RESEARCH COMMUNICATION

Essential role for the interaction between hnRNP H/F and a G quadruplex in maintaining p53 pre-mRNA 3'-end processing and function during DNA damage

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Following DNA damage, mRNA 3'-end formation is inhibited, contributing to repression of mRNA synthesis. Here we investigated how DNA-damaged cells accomplish p53 mRNA 3'-end formation when normal mechanisms of pre-mRNA 3'-end processing regulation are inhibited. The underlying mechanism involves the interaction between a G-quadruplex structure located downstream from the p53 cleavage site and hnRNP H/F. Importantly, this interaction is critical for p53 expression and contributes to p53-mediated apoptosis. Our results uncover the existence of a specific rescue mechanism of 3'-end processing regulation allowing stress-induced p53 accumulation and function in apoptosis.

Supplemental material is available for this article.

Received August 31, 2010; revised version accepted December 15, 2010.

The cellular response to DNA damage is a protective mechanism against disease. This involves a functional interaction of DNA repair and transcription machineries, resulting in a global decrease of mRNA levels and repair of the induced DNA lesions. Recently, it has been shown that pre-mRNA 3'-end processing plays an important role in the repression of mRNA synthesis.

mRNA 3'-end formation, a nuclear process consisting of cleavage of pre-mRNA 3' ends and poly(A) tail addition, is essential for mRNA functions, including stability, translocation to the cytoplasm, and translation. 3'-End processing proceeds through the recognition of *cis*-acting elements of the pre-mRNA (defined as the polyadenylation [pA] signal) by a complex machinery that is associated with a large number of protein factors regulating the efficiency and specificity of this process and mediating its

interaction with other nuclear events, including transcription and splicing (for recent reviews, see Danckwardt et al. 2008; Millevoi and Vagner 2010). Cleavage stimulation factor (CstF), an essential component of the basal 3'-end processing machinery (Takagaki et al. 1989), plays a coordinating role in the DNA damage response. Following DNA damage, CstF inhibits 3'-end processing by forming regulatory complexes with different factors, including RNA polymerase II (RNA Pol II), the BARD1/BRCA1 tumor suppressor, and PARN (Kleiman and Manley 1999, 2001; Kleiman et al. 2005; Kim et al. 2006; Mirkin et al. 2008; Cevher et al. 2010).

A paradigm of the DNA damage response is that transcripts of genes inducing cell cycle arrest, DNA repair, and apoptosis are expressed before the DNA repair process begins. This suggests that gene-specific compensatory mechanisms should be used on DNA damage-activated genes to ensure their transcription and proper formation of mRNA 3' ends, providing the cell with a way to react to genotoxic stresses and maintain genomic integrity. Among these, the p53 tumor suppressor protein is pivotal in regulating the biological response to DNA damage. Following genotoxic insult, p53 functions as a sequence-specific transcription factor that regulates the expression of downstream target genes required for cell cycle arrest, DNA repair, or apoptosis (Levine and Oren 2009). Although it is well accepted that the activity of p53 is controlled through post-translational modifications (Kruse and Gu 2009), recent studies have revealed that p53 expression is also regulated at the transcriptional (Bruno et al. 2006) and translational (Mazan-Mamczarz et al. 2003; Takagi et al. 2005; Ofir-Rosenfeld et al. 2008) levels in response to genotoxic stress. Of importance, these studies not only show that p53 activation occurs through specific mechanisms that allow the p53 gene to bypass general repression of gene expression, but also suggest that the 3'-end processing step must be tightly regulated to ensure proper formation of p53 mRNA 3' ends.

Here we investigated how DNA-damaged cells accomplish p53 mRNA 3'-end formation when normal mechanisms of pre-mRNA 3'-end processing regulation are inhibited. We show that p53 pre-mRNA resists general inhibition of 3'-end processing caused by DNA damage stresses. The underlying mechanism involves the interaction between hnRNP H/F and a G-quadruplex RNA structure at the p53 pA signal. We also show that hnRNP H/F depletion compromises p53 pre-mRNA 3'-end processing, protein expression, and p53-mediated apoptosis.

