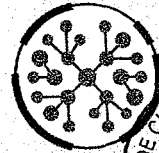


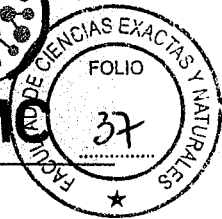


FCEyN

Departamento de Fisiología,
Biología Molecular
y Celular
"Profesor Héctor Maldonado"



FBMC



Buenos Aires, martes 24 de abril de 2018.-

Sr. Decano
Facultad de Ciencias Exactas y Naturales
Dr. Juan Carlos Rebores
S / D

Por la presente me dirijo a Ud. a fin de elevarle el informe de las actividades desarrolladas por el Dr. Alberto Kornblihtt durante su año sabático.-

Sin otro particular, saludo a Ud. con atenta consideración.-

REGISTRADO

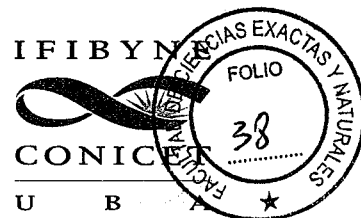
Pablo Wappner
Director DFBMC

UBA - FACULTAD DE CIENCIAS EXACTAS Y NATURALES DIRECCION DE MESA DE ENTRADAS Y DESPACHO	
ENTRO	SALIO
26 ABR 2018	

Nº 1884

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AGREGAR AL EXPEDIENTE 506.387



UNIVERSIDAD DE BUENOS AIRES
FACULTAD DE CIENCIAS EXACTAS Y NATURALES
DEPARTAMENTO DE FISIOLOGÍA, BIOLOGÍA MOLECULAR Y CELULAR
CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS
INSTITUTO DE FISIOLOGÍA, BIOLOGÍA MOLECULAR Y NEUROCIENCIAS (IFIBYNE-CONICET-UBA)

22 de abril de 2018

Sr. Decano de la Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires
Dr. Juan Carlos Reboreda

Tengo el agrado de dirigirme a usted con el fin de presentarle el informe de las actividades desarrolladas durante mi año sabático, comprendido: 1 de enero al 31 de diciembre de 2017.

1. Actividades en orden cronológico

MARZO

1.1 Seminario por invitación

Instituto de Biotecnología, UNAM, Cuernavaca, México (14/03/17).

1.2 Actividad docente

Seis horas de clase teórica sobre epigenética y procesamiento del ARN mensajero para alumnos avanzados de licenciatura en biotecnología de la Universidad Autónoma de México en Cuernavaca (13/03/17).

Invitación del Dr. Omar Coso a dictar la primera teórica de Introducción a la Biología Molecular y Celular de la FCEN-UBA (21/03/17).

1.3 Tesis doctoral aprobada

El 23 de marzo de 2017 mi discípula Micaela Godoy Herz defendió y aprobó con sobresaliente su tesis doctoral en la FCEN-UBA cuyo título es Regulación del splicing alternativo y de la elongación de la transcripción en plantas por acción de la luz.

1.4 Académico

Fui incorporado a la Academia de Ciencias de América Latina.

ABRIL

1.5 Evaluación institucional

Entre el 17 y el 19 de abril participé de la reunión del Consejo Científico Internacional del Instituto Pasteur de Montevideo, Uruguay, del cual soy miembro.

1.6 Seminario por invitación

Conferencia por el aniversario del Instituto Mercedes y Martín Ferreyra, Córdoba, Argentina (21/04/17).

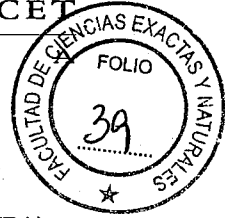


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1.7 National Academy of Sciences de EEUU

Entre el 28 de abril y el 1 de mayo participé en Washington de la reunión anual de la National Academy of Sciences de EEUU, de la cual soy miembro extranjero, acompañando el acto de incorporación del investigador argentino Gabriel Rabinovich.

MAYO-JUNIO

1.8 Visita a laboratorio extranjero

Entre el 8 de mayo y el 25 de junio trabajé en el laboratorio del Dr. Robert Singer en el Albert Einstein College of Medicine del Bronx, Nueva York. Durante mi estadía hice experimentos con el Dr. Adrien Sénécal, investigador post-doctoral del grupo del Dr. Singer, con el objeto de medir elongación de la RNA polimerasa II mediante técnicas de microscopía de avanzada. Además, participé de las reuniones de grupo semanales y el Dr. Singer me encargó realizar una evaluación de la actividad científica de los quince miembros de su grupo, por lo que me entrevisté durante al menos media hora con cada uno de ellos y elaboré un informe crítico sobre las fortalezas y debilidades de los proyectos de investigación individuales.

1.9 Seminarios por invitación

Mount Sinai General Hospital en Nueva York, invitado por el Dr. Julio Aguirre Ghiso (16/05/17).

Universidad de Nevada en Reno, invitado por la Dra. Patricia Berninson (17/05/17).

Albert Einstein College of Medicine, invitado por el Dr. Robert Singer (15/06/17).

JULIO

1.10 Recepción Comité Evaluador Externo del IFIBYNE

Entre el 12 y el 14 de julio, recibí, en mi función de Director del IFIBYNE, al comité evaluador formado por los Dres. Xosé Bustelo (Salamanca, España), Andrés Aguilera (Sevilla, España), Ramón Latorre (Valparaíso, Chile), Alfredo Cáceres (Córdoba, Argentina), José Luis Peña (Nueva York, EEUU) y Eduardo Blumwald (Davis, EEUU). Este comité entrevistó 32 candidatos a incorporarse como jefes de grupos de investigación al IFIBYNE (UBA-CONICET).

1.11 Seminario por invitación

Sociedade Brasileira de Bioquímica y Biología Molecular, Águas de Lindóia, Brasil. Simposio en honor del Dr. Héctor Terenzi (28/07/17).

AGOSTO

1.12 Organizador de reunión científica

Entre el 22 y el 26 de agosto asistí como organizador, junto con los Dres. Jean Beggs de Edimburgo, Reino Unido, y Jens Lykke-Andersen, de San Diego, EEUU, al



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meeting de Cold Spring Harbor "Eukaryotic mRNA processing". Este meeting se realiza cada dos años y soy coorganizador en las ediciones de 2017, 2019 y 2021.

1.13 Conferencia por invitación

Conferencia plenaria en el simposio anual de la Fundación Huésped de Buenos Aires (30/08/17).

SEPTIEMBRE

1.14 Conferencias por invitación

Conferencia en la Reunión Anual de FAME (Familias Atrofia Muscular Espinal de Argentina), Rosario (09/09/17).

Conferencia plenaria de clausura. Sociedad de Bioquímica y Biología Molecular de Chile. Puerto Varas, Chile (29/09/17).

OCTUBRE

1.15 Conferencia por invitación

Conferencia plenaria. Meeting "Forty years of mRNA splicing: from discovery to therapeutics". Cold Spring Harbor Laboratory, New York, EE.UU (24/10/17). Es de destacar que en esta reunión, organizada por el premio Nobel Philip Sharp, uno de los descubridores del splicing, hablamos los investigadores de todo el mundo que a lo largo de 40 años hicimos contribuciones relevantes al tema.

2. Trabajos publicados

Se publicaron 3 trabajos en revistas internacionales con referato:

2.1 Muñoz*, M. J., Nieto Moreno*, N., Giono*, L. E., Cambindo Botto, A. E., Dujardin, G., Bastianello, G., Lavore, S., Torres-Méndez, A., Menck, C. F. M., Blencowe, B., Irimia, M., Foiani, M. & Kornblihtt, A. R. Major roles of pyrimidine dimers, nucleotide excision repair and ATR in the alternative splicing response to UV irradiation. *Cell Reports* 12, 2868-2879 (2017). I.F.: 8,4.

2.2 Fiszbein, A. & Kornblihtt, A. R. Alternative splicing switches: Important players in cell differentiation. *BioEssays* 39, doi: 10.1002/bies.201600157 (2017). I.F.: 4,2.

2.3 Kornblihtt, A. R. Epigenetics at the base of alternative splicing changes that promote colorectal cancer. *J. Clin. Invest.* 127, 3281-3283 (2017). I.F.: 12,4.

El 2.1 es un trabajo de investigación original. El 2.2 es una revisión por invitación y el 2.3 es un comentario sobre un trabajo de otro grupo publicado en el mismo número de la revista. Se adjuntan copias de los trabajos y se destaca que el 2.1 fue tapa de la



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revista con una ilustración realizada por la co-autora e investigadora asistente de mi grupo, Dra. Luciana Giono.

3. Artículo sobre la situación de ciencia y técnica en Argentina

Fui invitado a escribir un artículo de actualidad en la revista Nature, que también adjunto:

Kornblihtt, A. R. Where science and nonsense collide. *Nature* 541, 135 (2017).

4. Dirección de discípulos

Durante todo 2017 continué dirigiendo las tesis doctorales de Nicolás Nieto Moreno y Luciano Marasco, la beca estímulo de Guillermina Kubaczka, la beca post-doctoral de Micaela Godoy Herz y la pasantía del estudiante de biología de la Universidad de Dundee, Escocia, Reino Unido, José Stigliano.

5. En los medios

5.1 Entrevista en Tiempo Argentino:

<https://www.tiempoar.com.ar/articulo/view/71549/kornblihtt-es-perverso-que-el-ministro-se-dedique-a-ningunear-pa-blicamente-al-conicet>

5.2 Artículo en La Nación:

<https://www.lanacion.com.ar/2058959-la-modificacion-genetica-de-embriones-humanos>.

5.3 Artículo en Página 12: <https://www.pagina12.com.ar/55336-responsabilizan-a-los-investigadores-para-justificar-el-ajus>


5.4 Perfil biográfico en el Journal of Cell Biology:

<http://jcb.rupress.org/content/early/2017/01/18/jcb.201701050>

5.5 Carta de lectores en La Nación:

<https://www.lanacion.com.ar/1974126-cartas-de-los-lectores>

Sin otro particular, saludo a usted muy atentamente.

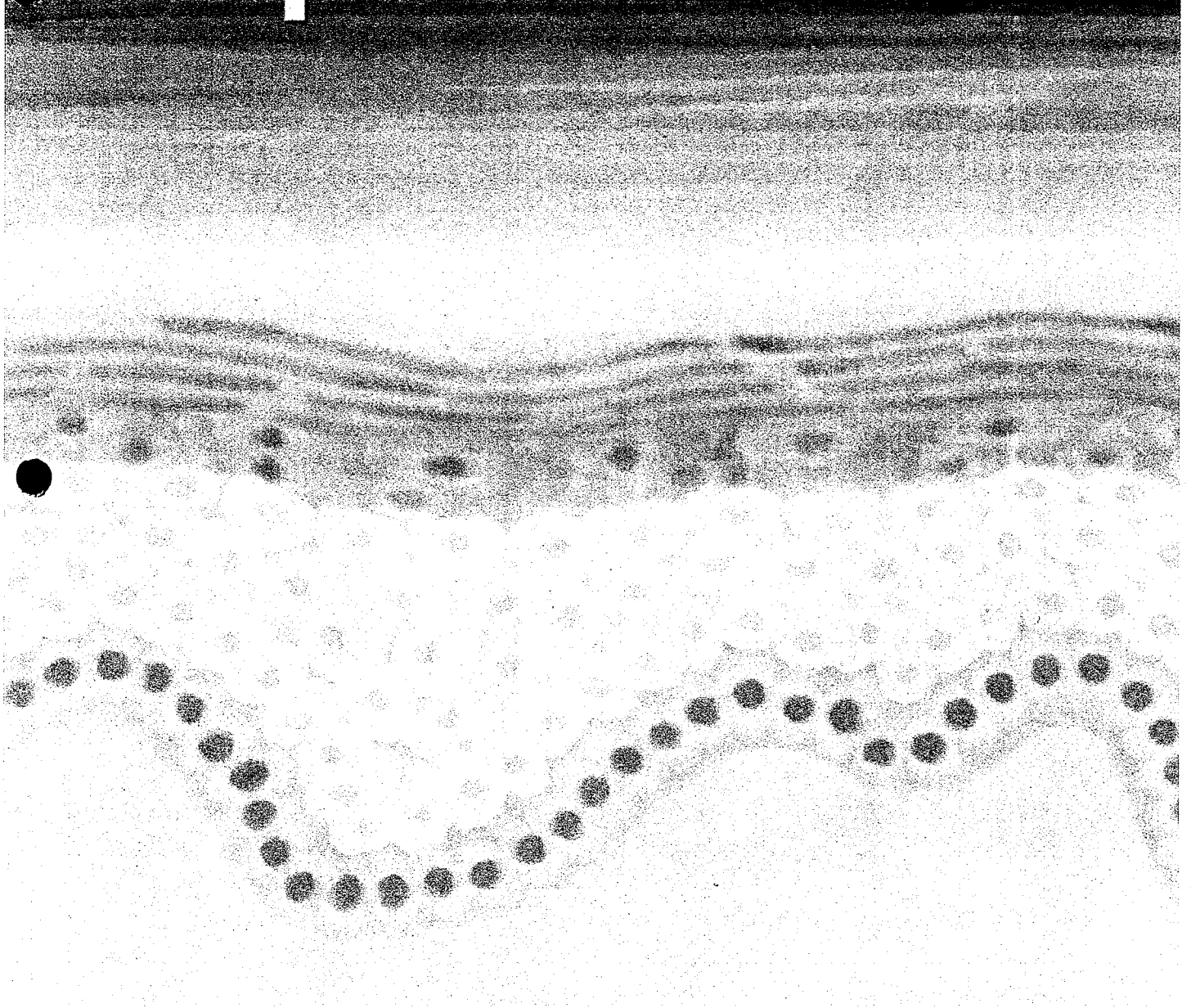

Dr. Alberto R. Kornblihtt
Profesor Titular FCEN-UBA
Investigador Superior CONICET

