A lncRNA to repair DNA

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Long non-coding RNAs (lncRNAs) have emerged as regulators of various biological processes, but to which extent lncRNAs play a role in genome integrity maintenance is not well understood. In this issue of *EMBO Reports*, Sharma *et al* [1] identify the DNA damage-induced lncRNA *DDSR1* as an integral player of the DNA damage response (DDR). *DDSR1* has both an early role by modulating repair pathway choices, and a later function when it regulates gene expression. Sharma *et al* [1] thus uncover a dual role for a hitherto uncharacterized lncRNA during the cellular response to DNA damage.

See also: V Sharma *et al* (November 2015)

When the genome gets damaged, cells have to initiate an efficient response to faithfully repair the lesion and maintain genome integrity. DNA double-strand breaks (DSBs) are among the most toxic lesions and two major repair pathways share the duty to safeguard the genome when chromosomes break: The non-homologous end-joining (NHEJ) pathway re-ligates broken DNA ends, whereas homologous recombination (HR) employs the homologous DNA sequence generated during replication as a template to restore the broken chromosome. Which pathway to choose is an important decision for cells, and making a wrong choice can lead to detrimental repair outcomes. Recent efforts have thus focused on identifying the cellular determinants involved in the regulation of this repair pathway choice. Interestingly, many RNA-binding proteins accumulate at sites of DNA damage, indicating that they may have important functions during the DDR. RNA itself, and the DNA transactions involved in its synthesis, can interfere with repair events and thereby cause problems.

On the other hand, specific RNA species might actively assist the DDR by regulating the extent and dynamics by which genome caretaker proteins gain access to the break sites. Indeed, small non-coding RNAs have been implicated in various facets of the DDR [2]. In this issue of *EMBO Reports*, Sharma *et al* [1] take an unbiased approach to reveal that also long non-coding transcripts are induced by DNA damage, and identify the DNA damage-sensitive RNA1 (*DDSR1*) as a novel regulator of DNA repair.

In order to identify differentially regulated lncRNAs in response to DNA breakage, Sharma *et al* [1] isolated RNA from immortalized human fibroblasts treated with three DNA-damaging agents (neocarzinostatin, camptothecin, and etoposide) and hybridized the reverse-transcribed material to microarrays containing known non-coding RNA sequences. While several non-coding RNAs were differentially regulated, only one lncRNA was up-regulated in both nuclear and total RNA extracts in response to all three treatments, and the authors named it *DNA damage-sensitive RNA1 (DDSR1)*. In order to elucidate the mechanism of *DDSR1* expression, Sharma *et al* [1] first tested whether the kinase ATM, which is activated by DSBs and regulates many downstream signaling events of the DDR, was required for *DDSR1* induction. Indeed, this turned out to be the case. Next, they noticed that the genomic region upstream of the *DDSR1* gene contains a putative NF- κ B consensus motif, and treating cells with an inhibitor of NF- κ B suppressed the induction of *DDSR1*. This suggests that ATM, via NF- κ B activation, drives the expression of *DDSR1*.

Since lncRNAs are known regulators of gene expression, Sharma *et al* [1] performed transcriptome analyses in the presence or absence of *DDSR1* and observed a deregulation of a subset of p53 target genes and of

