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PII: S0022-2836(16)00177-7
DOI: doi: 10.1016/j.jmb.2016.03.004
Reference: YJMBI 65022

To appear in: *Journal of Molecular Biology*

Received date: 10 December 2015
Revised date: 1 March 2016
Accepted date: 7 March 2016

Please cite this article as: Giono, L.E., Moreno, N.N., Botto, A.E.C., Dujardin, G., Muñoz, M.J. & Kornblihtt, A.R., The RNA response to DNA damage, *Journal of Molecular Biology* (2016), doi: 10.1016/j.jmb.2016.03.004

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The RNA response to DNA damage

Luciana E. Giono[1], Nicolás Nieto Moreno[1], Adrián E. Cambindo Botto[1], Gwendal Dujardin[1,2], Manuel J. Muñoz[1], and Alberto R. Kornblihtt[1]*

[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET) and Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, C1428EHA Buenos Aires, Argentina

[2]Centre for Genomic Regulation. Dr. Aiguader 88, E-08003 Barcelona, Spain.

* Corresponding author: ark@fbmc.fcen.uba.ar

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Abstract

Multicellular organisms must ensure genome integrity to prevent accumulation of mutations, cell death, and cancer. The DNA damage response (DDR) is a complex network that senses, signals and executes multiple programs including DNA repair, cell cycle arrest, senescence and apoptosis. This entails regulation of a variety of cellular processes: DNA replication and transcription, RNA processing, mRNA translation and turnover, and post-translational modification, degradation and relocalization of proteins.

Accumulated evidence over the past decades has shown that RNAs and RNA metabolism are both regulators and regulated actors of the DDR. This review aims to present a comprehensive overview of the current knowledge on the many interactions between the DNA damage and RNA fields.

Research highlights:

• The DNA damage response is essential for genome integrity in multicellular organisms.

• RNAs and RNA metabolism play a prominent role in the DNA damage response (DDR).

• Splicing factors and RNA binding proteins are regulators of and regulated by the DDR.

• Small and long non coding RNAs and miRNAs also participate in the DDR.

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Key words:

DNA damage response; p53; RNA processing; alternative splicing; ncRNAs

Abbreviations

ARE: AU-rich sequence elements

ARE-BP: ARE-binding protein

AS: Alternative splicing

BER: Base excision repair

CPT: Camptothecin

CS: Cockayne syndrome

CTD: C-terminal domain

DOX: Doxorubicin (Adriamycin)

DDR: DNA damage response

DDRNAs: DNA damage response RNAs

DSB: DNA double-strand break

ETO: Etoposide

GGR: Global genome repair

hnRNP: heterogeneous nuclear ribonucleoprotein

IR: Ionizing radiation

lincRNAs: Long intergenic non coding RNAs

NMD: Nonsense mediated decay

RNAi: RNA interference

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RNAPII: RNA polymerase II

TCR: Transcription-coupled repair

miRNA: microRNA

ncRNA: non-protein-coding RNA

NER: Nucleotide excision repair

RBP: RNA binding protein

SR proteins: serine-arginine proteins

UV^{s}S: UV-sensitive syndrome

XP: Xeroderma pigmentosum

1. The expanding universe of RNAs

Scientific breakthroughs often follow technical breakthroughs. Forty years ago, the isolation and application of restriction enzymes, the development RNA-DNA hybridization methods combined with electron microscopy and the Southern blot technique were among the tools that led to a "mini-revolution in molecular genetics" [1]: the discovery of split genes and RNA splicing that resulted in the 1993 Nobel Prize to Philip Sharp and Richard Roberts. In the following years, a handful of genes giving rise to different mRNA variants through alternative splicing were described and thought to be rare, exceptional events. We know today that, in humans, about 95% of genes display alternative splicing, multiplying the functional diversity of the mRNAs - and proteins- generated [2]. Alternative splicing can be both cause and result of natural

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and pathological cellular processes as diverse as cell proliferation and differentiation, cancer, cystic fibrosis, hemophilia, and spinal muscle atrophy, among many others [3].

In the past two decades, the development of RNA sequencing and other high-throughput-sequencing technologies, coupled with powerful computational analysis, has opened our eyes to an expanding universe of non-protein-coding RNAs (ncRNAs) [4]. These RNAs function in regulatory mechanisms affecting virtually every single cellular process. In the past few years, mounting evidence has shown an important role in the DDR of proteins involved in RNA metabolism. Moreover, recently identified ncRNAs termed diRNAs [DNA double-strand break (DSB-induced RNAs] and DDRNAs (DNA damage response RNAs) are generated at sites of damage, modulate the DDR, and promote DNA repair [5, 6].

This review will focus on how RNAs are regulated and, in turn, regulate the cellular response to DNA damage, with emphasis on alternative splicing.

2. The cellular DNA damage response

Maintenance of genome stability is an absolute requirement for cell survival and stable propagation of genetic information. Yet cells are constantly challenged by the generation of close to 10^5 spontaneous DNA lesions per cell on a daily basis, resulting from hydrolytic reactions, and reactive oxygen and nitrogen species and other metabolic products. DNA is also damaged by exogenous agents such as ionizing radiation (IR) and UV light and, for instance, sunlight exposure can increase that number by up to 10^5 additional lesions [7, 8].

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To deal with this constant assault, cells have evolved an intricate signaling network -collectively referred to as the DDR- that activates cell cycle checkpoints and DNA repair pathways, and regulates different aspects of cell biology such as DNA replication, transcription, recombination, chromatin remodeling, and differentiation. Ultimately, this signaling network will determine the outcome: DNA repair and cell survival, replicative senescence, or apoptosis [8]. Premature ageing, cancer predisposition, and an overwhelming list of human diseases associated with defects in the DDR highlight its vital significance [9].

Specific lesions or categories of lesions are detected by at least five independent sensor complexes that activate specialized signaling and DNA repair pathways. Central to the DDR are the transducers ATM, ATR, and DNA-PK, of the PI3K-like family of protein kinases, and the poly(ADP-ribose) polymerase members PARP1 and PARP2, which are recruited to sites of DNA damage, leading to the formation of large nuclear foci termed “DDR foci” [10-12]. There, post-translational modification of downstream substrates and extensive chromatin remodeling play prominent roles [13]. Most of the current knowledge about the DDR is centered on ATM and ATR and their downstream targets, the mediator kinases Chk1 and Chk2, and the more recently described p38MAPK and MK2, which activate numerous effector proteins [10]. Either ATM, ATR, or the downstream mediator kinases can phosphorylate the tumor suppressor p53, one of the most important and best characterized effectors of the DDR. p53 is tightly regulated in unstressed cells and becomes stabilized and activated through multiple post-translational modifications upon DNA damage and other types of stress [14].

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Integrating signals from many different pathways, p53 regulates the expression of an extensive list of targets genes involved in numerous cellular processes of the DDR: cell cycle progression, DNA repair, senescence, apoptosis, metabolism, and angiogenesis, among others [15]. The outcome of the DDR appears to be stimuli- and cell-type specific and the rules governing this life-or-death decision still remain elusive [16].