Cell Reports

Volume 18
Number 12

March 21, 2017

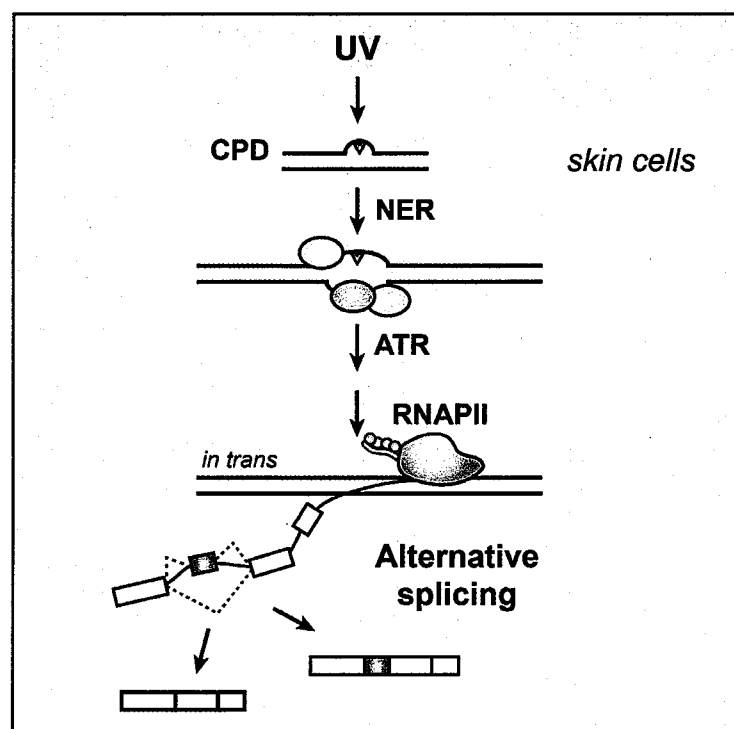
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Cell Reports

Major Roles for Pyrimidine Dimers, Nucleotide Excision Repair, and ATR in the Alternative Splicing Response to UV Irradiation

Graphical Abstract



Authors

Manuel J. Muñoz, Nicolás Nieto Moreno, Luciana E. Giono, ..., Manuel Irimia, Marco Foiani, Alberto R. Kornblihtt

Correspondence

ark@fbmc.fcen.uba.ar

In Brief

Muñoz et al. find that UV-induced DNA damage is the main determinant affecting gene expression in response to UV in skin cells. DNA repair and the subsequent activation of ATR modulate RNAPII phosphorylation and alternative splicing patterns specifically in keratinocytes.

Highlights

- DNA damage is sufficient to trigger a global alternative splicing (AS) response to UV
- Photolyase-mediated elimination of the DNA lesions (CPDs) abolishes the AS response
- RNAPII is the target, but not a sensor, of the signaling cascade initiated by CPDs
- GG-NER and ATR participate in the control of RNAPII phosphorylation and AS by UV

Accession Numbers

GSE85510



Muñoz et al., 2017, Cell Reports 18, 2868–2879
March 21, 2017 © 2017 The Author(s).
<http://dx.doi.org/10.1016/j.celrep.2017.02.066>

CellPress

Major Roles for Pyrimidine Dimers, Nucleotide Excision Repair, and ATR in the Alternative Splicing Response to UV Irradiation

Manuel J. Muñoz,^{1,2,7} Nicolás Nieto Moreno,^{1,7} Luciana E. Giono,^{1,7} Adrián E. Cambindo Botto,¹ Gwendal Dujardin,^{1,3,4} Giulia Bastianello,² Stefania Lavore,² Antonio Torres-Méndez,^{3,4} Carlos F.M. Menck,⁵ Benjamin J. Blencowe,⁶ Manuel Irimia,^{3,4} Marco Foiani,² and Alberto R. Kornblihtt^{1,8,*}

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<http://dx.doi.org/10.1016/j.celrep.2017.02.066>

SUMMARY

We have previously found that UV irradiation promotes RNA polymerase II (RNAPII) hyperphosphorylation and subsequent changes in alternative splicing (AS). We show now that UV-induced DNA damage is not only necessary but sufficient to trigger the AS response and that photolyase-mediated removal of the most abundant class of pyrimidine dimers (PDs) abrogates the global response to UV. We demonstrate that, in keratinocytes, RNAPII is the target, but not a sensor, of the signaling cascade initiated by PDs. The UV effect is enhanced by inhibition of gap-filling DNA synthesis, the last step in the nucleotide excision repair pathway (NER), and reduced by the absence of XPE, the main NER sensor of PDs. The mechanism involves activation of the protein kinase ATR that mediates the UV-induced RNAPII hyperphosphorylation. Our results define the sequence UV-PDs-NER-ATR-RNAPII-AS as a pathway linking DNA damage repair to the control of both RNAPII phosphorylation and AS regulation.

INTRODUCTION

As a first barrier between the body interior and the exterior, our skin is regularly exposed to UV radiation. The UV light that reaches the Earth's surface can damage cellular components, such as DNA, RNA, proteins, and lipids, and is, therefore, the most prominent and ubiquitous carcinogen in our natural environment (Meinikova and Ananthaswamy, 2005). Despite the high incidence of skin cancer in human health and the fact that gene expression is a key target of signaling cascades triggered

by UV exposure, little is known about gene expression mechanisms occurring in response to UV light.

Alternative splicing (AS) is the main process that amplifies DNA information by generating multiple mRNA and protein variants from each of the vast majority (95%) of mammalian genes (Barash et al., 2010; Kornblihtt et al., 2013). AS has been shown to be regulated by external cues through various mechanisms whose alterations cause hereditary disease and cancer (Hua et al., 2011; Srebrow and Kornblihtt, 2006). This, together with findings of important biological roles for tissue- and species-specific AS patterns (Ellis et al., 2012; Gracheva et al., 2011), places the regulation of AS at the same level of importance as the regulation of transcription in the determination of cell differentiation and fate. We have previously identified transcriptional regulation and AS as crucial targets of signaling cascades resulting from UV irradiation of human cells. We have shown that UVC irradiation (<280 nm) promotes the phosphorylation of the C-terminal domain (CTD) of RNAPII, which slows down transcriptional elongation and affects AS of several genes, some of which are key for survival/apoptosis decisions (Muñoz et al., 2009). These observations were confirmed and extended by other genome-wide studies (Ip et al., 2011), which reinforced the evidence for physiological roles of the kinetic coupling between transcription and splicing. In this context, it is now known that, for alternative cassette exons, slow elongation can promote either exon inclusion (de la Mata et al., 2003; Muñoz et al., 2009) or skipping (Dujardin et al., 2014; Fong et al., 2014), depending on the regulatory *cis*-acting sequences and *trans*-acting factors operating in each particular AS event. Given the multiple cellular components susceptible to be damaged by UV light, it was important to evaluate whether damage of DNA or of other cell molecules trigger the AS response. The most conspicuous UV-induced DNA lesions are cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone photoproducts ((6-4)PPs), both of which are, at least in placental mammals, mainly repaired by the nucleotide excision

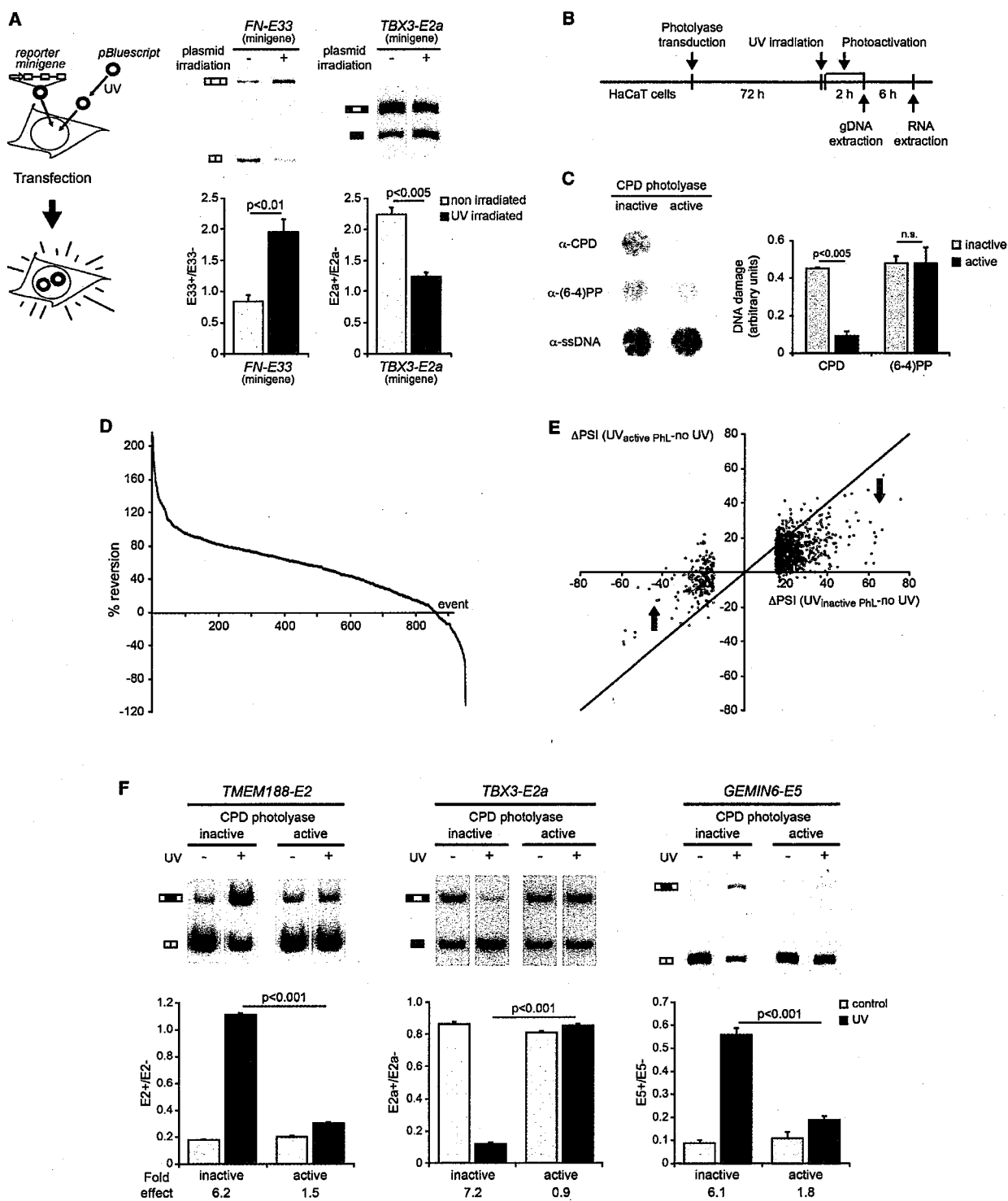


Figure 1. Cyclobutane Pyrimidine Dimers Are Responsible for the UV Effect on AS in Skin Cells

(A) HaCaT cells were co-transfected with pBluescript (pBS) or pBS irradiated in vitro with 1,500 J/m² UV together with *FN-E33* or *TBX3-E2a* reporter minigenes for 24 hr. AS patterns of *FN* or *TBX3* were assessed by radioactive RT-PCR with specific primers for the minigenes-derived mRNAs. The ratio inclusion/skipping is shown.

