RNA takes over control of DNA break repair

Francesca Storici and Ailone E. Tichon

Small RNAs generated at DNA break sites are implicated in mammalian DNA repair. Now, a study shows that following the formation of DNA double-strand breaks, bidirectional transcription events adjacent to the break generate small RNAs that trigger the DNA damage response by local RNA:RNA interactions.

Maintenance of DNA integrity is crucial for a cell to have a healthy life and for transmission of accurate genetic information to its progeny. Exogenous agents, including radiation or chemicals, as well as endogenous sources, such as reactive oxygen species or defects in DNA metabolism, pose threats to genome stability. Among the most dangerous DNA lesions are DNA double-strand breaks (DSBs), which if not properly and timely sealed can become sites of mutations or chromosomal rearrangements — well-known hallmarks of cancer and other genetic disorders[1]. The process of DSB repair is one of the most extensively studied mechanisms of DNA repair, yet much remains to be understood about its players and dynamics. The DNA damage response (DDR) is a complex network of cellular pathways that detect DNA lesions and organize a response signal cascade to repair the DNA. In this issue of *Nature Cell Biology*, Michelini *et al.*[2] uncover an aspect of the DDR in which RNAs are the directors. The study shows that sequence-specific RNA:RNA interactions orchestrate the DDR in response to DSBs.

RNA molecules are known to play central roles in the regulation of chromatin functions at different levels, including maintenance of genome stability. Examples include XIST, which coats one of the two X chromosomes leading to its transcriptional inactivation[3], FIRRE that can modulate the organization of intra-chromosomal subregions[4], and NORAD that controls the stability of genes responsible for proper chromosome segregation in cell division[5]. Moreover, actively transcribed DNA exhibits faster DSB repair than transcriptionally inactive DNA, and recruits proteins specific to repair through homologous recombination[6,7] and/or non-homologous end joining following a DSB[8]; whereas RNA transcripts, reversing the central dogma, can also be direct templates in DSB repair[9].

Another surprise in the DNA repair field was the finding by the d'Adda di Fagagna team and other groups that the double-stranded endoribonucleases Drosha and Dicer of the RNA interference system are actively involved in the DDR through generation of small RNAs (termed DDRNAs) directly at sites of DNA DSBs[10–12]. A more recent study reported that following DNA DSB induction, a phosphorylated form of Dicer accumulates in the nucleus and is recruited to DSBs in human embryonic kidney cells and mouse embryonic fibroblasts[13]. These findings have triggered a number of interesting questions. For instance, what is the function of DDRNAs generated by Drosha and Dicer in the nucleus of cells at sites of DSBs? How relevant are the small RNAs for DNA repair? By which mechanism do DDRNAs activate the DDR?

Evolving from the initial discovery of DDRNAs, with a remarkable set of experiments and sophisticated techniques of molecular imaging and intracellular RNA detection, Michelini *et al.*[2] investigated the significance of the DDRNAs in the context of DSB repair in mammalian cells. First, using synthetic DDRNAs that match sequences flanking an I-SceI-enzyme-induced DSB in mouse chromosomal DNA, the authors show that the DDRNAs colocalize with the DSB, and DDRNA presence is critical for recruitment of the p53-binding protein 1 (53BP1), an early participant in the DDR. Whereas the DDRNAs do not bind to the DNA ends of the break, their localization at the DSB site is mediated by RNA polymerase II (RNAPII), which is responsible for DDRNA biogenesis and function. By exploring the transcriptional landscape in the vicinity of the DSB site using single-molecule fluorescent *in situ* hybridization (smFISH) and strand-specific quantitative PCR with reverse transcription (RT-qPCR) following DSB induction, the authors found that damage-induced long non-coding RNAs (dilncRNAs) are bidirectionally transcribed from both sides of the lesion. Further experiments by RT-qPCR, using different enzymatic sources for DSBs in various mammalian cell lines, showed that dilncRNA transcription is sensitive to RNAPII inhibitors, similar to DDRNA. By incubating a plasmid cut by the I-SceI enzyme *in vitro* with transcription-competent human cell extracts in the presence of [α-^{32}P]UTP, the authors detected nascent transcripts that were sensitive to the RNAPII inhibitor α-amanitin. Deep sequencing of these dilncRNA products following 5′ rapid amplification of cDNA ends (5′ RACE) showed that the dilncRNA transcripts are homologous to plasmid sequences that start right at DSB ends. In addition, strand-specific RT-qPCR showed that dilncRNAs accumulate following Drosha knockdown in cells with a DSB, whereas a gel fraction containing RNAs with sizes corresponding to DDRNAs (15–40 nucleotides) increased after DNA damage and decreased following Dicer knockdown. Using these results, the investigators provide evidence that dilncRNAs are precursors of DDRNAs (Fig. 1).

