

RNF8 - The Achilles heel of DNA repair when splicing rules

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Double-strand breaks, one of the most lethal forms of DNA damage, must be repaired accurately to maintain normal cellular functions and prevent diseases, such as cancer. Accordingly, several systems for detecting DNA damage, signaling its presence and mediating its repair, involving groups of proteins that act in a coordinated fashion have evolved. Surprisingly, recent genome-wide proteomic and siRNA screens focused on identifying factors involved in the repair of DNA double-strand breaks indicate that splicing and RNA processing proteins are crucial players. For example, splicing factors are phosphorylated by ATM and ATR in response to DNA damage¹ and knockdown of these factors both enhances spontaneous DNA damage² and impairs the ability of cells to repair double-strand breaks by homologous recombination.^{3,4} Moreover, in line with this enhanced genomic instability, the genes encoding splicing-related factors are mutated in many cancers. Still, the manner in which splicing factors regulate DNA repair remains unclear.

To uncover the underlying mechanism, we examined whether the spliceosome participates directly or indirectly (e.g. via changes in gene expression) in DNA repair.⁵ Inhibition of spliceosome assembly with the small molecules pladienolide B and isoginkgetin for only 2 hours dramatically impaired the accumulation of several proteins at double-strand breaks, including WRAP53 β , RNF168, 53BP1, BRCA1 and RAD51, as well as reduced ubiquitinylation at these sites. Markedly, when splicing was inhibited for 16 h, less than 3% of the cells formed repair foci upon irradiation. Since accumulation of the upstream repair factors MDC1 and γ H2AX was unaltered, we concluded that the initial steps of the signaling cascade were functional and only factors downstream of MDC1 are sensitive to inhibition of splicing.

In agreement with their defective DNA repair, splicing-deficient cells displayed significant amounts of residual γ H2AX foci and direct measurement of repair efficiency confirmed that their repair by homologous recombination (HR) was reduced 60%.

To explore whether defective DNA repair was due to attenuated expression of repair factors because splicing of their transcripts was impaired, both mRNA and protein levels were measured and found to be reduced for most of the repair factors investigated in a manner correlated with the degree/

timing of splicing inhibition. Notably, the level of RNF8 protein, which acts directly downstream of MDC1, was reduced severely already 2 hours after splicing inhibition, indicating that it is turned over rapidly and particularly dependent on ongoing splicing for its expression.

Ubiquitylation of damaged chromatin by RNF8, the first E3 ligase to localize to DNA breaks, promotes the subsequent assembly of RNF168, 53BP1, BRCA1 and RAD51. Interestingly, when splicing was inhibited, overexpression of RNF8 fully restored the ubiquitylation of damaged chromatin, accumulation of downstream factors and subsequent repair by HR, as well as normal clearance of γ H2AX. Notably, DNA repair was not restored by overexpression of other repair factors whose levels are reduced by splicing inhibition, indicating that RNF8 is particularly important for this process.

Splicing factors were among the most frequent hits in several genome-wide screens. We found that knockdown of 3 splicing-related proteins (SF3B1, PRPF8 and RBMX) identified in these screens resulted in downregulation of RNF8 and reduced ubiquitylation of damaged chromatin. Importantly, introduction of GFP-RNF8 into these cells completely restored both ubiquitylation and downstream signaling. Our findings support a model in which ongoing splicing promotes the expression of several short-lived repair factors, including RNF8, RNF168 and RAD51, with adequate levels of RNF8 being particularly critical for the repair of double-strand breaks. In this manner, splicing controls repair of the most dangerous form of DNA damage (Fig. 1).

Although our observations indicate that loss of RNF8 when splicing is deregulated is a major cause of defects in DNA repair, alternative mechanisms have also been demonstrated. These include maintenance of the transcription of essential repair factors by the spliceosome⁶ and alternative splicing of specific factors, like BRCA2.³ In addition, transcriptionally active chromatin recruits factors involved in HR more efficiently through histone modifications, like H3K36me3,⁷ suggesting that ongoing splicing regulates DNA repair by influencing histones. Furthermore, splicing factors prevent the formation of R-loop structures at sites of ongoing transcription, which could explain the observation by ourselves and others, that certain splicing factors are recruited to DNA lesions.⁶

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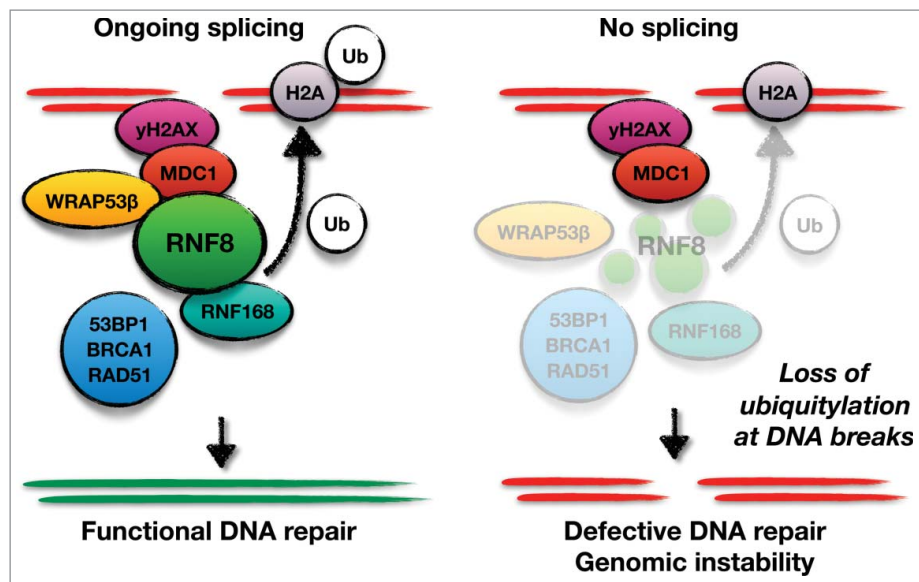


Figure 1. Model of splicing-regulated repair of DNA double-strand breaks.

However, since not all spliceosomal components are recruited to these lesions and some are even excluded, those recruited must act independently of the spliceosome.

Interactions between the spliceosome and DNA damage signaling appear to be reciprocal, since DNA damage influences the pattern of splicing and association of the spliceosome with chromatin. These latter phenomena have so far been shown to occur in response to UV but not ionizing radiation, indicating the importance of the site and type of lesion.

In summary, our discovery of RNF8 as a rate-limiting factor in DNA repair not only establishes a novel mechanism underlying the involvement of splicing in this repair, but also provides considerable insight into the pathogenesis of various diseases caused by dysregulation of splicing, including cancer, and may thus have far-reaching clinical and therapeutic implications.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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