(legend continued on next page)

repair (NER) system (Marteijn et al., 2014). NER is initiated by two distinct DNA-damage-sensing mechanisms that use the same machinery to repair the damage: transcription coupled repair (TCR or TC-NER) and global genome repair (GGR or GG-NER). TC-NER detects and removes damage from the template strand of genes that are being transcribed and depends on RNAPII elongation and on the Cockayne syndrome proteins CSA and CSB. Mutations in this pathway are associated with cell death probably due to deficiencies in transcriptional re-start after damage (Anindya et al., 2010). GG-NER, the repair mechanism that prevails in keratinocytes, the most abundant cell type in the skin (D'Errico et al., 2007), removes UV-induced damage present in both transcribed and non-transcribed regions of the genome and depends on the UV-DDB complex, in which XPE interacts with the lesion, and on XPC to detect the damage. Once the damage is recognized by either TC-NER or GG-NER factors, both pathways converge at the recruitment of the general transcription factor TFIIH, which opens the DNA helix around the damage. After the recruitment of XPA and the endonucleases XPF and XPG to excise the damaged strand, the resulting single-stranded DNA (ssDNA) gap is filled by DNA synthesis and ligation (Marteijn et al., 2014).

DNA damage not only induces DNA repair but triggers signaling pathways as well. Ataxia telangiectasia mutated (ATM) and ataxia telangiectasia mutated and Rad3 related (ATR), members of the PI(3)-like protein kinase family, are enzymes of paramount importance in the DNA damage response. Whereas ATR was originally identified as a key factor controlling DNA replication in S phase, it was later shown to be activated throughout the cell cycle by ssDNA generated during NER (Hanasoge and Ljungman, 2007; Marteijn et al., 2009; Matsumoto et al., 2007; Stiff et al., 2008; Vrouwe et al., 2011). Moreover, it was also shown that ATR activation by UV irradiation in turn activates ATM (Stiff et al., 2006).

Here, we provide direct proof that UV-induced CPDs are sufficient to initiate the AS response in skin cells without the involvement of any other type of UV-damaged molecules. Photolyase-mediated removal of CPDs abolished the UV-induced RNAPII phosphorylation and AS regulation, therefore demonstrating that CPDs are the main trigger in the modulation of two key steps of gene expression in UV-treated keratinocytes. Transfection experiments with irradiated plasmids that serve as templates for transcription suggest that RNAPII is a target,

but not a sensor, of the DNA lesion. Our results reveal that ATR acts as a bridging signal from the DNA lesion to the RNAPII phosphorylation/AS regulation in a NER-dependent manner.

RESULTS

Cyclobutane Pyrimidine Dimers Are Responsible for the UV Effect on RNAPII Phosphorylation and AS in Skin Cells

We have previously found that, in hepatoma cells, UV irradiation regulates co-transcriptional AS in a p53-independent manner, through the phosphorylation of RNAPII CTD and subsequent inhibition of transcriptional elongation (Muñoz et al., 2009). In order to determine whether damage of DNA, and not of other types of cell components, was sufficient to trigger RNAPII hyperphosphorylation and AS regulation in human keratinocytes (HaCaT cells), we used two different approaches: (1) transfection of UV-damaged naked plasmid DNA without irradiation of cells and (2) irradiation of cells followed by prompt removal of DNA lesions. In the first strategy, to avoid the concomitant damage of RNA, proteins, lipids, and other molecules after UV treatment of cells, a water solution of a plasmid with no relevant sequences for mammalian cells, pBluescript (pBS), was irradiated in vitro with UV light and co-transfected with two different AS reporter model minigenes: one carrying an alternative exon whose inclusion is upregulated by UV irradiation (*FN-E33*) and another with an alternative exon whose skipping is promoted (*TBX3-E2a*). Co-transfection of the minigenes with the in-vitro-irradiated pBS caused an increase in *FN-E33* inclusion and in *TBX3-E2a* skipping in the absence of cell irradiation, mimicking the UV effect (Figure 1A). It is important to point out that the in-vitro-irradiated plasmid preserved its circular integrity and that, as expected for the effects of UV irradiation, did not contain double-strand breaks (Figure S1A).

These experiments indicate that damaged DNA is sufficient to trigger a splicing response that is independent of the potential effects of damaging other cell molecules. It also confirmed, using a different experimental approach, our previous demonstration that the UV effect on AS is not a consequence of DNA damage in cis, i.e., on the actual template DNA encoding the alternative mRNA isoforms (Muñoz et al., 2009), but rather of a systemic or global cell response to damage. In the second approach, we reasoned that, if damaged DNA is the cause of AS modulation

(B) Diagram showing the adenovirus infection protocol. Immediately following UV irradiation, the photolyase was activated by treatment with white light for 2 hr. Genomic DNA (gDNA) and RNA were extracted at the indicated time points.

(C) Repair of DNA lesions in HaCaT cells transduced with the CPD photolyase as described in (B) was assessed by DNA western dot blot using specific antibodies against CPD, 6-4(PP), or total single-stranded DNA (ssDNA).

(D) HaCaT cells transduced with CPD photolyase-expressing adenovirus were left untreated (no UV) or UV irradiated. Irradiated cells were then exposed to white light to activate the photolyase (UV, active PhL) or kept in the dark (UV, inactive PhL). Global transcriptome analysis was performed to compare irradiated cells with an active or inactive CPD photolyase. y axis, percentage of reversion of UV-induced changes for alternatively spliced cassette exons following photolyase photoactivation. Positive percent values represent reversion of UV-induced AS changes toward control levels.

(E) Reversion of changes in inclusion levels induced by UV by expression of photolyase. The x axis represents the Δ PSI of each event between UV, inactive PhL and no UV samples, using a 15% threshold. The y axis shows the values of Δ PSI for the same events in respect to the control when the photolyase is active (UV, active PhL). The red line represents the relation between the Δ PSI of UV, inactive PhL minus control with respect to the Δ PSI of UV, active PhL minus control, i.e., the absence of photolyase effect on UV-induced changes. The blue arrows indicate the direction of the changes where reversion is happening.

(F) AS patterns in CPD photolyase-transduced HaCaT cells treated with 15 J/m² UV were assessed 6 hr after photolyase activation by radioactive RT-PCR. For all experiments, images of a representative experiment and mean, SEM, and p values (Student's t test) of three experiments are shown. See also Figures S1 and S2 and Tables S1 and S2.

upon UV irradiation, then treatment of cells with UV light followed by removal of DNA lesions should not produce AS changes. As mentioned earlier, the most abundant UV-induced DNA pyrimidine dimer (PD) lesions are CPDs. This type of lesion induces little distortion in the double helix and consequently is poorly repaired by the NER pathway (Garcin et al., 2007), the main system dealing with UV-induced DNA damage in humans. How can we dissociate UV treatment and formation of CPDs if these are slowly repaired but heavily induced by UV light? Photolyases are white-light-activated flavoenzymes that specifically revert the different lesions generated by UV irradiation. The fact that placental mammals do not possess photolyases offers an invaluable opportunity to use a CPD-specific photolyase from a marsupial mammal (*Potorous tridactylus*, the Australian rat kangaroo) as a tool to selectively abolish the contribution of CPDs. HaCaT cells were transduced with an adenovirus expressing the *Potorous* CPD photolyase and, 72 hr later, treated with 15 J/m² of UV. The photolyase was then activated by exposing the cells to white light for 2 hr or keeping them in the dark as a control, after which cells were harvested (Figure 1B). After total DNA purification, damage was assessed by DNA western dot blot using antibodies specific to CPD, (6-4)PP, or to ssDNA as control. Figure 1C shows that photolyase activation reduced the amount of CPDs by about 80%, keeping the levels of (6-4)PPs unaffected. Similar results were obtained by immunostaining of fixed cells with the same antibodies (Figure S1B).

In order to assess the global effects of UV irradiation on AS and the extent of the CPD contribution to these effects, we performed RNA sequencing (RNA-seq) analysis of duplicate samples of photolyase-transduced HaCaT cells, left untreated ("no UV"), UV irradiated and kept in the dark ("UV, inactive PhL"), or UV irradiated and photoactivated for 2 hr with white light ("UV, active PhL"). We analyzed the variations in the percentage of alternative exon inclusion (PSI [percent spliced in]) for each AS event (ASE) that were greater than 15% (Δ PSI > 15%; see Experimental Procedures). We observed that irradiation with 15 J/m² of UV light altered the inclusion levels of 1,551 ASEs (UV, inactive PhL versus no UV; Figure S1C). These events occurred in 1,174 unique genes, indicating that UV affects the splicing pattern of a considerable proportion of genes expressed in HaCaT cells (~11% of the total number of multixonic genes expressed in these samples). Approximately 64% (991) of the affected ASEs are cassette exons, in which we focused our analysis. Of these, 77.8% (771) show higher inclusion upon UV treatment. We found that removal of CPDs by photoactivation of the marsupial photolyase reverted the effects of UV light to different extents in the majority (87%) of the cassette exon events (Figure 1D), with 56% of the affected exons reverting their PSI at least by 50%.

To study the contribution of CPDs in more detail, we compared the Δ PSI between the UV-irradiated and control samples (UV, inactive PhL versus no UV) with their corresponding Δ PSI in samples from cells with active photolyase with respect to control (UV, active PhL versus no UV; Figure 1E). Each dot corresponds to one of the 991 alternative exons whose inclusion changes upon UV irradiation. Strikingly, most cassette exons with either higher or lower inclusion levels upon UV treatment (x axis) show a clear reduction in the Δ PSI caused by UV when the photolyase is active (y axis), observed by a general displace-

ment from the diagonal toward the horizontal axis (blue arrows). In the type of graphic shown in Figure 1E, if the photolyase activity had no effect, all dots would be aligned along the diagonal. These results strongly indicate that CPDs are the main cause of the AS response to UV light.

Figures 1F and S1D show the validation of the RNA-seq data (Table S1) by individual RT-PCR analysis of seven endogenous ASEs, which include the *TBX3-E2a* example used in Figure 1A. Consistent with results in Figure 1A, the change in AS observed in the *FN-E33* exon is also reverted by the photolyase (Figure S1E).

To study a putative contribution of (6-4)PPs in the cellular response to UV, we conducted a similar set of experiments using the specific (6-4)PP photolyase from *Arabidopsis thaliana* (Figures S2A–S2C). Figure S2C shows that, unlike CPDs, complete removal of (6-4)PPs does not alter the UV effect on AS. This may reflect the fact that CPDs are induced roughly five times more than (6-4)PPs (Besaratina et al., 2011) when using, as we do, a UVC lamp with an emission peak of 254 nm. Yet, the most harmful UV radiation that reaches the Earth's surface is not UVC but UVB (305 nm), because UV radiation below 305 nm is filtered by the ozone layer (Besaratina et al., 2011). We then compared the effect on AS and RNAPII phosphorylation of UVC and UVB. For both wavelengths (254 and 305 nm), we used doses that generated comparable amounts of CPDs but different amounts of (6-4)PPs (Figure S2D). Under these conditions, we found that UV-induced RNAPII phosphorylation, shown as RNAPIIo/RNAPIIa ratio (Figure S2E), and AS patterns (Figure S2F) were affected at comparable levels in UVC- or UVB-treated cells, further strengthening the role of CPDs in the response to UV light and suggesting that UVB acts in a similar fashion as UVC.

Although the importance of the effect of CPDs on gene expression has been addressed before (Boros et al., 2015), the crosstalk between CPD's impact in mRNA levels and AS has not been documented. We found that activation of the CPD photolyase, but not the (6-4)PP photolyase, prevented the UV-induced increase in the RNAPIIo/RNAPIIa ratio (Figures 2A and S3A), as well as both up- and downregulation of gene expression levels (Figure 2B). Moreover, the ASEs affected by UV in both directions (increased and decreased PSI) often correspond to genes whose expression is downregulated by UV (Figure 2C), and most importantly, photolyase-triggered reversion of AS changes clearly takes place in genes whose expression is downregulated by UV (Figure 2D), consistently with a role for a reduction in RNAPII elongation. In agreement with this, phosphomimetic CTD mutants and a slow elongation RNAPII mutant duplicate the UV effect on AS in skin cells, whereas non-phosphorylatable mutants prevent the UV effect (Figures S3B and S3C), confirming previous results from our group obtained in a different cell line (Muñoz et al., 2009). Therefore, results in Figure 2 suggest that the effect of CPDs on AS is mediated by RNAPII, which could act either as a sensor of damage or as an effector of signaling cascades triggered by CPDs.