Results and Discussion

p53 pre-mRNA 3'-end processing resists DNA damage-dependent inhibition of mRNA 3'-end formation

To assess whether p53 mRNA 3'-end formation is inhibited during DNA damage, we exposed different cell lines to DNA-damaging agents and quantified 3'-end processing efficiency of the p53 primary transcript or the control TBP pre-mRNA. Exposure of A549, HCT116, and MCF7 cells to DNA-damaging agents doxorubicin and UV irradiation induced significant p53 protein accumulation (Supplemental Fig. 1). Real-time PCR analysis of p53 3'-end processing efficiency measured by the ratio

[**Keywords:** DNA damage; pre-mRNA 3'-end processing; polyadenylation; p53 tumor suppressor; hnRNP H; hnRNP F]

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Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.607011>.

of uncleaved (not polyadenylated) (Supplemental Fig. 2) to total RNA (or the ratio of cleaved and polyadenylated to total RNA) (Supplemental Fig. 3) revealed that p53 mRNA 3'-end formation is maintained in all cell lines after doxorubicin exposure or UV irradiation (Fig. 1). In contrast, and as expected from previous studies, TBP pre-mRNA 3'-end processing significantly decreased after exposure to both DNA-damaging agents in the different cell lines, as revealed by the increased ratio of uncleaved to total TBP RNA (Fig. 1). Similarly, p53 target gene *p21*, but not *hprt*, has been shown previously to be properly transcribed and processed at the 3' end following DNA damage by a mechanism that bypasses the requirement for CTD phosphorylation and the presence of several known RNA Pol II elongation factors (Gomes et al. 2006). These findings support the hypothesis that DNA-damaged cells use specific alternative regulatory pathways to counteract general repression of mRNA synthesis.

G-rich sequences located downstream from the p53 pre-mRNA cleavage site contribute to enhance 3'-end processing efficiency

To address how p53 pre-mRNA is efficiently processed during DNA damage, we first focused on identifying regulatory sequence elements within the regions flanking the pA signal of the p53 pre-mRNA. This revealed the presence of two *cis*-elements known to modulate 3'-end processing efficiency (Millevoi et al. 2009 and references therein): a U-rich element located upstream of the pA signal, and a G-rich region positioned downstream from the cleavage site (Fig. 2A). The function of these elements was tested by using a luciferase reporter vector containing the p53 3'-end region inserted downstream from the luciferase gene (Rluc/p53), and two deletion constructs in which either the U-rich (Δ U) or the 3'-end-containing G-rich region (D1) was deleted (Fig. 2A). The luciferase reporter assay showed a 2.5-fold decrease in luciferase expression when deleting the G-rich region, while a minor decrease was obtained with the Δ U construct (Fig. 2B). To

Figure 1. p53 pre-mRNA 3'-end processing is not affected by DNA-damaging agents. Reverse transcription of nuclear RNA from A549, HCT116, and MCF7 cells left untreated (ctrl), UV-irradiated (UV), or treated with doxorubicin (doxo), followed by real-time PCR with primers specific to either p53 or TBP located on either side of the cleavage site to amplify the uncleaved RNA, or upstream of the cleavage site to amplify both the uncleaved and cleaved RNA, as depicted.