Super-resolution binding-activated localization microscopy and chromatin immunoprecipitation experiments revealed that active RNAPII colocalizes with γH2AX at

Francesca Storici and Ailone E. Tichon are at the School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332, USA.
e-mail: storici@gatech.edu

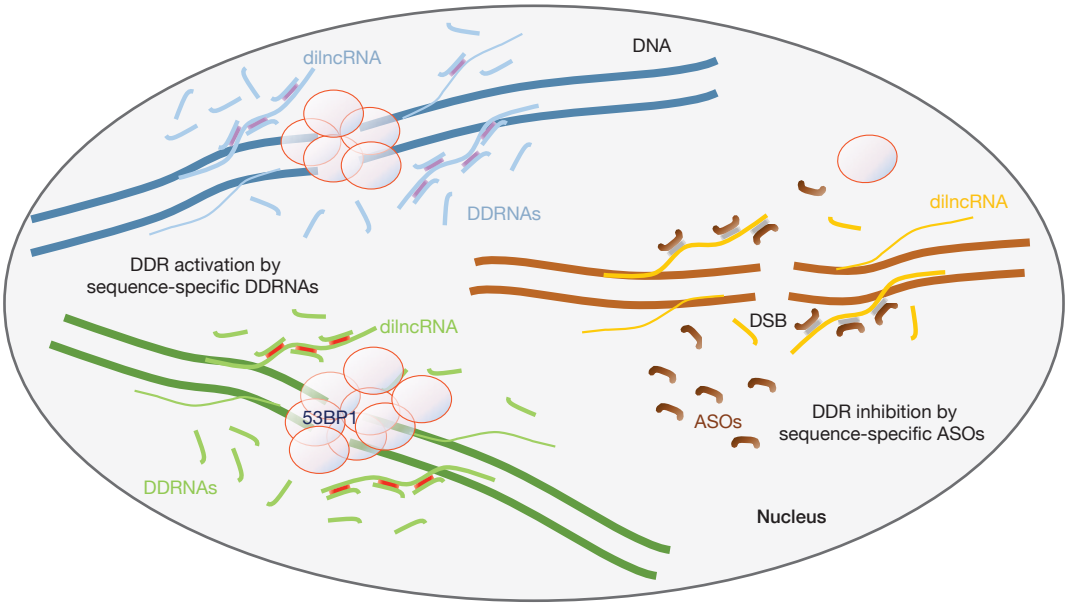

Figure 1 RNA:RNA interactions at DNA DSB sites amplify the stimulatory signal for activation of the DDR specifically at these sites. The drawing shows three different DNA DSB sites in a cell nucleus, depicted as interrupted, thick, parallel strands in blue, green and orange, respectively. The work by Michelini *et al.*[2] suggests that every different DNA DSB site has its unique set of RNA:RNA interactions (shown as small, sandwiched, purple or red rectangles at the two DSB sites on the left) between its specific dilncRNAs and small non-coding RNAs termed DDRNAs (both shown as light blue, green or yellow lines), which originate from dilncRNAs at each DSB site. Such local RNA:RNA interactions are signals to activate the DDR by recruitment of the early DDR factor 53BP1 (transparent ovals). The dilncRNAs generated from DSB ends (thicker lines) were found to be more abundant than those generated towards DSB ends (thinner lines). ASOs can be designed to specifically block DSB repair at a chosen locus, without interfering with DSB repair at other loci within the same nucleus. Here, ASOs (shown as short, thick, brown lines) are specific to dilncRNA sequences of the DSB site on the right and block formation and function of DDRNAs at this DSB site by interacting (small, sandwiched, grey rectangles) with the complementary dilncRNAs.