The AS Response to UV Is Not Triggered by an Active RNAPII nor by Transcription-Coupled Repair

It has been recently proposed that, in human fibroblasts, RNAPII stalling in UV-damaged genes modulates AS through

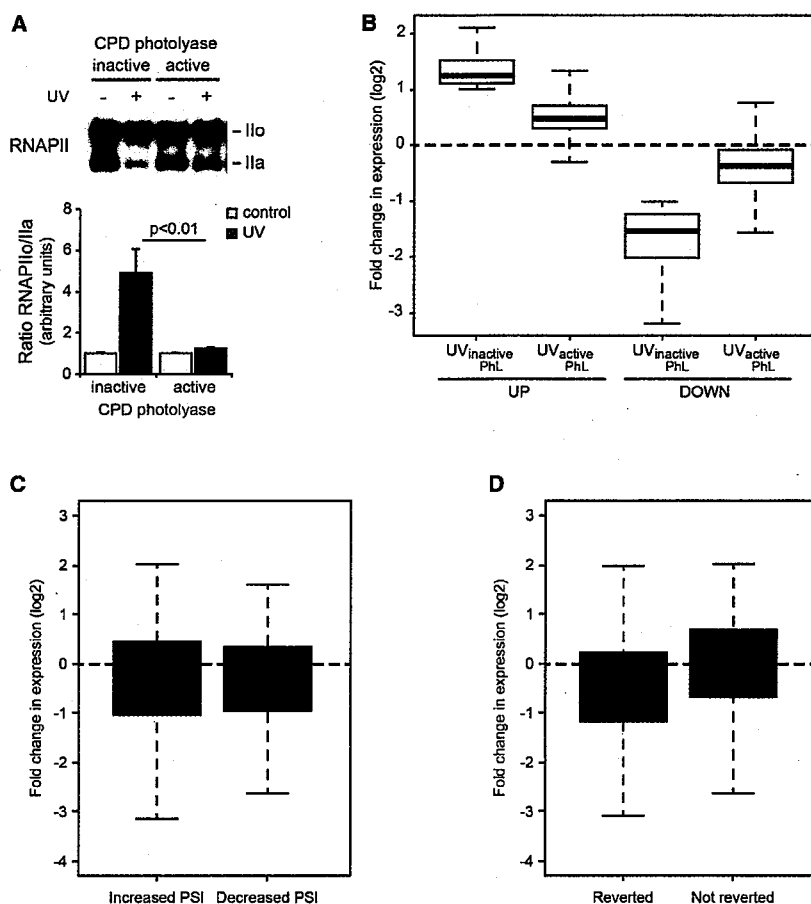


Figure 2. Cyclobutane Pyrimidine Dimers Modulate RNAPII Phosphorylation and Gene Expression Levels

(A) RNAPII phosphorylation status in CPD photolyase-transduced HaCaT cells treated with 15 J/m² UV was assessed 2 hr after photolyase activation. Global RNAPII phosphorylation pattern was determined by western blot using an antibody against the N-terminal part of RNAPII major subunit (Rbp1) and quantification of the phospho RNAPII (Ilo) to non-phospho RNAPII (IIa) ratio. Images of a representative experiment and mean, SEM, and p values (Student's t test) of two experiments are shown.

(B) Global transcriptome analysis of CPD photolyase-transduced HaCaT cells. Reversion of UV-induced changes in gene expression following photolyase photoactivation (UV, active PhL versus UV, inactive PhL) for genes increasing or decreasing their expression levels by at least 2-fold up and down, respectively.

(C) Distribution of changes in steady-state levels upon UV treatment for genes that contain cassette exons with increased (blue) or decreased (red) inclusion. For clarity, outliers are not plotted.

(D) Distribution of changes in steady-state levels for genes whose AS cassette exons are dependent on CPDs (reverted by photolyase expression by at least 50%) or not.

See also Figure S3.

an R-loop-dependent mechanism (Tresini et al., 2015). Results in Figure 1A suggest that, at least in keratinocytes, this is not the case because the pBS plasmid irradiated in vitro, which is effective in triggering the AS response, does not carry an RNAPII promoter. To further evaluate the potential role of an active RNAPII as a sensor of the CPD effect on AS, we replaced the pBS plasmid with a luciferase reporter vector with (pGL3-SV40; Promega pGL3-Control) or without (pGL3; Promega pGL3-Basic) the strong RNAPII promoter of the SV40 virus. Figure 3A shows that the presence of the SV40 promoter allows for transcription of the pGL3 plasmid, revealed by the elicited luciferase activity (compare bars 1 and 3). Although UV irradiation reduces transcription, irradiated pGL3-SV40 still displays about 5,000 times more luciferase activity than irradiated pGL3 (compare bars 2 and 4). Despite the qualitative difference in transcription capabilities, the in-vitro-irradiated pGL3 and pGL3-SV40 plasmids elicited similar effects on *FN-E33* and *TBX3-E2a* minigenes AS patterns (Figure 3B), indicating that transcription of the irradiated plasmid is not required for the AS response to damaged DNA. This evidence points to a lack of roles for RNAPII as a lesion sensor and therefore of the involvement of TC-NER in the AS response in keratinocytes. To confirm these predictions, we first investigated whether TC-NER is involved in the AS response to

UV light in keratinocytes. For this, we used small interfering RNA (siRNA) to knockdown expression of the TC-NER factor CSB and found that the UV effect was similar to the control siRNA-treated cells (Figure 3C), with a CSB mRNA and protein depletion of approximately 80% (Figures S4A and S4B). Similar results were obtained when irradiating patient-derived CSB mutant cells or a control counterpart stably expressing a wild-type version of CSB (Figure S4C).

To understand our results obtained in keratinocytes in the context of the observations made in fibroblasts (Tresini et al., 2015), we compared the UV response in both cell types. Results in Figure 3D show that fibroblasts and keratinocytes behave differently upon UV irradiation: whereas UV promotes inclusion of the *FN-E33* in keratinocytes, it induces skipping in fibroblasts. We then analyzed the effects of co-transfecting the *FN-E33* AS reporter minigene together with in-vitro-irradiated pGL3 or pGL3-SV40 in fibroblasts and found that the need for transcription of the irradiated plasmid to elicit the respective AS responses differs between the two cell types. Whereas in keratinocytes, the presence or absence of an RNAPII promoter in the pGL3 vector is dispensable to mimic the UV effect (Figure 3B), in fibroblasts, there is a clear need for transcription of the irradiated plasmid to duplicate the UV effect (Figure 3E).

These experiments independently indicate that, although RNAPII seems to be an important target of the signaling elicited by CPD lesions (Figure 2A), the enzyme is not acting as the main sensor for the CPD effect on AS in human keratinocytes,

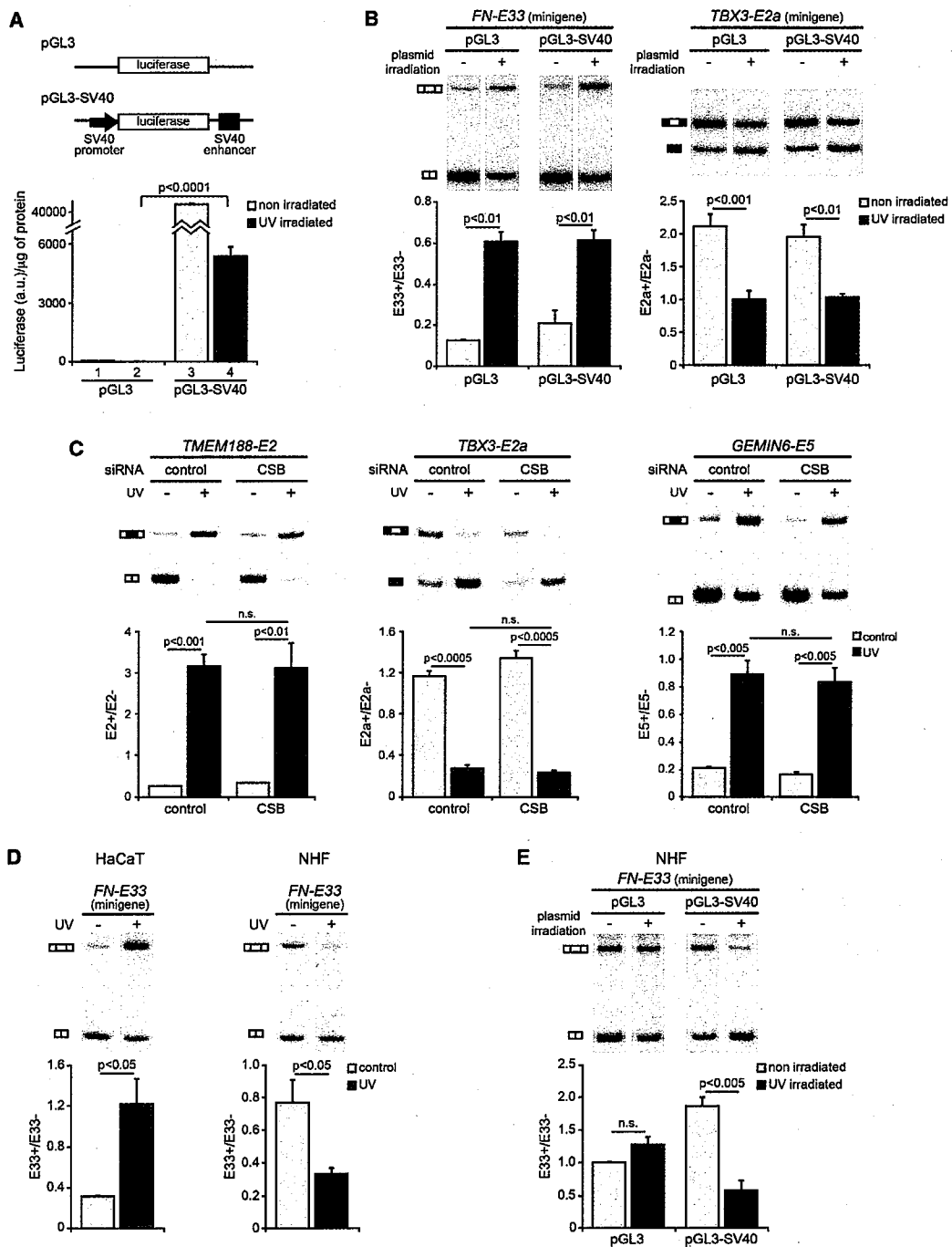


Figure 3. AS Regulation by UV Is Not Triggered by RNAPII nor TC-NER

(A) Expression of the luciferase reporter plasmids pGL3 basic (pGL3) or pGL3 control (pGL3-SV40) for untreated or in-vitro-irradiated vectors 24 hr after transfection.

(B) HaCaT cells were co-transfected with pGL3 or pGL3-SV40 vectors, either untreated or in vitro irradiated, together with *FN-E33* or *TBX3-E2a* minigene reporters for 24 hr. AS patterns of *FN* or *TBX3* were assessed as in Figure 1A.

(C) HaCaT cells were transfected with 25 nM of control or CSB siRNA oligonucleotides for 72 hr. Cells were irradiated with 15 J/m² UV, and AS of the indicated endogenous genes was assayed as described in Figure 1.

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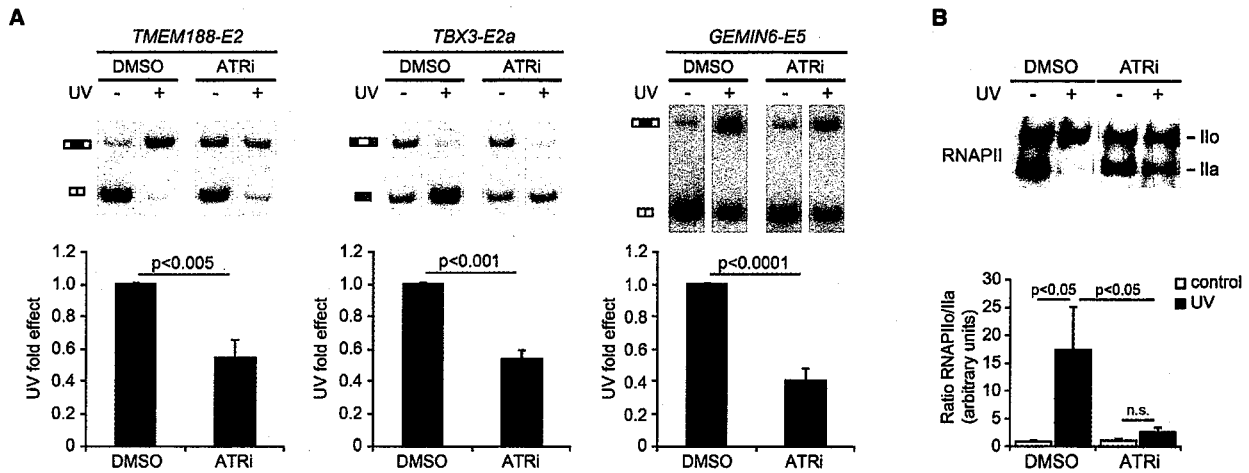


Figure 4. ATR Modulates AS Patterns and RNAPII Phosphorylation in Response to UV Light

(A) HaCaT cells were pre-treated with 10 μ M ATR inhibitor ETP-46464 (ATRi) 1 hr prior to UV irradiation (15 J/m²). After 6 hr, AS of the indicated endogenous genes was assayed as described.

