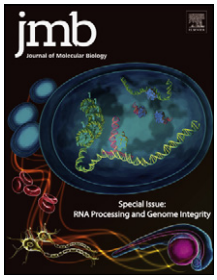

The G-Quadruplex-Specific RNA Helicase DHX36 Regulates p53 Pre-mRNA 3′-End Processing Following UV-Induced DNA Damage

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Abstract

Pre-mRNA 3′-end processing, the process through which almost all eukaryotic mRNAs acquire a poly(A) tail is generally inhibited during the cellular DNA damage response leading to a profound impact on the level of protein expression since unprocessed transcripts at the 3′-end will be degraded or unable to be transported to the cytoplasm. However, a compensatory mechanism involving the binding of the hnRNP H/F family of RNA binding proteins to an RNA G-quadruplex (G4) structure located in the vicinity of a polyadenylation site has previously been described to allow the transcript encoding the p53 tumour suppressor protein to be properly processed during DNA damage and to provide the cells with a way to react to DNA damage. Here we report that the DEAH (Asp-Glu-Ala-His) box RNA helicase DHX36/RHAU/G4R1, which specifically binds to and resolves parallel-stranded G4, is necessary to maintain p53 pre-mRNA 3′-end processing following UV-induced DNA damage. DHX36 binds to the p53 RNA G4, while mutation of the G4 impairs the ability of DHX36 to maintain pre-mRNA 3′-end processing. Stabilization of the p53 RNA G4 with two different G4 ligands (^{PNA}DOTASQ and PhenDC3), which is expected from previous studies to prevent DHX36 from binding and unwinding G4s, also impairs p53 pre-mRNA 3′-end processing following UV. Our work identifies DHX36 as a new actor in the compensatory mechanisms that are in place to ensure that the mRNAs encoding p53 are still processed following UV.

Introduction

Newly RNA polymerase II-transcribed precursor messenger RNAs (pre-mRNAs) undergo a series of interconnected RNA processing events including the formation of the RNA 3′-ends, a process known as pre-mRNA 3′-end processing or polyadenylation (pA). This nuclear process, through which almost all eukaryotic mRNAs acquire a poly(A) tail, is a two-step reaction involving endonucleolytic cleavage and subsequent addition of adenosine residues. The poly(A) tail is crucial for the regulation of mRNA stability, transport to the cytoplasm and translation [1,2].

The 3′-end processing machinery comprises more than 80 proteins which recognize conserved *cis*-acting elements of the pre-mRNA known as the pA signal [1,2]. The poly(A) signal is defined by (i) an hexameric sequence (most often AAUAAA) found 10–30 nt upstream of the cleavage site (generally a CA dinucleotide) and (ii) a downstream sequence element (DSE) (U/GU-rich) located 30 nt downstream of the cleavage site. The pre-mRNA 3′-end processing reaction is modulated by additional sequence elements located upstream (upstream sequence element) or downstream (auxiliary downstream sequence element) of the cleavage site. These sequences function by recruiting essential

3′-end processing factors (e.g., the cleavage stimulation factor cleavage stimulatory factor (CstF) complex to the DSE and the cleavage and polyadenylation specificity factor complex to the AAUAAA sequence) or regulatory RNA binding proteins (RBPs) to upstream sequence elements and auxiliary downstream sequence elements.