several other genes involved in genome maintenance and cell survival. Moreover, cell proliferation and DNA damage signaling were reduced in cells lacking *DDSR1*. To gain insight into how *DDSR1* affects DNA repair, the authors turned to DSB repair reporter cell lines and found that specifically, the HR pathway was compromised in *DDSR1*-deficient cells and that this entailed a modest increase in PARP inhibitor sensitivity. But how exactly does *DDSR1* promote repair by HR? To address this question, Sharma *et al* [1] decided to analyze protein interaction partners of *DDSR1* by mass spectrometry. Reassuringly, they identified several proteins implicated in the DDR, including a protein called hnRNPUL1 [1]. This member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family was previously shown to be recruited to sites of DNA damage in a manner that required the formation of poly(ADP-ribose) (PAR) by PARP enzymes [3]. hnRNPUL1 thus seems to belong to a growing number of RNA-binding proteins and transcription factors that respond to PAR signaling at sites of DNA breaks [4]. Interestingly, in the case of hnRNPUL1, the recruitment to break sites also depends on the DSB-sensing protein complex MRN, and one function of hnRNPUL1 is to promote DNA end resection downstream of MRN for subsequent repair by HR [5]. Sharma *et al* [1] therefore analyzed markers of DNA end resection and found a reduction in cells lacking *DDSR1*, suggesting that depletion of *DDSR1* inhibits the resection step of HR [1]. Consistent with such a scenario, the authors observed an increased accumulation of BRCA1 together with its binding partner RAP80 at sites of DNA damage [1]. This is interesting, because unlike other pro-recombinogenic BRCA1-containing complexes, the BRCA1/RAP80 complex was shown to restrict DNA

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Figure 1. The lncRNA DDSR1 regulates DSB repair.

(A) Upon DSB formation, the transient recruitment of hnRNPUL1 into a PAR-seeded protein assembly facilitates early sorting of repair factors. Sharma *et al* [1] show that these early pathway decisions are modulated by DDSR1, which sequesters the anti-recombinogenic BRCA1/RAP80 complex away for DSBs. (B) Later during the DDR, the ATM–NF-κB signaling axis stimulates DDSR1 transcription to reinforce its role in mediating repair pathway choice and to balance p53 target gene expression. (C) Through enhanced sequestration of the BRCA1/RAP80 complex, the increased levels of DDSR1 foster DSB repair by HR. The abundant DDSR1 also binds hnRNPUL1, which may facilitate the transition from early to advanced stages of the DDR.

end resection [6,7], and its enhanced recruitment in DDSR1-deficient cells might thus underlie the reduced HR efficiency.

Of note, the DNA damage-enhanced expression of DDSR1 took several hours to occur. In contrast, the recruitment of genome caretakers to sites of DNA damage takes place in the range of seconds to minutes. The role of DDSR1 to assist faithful DNA repair and promote cell survival could therefore be a dual one (Fig 1): Early upon DSB formation, DDSR1 sequesters the BRCA1/RAP80 complex and prevents it from undue binding to damaged chromatin. Concomitantly, PAR assembles numerous proteins around the break site, including hnRNPs and other RNA-binding proteins. Through multivalent interactions, such an

assembly can lead to a phase transition [8], and the resulting phase boundary can function as a selective barrier to allow or restrain access of proteins to the damaged site [8,9]. Sequestration of BRCA1/RAP80 by DDSR1 may prevent the unscheduled inclusion of the complex into the PAR-seeded protein assembly and thereby modulate repair pathway choice. Subsequent to this early role, DDSR1 expression is enhanced in an ATM- and NF-κB-dependent manner to inhibit the expression of p53 target genes—the second role of DDSR1 during the DDR.

The roles of non-coding RNAs in the DDR are only beginning to emerge and may be manifold. They could serve as recruitment platforms that initiate protein

assembly at DNA break sites, or prevent untimely accumulation of proteins around DNA lesions by sequestration. We are still far from understanding the dynamics of such processes and how different RNA species or PAR may compete for the same set of proteins. A binary view based on specificity versus non-specificity in protein interactions with nucleic acids is unlikely to suffice, and new quantitative models are currently being developed [10]. Such efforts will help to grasp the complexity and dynamics of interactions between proteins and nucleic acids and will allow additional insights into the function of coding and non-coding RNAs, long and short, and of molecules like PAR in the cellular response to DNA damage.

The identification of the lncRNA DDSR1 by Sharma *et al* [1] as a DNA damage-responsive transcript sheds new light on the role of lncRNAs in DNA repair. An exciting twist comes from the observation that DDSR1 functions both early, when the choice between DSB repair pathways is being made, and at later stages, when cells regulate the expression of DNA damage-responsive genes, highlighting the multifaceted nature of genome integrity maintenance.

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