(B) RNAPII phosphorylation status (phospho RNAPII [Ilo] to non-phospho RNAPII [Ila] ratio) in HaCaT cells pre-treated with ATRi was analyzed 2 hr after UV irradiation with 15 J/m². Images of a representative experiment and mean, SEM, and p values (Student's t test) of three experiments are shown. See also Figure S5.

in contrast to what has been reported by Tresini and co-workers in fibroblasts. Taken together, these results demonstrate the differences in the mechanisms controlling gene expression in different cell types and highlight the importance of using the most appropriate model when analyzing the effects of a natural carcinogen as UV light.

We then investigated the involvement of other systems capable of interacting with DNA lesions, such as DNA replication. To evaluate the role of an active DNA polymerase as a sensor of damaged DNA, we sorted control and UV-treated HaCaT cells, based on their DNA content, to analyze AS patterns in G1, S, and G2/M populations. The UV effect proved to be of comparable magnitude in all cell cycle phases (Figure S4D), with no particular increase in S phase, which suggests that the replisome is not a main sensor for the UV effect on AS.

Finally, and having in mind that GG-NER, but not TC-NER, is the main system in charge of DNA repair in keratinocytes (D'Errico et al., 2007), we reasoned that a reduced CPD recognition would elicit a reduced UV effect on AS. Using CRISPR-Cas9 technology, we found that ablation of XPE, the main factor in charge of CPD recognition, partially decreased the UV effect on AS (Figures S4E and S4F), strongly suggesting a role for DNA repair in the control of gene expression in skin cells.

Results so far suggest that, in keratinocytes, where GG-NER prevails, XPE, but not RNAPII, acts as one of the lesion sensors for the AS response.

ATR Modulates the Alternative Splicing Response to CPDs

In view of results in Figure 3, given that the UV effect is of comparable magnitude in G1, S, and G2/M (Figure S4D), the fact that NER is active throughout the cell cycle (Sancar et al., 2004) and the decreased UV effect on AS in XPE-null cells (Figure S4F), we thought that the repair system itself could be responsible for damage sensing and signaling. A key event that follows NER is the activation of ATR (Hanasoge and Ljungman, 2007; Marteiijn et al., 2009; Matsumoto et al., 2007; Stiff et al., 2008; Vrouwe et al., 2011), which, in turn, can activate ATM (Stiff et al., 2006). To investigate the contribution of these kinases in the AS response to CPDs, we irradiated HaCaT cells pre-treated with ATR or ATM inhibitors, whose specificities were verified by their abilities to inhibit Chk1 or Chk2 phosphorylation, respectively (Figure S5A). For the three ASEs used as models, ATR inhibition reduced the UV effect on AS patterns (Figure 4A) by at least 50%. A less pronounced inhibition was observed when inhibiting ATM (Figure S5B). We then analyzed the roles of ATR and ATM in the UV-induced RNAPII hyperphosphorylation. Figure 4B shows that the increase in RNAPII^o/RNAPII^a ratio caused by UV is prevented by the ATR inhibitor. Such a strong role for ATR in the phosphorylation of the CTD is unprecedented and likely indirect, considering that, to date, the main Ser/Thr kinases shown to directly phosphorylate the CTD are CDK7, CDK9, CDK12, and CDK13 (Bartkowiak et al., 2010; Muñoz et al., 2010). The actual

(D) HaCaT cells or normal human fibroblasts (NHF) were transfected with the *FN-E33* reporter minigene. After 4 hr, cells were UV irradiated (15 J/m²) and harvested 24 hr later. AS patterns of *FN* were assessed by radioactive RT-PCR with specific pairs of primers for the minigene-derived mRNAs.

(E) Normal human fibroblasts were co-transfected with pGL3 or pGL3-SV40 vectors, either untreated or in vitro irradiated, together with the *FN-E33* minigene reporter for 24 hr. AS patterns of *FN* were assessed as described.

For all experiments, images of a representative experiment and mean, SEM, and p values (Student's t test) of three experiments are shown. See also Figure S4.

target(s) of ATR remain yet to be identified. Similar to what happened with AS regulation, ATM inhibition impaired the UV-induced hyperphosphorylation of RNAPII but to a much lesser extent (Figure S5C).

NER Links CPD Repair to ATR Activation and the AS Response in Keratinocytes

It has been shown that ATR activation is triggered by ssDNA segments generated after incision by NER nucleases to remove the damaged strand and before the participation of DNA polymerases to copy the undamaged strand (Marteijn et al., 2014). Figure 5A shows that photolyase-dependent removal of CPDs inhibits the UV-induced ATR activation, indicated by the increase in the phosphorylation levels of Chk1. To further evaluate the role of the NER pathway on ATR activation in conditions in which general DNA replication does not occur, we first obtained non-cycling HaCaT cells through a serum deprivation protocol reported by the Sancar group (Kemp and Sancar, 2016; Figure S5D). As shown in Figure 5B (compare lanes 1 and 2), UV irradiation of non-cycling HaCaT cells activates ATR as indicated by Chk1 phosphorylation. Moreover, this activation also takes place in the presence of actinomycin D, a general inhibitor of transcription (lanes 3 and 4; Figure S5E), thus favoring the idea that ATR can be activated in response to UV but in the absence of both replication and transcriptional stress. More interestingly, and in agreement with published evidence that UV-induced photolesions elicit ATR kinase-dependent signaling in non-cycling cells (Vrouwe et al., 2011), inhibition of repair synthesis by aphidicolin resulted in ATR over-activation (Figure 5C). More importantly, aphidicolin treatment greatly enhanced the UV effect on AS (Figure 5D) and increased the RNAPII α /RNAPII β ratio (Figure 5E). Unlike previous experiments where UV irradiation was performed at 15 J/m², here we used a lower dose (5 J/m²) in order to better visualize the enhancement of all three processes by aphidicolin. We propose that prevention of gap-filling to replace the damaged strand keeps ssDNA segments exposed, which then activates ATR, causing in turn changes in AS via RNAPII phosphorylation.

DISCUSSION

DNA, RNA, proteins, and lipids, as well as many other molecules within the cell, can function as UV light receptors, as they absorb UV radiation and produce signals in response to it. Whereas the global consequences of skin exposure to sunlight are clear, ranging from inflammation, erythema, or photoaging to skin cancer, the molecular pathways triggering such responses are only partially understood.

In the present work, we show that, upon UV irradiation, DNA damage is not only necessary but sufficient to modulate AS in human keratinocytes. By transfecting in-vitro-damaged plasmid DNA, we have recapitulated the UV effect on AS. It is worth noting that, unlike plasmid DNA, transfection of irradiated small double-stranded oligo-deoxynucleotides (20 mers) into HaCaT cells had no effect on AS (data not shown). We attribute this failure to the reported evidence that short double-stranded DNA is not a good substrate for NER (Kulaksiz et al., 2005; Shell et al., 2013), the pathway we report here as responsible for the effects

of UV light on AS. Our results rule out roles for RNA, protein, or lipid damage in the AS response. This does not imply that RNA damage, for example, is exempt from playing other roles, like the reported stimulation of the production of the inflammatory cytokines tumor necrosis factor α (TNF- α) and interleukin-6 (IL-6) (Bernard et al., 2012), which in turn modulate gene expression patterns in a skin system.

Using a photoactivable marsupial photolyase in placental mammalian (human) skin cells, we demonstrate that the AS and RNAPII responses are triggered by the CPD photoproducts generated upon UV irradiation. According to their functional category or gene ontology terms, the RNA-seq analysis revealed more than 170 genes whose AS patterns are affected by UV and reverted by the CPD photolyase by at least 50% are involved in apoptosis, cell cycle, or DNA damage (Table S2). This confirms the functional relevance of the AS response to UV, studied in more depth in our previous report (Muñoz et al., 2009). The photolyase also reverted RNAPII CTD hyperphosphorylation (Figure 2A), strengthening our previous evidence that this event mediates the change in AS. On the contrary, under the experimental conditions used in this work, we did not observe changes in RNAPII phospho status, nor in the AS events analyzed herein, when expressing the (6-4)PP photolyase. A possible explanation is that CPDs are heavily induced, whereas (6-4)PPs are only mildly formed, upon UV irradiation. In any case, we cannot rule out a role for (6-4)PPs in the AS response to UV light.

RNA-seq analysis showed that CPDs are the main cause of the UV effect on AS because 87% of the ASEs affected by UV showed total or partial reversion with the photolyase. UV treatment increased inclusion of 78% and skipping of 22% of cassette exons, the prevalent modality of AS in mammalian keratinocytes (Figure S1C). Abrogation of CPDs by the photolyase reverts both types of changes but, most importantly, when gene expression and AS analyses are combined, it becomes clear that UV regulation of both “up” and “down” cassette exons (Figure 2C) and photolyase reversion (Figure 2D) occur in genes whose expression is reduced by UV. We interpret this as an indicator that CPDs are acting through the modulation of RNAPII elongation rates because most reversions occur when there are changes in expression levels.

Three different experiments support the conclusion that, in contrast to what was reported for fibroblasts (Tresini et al., 2015), in keratinocytes, the cell type normally exposed to sunlight, RNAPII is the target, but not the sensor, of the UV effect. First, the effects of in-vitro-damaged plasmid DNA on AS were identical whether the plasmid sequences were being transcribed or not (Figure 3B). Second, in this case, in agreement with evidence provided by Tresini et al. (2015), depletion of the TC-NER factor CSB had no influence on the AS response to UV irradiation (Figures 3C and S4C). Third, and unlike to what happened in keratinocytes, the use of an in-vitro-irradiated plasmid carrying a strong RNAPII promoter, but not the same vector without the promoter, mimicked the UV effect on AS in fibroblasts (Figure 3E), confirming the role of RNAPII as a sensor of damage in this cell type. This experiment highlights differences in the mechanism controlling gene expression between keratinocytes, the most common cell type naturally affected

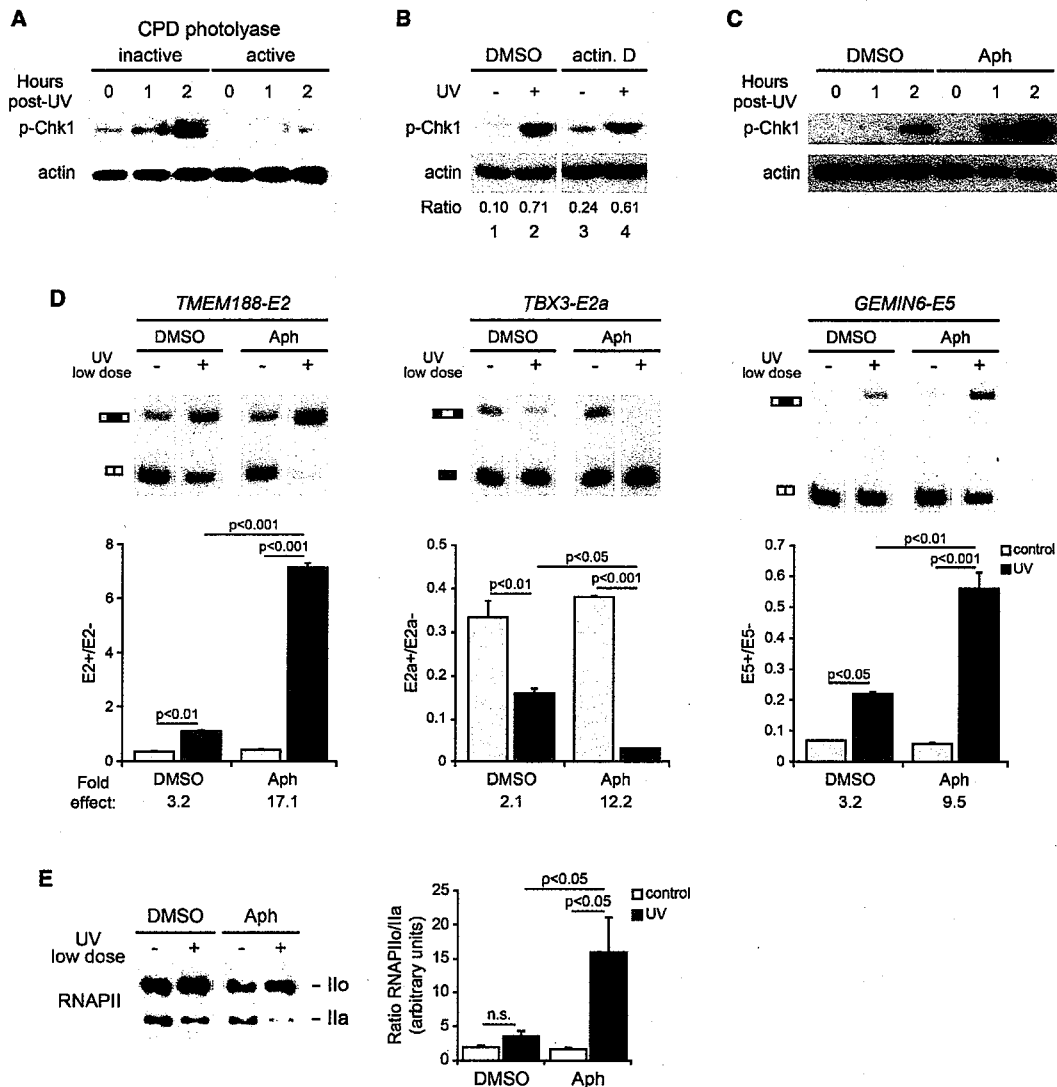


Figure 5. Inhibition of DNA Gap-Filling Enhances the UV-Induced RNAPII Hyperphosphorylation and AS Regulation

(A) Levels of phosphorylated Chk1 (Ser317) were analyzed in CPD photolyase-transduced HaCaT cells at the indicated times after UV irradiation (15 J/m²) followed by 1 hr of photolyase photoactivation.