The DDR can be viewed as a two-step process: a first and fast initiation response is mediated by post-translational modifications, regulating activity, localization, stability and protein-protein interactions of numerous substrates [13]. A second -and necessarily slower- maintenance response involves newly synthesized mRNAs that can be regulated at the level of transcription, splicing, and 5′- and 3′-end processing. In between, pre-existing mRNAs can be post-transcriptionally regulated through modulation of their stability and translation, allowing cells a rapid adjustment [17].

As will be discussed in the following sections, the RNA response to DNA damage is affected by both post-translational modifications and the elicited transcriptional programs.

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3. Transcription and mRNA processing during the DDR

3a. RNA polymerase II

In eukaryotes, all mRNAs and many ncRNAs are transcribed by RNA polymerase II (RNAPII), a holoenzyme composed of 12 subunits. Rpb1, the largest and catalytic subunit, contains an evolutionarily conserved C-terminal domain (CTD) composed, in mammalian cells, of 52 tandem repeats of a heptad (YSPTSPS) containing five phospho-

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acceptor residues. In normal cells, the CTD undergoes cycles of phosphorylation/dephosphorylation and plays critical roles during the cycle of transcription initiation, elongation, and termination, and in many other aspects of RNA processing [18]. In cells that have sustained DNA damage, the RNAPII and its CTD are also critical for sensing the damage and contribute to organizing the DDR [19, 20].

For many years it has been known that some DNA lesions constitute a potent block to transcription by elongating RNAPII. This is particularly the case for the bulky UV-induced DNA lesions. Because transcription blocks may disturb cellular homeostasis with potentially fatal outcome, cells have evolved a dedicated repair pathway: transcription-coupled repair (TCR), a subpathway of nucleotide excision repair (NER) [21]. Different models have been proposed as to how cells deal with the stalled RNAPII, the common denominator being that the polymerase must be displaced in order to allow access of repair factors to the lesion. Within minutes of treating cells with UV light, cisplatin (CIS) or camptothecin (CPT), all agents that generate DNA lesions that are repaired by NER, a fraction of Rpb1 becomes hyperphosphorylated, ubiquitinated and degraded by the proteasome [22, 23]. Alternatively, RNAPII may reverse translocate (backtracking) or, less frequently, bypass the lesion, with the possibility of transcriptional mutagenesis [24]. More recent studies indicate that RNAPII degradation occurs only as “a last resort”, favoring the backtracking model [25]. In either case, all three scenarios require the assistance of TCR specific factors that are recruited to the chromatin by the arrested RNAPII, mainly CSA, CSB, UVSSA, XAB2, HMGN1 [21]. Following the initial recognition, the core NER proteins, common to the global genome

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repair (GGR) NER subpathway, are then recruited and the lesion is removed (for a review on NER see ref. [26]). The importance and complexity of the NER pathways is underscored by hereditary diseases of varied phenotypes and severity associated to mutations of the involved factors observed in patients with Cockayne and UV-sensitive syndromes (CS and UV[s]S, TCR-deficient), and Xeroderma pigmentosum (XP, GGR- or GGR/TCR-deficient) [27].

The half-life of RNAPII arrested at a UV-induced cyclobutane pyrimidine dimer (CPD) is ~ 20 hours *in vitro*, making RNAPII the most sensitive damage detector "by proxy" known to date. Moreover, the recent realization of the extent of pervasive transcription gives RNAPII and TCR a significantly more important role in DNA repair than previously acknowledged [28]. In addition to initiating TCR, and no less importantly, stalled RNAPII has been reported to trigger the activation of DNA damage checkpoints, causing phosphorylation and accumulation of p53 in an RPA- and ATM/ATR-dependent manner [19].

As mentioned above, treatment with UV light or CPT induces hyperphosphorylation of RNAPII and the positive transcription elongation factor b (P-TEFb) has been implicated in this modification. P-TEFb, composed of cyclin-dependent kinase 9 (Cdk9) and a regulatory cyclin T subunit, is found in cells in two different forms: an active kinase, P-TEFb small complex, or an inactive large complex composed of P-TEFb, the snRNA 7SK, two HEXIM subunits, LARP7, and MePCE [29]. UV, CPT, and other stimuli have been reported to release P-TEFb from the 7SK snRNP complex, and this is concurrent

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with RNAPII hyperphosphorylation by Cdk9, reduced elongation, and transcriptional repression [30]. Despite the similarities, pre-treatment of cells with caffeine, a broad inhibitor of the PI3K family of kinases, prevented P-TEFb release following UV but not CPT. The implications of this observation remain unclear, as specific inhibitors of the DDR kinases ATR, ATM and DNA-PK had no effect on P-TEFb release or transcription inhibition. This observation suggests the involvement of another signaling protein and different signaling pathways activated by UV and CPT [31]. Hexamethylene bisacetamide (HMBA), a differentiation inducing agent, was also shown to release P-TEFb from the 7SK snRNP complex and this was accompanied by p53 activation, p21 induction, and cell cycle arrest. p53 was co-immunoprecipitated with Cdk9 and cyclin T, but not HEXIM, suggesting its association with the catalytic active P-TEFb complex. This interaction was proposed to allow p53 to recruit P-TEFb to the p21 gene [32].

In yeast, interfering with the binding of a number of phospho CTD-associating proteins (PCAPs), or inducing aberrant phosphorylation of RNAPII's CTD, led to increased DNA damage sensitivity. Based on these results, a CTD-associated DNA damage response (CAR) system was proposed, that is composed of PCAPs and organized around the phospho CTD of elongating RNAPII. Most of the components of the CAR system are evolutionarily conserved and although an equivalent role in humans awaits confirmation, some of these proteins had already been linked to cancer [20].

3b. Gene transcription

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Damaged DNA poses a threat to transcription: RNAPII may stall at lesions or bypass them, with the potential risk of transcriptional mutagenesis. Preventing this, UV irradiation and other types of DNA damage have been shown to transiently inhibit the synthesis and processing of mRNAs. Although it has been reported that the repression of RNA synthesis by DNA damage is a global phenomenom [33, 34], some studies suggest that it might be in fact a local effect [35-38]. Regardless, cells rely heavily on gene transcription to mount an appropriate DDR and ensure checkpoint maintenance and genome stability. The best example of this is the DNA damage transcriptional response mediated by p53 [39], but other transcription factors contribute also to the DDR. In cells that have sustained a limited amount of damage, NF-κB activation would regulate cell survival pathways, tipping the balance towards cell cycle arrest and DNA repair rather than apoptosis [40].

This requirement for transcription amid transcription inhibition represents a paradox, and the transcription-coupled DNA repair pathway is only one of the mechanisms that cells have evolved in order to face this situation. Failure to recover from transcription inhibition following UV irradiation is one of the hallmarks of TCR-deficient CS cells. However, evidence suggests that this is not solely due to impaired DNA repair of actively transcribed genes, and that in addition to the physical block imposed by lesions, other mechanisms are set in place that inhibit transcription [33, 41].

One study addressed this transcription paradox while providing an original perspective on p53 life-or-death decisions. An analysis of p53-induced genes following UV irradiation showed that increasing the dose of UV light caused a decrease in the number

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of transcribed genes but also a shift towards the transcription of more compact genes, with fewer and smaller introns. Interestingly, these compact genes were enriched for initiators of apoptosis. This is consistent with the fact that the probability of transcription blocking by DNA lesions depends on the amount of lesions and the size of the transcribed genes and suggests a passive mechanism that would promote apoptosis in cells that have sustained levels of DNA damage beyond repair [42].