Pre-mRNA 3′-end processing is regulated during cell growth and differentiation and is often found altered in diseases (e.g., cancer and viral infection) [3–5] leading to a profound impact on the level of protein expression since unprocessed transcripts at the 3′-end will be degraded or unable to be transported to the cytoplasm. More specifically, it has been shown that pre-mRNA 3′-end processing plays a role in the cellular DNA damage response (DDR). In response to DNA damage, BARD1 (BRCA1-associated protein) interacts directly with CstF [6,7]. As a result, the pre-mRNA 3′-end processing is transiently inhibited by enhanced formation of CstF/BARD1/BRCA1 complexes [6,7]. Furthermore, the ribonuclease PARN, which facilitates mRNA degradation *via* deadenylation, also associates with one of the CstF subunits and BARD1 following DNA damage [8,9]. The BARD1-mediated inhibition of the pre-mRNA 3′-end processing has been proposed to prevent the processing of potential aberrant RNAs that may arise after DNA damage induction. However, transcripts of genes implicated in the DDR should be properly processed to provide the cells with a way to react to DNA damage. This suggests that compensatory mechanisms must be used on pre-mRNAs of genes implicated in the DDR. It has been demonstrated that in the case of the prothrombin mRNA, genotoxic stress can activate p38MAPK signalling, resulting in the phosphorylation of inhibitory proteins, facilitating the 3′-end processing of the pre-mRNA and the subsequent increase in mature mRNA and protein levels [10]. In addition, despite the general inhibition of pre-mRNA 3′-end processing following UV-induced DNA damage [6,7,11], 3′-end processing of the p53 pre-mRNA is specifically maintained [12]. This maintenance requires a G-rich sequence located downstream of the p53 pA signal, as mutation of this sequence results in an inhibition of processing following UV irradiation [12]. G-rich RNA sequences can be specifically recognized by several RBPs including the heterogeneous nuclear ribonucleoprotein (hnRNP) F/H family of proteins [12–14]. We have found that hnRNP H/F interacts with the p53 G-rich sequence and regulates p53-mediated apoptosis under DNA damaging conditions [12].

The p53 G-rich sequence has the capacity to form an RNA G-quadruplex (G4) [12], a non-canonical structure that occurs between guanines, creating planar stacks of G-quartets [15]. These stable structures are found in many facets of RNA biology, including transcription termination, splicing and translation [15]. The RNA G4 located downstream of the p53 pA signal has been shown to be involved in hnRNP H/F binding to and the maintenance of p53 pre-mRNA 3′-end processing [12]. G4 RNA structures can also be specifically bound and resolved by RNA helicases, including the G4-specific ATP-dependent DEAH-box helicase DHX36 (RHAU; GR1) [16–21]. DHX36 is known as the major source of unwinding activity in human cell lysates [21,22]. Moreover, *in vitro* studies revealed that DHX36 is able to bind to G4 with high affinity and catalyses its resolution [16]. However, DHX36 has not been implicated in pre-mRNA 3′-end processing or in the DDR.

Here we report that DHX36 is involved in maintaining p53 pre-mRNA 3′-end processing following UV-induced DNA damage through its direct binding to the G4 RNA structure located downstream of the p53 pA signal.

Results and Discussion

DHX36 is required for p53 pre-mRNA 3′-end processing in response to UV-induced DNA damage

An RNA pull-down experiment performed to identify proteins that showed a preference for RNA G4s compared to G-rich sequences detected the RNA helicases DHX36 and DHX9, where DHX36 displayed the strongest affinity for RNA G4s [13]. Therefore, we sought to determine if DHX36 is involved in the regulation of p53 pre-mRNA 3′-end processing following DNA damage. Pre-mRNA 3′-end processing efficiency was assessed using a real-time quantitative PCR (RT-qPCR) approach in which we measured the ratio of uncleaved (and therefore unprocessed RNA) to total RNAs (unprocessed and processed RNAs) present in the nuclear pool of RNAs (Fig. 1a), as previously described [12]. We used UV irradiation (50 J/m^2–254 nm) as a source of DNA damage to A549 cells which do not contain p53 mutations, and which we have demonstrated previously to exhibit maintenance of p53 pre-mRNA 3′-end processing following UV [12]. Assessment of the effect of the UV exposure on proliferation showed approximately a 50% reduction in proliferation (Supplementary Fig. 1), indicating that cells were responding to the UV exposure. Consistent with our previous study [12], UV irradiation did not alter the uncleaved/total ratio for p53 RNAs, while it increased the uncleaved/total ratio for TATA-binding protein (TBP) RNAs (used as a control) (Fig. 1b) confirming that p53 pre-mRNA 3′-end processing is specifically maintained in response to UV.

In UV-irradiated cells but not in control cells, siRNA-mediated depletion of DHX36 (Supplementary Fig. 2a) increased the uncleaved/total ratio for p53 RNAs (Fig. 1b), while it had no effect on the uncleaved/total ratio for TBP RNAs (Fig. 1b). This result indicated that DHX36 is necessary for the maintenance of p53 pre-mRNA 3′-end processing