Figure 2. p53 pre-mRNA 3'-end processing is regulated by a downstream G-rich element. (A) Illustration of the luciferase reporter vector (Rluc/p53) containing the p53 pA signal and 3'-end-flanking regions inserted downstream from the Renilla luciferase gene (Rluc). Individual clones carrying deletion of the U-rich element (Δ U) or the G-rich region (D1–D4) are depicted, and their positions are indicated as positive and negative values relative to the cleavage site (CA). (CMV) Cytomegalovirus promoter. (B) The ratio of Renilla/firefly luciferase activities (Rluc/Fluc) was determined using HeLa cells cotransfected with the wild-type, Δ U, and D1 Rluc/p53 reporter plasmids and an internal control plasmid encoding the firefly luciferase (Fluc). (C) RPA of nuclear RNA isolated from HeLa cells transiently transfected with the indicated Rluc/p53 reporter plasmids. Protected bands representing the uncleaved (UN) and cleaved (CL) transcripts are shown. (Ctrl1) Without RNase; (Ctrl2) yeast RNA. Note that the length of the bands corresponding to uncleaved transcripts differs in each lane because the introduced deletions overlap with the protection probe, which contains wild-type 3'-flanking sequences. The cleavage efficiency—measured by the ratio of cleaved to uncleaved RNA and normalized to the wild-type construct—is shown below.

better characterize the functional role of this region in pre-mRNA 3'-end processing, we created a series of Rluc/p53 constructs in which the G-rich region was progressively deleted in a 3'-to-5' direction and analyzed mRNA 3'-end formation by RNase protection analysis (RPA). RPA of the transfected reporter plasmids showed that, whereas the D4 construct did not lead to a major effect on 3'-end processing efficiency, a fivefold decrease in 3'-end processing efficiency was observed with the D3 construct (Fig. 2C). This suggests that the sequence encompassing nucleotides +108 to +145 contains sequence or structural elements that are critical for p53 pre-mRNA 3'-end processing.

A G quadruplex in the p53 pre-mRNA 3'-flanking region is critical for maintaining p53 3'-end processing efficiency following UV irradiation

G-rich sequences are potential candidates to form G-quadruplex structures (G4), consisting of planar arrays of four guanines (G quartets), each guanine pairing with two neighbors by Hoogsteen bonding. These are specific DNA/RNA architectures adopted in telomeres, promoter regions, and 5' and 3' ends of the primary transcripts, and play a role in telomere lengthening, transcription, and translation (for a recent review, see Lipps and Rhodes 2009). Biocomputing analysis of the previously identified p53 pre-mRNA regulatory region using the QGRS Mapper program (<http://bioinformatics.ramapo.edu/QGRS/index.php>) revealed that the sequence encompassing

nucleotides +108 to +142 folds in a G4 structure. Since planar layers of G quartets are stabilized preferentially by K^+ in comparison with Na^+ (Sundquist and Klug 1989), we investigated the presence of a G4 by analyzing the influence of the nature of the cation on the pauses of reverse transcription catalyzed by the avian myeloblastosis virus (AMV) reverse transcriptase that has no monovalent cation requirement. As shown in Figure 3A, when NaCl was substituted for KCl in the reverse transcription buffer, the strong pauses observed with K^+ disappeared. This result strongly suggests the occurrence of a G4 in the G-rich (+108 to +142) region. A GGGG-to-CACA mutation in the last G stretch of the predicted G4 (G4M) abolished the K^+ -dependent reverse transcriptional pausing (Fig. 3A), further demonstrating the presence of a G4 in this G-rich sequence element.

To determine whether this structure is involved in the mechanism allowing p53 pre-mRNA to be properly processed at the 3' end during DNA damage, we introduced the G4M mutation in the Rluc/p53 vector. The G4M mutant induced a sixfold and fourfold inhibition of 3'-end processing efficiency compared with the wild-type sequence in untreated (Fig. 3B, graph i) and UV-irradiated (Fig. 3B, graph ii), cells respectively, suggesting that the ability to form a G4 at this position is critical for p53 pre-