More recently, the general transcription factor TFIIB was revealed as a key element allowing a general attenuation of cellular transcription while leaving the p53 transcriptional response intact. TFIIB is an essential component of the RNAPII pre-initiation complex but also plays a role in transcription termination through interactions with the 3'-end processing factors CPSF and CstF-64 in a phosphorylation-dependent manner. Although modification of TFIIB and, consequently, gene transcription, is reduced upon DNA damage, p53 was shown to directly interact with CstF, providing an alternative mechanism for the recruitment of termination factors to p53 target genes [43]. In line with this observation, some p53 target genes were reported to have different requirements regarding transcription. As mentioned above, P-TEFb activation by HMBA was shown to induce the prototypical p53 target gene *p21* [32]. However, although P-TEFb activity appears to be sufficient for p21 induction, it also appears to be dispensable for activation in certain contexts. Indeed, and unlike most genes, *p21* did not require P-TEFb kinase activity, phosphorylation of RNAPII at serine 2 nor recruitment of the elongation factor FACT. Moreover, pharmacological inhibition of P-

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TEFb Cdk9 that led to global transcription inhibition resulted in p53 accumulation and activation, p53 target gene induction, and p53-dependent apoptosis [44].

3c. Post-transcriptional regulation

With transcription inhibition, the regulation of the pool of preexisting transcripts becomes a key controlling point during the DDR. Reports have shown that mRNAs can be regulated at the level of maturation, nuclear export, stability and translational activity.

With the exception of histone mRNAs, all eukaryotic mRNAs undergo 3′ end endonucleolytic cleavage followed by addition of some 200 adenosine residues, the poly(A) tail. 3′-end formation and 3′ processing factors have been shown to play a significant role in the DDR through the global and gene-specific regulation of the synthesis and stability of mRNAs [45].

Upon UV irradiation, two proteins involved in DNA repair and checkpoint activation, BRCA1 and BARD1, regulate 3′-end formation. BRCA1 and an ATM-phosphorylated BARD1 form a complex that binds to CstF-50 and inhibits mRNA 3′-end cleavage and polyadenylation [46]. Similarly, p53 negatively regulates 3′ processing through binding to the CstF/BARD1 complex [47].

THOC5 is a member of the TREX (transcription/export) family and a component of the THO complex that is recruited to protein coding genes, coupling transcription with mRNA export. Upon DNA damage, p53- and ATM-dependent phosphorylation of

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THOC5 impaired its ability to bind mRNA, causing a drastic reduction in the cytoplasmic pool of a set of THOC5-dependent mRNAs. Of note, p53 effectors and regulators were among the THOC5-independent mRNAs whose export remained unaffected [48].

Microarray analysis of polysome-bound vs. total RNA showed that, both in human astrocytes and brain tumor cells, IR affects gene expression primarily at the level of translation: 10 times more genes were found regulated through their recruitment to and away from polysomes compared to those modulated through transcription. Interestingly, a considerable fraction of these genes were involved in transcription regulation and RNA metabolism [49]. In human breast cells, IR causes an early and transient increase in translation through an ATM-, ERK-, and mTOR-dependent but p53-independent inhibitory phosphorylation of the 4E-BP1 factor. Following this initial wave, 4E-BP1 shifts to the hypophosphorylated form, becomes stabilized and, by associating with eIF4E, inhibits protein synthesis [50]. Finally, in mouse embryonic stem cells, CIS treatment led to mRNA translation arrest, protecting cells from genotoxic stress [51].

In addition to these global regulatory mechanisms, the DDR can also affect the stability and translation of specific mRNAs through the association of RNA binding proteins (RBPs) to sequence elements that are frequently, but not exclusively, found in the 3'UTRs of mRNAs. Different classes of AU-rich sequence elements (AREs) have been described that regulate mRNA stability and translation. The recognition of theses motifs by ARE-binding proteins (ARE-BPs) is promiscuous: the same ARE can bind different

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ARE-BPs and many ARE-BPs have been shown to interact with multiple ARE-containing mRNAs. This sets a scenario for cooperative and antagonistic behaviors between ARE-BPs towards a given mRNA [52]. In many cases, these ARE-containing mRNAs encode effectors of the DDR and allow cells a rapid response [53, 54].

The first observation linking the DDR with RBPs was reported over two decades ago when UV and other DNA damaging agents were shown to upregulate and/or activate up to 13 RBPs [55]. Subsequently, transcripts from the five growth arrest and DNA damage-inducible (*GADD*) genes, all of which contain ARE elements in their 3'UTR, were found to become stabilized following DNA damage [56].

In particular, *GADD45α* is a p53 target gene that contributes to G2/M cell cycle arrest, apoptosis, and DNA repair [57]. In unstressed cells, *GADD45α* expression is negatively regulated by a dual mechanism involving AREs present in the mRNA 3'UTR: 1) binding of the ARE-BP AUF1 promotes its decay, and 2) binding of the TIA1-related protein TIAR suppresses its translation [58]. Upon genotoxic stress, the association of AUF1 and p38-phosphorylated TIAR to *GADD45α* mRNA is dramatically reduced. Conversely, MK2 phosphorylation of hnRNPA0 stimulates its binding to the *GADD45α* 3'UTR, stabilizing the mRNA [59]. In addition to AREs, TIAR can bind to C-rich motifs that, when inserted into heterologous reporters, were able to suppress their translation. Treatment of cells with UV caused several mRNAs to dissociate from TIAR, upregulating the levels of the encoded proteins that included effectors/modulators of the DDR like Apaf-1 and TCF3 [60], and translation factors PABPN1, ETF1, TUFM, EIF5a, and RPL24 [61].

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p21 mRNA stability is also regulated by RBPs that bind elements present in its 3'UTR. Like *GADD45α*, *p21* mRNA is antagonistically regulated by AUF1 and HuR, and UV irradiation reverses the ratio of binding of both proteins to the mRNA, resulting in elevated *p21* mRNA levels [62]. RNPC1, an RBP and p53 target, cooperates with HuR in enhancing *p21* mRNA stability [63, 64]. In contrast, PCBP4, another RBP and p53 target gene, reduces *p21* mRNA stability and promotes apoptosis and G2 arrest [65, 66]. Finally, following UV irradiation, HuR enhances translation of the *p53* mRNA through binding to its 3'UTR [67], whereas RNPC1 prevents cap-binding protein eIF4E from binding *p53* mRNA, repressing its translation and creating an autoregulatory loop [68].

Accompanying the rapid degradation of cell cycle-promoting proteins, the DDR can also accelerate mRNA decay. UVC irradiation caused the rapid degradation of cyclin D1 (*CCND1*) mRNA through an antagonistic regulation similar to that of *p21* mRNA but with opposite effects: UV light decreased the association of the *CCND1* transcript to HuR while increasing its binding by AUF1 [62]. TIS11D, a member of the TTP family of ARE-BPs that destabilizes ARE-containing mRNAs, was identified as a candidate p53 target gene and its overexpression inhibited cell proliferation and apoptosis [69]. BTG2 is also induced by p53 and its disruption impaired cell cycle arrest at the G2/M phase following DNA damage [70]. Later studies identified BTG2 as a general activator of mRNA decay through enhanced deadenylation of transcripts [71]. These observations suggest that the p53 response to DNA damage appears to involve promoting the decay of specific mRNAs.

