulation by long ncRNA have been described from X inactivation, DNA looping, chromosome pairing and epigenetic regulation [6]. Concerning small ncRNA, previous work on the filamentous fungus *Neurospora crassa* revealed the existence of short ncRNA (qiRNA), expressed from the rRNA locus, associated with argonaute protein and induced upon DNA damage [7].

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However, if the biogenesis of qiRNAs has started to be deciphered and required the RNAi pathway, their precise function remains unknown and they are still considered as aberrant siRNA [8].

The team of Fabrizio d'Adda di Fagagna at IFOM, seeking for factors involved in DNA damage signaling pathways, explored why diverse human cells became sensitive to DNA damage when DICER and DROSHA, the two enzymes processing small ncRNA, are absent. Using high-throughput sequencing approaches in collaboration with the RIKEN institute in Japan, combined with appropriate genomic engineering on human and invertebrate systems, they identified the presence of novel small ncRNA, that they coined the DNA Damage RNA (DDRNA).

These RNAs are produced from the DNA damage site [9] and necessary for signaling the damage to the DNA repair machinery. Indeed, impairing the biogenesis of the DDRNA results in cells enabled to repair and cell-cycle arrested. Strikingly, the very same damaged cells when grown in presence of a set of artificially produced DDRNA corresponding to the DNA damage site, recover their ability to repair their broken DNA.

The authors propose that the DDRNA could trigger the molecular alarms through which the cell detects the problem and resolves it by repairing the damage. The exact molecular mechanism behind this DDRNA signaling pathway should be explored in the future.

Site-specific DICER and DROSHA RNA products control the DNA-damage response

Non-coding RNAs (ncRNAs) are involved in an increasingly recognized number of cellular events. Some ncRNAs are processed by DICER and DROSHA RNases to give rise to small double-stranded RNAs involved in RNA interference (RNAi). The DNA-damage response (DDR) is a signalling pathway that originates from a DNA lesion and arrests cell proliferation³. So far, DICER and DROSHA RNA products have not been reported to control DDR activation. Here we show, in human, mouse and zebrafish, that DICER and DROSHA, but not downstream elements of the RNAi pathway, are necessary to activate the DDR upon exogenous DNA damage and oncogene-induced genotoxic stress, as studied by DDR foci formation and by checkpoint assays. DDR foci are sensitive to RNase A treatment, and DICER- and DROSHA-dependent RNA products are required to restore DDR foci in RNase-A-treated cells. Through RNA deep sequencing and the study of DDR activation at a single inducible DNA double-strand break, we demonstrate that DDR foci formation requires site-specific DICER- and DROSHA-dependent small RNAs, named DDRNAs, which act in a MRE11–RAD50–NBS1-complex-dependent manner (MRE11 also known as MRE11A; NBS1 also known as NBN). DDRNAs, either chemically synthesized or in vitro generated by DICER cleavage, are sufficient to restore the DDR in RNase-A-treated cells, also in the absence of other cellular RNAs. Our results describe an unanticipated direct role of a novel class of ncRNAs in the control of DDR activation at sites of DNA damage. [PMID: 22722852]

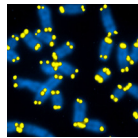
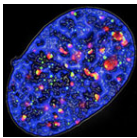
Interestingly, two other teams, using two different model organisms, drosophila and Arabidopsis thaliana reached independently similar conclusions. Thus this novel DDRNA signaling pathway may have broad implications in various organisms.

In drosophila, DDRNA are generated from the DNA double-strand breaks at the end of chromosome, resulting from an active sense/antisense transcription and leading to gene silencing at the vicinity of the damage [10]. In plants, DSB-induced small RNAs, similar to DDRNA, are potentiated by argonaute for an unknown process [11].



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Although in both studies the direct roles of DDRNA have not been explored in details, these works suggest that DDRNA is a conserved and widespread mechanism that relays the DNA repair machinery, opening new avenues for the function of small ncRNA in DNA maintenance regulation.



*DNA Damage Response and Cellular Senescence,
Fabrizio d'Adda di Fagagna*

The notion that the dark matter of the genome represents a tremendous resource for various functions is now coming into a reality. It would not be surprising to soon realize that beyond DNA processes even unsuspected cellular functions will be demonstrated to be modulated by ncRNA. (nc) RNA should thus take a full space as a major actor in the molecular biology of the cell and the genetic world [12, 13].

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Geneviève Almouzni is director of research at the CNRS, head of the Nuclear Dynamics and Genome Plasticity unit, a mixed structure between the Institut Curie and the CNRS. Her team, chromatin dynamics, over the years has conducted research on chromatin, histone variants, histone chaperones and nuclear organization with a particular interest on heterochromatin. Her general objective has been to dissect the mechanisms

of chromatin assembly, from the basic structural unit, the nucleosome, up to the higher-order structures in the nucleus.

In addition, her work on specific nuclear domains has unveiled the importance of non-coding centromeric heterochromatic regions, which are of major importance for chromosome segregation and relevant for cancer. Her lab has exploited a variety of tools and models to

understand the in vivo functions of chromatin dynamics (e.g. *Xenopus*, mouse) that combine biochemistry, cell biology, and developmental biology. These integrated approaches from molecules to tissue and a whole organism are actively pursued in particular within the network of excellence EpiGeneSys, to move epigenetics towards system biology (www.epigenesys.org) for which she acts as the scientific coordinator.

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epigenetic functions of long non-coding (lnc)RNAs in eukaryotes, especially those antisense to genes. Using RNA-seq technology, his team defined a novel class of cryptic lncRNA in yeast, termed XUTs with a regulatory function on gene expression that might

be conserved throughout the eukaryotic kingdom and might have a role on genomic instability. The activity of the team is mostly dedicated to yeast genetics but also to genomics in human cancer cells.