mRNA 3'-end processing during DNA damage. The relationship between the G4 structure and its function in p53 3'-end processing before and after UV irradiation was further confirmed by using two additional G4 mutants (Supplemental Fig. 4). To further show the importance of G4 formation in p53 3'-end processing, we used TMPyP4, a compound known to bind and stabilize G4 structures (Lipps and Rhodes 2009). Cation-dependent reverse transcription showed that TMPyP4 increases the ability of the G4 formed at the +108 to +142 region to pause the AMV reverse transcriptase, suggesting an improved stability of the G4 in the presence of this compound (Fig. 3C). Treatment of A549 cells with TMPyP4 not only induced p53 protein expression, but also increased 3'-end processing of the p53 transcripts compared with TBP (Fig. 3D), suggesting that stabilization of the p53 G4 plays a role in stimulating p53 gene expression by stimulating the 3'-end processing step. Extending the role of G4 in gene expression, this set of results shows that a functional G-rich element at the p53 pA signal is structured in a G4 conformation that is not only critical to stimulate p53 3'-end processing, but also necessary to counteract 3'-end formation inhibition induced by DNA damage.

The G4 at the p53 pA signal interacts with hnRNP H/F in a UV-dependent manner

In order to determine the protein factors that bind to the p53 G4 and may be involved in the mechanism by which the G4 affects 3'-end processing during DNA damage, we performed RNA affinity chromatography experiments. Since hnRNP H/F was shown by us (Millevoi et al. 2009) and others (Bagga et al. 1998; Alkan et al. 2006; Dalziel et al. 2007) to bind G-rich sequences and plays a role in 3'-end processing, we analyzed whether this factor was able to bind the G-rich regulatory element at the p53 pA signal. The G-rich sequence located downstream from the SV40 pA signal that binds hnRNP H/F (Bagga et al. 1998; Alkan et al. 2006) and forms a G4 (Supplemental Fig. 5) served as positive control. As shown in Figure 4A, hnRNP H/F was pulled down by both the p53 and SV40 G-rich RNAs. This interaction was almost completely abolished when using the G4M mutation of the p53 G-rich element or a SV40 RNA containing a G-to-C mutation in a G stretch that hampers G4 formation. Conversely, hnRNP A1, which has been shown previously to bind RNA G4 structures (Abdul-Manan et al. 1996), was not retained by either of the two RNAs. Using UV cross-linking assays followed by immunoprecipitation (IP) with the hnRNP H/F antibody, we showed that the hnRNP H/F–G4 interaction is the result of a direct contact between the protein factor and the RNA folded in a G4 (Fig. 4B). This effect is not specific of the p53 G4 since it occurred also for the SV40 RNA (Fig. 4B). Addition of G4-stabilizing compound TMPyP4 to the nuclear extract increased the interaction between hnRNP H/F and the p53 G4, further confirming that the functional G-rich element at the p53 pA signal, when structured in a G4 conformation, interacts with hnRNP H/F in a specific manner (Fig. 4C). Finally, we tested whether DNA damage inducers affect the hnRNP H/F–G4 interaction at the p53 pA signal. Using UV cross-linking/IP assays with nuclear extract from A549 exposed or not to UV radiation, we showed that DNA damage increased the binding of hnRNP H/F to the p53 G4 (Fig. 4C). This effect is specific to the p53 G4 only, since it did not occur for the TBP RNA (Fig. 4C). To determine whether DNA damage stimulates

Figure 3. A G4 structure at the p53 pA signal is required to counteract UV-induced 3'-end processing inhibition. (A) Cation-dependent termination of reverse transcription at p53 RNA wild-type or mutated G4 (G4M). Strong pauses of reverse transcriptase are indicated by arrowheads, with their positions within the p53 RNA 3'-end sequence. Full-length extension products are indicated by open triangles. (B) Transfection of the wild-type or G4M Rluc/p53 reporter plasmids in A549 cells left untreated (graph i) or UV-irradiated (graph ii), followed by analysis of 3'-end processing efficiency by RT-qPCR. (C) Cation-dependent pauses of reverse transcriptase at the p53 G4 (indicated by arrowheads), depending on TMPyP4 addition. (D) Western blot analysis of p53 protein accumulation (graph i) and RT-qPCR analysis of endogenous p53 3'-end processing efficiency (graph ii) in either TMPyP4-treated or untreated A549 cells.