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4. Constitutive and alternative splicing

Splicing consists of the removal of non-coding intronic sequences from pre-mRNAs by the spliceosome, a large ribonucleoprotein complex. Splicing proceeds through a multi-step assembly of components that interact with the boundaries of the intron: the 5′ and 3′ splice sites (ss), as well as the branch point located near the 3′ splice site. Many of the interactions between the pre-mRNA and spliceosomal components are weak and require stabilization by auxiliary splicing factors, a feature that is crucial for the flexibility of the spliceosome and the splicing reaction [72]. Moreover, 5′ and 3′ splice sites often deviate from the consensus sequences (i.e. are weak sites, as opposed to strong sites) posing a challenge to their recognition by the spliceosome, and requiring the assistance of additional elements. Indeed, most pre-mRNAs contain *cis*-regulatory sequences, termed exonic or intronic splicing enhancers or exonic or intronic splicing silencers depending on their location and whether they stimulate or repress splicing. These regulatory elements bind a variety of *trans*-acting splicing factors that stabilize or hinder spliceosomal interactions. Taken together, the flexibility of the spliceosomal interactions, the degeneracy of 5′ and 3′ splice sites, and the action of regulatory *cis*-acting elements and their binding factors set the stage for a highly versatile regulation of pre-mRNA splicing, whose outcome is alternative splicing (AS) [73]. Finally, AS is influenced by numerous factors such as chromatin structure - DNA and histone modification, and nucleosome positioning-, mRNA secondary structure, tissue- and

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developmental stage-specific factors, promoters, transcription elongation rate and pauses, and ncRNAs [73-75].

4a. Coupling of transcription and splicing

mRNA splicing takes place cotranscriptionally. This links both processes -splicing and transcription- in time and place. The other main link is RNAPII and more specifically its CTD [18]. The connection between RNAPII and splicing is two-fold: the elongation rate of the polymerase determines the time available for upstream sequences in the mRNA to interact with splicing factors before downstream sequences are transcribed, in what is known as the *kinetic coupling model*. Additionally, the CTD of human RNAPII can function as a scaffold for the binding of mRNA processing factors. The *recruitment coupling model* posits that 5′ and 3′ processing in particular, but also mRNA splicing, can be affected by the phosphorylation status of RNAPII and the recruited factors. Far from being mutually exclusive, these two models are actually two faces of the same coin as kinetics of RNAPII can affect factor recruitment and recruited factors can, in turn, affect elongation rate [73].

As mentioned above, several DNA damaging agents have been reported to affect RNAPII phosphorylation, transcription elongation rate and, consequently, AS. In this way, the DDR can regulate the AS of its own effectors and of genes involved in mRNA metabolism, indirectly affecting a wider array of genes (Tables 1 and S1).

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Hyperphosphorylation of RNAPII was observed following UV irradiation and was accompanied by reduced transcription elongation rate and shifts in the patterns of AS of numerous genes [76, 77]. Consistent with this, phospho-mimetic RNAPII mutants recapitulated the effect of UV on AS. Conversely, Cdk9 inhibition or a phospho-deficient mutant RNAPII prevented this regulation. The effect on transcription elongation and AS did not result from DNA lesions physically hindering RNAPII progression *in cis*. Instead, a systemic cellular response appears to be triggered by DNA damage that affects intact genes, as was demonstrated by transfecting an AS reporter minigene after the cells were irradiated [76].

Similarly to UV irradiation, CPT treatment has also been shown to promote RNAPII hyperphosphorylation [78] and reduced elongation rate [79]. In a genome wide analysis of AS events, CPT treatment caused significant changes in splicing in 5% of the genes analyzed, and increased in frequency with the length of the transcript [78]. Similarly, slowing down RNAPII transcription elongation with CPT resulted in coupled decreased mRNAs levels and increased exon inclusion, consistently with the kinetic model [80].

In both studies, CPT-induced changes showed a significant enrichment of genes involved in splicing and RNA binding and processing [78, 80].

Exon arrays were used to examine both expression and splicing changes induced by IR in a comprehensive manner. Although a significant fraction of the observed variations awaits proper validation by PCR or similar techniques, this approach led to the identification of a number of IR-regulated genes producing multiple isoforms, using

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alternative promoters and some genes whose pattern of AS was affected following IR, such as *GADD45G* and *VWCE* [81].

More recently, UV irradiation was shown to displace late-maturation stage core splicing factors U2/U5/U6 from chromatin. The mobilization of splicing factors appeared to be triggered by both transcription inhibition and ATM signaling. The proposed mechanism involves the formation of R-loops (pairing of nascent mRNA with the complementary unwound DNA template) as a result of RNAPII arrest at DNA lesions, local displacement of splicing factors, and the negative supercoiling behind RNAPII. R-loop formation would then activate ATM, amplifying the mobilization signal. This led to an increase in intron retention and exon skipping, an expected outcome of splicing impairment. However, an important proportion of increased exon inclusion was also observed among the UV-induced AS changes [82]. Transcription-dependent CPT-induced DSBs, which trigger R-loop formation and ATM activation, were also shown to promote spliceosome mobilization [82, 83].

With 95% of human genes undergoing AS, it is to be expected that many of these genes will be involved in the DDR. p53, TRAF2, Bcl-x, Bcl-2, Bak, Mcl-1, Apaf-1, caspases 2, 8, and 9, survivin, and PIG3 are among the many DDR effectors making cell survival/apoptosis decisions whose activity is regulated at the level of AS [84, 85]. The regulation of some of these AS events has been described in the context of the DDR (Tables 1 and S1, and references therein).

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A panel of 20 anticancer agents, many of which are known to trigger DNA damage, was shown to affect AS of a set of apoptotic genes in multiple cell lines, with a significant proportion of the observed changes being cell type-specific. In many, although not every case, splicing shifts occurred towards the proapoptotic forms of the different proteins [86].

Among the most studied AS events are those of the caspase 9, Bcl-x and Mdm2 genes.

Two variants of caspase-9 of the intrinsic apoptotic pathway are found in cells through inclusion/exclusion of an exon 3-4-5-6 cassette: the pro-apoptotic caspase-9a (L) and its dominant negative anti-apoptotic caspase-9b (S) splice variant. DNA damage upon treatment with UV and a panel of 20 anticancer drugs favored splicing of the pro-apoptotic caspase-9a form [76, 86].

The Bcl-x gene gives rise to several alternative splice products with antagonistic functions. The anti-apoptotic Bcl-xL and the pro-apoptotic Bcl-xS are the best characterized isoforms and their ratio influences cell susceptibility to apoptosis [85]. Genotoxic stress induced by oxaliplatin or CIS activated the ATM/Chk2/p53 pathway favoring the production of the proapoptotic Bcl-xS variant, possibly through the phosphorylation of a splicing repressor that binds a regulatory element in the *Bcl-x* transcript [87]. UV irradiation also increased the ratio Bcl-xS/Bcl-xL in a variety of human cells, but this was independent of their p53 status [76].

Different DNA damaging agents were shown to induce AS of Mdm2 and Mdm4, two of the main negative regulators of p53. The Mdm2 alternatively spliced variants lack various exons that include the p53 binding domain and nuclear localization signal.