DSB sites in mammalian cells. Recent work in *Schizosaccharomyces pombe* also showed that RNAPII transcripts are generated at the site of a DNA DSB[14]. However, whereas Dicer is active in *S. pombe* cells, Dicer was not found to be involved in the activation of the DDR in fission yeast. It would be interesting to investigate whether formation of DDRNAs at DSB sites by Drosha and Dicer is exclusive to multicellular systems.

To elucidate the upstream events in the cascade of dilncRNA formation, the authors examined a possible interaction between RNAPII and the Mre11–Rad50–Nbs1 (MRN) complex, which was previously found to be required for DDRNA-driven DDR activation[10] following DNA damage in mammalian cells. The authors conducted a set of pull-down experiments and showed that RNAPII was immunoprecipitated with each of the MRN components, and the signal was markedly enhanced after ionizing irradiation. Furthermore, they found that dilncRNA levels were reduced after MRN silencing or inhibition with the specific inhibitor Mirin, and that the presence of transcribing RNAPII at the DSB was also reduced following MRN inhibition. Overall, these results support an interaction model between RNAPII and MRN following DNA damage. More studies are needed to better characterize the mechanism by which RNAPII and MRN interact and how such contact affects the downstream steps in the DDR.

The role of RNAPII was further explored for its activity in DDR focus formation. Following inhibition of RNAPII, irradiation- or I-SceI-induced DSBs led to impaired focus formation of 53BP1 and ATM (and other DDR factors), while their mRNA levels and focus formation of the γH2AX were unchanged. These results suggest that RNAPII activity at a DSB precedes DDR activation. An intriguing finding in this study was that following DSBs generated by the restriction enzyme AsiSI in human fibroblasts, the accumulation of 53BP1 on damaged chromatin was reduced after inhibition of RNAPII within and, surprisingly, even outside the transcriptional region. It remains unclear how RNAPII generates DSB-associated transcripts in such intergenic regions. Of note, by using a similar system to induce DSBs in chromosomal DNA, the Legube lab has shown that the preexisting chromatin status has a profound impact on the repair pathway that is activated for DSB repair[6]. Clearly, further investigations are needed to better understand how chromatin affects the DDR in response to DSBs and how RNA:RNA interactions respond to chromatin status of the damaged DNA. For example, it would be interesting to determine how the chromatin environment affects the synthesis of dilncRNAs and DDRNAs. It may be expected that open chromatin in actively transcribed regions generates a larger set of DDRNAs than silent chromatin as the transcribed sites may have more abundant precursor dilncRNAs, especially those towards the DSB ends. A question is therefore whether the quantity of DDRNAs at a specific DSB locus affects the response to DNA damage at that locus.

Finally, the authors studied the relevance of the dilncRNA to the DDR by using locked nucleic acids as antisense oligonucleotides (ASOs) to target the dilncRNA sequences and impair their interaction with DDRNAs. ASOs specific to sequences flanking the I-SceI or I-PpoI DSBs reduced 53BP1 focus formation but had no effect on 53BP1 in irradiated cells. When two DSB foci were visualized in mouse cells stably expressing GFP-LacR or Cherry-TetR, respectively, Tet-specific ASOs

impaired 53BP1 focus formation and repair at the Tet locus, but not at the Lac locus in the same nucleus. These data corroborate the findings that small RNAs specific to each damaged DNA locus are generated and play a role in activating the DDR at each specific site. In a case of multiple DNA lesions per cell, could the specificity and abundance of DDRNAs guide a cell to 'make decisions' on which damaged DNA locus should have priority of repair? The results of Michelini *et al.*[2] may also stimulate the development of gene editing/therapy strategies to modulate the DDR at chosen sites of induced DNA DSBs. By exploiting the use of synthetic DDRNAs and/or inhibitory ASOs, it may be possible to manipulate the DDR by enhancing it at certain loci and/or diminishing it at others within the same cells (Fig. 1). Most certainly, this study will generate new avenues to pursue in the quest to understand the DNA damage response.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Capturing endosomal vesicles at the Golgi

J. Christopher Fromme and Mary Munson

Membrane trafficking specificity between distinct compartments ensures that cargo proteins and lipids are delivered to their target organelle. However, accurate recognition of cargo carriers by tethering factors on target membranes is poorly understood. TBC1D23 is now identified as an adaptor that links endosome-derived vesicles with golgins at the *trans*-Golgi.