Figure 4. The G4 at the p53 pA signal interacts with hnRNP H/F in a UV-dependent manner. (A) RNA affinity chromatography using the G-rich element at the p53 or SV40 late pA signal, either wild-type or mutated (G4M), in the last G stretch and HeLa nuclear extracts, followed by Western blot analysis with the hnRNP H/F or hnRNP A1 antibodies. (B) UV cross-linking assays using the various RNAs as in A, followed by IP with the hnRNP H/F antibody. (C, top panel) UV cross-linking of the p53 or TBP RNAs in HeLa nuclear extracts, either TMPyP4-treated or untreated, followed by IP with the hnRNP H/F antibody. (Bottom panel) UV cross-linking of p53 or TBP RNA in nuclear extracts from A549 cells, either UV irradiated or left untreated, followed by IP with the hnRNP H/F antibody. (D) IP of the in vivo cross-linked RNA–protein complexes (RIP) in A549 cells, UV-irradiated or mock-treated, using the hnRNP H/F antibody followed by RT-qPCR analysis of the p53 pre-mRNA in the input and the RIP samples.

hnRNP H/F binding to the p53 transcript in vivo, we performed an RNP IP (RIP) assay using cell extracts from A549 cells UV-irradiated or left untreated and monitored the endogenous p53 primary transcript contained in the IPs. For this purpose, IPs were carried out with A549 cell lysates after formaldehyde cross-linking to specifically assay for in vivo RNA–protein interactions, with antibodies directed against hnRNP H/F. As shown in Figure 4D, endogenous p53 pre-mRNA was enriched in IP samples derived from UV-irradiated cells compared with mock-treated samples. Taken together, these results reveal that, following DNA damage, hnRNP H/F more closely associates with the G4 at the 3' end of the p53 primary transcript. Our results also show that TMPyP4 increases p53 3'-end processing efficiency through the stabilization of the p53 G4 and, as a consequence, the hnRNP H/F–p53 G4 interaction, contributing to p53 protein accumulation (Figs. 3, 4). These findings, together with previous observations showing increased p53 expression by G4 ligands (Lixia et al. 2008), prompt us to propose that, in addition to inhibiting telomerase activity, G4 ligands stimulate p53 accumulation via the 3'-end processing step, and p53 in turn represses telomerase activity directly (by interacting with the telomerase) (Li et al. 1999) or indirectly (by inhibiting telomerase mRNA synthesis) (Kanaya et al. 2000; Xu et al. 2000). Thus, the process generating mRNA 3' ends could be involved in the anti-tumor action of G4-interacting ligands via the p53 pathway.

hnRNP H/F regulates p53 expression and function in apoptosis during DNA damage

We next investigated the functional importance of the DNA damage-dependent interaction between hnRNP H/F and the G4 at the p53 pre-mRNA 3' end. To this end, we

performed siRNA-mediated depletion of hnRNP H, hnRNP F, or both proteins in A549 cells exposed or not to UV irradiation, and measured p53 expression at the protein and 3'-end processing levels. As shown in Figure 5A, siRNA-mediated silencing of hnRNP H and/or hnRNP F reduced p53 protein expression in UV-irradiated but not mock-treated cells. Similar results were obtained by exposing different cell lines to doxorubicin (Supplemental Fig. S6), suggesting that hnRNP H/F depletion counteracts DNA damage-dependent p53 accumulation regardless of cell type and the DNA-damaging agent. It is important to note that, in agreement with previous studies (Decker et al. 2003; Fox and Stover 2009), exposure of A549 cells to UV irradiation resulted in an increased hnRNP H/F expression (Fig. 5A). Importantly, loss of p53 protein expression in DNA-damaged A549 cells depleted of hnRNP H