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These isoforms are expected to increase p53 protein levels and induce the p53 pathway [88, 89]. Similarly, CPT treatment caused skipping of different sets of Mdm2 exons. Although CPT treatment induced RNAPII hyperphosphorylation, its effect on Mdm2 exon skipping was unaffected by the CDk9 inhibitor DRB and Cdk7/9 inhibitor H8, suggesting that it is independent of RNAPII phosphorylation [90]. In a different report, retention of the 108 bp intron 10 (Mdm2^{+108}) upon treatment with doxorubicin (DOX) correlated with increased p53 accumulation [91].

Other p53 targets involved in the DDR and regulated at the level of AS include PIG3, Fas, Bax, and survivin [86, 92, 93].

4b. Regulation of splicing factors

mRNA AS is regulated by serine-arginine (SR) proteins that bind splicing enhancers and heterogeneous nuclear ribonucleoproteins (hnRNPs) that interact with splicing silencers, as well as other tissue specific splicing factors. Most "classical" SR proteins are subjected to a phosphorylation/dephosphorylation cycle that regulates, among other things, their shuttling between the nucleus and cytoplasm [94]. Moreover, in addition to both constitutive and alternative splicing, SR proteins participate in other aspects of mRNA metabolism including transcription, nuclear export, stability and translation, and even microRNA processing [95, 96]. hnRNPs are modular proteins characterized by various types of RNA recognition motifs and a highly varied set of domains that result in a vast functional diversity. They are generally involved in mRNA splicing but, as SR

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proteins, also participate in mRNA processing, editing, trafficking, stability, and translation, and undergo nucleocytoplasmic shuttling [97].

During the last decade, multiple large scale proteomics analysis and genome-wide screens have been uncovering a remarkably strong connection between mRNA processing and the DDR. Different studies looked into ATM and/or ATR phosphorylation targets and dynamics following IR or the ATM-activating radiomimetic drug neocarzinostatin [98-100] and siRNA screens were used to identify genes whose downregulation increased IR sensitivity or resulted in DNA damage through alterations in mRNA metabolism [101, 102]. In these reports, an unexpectedly vast proportion of the identified candidate proteins and genes belonged to the "RNA processing" category. Together, these results showed that RNA metabolism is a major hub of the DDR and illustrate the importance of the strong connection between the DDR and the RNA field.

The activity of splicing factors is regulated during the DDR at the level of transcription, splicing, post-translational modification, subcellular localization, and protein-protein interaction (Tables 2 and S1) [103]. p53 has been shown to directly and indirectly regulate several RBPs, including the already mentioned RBPs RNPC1 and PCBP4, that are upregulated in response to DNA damage, but also hnRNP D/AUF1 and CPEBP4, among others [104]. The transcription factor E2F1 is known for its seemingly contradictory ability to promote cell cycle progression at the S phase and induce apoptosis during the DDR [105]. Among its many targets, E2F1 has been shown to

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regulate the expression of numerous RNA processing factors [106]. In particular, in the context of the DDR, cell treatment with cyclophosphamide was shown to regulate AS of several apoptotic genes in an E2F1- and SRSF2-dependent manner. In these cells, E2F1 associated to the *SRSF2* gene promoter activating its transcription [107].

SRSF2 is also regulated by post-translational modification coupled to degradation: in normal cells, Tip60 acetylates SRSF2 and prevents nuclear translocation of the SRSF2 kinases SRPK1 and 2. The resulting hyperacetylated/hypophosphorylated SRSF2 is degraded through the proteasome. Following DNA damage, Tip60 mRNA and protein levels are downregulated, hypoacetylated/hyperphosphorylated SRSF2 accumulates and regulates caspase-8 splicing, resulting in increased apoptosis and reduced growth arrest [108].

The AS of many RBPs and splicing factors was altered by DNA damage, including SRSF1, RBM8A, ZRANB2, SF3B3, TIA-1, and TIAL1, among others. Interestingly, in some of these cases, included exons introduced premature termination codons that could elicit nonsense-mediated mRNA decay [78, 80, 109].

The hSlu7 splicing factor has been suggested to hold the 5′ exon within the spliceosome complex while the correct 3'ss is selected. hSlu7 accumulated in the cytoplasm of UV irradiated and heat shock treated cells, via a JNK- and p38-dependent pathway. Intriguingly, neither hydrogen peroxide, CIS nor neocarzinostatin led to this cytoplasmic accumulation. The altered subcellular distribution of hSlu7 affected the AS of a splicing reporter minigene (adenosine deaminase *ADAR2*) and the endogenous D-aspartate-oxidase *DDO* gene. The AS patterns were recapitulated by downregulation of

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hSlu7 suggesting that they result from reduced nuclear levels of the splicing factor [110].

The p38 pathway was also involved in the regulation of hnRNP A1 localization. Like hSlu7, hnRNP A1 cytoplasmic accumulation was observed in cells exposed to UV light or osmotic stress and its reduced nuclear levels affected the AS of the E1A splicing reporter minigene [111].

EWS, a member of the TET family, interacts with components of both the transcription (TFIID and RNAPII subunits) and splicing (U1 snRNP protein U1C, YB-1) machineries. UV irradiation was shown to release EWS from its target mRNAs, transiently relocalizing to nucleoli, and resulting in changes in the AS of many genes involved in the DDR [77, 112]. Similarly, CPT impaired the interaction of EWS and the splicing factor YB-1, reducing the recruitment of YB-1 to the Mdm2 gene and promoting exon skipping for Mdm2 and other genes [90]. FUS/TLS (for fused in sarcoma/translocated in liposarcoma), another RBP and TET family member, is a target of ATM and has also been linked to the DDR (see below) [113]. Of note, FUS/TLS and the splicing regulator TDP-43 are mislocalized in neurogenerative disorders like Amyotrophic lateral sclerosis and frontotemporal lobar degeneration and other diseases [114].

DNA damage by the topoisomerase II inhibitor mitoxantrone or CIS caused accumulation of Sam68 in ring-shaped structures surrounding the nucleoli at early time points and large granules at later times. hnRNP A1, TIA-1, and SR proteins SRSF1 and 2 colocalized with Sam68 in these nuclear granules that contained phosphorylated RNAP II transcriptionally active and the relocalization of Sam68 correlated with changes in

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alternative splicing of *CD44* mRNA [115]. Similarly, UV and other DNA damaging agents caused redistribution of SRSF2, 9G8, and other proteins involved in mRNA metabolism to perinucleolar regions termed DNA damage-induced NOR-associated patches (d-NAPs). A temporal correlation between d-NAP formation and changes in the AS of PIX3, Bcl-x and Smac/DIABLO apoptotic genes was observed [116].

hnRNPs are also regulated by the DDR through different mechanisms and in a damage-specific manner [117]. hnRNPA1 transcript or protein levels were found altered following IR or CIS treatment in different types of cells [118-120], and, as mentioned earlier, hnRNP A1 localization was altered by UV [111]. hnRNP B1 was downregulated by IR [121] and upregulated by UV [122]. hnRNP K was identified as a target of ubiquitin-dependent degradation by Mdm2. Following DNA damage, hnRNP K was sumoylated, preventing its degradation, and the protein became rapidly and transiently stabilized. hnRNP K was further shown to act as a transcriptional coactivator of p53 and to be required for efficient G1 and G2 arrest [123-125].