(B) Non-cycling HaCaT cells were pre-treated with 5 ng/μL actinomycin D (actin. D) for 4 hr prior to UV irradiation (15 J/m²). Two hours later, the levels of phosphorylated Chk1 (Ser317) were analyzed by western blot. Numbers represent the p-Chk1 to actin ratio.

(C–E) Non-cycling HaCaT cells were treated with 5 μg/mL aphidicolin (Aph) for 1 hr prior to low UV dose irradiation (5 J/m²). (C) Two hours later, the levels of phosphorylated Chk1 (Ser317) were analyzed by western blot. (D) AS patterns of the indicated endogenous genes were determined as described. Mean, SEM, and p values of a representative experiment are shown. (E) Two hours after UV treatment, the phospho RNAPII (Ilo) to non-phospho RNAPII (Ila) ratio was determined by western blot. Images of a representative experiment and mean, SEM, and p values (Student's t test) of two experiments are shown. See also Figure S5.

by sunlight, and fibroblasts. We would like to point out that many experiments in the published literature were performed using patient-derived fibroblasts with defects in repair mechanisms (TC-NER, GG-NER, etc.). In view of results in this work, some of the observations derived from fibroblasts-based experiments would need revision when drawing conclusions about the effects of UV light in the skin.

A lack of role for TC-NER in our cell system coincides with evidence that GG-NER prevails in keratinocytes (D'Errico et al., 2005, 2007; Mouret et al., 2008). In any case, both mechanisms repair CPDs throughout the cell cycle and involve the generation of ssDNA, which was shown to activate the PI(3)-like kinase ATR (Hanasoge and Ljungman, 2007; Martijn et al., 2009; Matsumoto et al., 2007; Stiff et al., 2008; Vrouwe et al., 2011). Using

a specific inhibitor, we show here that ATR participates in the CPD signaling both at the levels of RNAPII phosphorylation (Figure 4B) and AS (Figure 4A). In agreement with these results, and using the photolyase strategy, we showed that CPDs lead to activation of the ATR protein kinase (Figure 5A) independently of replication or transcriptional stress (Figure 5B). Because CPD repair by NER generates ssDNA intermediates that activate ATR, we focused on the inhibition of the last step in NER and found that inhibition of the gap-filling DNA synthesis by aphidicolin increased the UV effect on AS, RNAPII phosphorylation, and ATR activation (Figures 5C–5E).

We believe that ATR promotes RNAPII CTD phosphorylation indirectly because there are no recognizable ATR target sequences in the CTD. We cannot rule out effects of ATR at the level of phosphorylation of other proteins, apart from RNAPII, such as splicing factors (Matsuoka et al., 2007). These results unveil a new role for ATR in the control of gene expression at the transcription and splicing levels, with the signaling cascade UV-PD-ATR-RNAPII-AS as the most likely scenario. Moreover, if ATR activation by any means was similar, then the effect in S phase (in which ATR can be activated not only by NER but also by a stalled DNA polymerase) should be different than the effect in G1 or G2/M. Because results in Figure S4D showed that the UV effect on AS was similar in all cell cycles phases, we speculate that different modes of ATR activation, i.e., replication dependent or independent, would activate ATR in a different way, thus affecting different substrates or pathways. In any case, more research is needed to deeply understand the modes and consequences of ATR activation.

Tresini et al. (2015) reported that UV modulates AS in fibroblasts through an RNAPII and R-loop-dependent mechanism that activates ATM. In our keratinocyte model, we observed that ATM modulates RNAPII phosphorylation and AS (Figures S5B and S5C) but to a lesser extent than ATR. Despite this, it is unlikely that the role of ATM in the control of AS in keratinocytes can be explained by the mechanism proposed by Tresini et al. Results in Figure 3 demonstrate not only that RNAPII is not the lesion sensor but also that the mechanisms acting in fibroblasts and keratinocytes to modulate gene expression in a genotoxic scenario are different. A possible explanation for ATM's participation is its crosstalk with ATR, as it has been shown that, upon UV, ATR can activate ATM (Stiff et al., 2006). Finally, and in good agreement with the above-proposed mechanism, we found that ablation of GG-NER and CPD recognition factor XPE decreased the UV effect on AS. In view of this, it is tempting to speculate that repair-deficient cells from xeroderma pigmentosum patients would exhibit gene expression defects.

In summary, we described a pathway connecting UV-induced DNA lesions and their repair to ATR activation. This work highlights the importance of ATR in the control of gene expression programs in a genotoxic scenario throughout the cell cycle.

EXPERIMENTAL PROCEDURES

Cell Culture and Treatments

HaCaT cells and normal human fibroblasts (NHf) were cultured as indicated by ATCC. For experiments performed with non-cycling cells, HaCaT cells were grown to confluence and further incubated for 3 days in low serum (0.5% fetal

bovine serum [FBS]) medium as described (Kemp and Sancar, 2016). When indicated, cells were pre-incubated for 1 hr before UV irradiation with the following drugs: ATR inhibitor 10 μ M (ETP-46464; CNIO); aphidicolin 5 μ g/mL (Sigma); or for 4 hr with actinomycin D 5 ng/ μ L (Sigma).

UV Irradiation

Cells were washed once with PBS prior to UVC (254 nm) irradiation. UV irradiation was performed with a CL-1000 Shortwave Crosslinker (UVP) with an emission peak at 254 nm. The doses were quantified by its internal sensor. Unless otherwise indicated, the UV dose was 15 J/m² whereas low UV dose was 5 J/m². For in vitro irradiation of plasmids, drops of no more than 20 μ L were deposited on Parafilm and irradiated with 1,500 J/m² of UV.

DNA Western Dot Blot

Cells were harvested and total DNA was extracted with QIAmp DNA Mini Kit (QIAGEN) according to the manufacturer's protocol. DNA was quantified, denatured, and equal amounts were blotted on a nitrocellulose Hybond N+ membrane (GE Healthcare). DNA western dot blot was performed according to Perdiz et al. (2000), with minor changes. To assess the amount of CPDs, (6-4)PPs, and ssDNA, membranes were incubated with appropriate dilutions of anti-CPD or anti-(6-4)PP antibodies (TDM-2 and 64M-2; Cosmo Bio) and anti-ssDNA (MAB3034; Millipore). Signal was detected using the LI-COR Biosciences Odyssey Imager with the appropriate secondary antibodies and quantified with the ImageStudio software (LI-COR Biosciences).

Transfections

Transfection of siRNAs and AS reporter minigenes was performed using Lipofectamine 2000 (Thermo Scientific) according to manufacturer's instructions and as described in Muñoz et al. (2009). siRNAs were purchased from GE-Dharmacon: SMARTpool siGENOME ERCC6 (CSB; cat. no. M-004888) and siNegative-Control no. 2 (cat. no. D-001206-14).

AS Reporter Minigenes

FN-E33 reporter minigene was previously described (de la Mata and Kornblihtt, 2006). *TBX3-E2a* minigene was obtained by cloning a DNA fragment containing the alternative exon E2a, its flanking introns, and part of the upstream and downstream exons into the BstEII sites of the pUHC-*CFTR* minigene (Dujardin et al., 2014). The *FN-E33* and *TBX3-E2a* reporter minigenes were co-transfected with a plasmid expressing the tetracycline-controlled transactivator (tTA)-VP16. Transcription of the minigenes was allowed by removal of tetracycline following UV irradiation.

RNA Extraction and Radioactive RT-PCR Analysis

RNA was purified using TriPure reagent (Roche Life Science). Conditions and primers for radioactive RT-PCR of endogenous or reporter minigenes are described in the Supplemental Experimental Procedures.

Adenoviral Photolyase Transduction and Photoactivation

HaCaT cells plated on 12-well dishes were transduced with adenoviruses expressing the CPD photolyase from *Potorous tridactylus* or the (6-4)PP photolyase from *Arabidopsis thaliana* (de Lima-Bessa et al., 2008) at a MOI of 15,000 in 260 μ L of DMEM with 2% FBS. After 2 hr, 0.5 mL of growth medium were added and cells were additionally incubated for 72 hr. The expression of the photolyases was indirectly monitored by examining EGFP expression through fluorescence microscopy. For photoactivation, cells were washed with PBS, UV irradiated, and covered with 0.5 mL of DMEM without phenol red, with 10% FBS. Dishes were placed on a 4-mm-thick glass placed above a fluorescent lamp (GE FCL 22W/D Daylight 22W 12K). Companion dishes were wrapped in aluminum foil and treated similarly.

Global Transcriptomic Analysis of Alternative Splicing

HaCaT cells transduced with CPD photolyase-expressing adenovirus were left untreated (no UV) or UV irradiated. Irradiated cells were then exposed to white light to activate the photolyase (UV, active PhL) or kept in the dark (UV, inactive PhL). Cells were harvested 6 hr after photoactivation, and total RNA was extracted from two biological replicates of each condition, prepared using

the Illumina TruSeq mRNA kit, and sequenced on an Illumina HiSeq2000. For details, see Supplemental Experimental Procedures.

Luciferase Assay

Cells were transfected with 250 ng of pGL3 vectors (Promega). Twenty-four hours after transfection, luciferase activity was measured using the Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

ACCESSION NUMBERS

The accession number for the RNA-seq data reported in this paper is GEO: GSE85510.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2017.02.066>.

AUTHOR CONTRIBUTIONS

M.J.M., L.E.G., and N.N.M. designed and performed most experiments. A.E.C.B., G.D., G.B., and S.L. performed some experiments. M.I., A.T.-M., and B.J.B. performed the bioinformatics analysis of the RNA-seq experiments. C.F.M.M. participated in the design of the photolyase experiments. M.F. critically read the manuscript and contributed with scientific advice. M.J.M. and A.R.K. wrote the manuscript and supervised the whole work.

ACKNOWLEDGMENTS

We thank F. Coin, V. Gottifredi, and G. Soria for useful discussions and reagents as well as members of the lab, especially V. Buggiano and A. Srebrow, for their help and support. We also thank E. Cafferata for his invaluable help with the adenovirus purification and D. González Maglio for HaCaT cells. A.R.K. and M.J.M. received support from the Agencia Nacional de Promoción Científica y Tecnológica de Argentina (PICT-2011 1617, PICT-2014 2582), the Universidad de Buenos Aires (UBACYT 20020130100152BA), and the Alberto J. Roemmers Foundation. G.D. was supported by Marie Curie International Outgoing Fellowship within the EU Seventh Framework Programme for Research and Technological Development (FP7/2007-2013) under grant agreement 275632. B.J.B. is supported by grants from the Canadian Institutes of Health Research (FDN 148434). M.I. is supported by grants from the European Research Council (ERC-StG-LS2- 637591) and from Ministerio de Economía y Competitividad (BFU2014-55076-P; MINECO). A.T.-M. is supported by an FPI-SO fellowship from the Spanish Ministry of Economy and Competitiveness. M.F. is supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC) and Telethon-Italy. G.B. is recipient of a fellowship from the University of Milan. S.L. is an employee of IFOM Cell Biology Unit. M.J.M., L.E.G., and A.R.K. are career investigators and N.N.M. and A.E.C.B. are recipients of a graduate student fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (CONICET). A.R.K. is a Senior International Research Scholar of the Howard Hughes Medical Institute.

Received: November 14, 2016

Revised: January 11, 2017

Accepted: February 21, 2017

Published: March 21, 2017

REFERENCES

Anindya, R., Mari, P.-O., Kristensen, U., Kool, H., Giglia-Mari, G., Mullenders, L.H., Fouteri, M., Vermeulen, W., Egly, J.-M., and Svejstrup, J.Q. (2010). A ubiquitin-binding domain in Cockayne syndrome B required for transcription-coupled nucleotide excision repair. *Mol. Cell* 38, 637–648.

Barash, Y., Calarco, J.A., Gao, W., Pan, Q., Wang, X., Shai, O., Blencowe, B.J., and Frey, B.J. (2010). Deciphering the splicing code. *Nature* 465, 53–59.