Regulators of eukaryotic membrane trafficking pathways ensure that proteins residing in the membranes and lumens of intracellular organelles are delivered to the correct cellular destination. Long coiled-coil tethering proteins and large multi-subunit tethering complexes (MTCs) resident on the destination organelle membranes specifically recognize vesicle and tubular cargo carriers. The recognition and tethering processes are poorly understood at the molecular level, as only a few proteins that bridge the tethers and carriers have been identified. In this issue of *Nature Cell Biology*, Shin *et al.* use a combination of ectopic mitochondrial localization of the *trans*-Golgi tethers, called golgins, with proximity biotinylation to discover a golgin-interacting protein that captures endosomal vesicles *en route* to their destination at the *trans*-Golgi[1]. This adaptor protein is TBC1D23, a presumed non-catalytic member of the TBC (Tre-2/Bub2/Cdc16)-domain family of Rab GTPase-activating proteins (GAPs). TBC1D23 provides a direct link between golgin-97/245 and the WASH (Wiskott–Aldrich syndrome protein and SCAR homologue) complex on endosomal vesicles.

Once transport carriers arrive at the correct target compartment, SNARE proteins mediate fusion reactions between membranes to safely deliver the cargo. SNAREs provide some degree of specificity, but are not uniquely sufficient to prevent inappropriate or premature fusion events[2]. Instead, this specificity is thought to be achieved primarily through the action of vesicle-tethering factors. There are two broad classes of vesicle tethers. The first class are the multi-subunit tethering complexes, including exocyst, COG, GARP, HOPS, CORVET and DSL; these complexes bind to SNARE proteins and appear to regulate fusion by ensuring proper SNARE pairing on opposing membranes through an unknown mechanism[2]. The second class of vesicle tethers are long coiled-coil proteins, including a group named golgins[3–5]. The precise function(s) of golgins remains the subject of debate, but previous work from the Munro group solidified the idea that most golgins function as specific tethers on the Golgi complex, capturing vesicles originating from other organelles or from other areas of the Golgi[6].

Golgins are localized to different regions of the Golgi and capture distinct populations of vesicles. For example, golgins at the *cis*-Golgi capture vesicles derived from the endoplasmic reticulum and from later Golgi compartments, whereas golgins at the *trans*-Golgi capture vesicles derived from endosomal compartments[6]. The basis for this specificity has not been fully defined[3–5]. The golgin GMAP-210 was shown to bind to incoming vesicles through its affinity for the highly curved surface of transport vesicles[7], but this mechanism does not appear to be sufficient to explain the full spectrum of golgin-mediated tethering events. Two recent studies reported that the yeast golgins Uso1 (p115 homologue) and Coy1 (CASP homologue) bind to SNAREs, providing a potential specificity mechanism[8,9], but given that specific SNAREs are found on multiple membranes, it is not clear if these SNARE interactions can provide a sufficient level of docking specificity. Instead, it is probable that the tethers act directly on the SNAREs to facilitate SNARE assembly and fusion, as has been suggested for several MTCs[2]. Many golgins bind small GTPases of the Arf, Arl and Rab families, and these interactions provide specificity due to the localization of these GTPases to distinct organelles and vesicles. In addition, several golgins interact with cargo adaptors, other GTPase effectors that are present only on specific vesicles, and MTCs such as the COG complex[3–5].

J. Christopher Fromme is in the Department of Molecular Biology and Genetics, Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, New York 14853, USA. Mary Munson is in the Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, Massachusetts 01605, USA.
e-mail: mary.munson@umassmed.edu