5. Non-coding RNAs

Up until recently, the DDR was thought to rely exclusively on proteins for damage detection, signaling generation and amplification, and execution [6]. However, it has become clear that 1) the genome is pervasively transcribed, 2) much of this transcription generates ncRNAs that are often biologically functional, and 3) some of these ncRNAs are regulated in response to DNA damage and participate in the DDR [6, 126].

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5a. miRNAs

RNA interference (RNAi) is an evolutionarily conserved pathway that uses different kinds of small non-coding RNAs to regulate gene expression through various mechanisms: translational repression, mRNA degradation and, more recently described, transcription regulation by epigenetic modification and interaction with the transcriptional machinery [127].

microRNAs (miRNAs) are as abundant as transcription factors or RBPs, and they are similarly expressed in a cell type- or developmental stage-specific manner and subjected to regulation by environmental stimuli [128]. Just as splicing factors and RBPs, microRNAs and DDR factors are also in a reciprocal regulatory relationship.

Specific miRNAs have been identified that control the expression of a variety of DDR factors, including p53, ATM and several DNA repair proteins, checkpoint and cell cycle regulators (see [129, 130], and references therein).

Conversely, DDR factors regulate miRNA expression and biogenesis. In 2007, several groups reported that p53 can induce the transcription of miR-34s, a family of miRNAs that control a variety of targets involved in DNA repair, cell cycle regulation and apoptosis (reviewed in [131]). Subsequently, additional miRNAs regulated by p53 and affecting the outcome of the DDR were identified [132]. In addition, following DNA damage, p53 directly associates with the RNA helicase DDX5, a cofactor of DROSHA, to stimulate the post-transcriptional processing of a subset of pri-miRNAs also involved in cell proliferation control [133].

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Other DDR players also modulate miRNA biogenesis, including ATM and BRCA1. The analysis of the ATM phosphoproteome included components of the DROSHA-DGCR8 complex and ATM was shown to phosphorylate KSRP, a splicing regulatory protein also involved in the maturation of a subset of miRNAs [99, 134]. In response to IR, ATM reduced CREB-mediated transcription of miR-335, a miRNA that targets the homologous recombination repair protein CtIP [135]. BRCA1 was reported to interact with the DROSHA microprocessor complex and enhance processing a subset of pri-miRNAs [136].

Silencing of key components of the miRNA processing pathway severely impaired the cellular response to UV irradiation, as reflected by a reduced G1 phase arrest and cell survival. UV treatment was also shown to upregulate miR-16 expression, targeting the Cdc25A, cyclin D1 and cyclin E mRNAs [137].

The RNAi pathway presents multiple opportunities for regulation and fine-tuning of gene expression. In addition to the numerous steps required for miRNA biogenesis, their ultimate effect is also influenced by the ratio of miRNA/target mRNA, the expression of “sponges” or target mimics, the regulation of miRNA-mRNA association by RBPs, the subcellular localization of miRNA and RNAi proteins, and the availability of miRNA binding sites, which can be altered by RNA editing, AS and polyadenylation sites [138]. All of these are susceptible to regulation by DDR components and to modulate its outcome.

5b. ncRNAs and lncRNAs

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Other classes of ncRNAs that do not participate in the RNAi pathway have also been shown to play a role during the DDR.

In a series of elegant experiments, the d'Adda di Fagagna lab showed that RNAPII-, DROSHA-, and DICER-dependent 20-35 nt ncRNAs were generated at sites of DNA damage in cells treated with IR. These RNAs termed DDRNAs were required for DDR foci formation, and ATM recruitment and activation but did not involve the canonical translational repression by miRNAs. DICER or DROSHA knockdown impaired checkpoint activation and cell cycle arrest in both irradiated cells and a model of oncogene-induced senescence [139]. A similar production of DSB-induced RNAs (diRNAs) was observed in *Arabidopsis thaliana* plants through a pathway that required ATR and Dicer-like proteins. These diRNAs were recruited to DSBs by Ago2 to mediate DNA repair [5].

Several lncRNAs have been implicated in the transcriptional regulation of cell cycle genes during the DDR. IR induced the transcription of ncRNAs from multiple regulatory regions upstream of the *cyclin D1* gene promoter. At the *cyclin D1* promoter, $ncRNA_{CCND1}$s recruited a complex containing FUS/TLS, an RBP with reported functions in transcription, RNA processing and DNA repair. Allosteric modulation of TLS by $ncRNA_{CCND1}$s resulted in inhibition of CBP acetyltransferase activity and, consequently, transcriptional repression of the *cyclin D1* gene [140].

p53 was reported to regulate the transcription of several long intergenic non coding RNAs (lincRNAs). The *lincRNA-p21* gene is located upstream of the cdk inhibitor *p21* gene and is independently transcribed by a promoter containing highly conserved p53

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response elements. *lincRNA-p21* represses hundreds of genes normally regulated by p53 and is required for proper apoptosis induction. *lincRNA-p21* interacts with hnRNP K, recruiting it to its target genes to inhibit transcription through an unknown mechanism [141]. These and other studies prompted a systematic search for transcription of ncRNAs near and within cell cycle genes. A total of 216 discrete transcribed regions were identified, of which at least 12 were upregulated upon DNA damage, and two were located 1-5 kb upstream of the *p21* gene. PANDA (for p21 associated ncRNA DNA damage activated) is also transcriptionally regulated by p53. However, unlike *lincRNA-p21*, PANDA appears to restrict apoptosis through binding to NF-YA, a transcription factor that typically regulates apoptotic but not other p53 target genes [142].

Finally, ANRIL is a lncRNA-encoding gene located at the INK4b/ARF/INK4a locus. Its transcription is induced by DNA damage in an ATM/E2F-dependent and p53-independent manner, and generates an RNA complementary to the mRNAs of the cell cycle regulators $p15^{INK4b}$, $p16^{INK4a}$ and $p14^{ARF}$ that suppresses their expression and checkpoint activation. The authors propose that ANRIL alleviates the pRB and p53 pathways at the late stage of the DDR [143]. In prostate cancer tissues, ANRIL has been shown to mediate epigenetic silencing of the INK4b/ARF/INK4a locus by recruiting the Polycomb repressor complex [144].

Considering that other yet uncharacterized lncRNAs induced by genotoxic agents have been identified, that RBPs are frequently modulated by the DDR, and that many DDR factors associate with RNA, it is likely that new lncRNAs involved in the DDR will

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continue to be uncovered, functioning as signals, decoys, guides, or scaffolds to regulate gene expression [145, 146].

6. Conclusions and future perspectives

The ability of cells to respond to DNA damage is a matter of life or death, not only regarding the handling of naturally occurring DNA lesions, but also as a central determinant in tumor progression and, were that to happen, chemotherapy outcome (apoptosis vs. growth arrest) and resistance. Despite the prominent role of p53 in these decisions, there are many p53-independent components in the DDR that can have important effects on shifting the balance one way or the other. In the past decades, mounting evidence has shown the important role that RNAs and proteins involved in RNA metabolism play in the DDR (Fig. 1). Sensors, modulators and effectors of the DDR are regulated at the level of transcription and AS, as well as mRNA 3'-end processing, export, stability and translation. Much of this regulation is a consequence of the activity of RNAPII, splicing factors and RBPs, which are also modulated by the DDR at the level of protein post-translational modification, stability and localization. Moreover, a variety of ncRNAs including miRNAs, lncRNAs and small ncRNAs generated at the sites of DNA damage have also been shown to participate in the DDR [6, 129, 146].