Bartkowiak, B., Liu, P., Phatnani, H.P., Fuda, N.J., Cooper, J.J., Price, D.H., Adelman, K., Lis, J.T., and Greenleaf, A.L. (2010). CDK12 is a transcription elongation-associated CTD kinase, the metazoan ortholog of yeast Ctk1. *Genes Dev.* 24, 2303–2316.

Bernard, J.J., Cowing-Zitron, C., Nakatsuji, T., Muehleisen, B., Muto, J., Borkowski, A.W., Martinez, L., Greidinger, E.L., Yu, B.D., and Gallo, R.L. (2012). Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. *Nat. Med.* 18, 1286–1290.

Besaratinia, A., Yoon, J.-I., Schroeder, C., Bradforth, S.E., Cockburn, M., and Pfeifer, G.P. (2011). Wavelength dependence of ultraviolet radiation-induced DNA damage as determined by laser irradiation suggests that cyclobutane pyrimidine dimers are the principal DNA lesions produced by terrestrial sunlight. *FASEB J.* 25, 3079–3091.

Boros, G., Miko, E., Muramatsu, H., Weissman, D., Emri, E., van der Horst, G.T., Szegedi, A., Horkay, I., Emri, G., Karikó, K., and Remenyik, É. (2015). Identification of cyclobutane pyrimidine dimer-responsive genes using UVB-irradiated human keratinocytes transfected with in vitro-synthesized photolyase mRNA. *PLoS ONE* 10, e0131141.

D'Errico, M., Teson, M., Calcagnile, A., Nardo, T., De Luca, N., Lazzari, C., Soddu, S., Zambruno, G., Stefanini, M., and Dogliotti, E. (2005). Differential role of transcription-coupled repair in UVB-induced response of human fibroblasts and keratinocytes. *Cancer Res.* 65, 432–438.

D'Errico, M., Lemma, T., Calcagnile, A., Proietti De Santis, L., and Dogliotti, E. (2007). Cell type and DNA damage specific response of human skin cells to environmental agents. *Mutat. Res.* 614, 37–47.

de la Mata, M., and Kornblihtt, A.R. (2006). RNA polymerase II C-terminal domain mediates regulation of alternative splicing by SRp20. *Nat. Struct. Mol. Biol.* 13, 973–980.

de la Mata, M., Alonso, C.R., Kadener, S., Fededa, J.P., Blaustein, M., Peisch, F., Cramer, P., Bentley, D., and Kornblihtt, A.R. (2003). A slow RNA polymerase II affects alternative splicing in vivo. *Mol. Cell* 12, 525–532.

de Lima-Bessa, K.M., Armelini, M.G., Chiganças, V., Jacysyn, J.F., Amarante-Mendes, G.P., Sarasin, A., and Menck, C.F. (2008). CPDs and 6-4PPs play different roles in UV-induced cell death in normal and NER-deficient human cells. *DNA Repair (Amst.)* 7, 303–312.

Dujardin, G., Lafaille, C., de la Mata, M., Marasco, L.E., Muñoz, M.J., Le Jossic-Corcos, C., Corcos, L., and Kornblihtt, A.R. (2014). How slow RNA polymerase II elongation favors alternative exon skipping. *Mol. Cell* 54, 683–690.

Ellis, J.D., Barrios-Rodiles, M., Colak, R., Irimia, M., Kim, T., Calarco, J.A., Wang, X., Pan, Q., O'Hanion, D., Kim, P.M., et al. (2012). Tissue-specific alternative splicing remodels protein-protein interaction networks. *Mol. Cell* 46, 884–892.

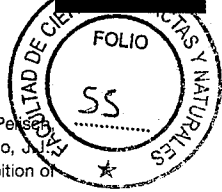
Fong, N., Kim, H., Zhou, Y., Ji, X., Qiu, J., Saldi, T., Diener, K., Jones, K., Fu, X.D., and Bentley, D.L. (2014). Pre-mRNA splicing is facilitated by an optimal RNA polymerase II elongation rate. *Genes Dev.* 28, 2663–2676.

Garcin, G., Douki, T., Stoebner, P.E., Guesnet, J., Guezennec, A., Martinez, J., Cadet, J., and Meunier, L. (2007). Cell surface expression of melanocortin-1 receptor on HaCaT keratinocytes and alpha-melanocortin stimulation do not affect the formation and repair of UVB-induced DNA photoproducts. *Photochem. Photobiol. Sci.* 6, 585–593.

Gracheva, E.O., Cordero-Morales, J.F., González-Carcacia, J.A., Ingolia, N.T., Manno, C., Aranguren, C.I., Weissman, J.S., and Julius, D. (2011). Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats. *Nature* 476, 88–91.

Hanasoge, S., and Ljungman, M. (2007). H2AX phosphorylation after UV irradiation is triggered by DNA repair intermediates and is mediated by the ATR kinase. *Carcinogenesis* 28, 2298–2304.

Hua, Y., Sahashi, K., Rigo, F., Hung, G., Horev, G., Bennett, C.F., and Krainer, A.R. (2011). Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. *Nature* 478, 123–126.



Ip, J.Y., Schmidt, D., Pan, Q., Ramani, A.K., Fraser, A.G., Odom, D.T., and Blencowe, B.J. (2011). Global impact of RNA polymerase II elongation inhibition on alternative splicing regulation. *Genome Res.* 21, 390–401.

Kemp, M.G., and Sancar, A. (2016). ATR kinase inhibition protects non-cycling cells from the lethal effects of DNA damage and transcription stress. *J. Biol. Chem.* 291, 9330–9342.

Kornblihtt, A.R., Schor, I.E., Alló, M., Dujardin, G., Petrillo, E., and Muñoz, M.J. (2013). Alternative splicing: a pivotal step between eukaryotic transcription and translation. *Nat. Rev. Mol. Cell Biol.* 14, 153–165.

Kulaksiz, G., Reardon, J.T., and Sancar, A. (2005). Xeroderma pigmentosum complementation group E protein (XPE/DDB2): purification of various complexes of XPE and analyses of their damaged DNA binding and putative DNA repair properties. *Mol. Cell. Biol.* 25, 9784–9792.

Marteijn, J.A., Bekker-Jensen, S., Mailand, N., Lans, H., Schwertman, P., Gourdin, A.M., Dantuma, N.P., Lukas, J., and Vermeulen, W. (2009). Nucleotide excision repair-induced H2A ubiquitination is dependent on MDC1 and RNF8 and reveals a universal DNA damage response. *J. Cell Biol.* 186, 835–847.

Marteijn, J.A., Lans, H., Vermeulen, W., and Hoeijmakers, J.H. (2014). Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat. Rev. Mol. Cell Biol.* 15, 465–481.

Matsumoto, M., Yaginuma, K., Igarashi, A., Imura, M., Hasegawa, M., Iwabuchi, K., Date, T., Mori, T., Ishizaki, K., Yamashita, K., et al. (2007). Perturbed gap-filling synthesis in nucleotide excision repair causes histone H2AX phosphorylation in human quiescent cells. *J. Cell Sci.* 120, 1104–1112.

Matsuoka, S., Ballif, B.A., Smogorzewska, A., McDonald, E.R., 3rd, Hurov, K.E., Luo, J., Bakalarski, C.E., Zhao, Z., Solimini, N., Lerenthal, Y., et al. (2007). ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316, 1160–1166.

Melnikova, V.O., and Ananthaswamy, H.N. (2005). Cellular and molecular events leading to the development of skin cancer. *Mutat. Res.* 577, 91–106.

Mouret, S., Charveron, M., Favier, A., Cadet, J., and Douki, T. (2008). Differential repair of UVB-induced cyclobutane pyrimidine dimers in cultured human skin cells and whole human skin. *DNA Repair (Amst.)* 7, 704–712.

Muñoz, M.J., Pérez Santangelo, M.S., Paronetto, M.P., de la Mata, M., Peláez, F., Boireau, S., Glover-Cutter, K., Ben-Dov, C., Blaustein, M., Lozano, J.J., et al. (2009). DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. *Cell* 137, 708–720.

Muñoz, M.J., de la Mata, M., and Kornblihtt, A.R. (2010). The carboxy terminal domain of RNA polymerase II and alternative splicing. *Trends Biochem. Sci.* 35, 497–504.

Perdiz, D., Grof, P., Mezzina, M., Nikaido, O., Moustacchi, E., and Sage, E. (2000). Distribution and repair of bipyrimidine photoproducts in solar UV-irradiated mammalian cells. Possible role of Dewar photoproducts in solar mutagenesis. *J. Biol. Chem.* 275, 26732–26742.

Sancar, A., Lindsey-Boltz, L.A., Unsal-Kaçmaz, K., and Linn, S. (2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.* 73, 39–85.

Shell, S.M., Hawkins, E.K., Tsai, M.S., Hlaing, A.S., Rizzo, C.J., and Chazin, W.J. (2013). Xeroderma pigmentosum complementation group C protein (XPC) serves as a general sensor of damaged DNA. *DNA Repair (Amst.)* 12, 947–953.

Srebrow, A., and Kornblihtt, A.R. (2006). The connection between splicing and cancer. *J. Cell Sci.* 119, 2635–2641.

Stiff, T., Cerosaletti, K., Concannon, P., O'Driscoll, M., and Jeggo, P.A. (2008). Replication independent ATR signalling leads to G2/M arrest requiring Nbs1, 53BP1 and MDC1. *Hum. Mol. Genet.* 17, 3247–3253.

Stiff, T., Walker, S.A., Cerosaletti, K., Goodarzi, A.A., Petermann, E., Concannon, P., O'Driscoll, M., and Jeggo, P.A. (2006). ATR-dependent phosphorylation and activation of ATM in response to UV treatment or replication fork stalling. *EMBO J.* 25, 5775–5782.

Tresini, M., Warmerdam, D.O., Kolovos, P., Snijder, L., Vrouwe, M.G., Demmers, J.A., van IJcken, W.F., Grosveld, F.G., Medema, R.H., Hoeijmakers, J.H., et al. (2015). The core spliceosome as target and effector of non-canonical ATM signalling. *Nature* 523, 53–58.

Vrouwe, M.G., Pines, A., Overmeer, R.M., Hanada, K., and Mullenders, L.H. (2011). UV-induced photolesions elicit ATR-kinase-dependent signaling in non-cycling cells through nucleotide excision repair-dependent and -independent pathways. *J. Cell Sci.* 124, 435–446.

WORLD VIEW

A personal take on events

OLIVER KORNBLIHT



Where science and nonsense collide

After a decade of progress, Argentina's scientists are battling a government bent on twisting public conceptions of their role, writes **Alberto Kornbliht**.

The government of President Mauricio Macri in Argentina marked its first birthday last month, but there is little to celebrate for scientists.

Unrest in the country made headlines around the world last month when thousands of investigators, graduate students and postdocs occupied the science ministry for five days. That protest ended with palliative concessions from the authorities — the offer of 500 postdoctoral fellowships to those who should have been granted junior-investigator positions — but the problems run much deeper.

Macri is the son of a powerful industrialist and a former business partner of US president-elect Donald Trump in property development. The Panama papers, a huge cache of tax files leaked last April, showed that he (and his father and siblings) owned several offshore companies. He is bluntly applying the counter-Keynesian plan of opening the economy, reducing the role of the state, increasing foreign debt and creating unemployment to reduce the cost of wages. And — despite his campaign promises to invest — the latest national budget bill pushed by Macri and approved by the Congress for 2017 cut funds for science and technology by 30%.

These brutal cuts are set to reverse a decade of solid investment and progress in Argentinian science. Under previous governments, more than 1,300 young investigators returned to the country and two homemade communications satellites were put into orbit. These administrations also created a ministry for science, technology and productive innovation and built 150,000 square metres of research institutes to house the growing numbers of investigators, graduate students, postdocs and technicians working for the National Council for Scientific and Technological Research (CONICET), the flagship national agency.

The most conspicuous evidence of the current government's approach has been the threat to reduce the number of young-investigator positions funded by CONICET; it was this that prompted the sit-in protest and it has been only partly addressed.

At the same time, communications satellite programmes have been suspended, and inflation and currency devaluation have reduced the buying power of salaries and research grants.

Scientists in Argentina fear a repeat of the brain drains that the country experienced during times of military rule and economic crisis. Already, colleagues report that young Argentinian scientists in Europe and the United States are having second thoughts about coming home.

The arguments used by ministers and officials to justify the budget cuts are disingenuous and fallacious. Widespread poverty in Argentina, we are now told, makes it unfair and unethical to divert the same amount of money as before to science. (As if poverty did not exist before!) Unlike some countries, Argentina owes its structural poverty

not to limited natural or human resources, but rather to a perversely uneven distribution of wealth and a regressive tax system. It seems unfair to punish scientists for such a system, particularly given that President Macri has eliminated export taxes for agriculture and mining, perhaps the two most profitable industries in the country.