Figure 5. hnRNP H/F regulates p53 expression and function in apoptosis. (A) Western blot analysis of p53, hnRNP H/F, and CFI 68 (loading control) in A549 cells treated or not with siRNA control or siRNA against hnRNP H and/or hnRNP F, followed or not by UV irradiation. (B) RT-qPCR analysis of p53 and TBP pre-mRNA 3'-end processing efficiency in A549 cells treated or not with siRNA against hnRNP H and/or hnRNP F, followed by UV irradiation. (C) Western blot analysis of PARP un-cleaved (UN) and cleaved (CL) p53, p21, and CFI 68 (loading control) in UV-irradiated HCT116 cells expressing p53 (HCT116 p53^{+/+}) or not (HCT116 p53^{-/-}) and treated with siRNA control or siRNA against hnRNP H and/or hnRNP F. (D) FACS analysis of HCT116 p53^{+/+} and p53^{-/-} cells treated with siRNA control or siRNA against hnRNP H and/or hnRNP F, followed by UV irradiation. The percentage of cells with a sub-G1 DNA content is indicated.

and/or hnRNP F appeared to be due, at least in part, to a significant reduction in p53 pre-mRNA 3'-end processing efficiency, which reached a 15-fold maximum when both hnRNPs were silenced (Fig. 5B; Supplemental Fig. 7). This effect is specific to the p53 pre-mRNA, since processing of TBP 3' ends was not affected by siRNA-mediated depletion of hnRNP H/F (Fig. 5B). It is important to note that the effect of hnRNP H/F depletion on p53 3'-end processing is not due to altered p53 transcription (Supplemental Fig. 8) and does not depend on the p53 mutational status (Supplemental Fig. 9). These findings support a model whereby DNA damage-induced hnRNP H/F expression promotes its association with the p53 G4, resulting in maintained 3'-end processing efficiency. This can occur by recruiting factors that are essential for the cleavage and pA reaction; namely, CstF (Bagga et al. 1998; Chen and Wilusz 1998) and poly(A) polymerase (PAP) (Millevoi et al. 2009). Since DNA damage induces CstF sequestration in complexes with BRCA1/BARD1 (Kleiman and Manley 1999, 2001), RNA Pol II (Kleiman et al. 2005), and PARN (Cevher et al. 2010), which inhibit 3'-end processing, the function of hnRNP H/F in DNA-damaged cells could be to prevent CstF from being hijacked in alternative protein complexes. This hypothesis is strongly supported by our findings showing that hnRNP H increases CstF 64 binding to the p53 pA signal (Supplemental Fig. 10). Since hnRNP H/F affected p53 protein expression, and p53 has well-documented proapoptotic activities, we asked whether this effect had consequences on p53 function in apoptosis. To test this hypothesis, we monitored PARP cleavage, a hallmark of apoptotic cell death, in the human HCT116 colon carcinoma cell line expressing (HCT116 p53^{+/+}) or not (HCT116 p53^{-/-}) p53. The isogenic cell lines were first treated with the siRNAs against hnRNP H and/or hnRNP F—or with a negative control siRNA—and then exposed to UV irradiation. As shown in Figure 5C, depletion of hnRNP H and/or hnRNP F in UV-irradiated HCT116 cells (+/+) attenuated PARP cleavage and decreased expression of p53 and p21, a main transcriptional target of p53. Importantly, the effect of hnRNP H/F inhibition on PARP processing is p53-dependent, as it was not observed in p53-null HCT116 cells. The proapoptotic effect of hnRNP H/F silencing in HCT116 (p53^{-/-}) cells (Fig. 5C) is in agreement with previous data showing that hnRNP H is able to block MST2-dependent apoptosis by controlling the splicing of the A-Raf transcription factor (Rauch et al. 2010). The role of hnRNP H/F in UV-induced apoptosis was also evident by the increased percentage of cells with sub-G1 DNA content in HCT116 (p53^{+/+}) cells treated with siRNAs against hnRNP H and/or hnRNP F, indicative of apoptosis (Fig. 5D). Again, this effect is absent in HCT116 (p53^{-/-}) cells, further supporting the importance of hnRNP H/F in p53-dependent apoptosis. Overall, these results reveal that hnRNP H/F depletion reduced p53 expression mostly by affecting the 3'-end processing step, and this contributes to halting p53-dependent apoptosis induced by UV irradiation. Notably, hnRNP H/F has been shown to influence the expression of apoptotic genes by acting mainly on the splicing process (Garneau et al. 2005; Camats et al. 2008; Fox and Stover 2009; Rauch et al. 2010), and its expression is modified in response to genotoxic agents (Decker et al. 2003; Fox and Stover 2009; Rauch et al. 2010). Although the mechanistic details of hnRNP H/F activation under DNA damage awaits further investigations, our study extends the notion of a link between hnRNP H/F and apoptosis to the process