AS is of particular interest because it affects both the quantity and the quality of the expressed proteins. Alterations in the splicing machinery and regulatory proteins have been implicated in cancer [3] and chemoresistance [147], and different approaches to

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correct these alterations or manipulate splicing outcomes have been addressed [148-150].

Extensive basic research has given us proof of the expanse and versatility of the regulatory mechanisms set in place to ensure genome integrity. The abundance of nodes, links, and loops with their synergies, antagonisms, and (apparent) redundancies provide as many targets of intervention, and will hopefully lead to the rational development of drugs and strategies for the treatment of cancer and other pathological conditions.

Acknowledgements

The authors apologize for selective citations which, by necessity, resulted in the work of many researchers remaining uncited or hidden in reviews.

L.E.G., M.J.M. and A.R.K. are career investigators and N.N.M is recipient of a graduate student fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (CONICET). A.E.C.B. is recipient of an undergraduate research fellowship from the Universidad de Buenos Aires. G.D. is supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 661051. A.R.K. and M.J.M received support from the Agencia Nacional de Promoción de Ciencia y Tecnología of Argentina, the Universidad de Buenos Aires. A.R.K. is a Senior International Research Scholar of the Howard Hughes Medical Institute.

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Figure legends

Figure 1: Reciprocal regulatory interactions between the DNA Damage Response and RNA metabolism. A schematic model of the DDR centered on the Mdm2-p53-p21 pathway illustrates the multiple levels of regulation involving RNAs and RNA metabolism.

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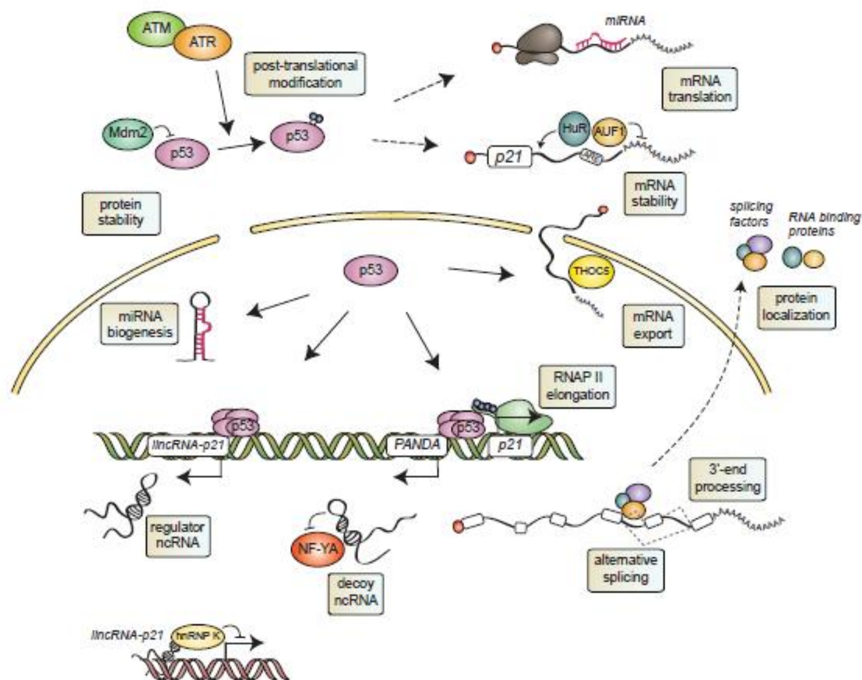

Figure 1

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Table 1: Regulation of alternative splicing by the DNA damage response

Gene-AS Event	Stimulus	Effect	Ref.
a) AS of genes known to be involved in the DDR			
Mdm2	UV, IR, CIS	Exons 4-11 skipping. p53 stabilization	[88]
	CPT, DOX, CIS	Exon(s) skipping. p53 stabilization	[90]
	DOX, actinomycin D	Intron 10 retention, truncated inactive variant. p53 stabilization	[91]
Mdm4	UV	Exon(s) skipping. p53 stabilization	[88]
Fas receptor (CD95)	mitomycin C	Increased soluble Fas isoform (exclusion of E6)	[93]
Caspase 2	ETO, CPT, DOX, mitoxantrone	Increased anti-apoptotic 2S form (inclusion of E9)	[151]
Caspase 8	CIS	Reduced anti-apoptotic form (inclusion of E8), via SRSF2	[108]
Caspase 9	UV	Increased pro-apoptotic caspase-9a form (inclusion of E3-4-5-6 cassette)	[76]
	multiple drugs, cyclophosphamide	Increased pro-apoptotic caspase-9a form via E2F1/SRSF2	[86, 107]
Bcl-x	UV, CIS	Increased pro-apoptotic Bcl-x_S form	[76, 116]
	CIS, oxaliplatin	Increased pro-apoptotic Bcl-x_S form via ATM/Chk2/p53	[87]
	cyclophosphamide	Increased pro-apoptotic Bcl-x_S form via E2F1/SRSF2	[107]
Bax, BRCA1	multiple drugs		[86]
survivin	multiple drugs	Increased pro-apoptotic form	[86]
GADD45G	IR	Reduced inclusion of E2	[81]
PIG3 (TP53I3)	UV, CIS	Increased exon E4 skipping (PIG3AS). Increased degradation	[92, 116]
Smac/DIABLO	UV, CIS	Increased exon E4 skipping (Smac3). Proapoptotic	[116]
NBS1	IR, MMS	DSB sensor complex. Decreased inclusion of exon with premature termination codon	[152]
Mcl-1	IR	Increased anti-apoptotic Mcl-1L form. Radioresistance	[153]
Abl1, Chk2, MAP4K2	UV	Increased exon skipping via relocalization of EWS	[112]
DHX9	UV	Increased inclusion of exon 6A (NMD+ form)	[77]
b) AS of genes involved in mRNA metabolism			
SRSF1	UV	Splicing factor, reduced intron inclusion (NMD+ form)	[154]
SF1, RBM8A, ZRANB2, BAT1	CPT	Splicing factors, increased exon skipping	[78]
HNRPDL, HNRPLL, SRSF2, SF3B3, SNRPB, MBNL2, PRPF3, PRPF38B	CPT, UV	Splicing factors, increased exon inclusion	[80]
FUBP1	UV	DNA and RNA binding protein, increased exon inclusion	[80]
TIA-1, TIAL1	UV	RNA binding proteins, increased exon inclusion	[80]
TIA-1, TIAF1	CIS, topotecan	RNA binding proteins	[86]
c) AS of other genes			
CD44	mitomycin C		[155]
	mitoxantrone	Increased inclusion of vE5, via Sam68	[115]
Fibronectin FN	UV	Increased inclusion of EDI	[76]
Elastin ELN	UV	Increased inclusion of E26A	[156]
CRH-R1	UV	Exon(s) skipping	[157]
VWCE	IR	Reduced inclusion of E4.	[81]
IVNS1ABP, NT5C3, PTPRC, TCF12	CPT, UV	Increased exon inclusion	[80]
SPPL2A	CPT	Decreased exon inclusion	[80]
RC3H2, ZCCHC8, THUMPD2, EED, CHD2, PAPOLG, KIAA0232	CPT	Decreased exon inclusion, via EWS/YB-1	[90]

