Review

A direct role for small non-coding RNAs in DNA damage response

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Historically, the role of cellular RNA has been subordinate and ancillary to DNA. Protein-coding mRNA conveys the information content of DNA, and transfer RNAs and ribosomal RNAs allow the polymerization of amino acids into proteins. The discovery of non-protein-coding RNAs (ncRNAs) provided an additional role for RNA in finely tuning DNA expression. However, it has recently become apparent that the safeguard of DNA integrity depends on small ncRNAs acting at the site of DNA lesions to signal the presence of DNA damage in the cell, and on the genes involved in their biogenesis to achieve accurate DNA repair. I review here evidence supporting a role for small ncRNAs, termed DNA damage-response RNAs (DDRNAs) or double-strand break (DSB)-induced RNAs (diRNAs), that are generated at sites of DNA damage and control the DNA damage response (DDR). I also discuss their biogenesis, potential mechanisms of action, and their relevance in cancer.

DNA damage response: a role for RNA

With the exception of RNA-based viruses and their DNA-encoded evolutionary remnants such as the specialized RNA-dependent DNA polymerase telomerase, the information necessary to life is stored in DNA. To avoid risks of information loss or corruption, it is imperative for a cell to preserve the structural integrity of its DNA. To this end, upon DNA damage cells mount a prompt DDR that prevents cell cycle progression of the damaged cell by enforcing cell cycle checkpoints and coordinating DNA damage repair (Box 1 and Figure I).

DDR activation relies on the coordinated recruitment of specialized DDR proteins at sites of DNA damage. DDR factors initially associate directly with DNA lesions. Subsequent protein modifications and additional interactions promote the accumulation of DDR factors, which form nuclear DDR foci large enough to be visualized by standard immunofluorescence techniques. DDR-mediated cellular outcomes may be cell death by apoptosis, a transient cell cycle arrest (checkpoint) followed by repair of DNA damage and resumption of proliferation, or cellular senescence caused by the persistence of unrepaired DNA damage [1,2].

The prevailing view has been that the DDR involves only proteins, and signal generation and amplification is achieved by protein interactions regulated by various protein modifications. RNA becomes involved only in the form of transcriptional outcome of DDR activation at the bottom of the signal cascade. Recently, a paradigmatic shift has changed this view and DDR activation has been shown to depend on small ncRNAs generated at sites of DNA damage. This review will discuss the biogenesis and roles of these RNAs, how they may act in the control of the DDR, and the potential relevance of these findings in cancer.

ncRNAs

It is now realized that the vast majority of the genome is transcribed [3]. Often these transcripts do not code for proteins, but are, nevertheless, biologically functional. Some of these ncRNAs are nuclear and may remain associated with chromatin in a sequence-specific manner to control epigenetic modifications of chromatin [4]. Some ncRNAs may aggregate in membrane-less subcellular structures where they regulate the localization and the activity of proteins or provide structural support, which leads to the regulation of a variety of cellular functions (Box 2). Some long ncRNAs [such as LincRNA-p21, PANDA (promoter of CDKN1A antisense DNA damage activated RNA), and a long ncRNA associated with the cyclin D1 (CCND1) gene], have also been reported to respond to genotoxic stress [5–7]. However, they have not been shown to localize directly and act at sites of DNA damage, suggesting they may act as downstream modulators of gene expression during the DDR.