generating 3' ends, and unraveled a new level of complexity in the p53 regulation of expression in response to genotoxic stress.

In conclusion, in this study, we provide evidence for the existence of a specific compensatory mechanism that (1) enables tumor suppressor p53 pre-mRNA to be properly processed at the 3' end following DNA damage stress, and (2) contributes to p53-dependent apoptosis. The proposed rescue mechanism of p53 pre-mRNA 3'-end processing and function by the hnRNP H/F-G4 interaction provides fertile ground for further investigation and, together with previous findings (Burns and Richter 2008), supports the notion that p53 3'-end processing can control its expression and biological activity.

Materials and methods

Cell culture, treatment, and transfection

Subconfluent A549, MCF-7, or HCT116 cells cultured in RPMI, DMEM, and McCoy's media, respectively, with 10% fetal bovine serum were exposed to UV light (254 nm) at 50 J/m² or 1 mg/mL doxorubicin for 16 h. TMPyP4 was added at a final concentration of 20 μ M for 24 h. Plasmid DNA (1 μ g) was transfected for 24 h in 60-mm plates with the JetPEI transfection reagent (Polyplus Transfection). siRNAs (2.5 nM) against hnRNP H, hnRNP F (Alkan et al. 2006), and a control siRNA (5'-GGUCCGGCUCCCCAAUGtt-3') (Eurogentec) were transfected with Lipofectamine RNAiMAX (Invitrogen) for 72 h.

pre-mRNA 3'-end processing efficiency analysis

RNA (5 μ g) from nuclei pellet isolated with the TRIzol RNA reagent (Invitrogen) was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen) and the random hexamer protocol. Real-time PCR was performed using a LightCycler system (Roche) with DNA MasterPLUS SYBR Green I kit (Roche). Data were then analyzed as 2exp(-Ct total RNA - Ct uncleaved). RPA was performed as in Dalziel et al. (2007) using 5 μ g of nuclear RNA and ~60,000 cpm (counts per minute) [³²P]UTP radiolabeled probe.

Cell cycle analysis

Attached cells were trypsinized, combined with floating cells, stained with propidium iodide (Sigma), and analyzed in a FACScan flow cytometry system using the CellFit software (Becton Dickinson). Data were obtained from 10⁴ viable cells.

Acknowledgments

We thank Sandra Bernat for technical help, and Olivier Sordet, Anne Pradines, and Anne-Françoise Tilkin-Mariamé for materials and discussions. We thank INSERM, Institut Claudius Regaud, FRM (Equipe FRM, Soutenue par la Fondation Recherche Médicale), and Fondation RITC (Recherche et Innovation Thérapeutique en Cancérologie) for financial support. A.D. was a recipient of a studentship from ARC (Association pour la Recherche sur le Cancer) and FRM.

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Erratum

Genes & Development 25: 220–225 (2011)

Essential role for the interaction between hnRNP H/F and a G quadruplex in maintaining p53 pre-mRNA 3'-end processing and function during DNA damage

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In the above-mentioned paper, Stéphan Vagner, instead of Stefania Millevoi, should have been listed as the corresponding author.

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Genes Dev. 2011, **25**:

Access the most recent version at doi:[10.1101/gad.607011](https://doi.org/10.1101/gad.607011)

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