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Table 2: Regulation of RBPs by the DDR

Name	Stimulus	Effect	Ref
a) Translation regulation			
AUF1 (hnRNP D)		Altered target mRNA binding	[62]
	DOX, IR	Downregulation, p53 target gene	[104, 118]
HuR	UV	Cytoplasmic accumulation; altered target mRNA binding	[62]
HuR	IR	Phosphorylation by p38; cytoplasmic accumulation	[63]
TIA-1	mitoxantrone	Accumulation in nuclear and cytoplasmic granules	[115]
TIAR	DOX	Phosphorylation by p38	[59]
	UV	Dissociation from mRNAs C-rich motifs	[61]
CPEBP4	DOX	Upregulation, p53 target gene	[104, 158]
Wig-1	5-FU	Upregulation, p53 target gene	[159]
PCBP4 (MCG10)	CPT	Upregulation, p53 target gene	[65]
b) Splicing factors			
SRSF1 (ASF, SF2, SRp30a)	UV, ETO, aphidicolin	Reduced phosphorylation	[154]
	mitoxantrone	Accumulation in nuclear granules	[115]
SRSF2 (SC35, PR264, SRp30b)	cyclophosphamide	Upregulation by E2F1	[107]
	CIS	Phosphorylation by SRPK2, acetylation by TIP60	[108]
	mitoxantrone	Accumulation in nuclear granules	[115]
	IR	Upregulation	[118]
SRSF1, 3, 6, 10	IR	Downregulation	[118]
SRSF5 (SRp40, HRS) SRSF6 (SRp55, B52)	mitomycin C	Upregulation	[93, 155]
hnRNP A1	mitoxantrone	Accumulation in nuclear and cytoplasmic granules	[115]
	UV	Cytoplasmic accumulation, via MKK3/6-p38	[111]
hnRNP A1, E2	IR	Upregulation	[118, 119]
hnRNPC, H3, M, R, U	IR	Downregulation	[118]
hnRNP K	ETO, DOX, UV	Sumoylation - p53 coactivator	[124]
	IR, phleomycin, UV	Protein stabilization	[123]
hnRNP A0	DOX	Phosphorylation by MK2	[59]
PRP19 (Pso4 complex)		Upregulation, ubiquitination	[160]
Sam68	mitoxantrone, CIS	Accumulation in nuclear and cytoplasmic granules	[115]
SIPP1	UV, IR	Accumulation in the cytoplasm	[108]
PSF/SFPQ	laser-induced DSBs	Relocalization to sites of DNA damage	[161]
THRAP3	ETO	Phosphorylation, excluded from sites of damage	[162]
RBM38 (RNPC1)	DOX	Upregulation, p53 target gene	[163]
hSlu7	UV	Nuclear export; JNK-dependent after UV	[110]
YB-1	CPT	Disrupted interaction with EWS	[90]
SLBP, NOL5A	IR	Upregulation	[118]
c) Splicing factor regulators			
SRPK1, SRPK2	CIS	Nuclear accumulation	[108]
SRPK2	CIS, IR	Phosphorylation, nuclear accumulation	[164]
EWS	UV	Relocalization to nucleolar compartment	[112]
	CPT	Disrupted interaction with YB-1	[90]
PPM1G	ETO, IR	Phosphorylation, recruitment to sites of damage	[162]

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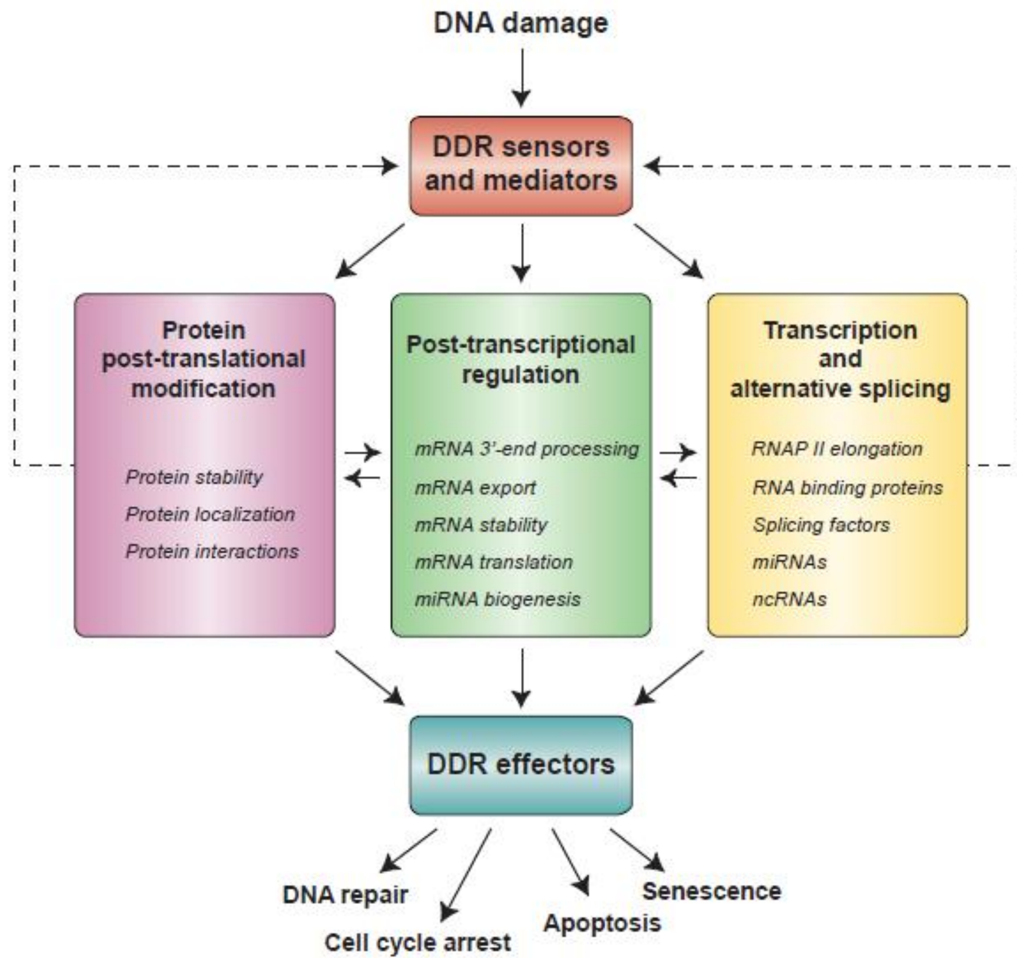

Graphical Abstract

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Highlights

- The DNA damage response is essential for genome integrity in multicellular organisms.
- RNAs and RNA metabolism play a prominent role in the DNA damage response (DDR).
- Splicing factors and RNA binding proteins are regulators of and regulated by the DDR.
- Small and long non coding RNAs and miRNAs also participate in the DDR.

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