Various small ncRNAs act in the RNA interference (RNAi) pathway, an evolutionarily conserved machinery whose components are thought to have originally evolved to preserve genomes from attacks by viruses and mobile genetic elements [8]. RNAi precursors are processed by RNases such as DROSHA and DICER (double-stranded RNA-specific endoribonucleases type III) to generate small double-stranded (ds) RNA products [9]. According to their biogenesis and functions, mature RNA products are classified as either small interfering RNAs (siRNAs), repeat-associated small interfering RNAs (rasiRNAs), Piwi-interacting RNAs (piRNAs) [QDE-2 interacting RNAs (qiRNA) in *Neurospora crassa*], and microRNAs (miRNAs)

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Box 1. Mechanisms of DDR

DDR is triggered by the recognition of DNA discontinuities in the form of DSBs or exposed regions of single-stranded DNA that are respectively recognized by two specialized DNA damage sensors: the MRN complex [meiotic recombination 11 homolog B (MRE11)–RAD50–Nijmegen breakage syndrome 1 (nibrin/NBS1)] and replication protein A (RPA), together with the 911 complex [RAD9–RAD1–HUS1 (HUS1 checkpoint homolog)] [51]. These DNA damage sensors recruit the apical protein kinases ATM through interaction with the MRN complex, and ATR (ataxia telangiectasia and Rad3-related) through interaction with RPA and the 911 complex, which is associated with the ATR-interacting protein (ATRIP). The activation of these protein kinases in turn modifies a variant of histone H2A known as H2AX on Ser139 (generating the so-called γH2AX) on the chromatin in *cis*, starting from the region most proximal to the DNA lesion and spreading distally for up to hundreds of kb. This amplification mechanism relies on the recruitment of the mediator of DNA damage checkpoint 1 (MDC1) which, together with 53BP1, sustains and amplifies DDR signaling by enforcing further accumulation of the MRN complex and activation of ATM. DDR signal amplification relies on additional mechanisms based on ubiquitylation of DDR factors [52]. Passed its threshold, DDR signaling spreads away from the damaged locus, and this is dependent on engagement of diffusible kinases CHK1 and CHK2 (checkpoint kinases 1/2). Ultimately, signals converge on cell cycle progression regulators such as p21, whose induction is mediated by p53, and on the protein phosphatase CDC25 (cell division cycle 25C). In parallel to checkpoint enforcement, DNA repair mechanisms are activated including homologous recombination (HR) and non-homologous DNA end joining (NHEJ). During HR, a single-stranded 3′ DNA end of the broken DNA invades a dsDNA with a homologous sequence, and restores the damaged DNA by using the intact homologous sequence as a template. Alternatively, during NHEJ, DNA ends are sealed back together by a DNA ligase. The choice between the two repair mechanisms is cell cycle regulated [53] and only upon full DNA repair is cell proliferation allowed to proceed [2] (Figure I).

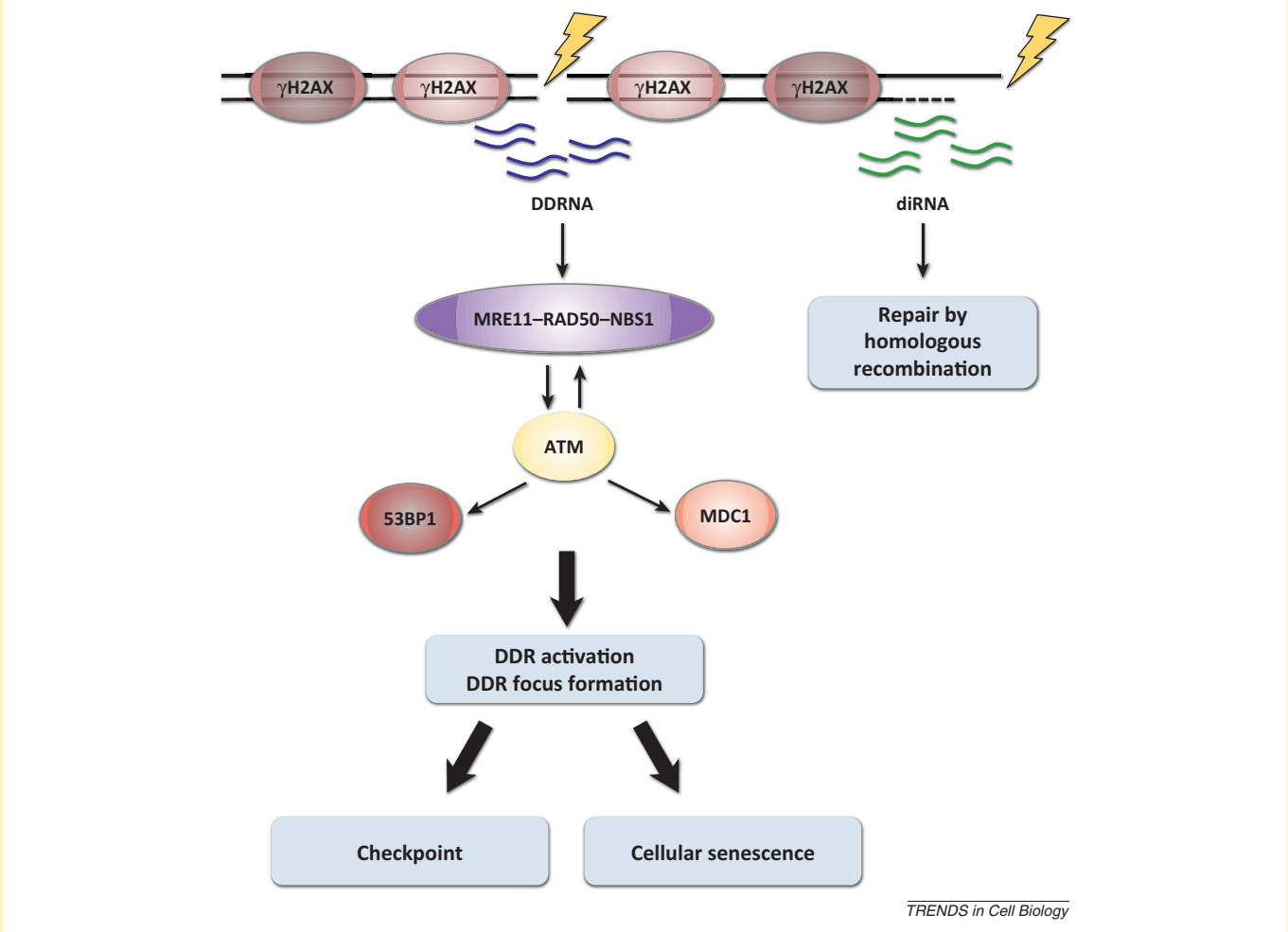

Figure I. Mechanisms of DNA damage response (DDR). Abbreviations: ATM, ataxia telangiectasia mutated; 53BP1, tumor protein p53 binding protein 1; DDRNA, DNA damage-response RNA; diRNA, double-strand break (DSB)-induced RNAs; γH2AX, γ-H2A histone family, member X; MDC1, mediator of DNA-damage checkpoint 1; MRE11, meiotic recombination 11 homolog B; NBS1, Nijmegen breakage syndrome 1 (nibrin); RAD50, DNA repair protein RAD50 homolog.

[9]. Only miRNA maturation is considered dependent on both DROSHA and DICER endoribonucleases. In mammals, miRNAs modulate gene expression by their ability to regulate mRNA translation and stability through their Argonaut (Ago) effector proteins.

There is evidence of interplay between small ncRNA and DDR. miRNAs control, among several targets, the expression of DDR genes including *ATM* (ataxia telangiectasia mutated) [10], *PRKDC* (protein kinase, DNA-activated, catalytic polypeptide, also known as DNA-PKcs) [11], *BRCA1* (breast cancer 1) [12], *H2AX* (H2A histone family, member X) [13] and *RAD51* (RAD51 recombinase) [14]. Furthermore, DDR factors can directly modulate the biogenesis of miRNAs by controlling their maturation: ATM phosphorylates KSRP (KH-type splicing regulatory protein), a DROSHA interactor [15], BRCA1 interacts with DROSHA