It gets worse. Officials have produced a series of provocative statements that threaten the accepted social values of science, research and scholarly pursuits. These statements include: "Investigators should be evaluated by the number of jobs they create and not by the number of papers they publish"; "Each PhD should be encouraged to create his/her own company"; and "CONICET scientists are merely 'paper publishers' who do not return to the society useful applications". One statement even reads: "Young scientists must leave

the country", without offering a concomitant government programme to support foreign postdoctoral training.

With such statements, the government is trying to exploit conflict between basic and applied research to sow public distrust of scientists and their work, and to attack the social sciences. This failure to distinguish between the generation of knowledge and the generation of technology is not innocent, and it creates false conceptions about the role of science in society.

Argentina's scientists are proud of their nation's contributions: a strong, fee-less, public university network; two Nobel laureates in science who made their discoveries in Argentina; and seven foreign associates of the US National Academy

of Sciences. To call such scientists "paper publishers" is offensive, as if scientific papers were the final aim of research rather than the means through which to make relevant findings public.

To complete the landscape of nonsense, the chief of the cabinet of ministers, Marcos Peña, attacked one of the fundamentals of science by saying that "critical thinking has done too much damage to our country". He continued: "Some people in Argentina think that being critical is being smart. Our government believes that being smart is being enthusiastic and optimistic."

This is gobbledygook, yet it neatly fits the New Age concept of the "revolution of happiness" proclaimed by Macri as a lubricant for social conflicts. Colleagues around the world should know that, in this new Argentina, science and technology could become dispensable. More demonstrations are sure to follow. We will not give up our scientific heritage and future without a fight. ■

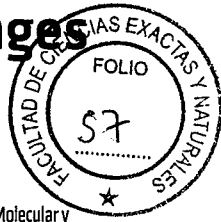
THE ARGUMENTS
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Epigenetics at the base of alternative splicing changes that promote colorectal cancer

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(1. AUTHOR: Please confirm that all author names are correct and complete.)

Chromatin modification influences gene expression by either repressing or activating genes, depending on the specific histone mark. Chromatin structure can also influence alternative splicing of transcripts; however, the mechanisms by which epigenetic marks influence splicing are poorly understood. A report in the current issue of the *JCI* highlights the biological importance of the coordinated control of alternative pre-mRNA splicing by chromatin structure and transcriptional elongation. Yuan et al. found that mutation of the histone methyl transferase *SEDT2* affects alternative splicing fates of several key regulatory genes, including those involved in Wnt signaling. As a consequence, loss of *SEDT2* in the intestine aggravated Wnt/ β -catenin signaling effects, thereby leading to colorectal cancer.

One gene, multiple products

Human genes, as well as those of most multicellular eukaryotes, are organized as mosaics of exons and introns. RNA polymerase II (RNAPII) transcribes the DNA sequence of each gene to generate a precursor mRNA molecule (pre-mRNA) that contains both exons and introns and serves as the substrate for splicing, the process by which introns are removed and exons are joined, yielding the mature mRNA that will eventually be translated to protein. One of the evolutionary advantages of pre-mRNA splicing is the ability to alter mature mRNA via alternative splicing, which allows generation of multiple mRNA and protein variants from each gene, thereby greatly expanding the coding capacity of the genome. Indeed, the number of protein-coding genes in humans is not radically different from that in the worm *Caenorhabditis elegans* (~20,000). However, alternative splicing is prevalent in vertebrates (1), strongly suggesting a key role in the higher complexity of these organisms. As a compelling example, alternative splicing occurs

in more than 95% of mammalian genes (2). In alternative splicing, a single type of pre-mRNA can be spliced in different ways, leading to similar but not identical mature mRNA species. A simple metaphor is that of a textile industry (transcription) that generates many copies of a fabric piece of the same length and quality (pre-mRNAs) and the tailor (the spliceosome) who makes different suits (mRNAs) with each piece of fabric, depending on where he/she cuts and sews (splicing) and on the remnants (introns) that are discarded.

Splicing is carried out by the spliceosome, a multimolecular complex composed of dozens of proteins and ribonucleoproteins. A single spliceosome assembles at every intron to be excised on each pre-mRNA as soon as it emerges from RNAPII during transcription (3). Indeed, accumulating evidence indicates that splicing, or at least the binding of spliceosome and splicing factors to the pre-mRNA that enables it, occurs cotranscriptionally (4). In turn, this cotranscriptionality allows for a complex molecular interplay between the transcrip-

tion and splicing machineries so that both processes are mutually coordinated and the kinetics and molecular mechanisms intrinsic to each of them influence each other. As a consequence of this coupling, splicing, like transcription, is highly regulated by chromatin structure and the quality and distribution of posttranslational histone modifications (histone marks).

Two models

Two nonmutually exclusive models have been proposed for the role of chromatin in alternative splicing. In the transcription kinetics model, the preferential positioning of nucleosomes in exons (5, 6) or deployment of specific histone marks within the gene body hinder or facilitate RNAPII progression. Changes in overall elongation rates of RNAPII have been shown to promote or inhibit the inclusion of alternative exons in the mature mRNA, depending on the identity of the particular alternative splicing event (7–9). Intragenic histone modifications, including H2B monoubiquitylation (10), H3K9 acetylation and methylation (11, 12), and H3K4 trimethylation (13), that affect nucleosome organization have been shown to correlate with changes in elongation. Independently of their role in elongation, certain histone modifications, such as H3K79me1, H2BK5me1, H3K27me1, H3K27me2, and H3K27me3, are more abundant throughout internal exons compared with introns and clearly correlate to exon expression (see ref. 14 for a review). In the second model, histone marks are thought to affect alternative splicing by recruiting splicing factors through chromatin-binding proteins (15). For example, H3K36me3 has been shown to recruit MRG15, a chromatin-containing protein that is part of several histone-modifying complexes. MRG15, in turn, drafts the splicing factor polypyrimidine tract binding protein 1 (PTB) (2. AUTHOR: Definition added

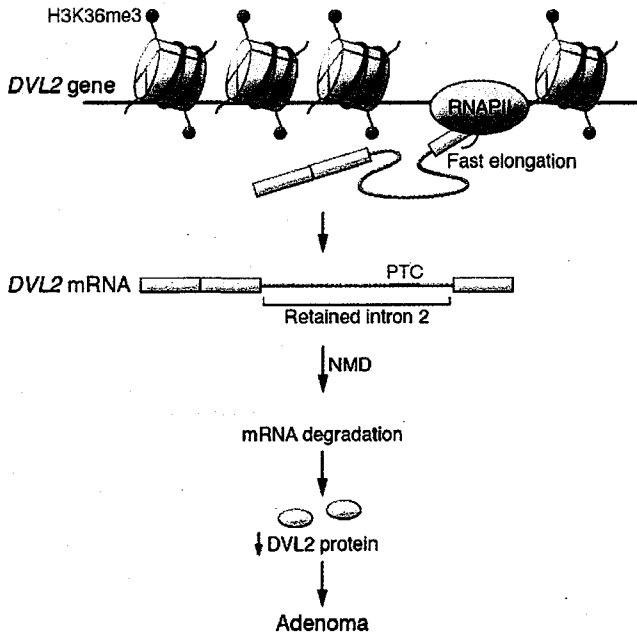
► Related Article: <https://doi.org/10.1172/JCI94292>

Conflict of interest: The author has declared that no conflict of interest exists.

Reference information: *J Clin Invest.* 2017;127(9):xxxx–yyyy. <https://doi.org/10.1172/JCI96497>.



A Intestinal cell with *Apc* mutated



B Intestinal cell with *Apc* and *SEDT2* mutated

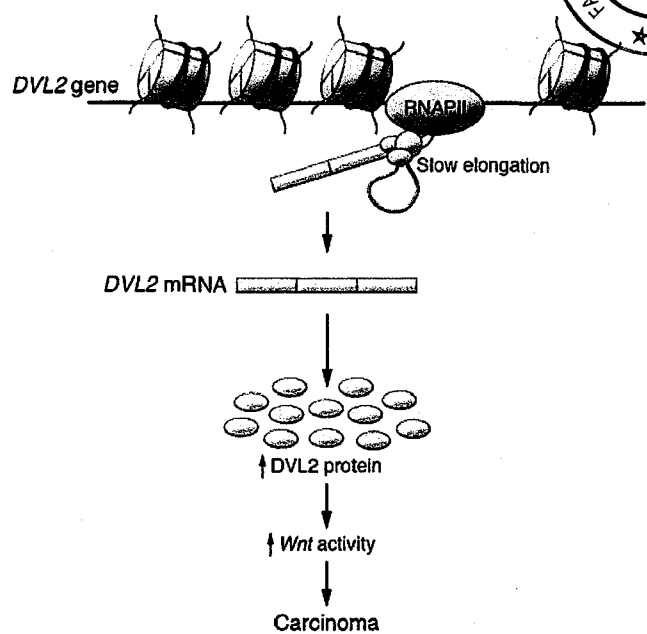


Figure 1. Model for the role of *SEDT2* in CRC. Cells in which only the *Apc* gene is mutated (**A**) develop adenoma, but have normal levels of H3K36 trimethylation. In the case of the *DVL2* gene, normal H3K36me3 levels cause retention of intron 2 in the mature mRNA, promoting degradation of this transcript by NMD as the result of the presence of a premature termination codon (PTC) in intron 2. Subsequent mutation of the *SEDT2* gene (**B**) globally decreases H3K36 trimethylation and reduces intron 2 retention in the *DVL2* mRNA. Higher levels of DVL2 protein aggravate Wnt/ β -catenin-dependent carcinoma progression.

correctly²), which controls alternative splicing decisions (16). There are many additional examples in the recent literature of histone modification–splicing factor associations that affect alternative splicing and evidence of a reverse process through which splicing factors and the splicing reaction itself participate in the writing and stabilization of histone marks that affect chromatin structure (14, 15).

Colorectal cancer

Despite an overwhelming accumulation of evidence, there are two persistent questions about the role of chromatin in alternative splicing. One, do changes in chromatin only correlate with changes in splicing, or is there a clear cause and effect relationship between chromatin structure and splicing? Two, most importantly, what are the physiopathological roles of such a complex coupling mechanism? In this issue, Yuan and colleagues (17) present compelling evidence that answers both questions. Specifically, the authors focus on the histone H3K36 methyltransferase SETD2, which has been shown to be mutated in a variety of human tumors. In general, H3K36 methylation is a permissive mark that pro-

motes gene transcription, while repressive marks, such as H3K9me and H3K27me, inhibit transcription. Yuan et al. showed that *SEDT2* expression and H3K36 methylation are both downregulated in human colorectal cancer (CRC) tissues compared with normal counterparts. Reduction of *SEDT2* expression in cultured cell lines promoted tumorigenesis, revealing that *SEDT2* is a tumor-suppressor gene whose role depends on the catalytic activity of the enzyme. Furthermore, intestinal cell-specific inactivation of the *Setd2* gene in an engineered mouse model surprisingly had no detectable phenotypic or viability effects, suggesting that *SEDT2* is dispensable for intestinal physiology. However, in animals harboring one mutated allele of the tumor-suppressor gene *Apc* (*Apc^{min/+}* mice), intestinal *SEDT2* ablation aggravated the adenoma-promoting role of APC deficiency, significantly shortening life span and greatly increasing the number of CRC tumors. These effects were determined to be the consequence of stimulation of Wnt-dependent transformation and stemness programs, as revealed by the up- and downregulation of the abundance of several signature mRNAs of

Wnt signal transduction pathways. Many studies would have stopped at this result and assumed that perturbation of gene homeostasis in the absence of *SEDT2* was exclusively due to changes in the transcription of key Wnt genes. However, Yuan and colleagues investigated global changes in mRNA-splicing variants and discovered more than 700 genes, many of which were syndicated as cancer associated, in the intestines of *SEDT2*-deficient animals with significant alterations in alternative splicing compared with intestines of control mice. ChIP followed by deep sequencing analyses (ChIP-seq) showed that, upon *SEDT2* depletion, there is an outstanding overlap between genes with a reduction of the H3K36me3 mark in their bodies and those with changes in alternative splicing. Moreover, a subset of these genes displayed intragenic enrichment of RNAPII at alternative splicing sites, consistent with a reduction in RNAPII elongation.

One of the prevalent alternative splicing modes regulated by *SEDT2* is intron retention. In particular, retention of intron 2 of the mRNA encoding *DVL2* decreases with *SEDT2* ablation. *DVL2* is a member of the dishevelled (*dsh*) protein family,



which is a key component of Wnt pathway signaling. This increase in DVL2's intron 2 excision is presumably a consequence of reduced H3K36me3 around intron 2, which slows RNAPII elongation. As intron 2 contains a premature termination codon of translation, the retention of this intron in DVL2 mRNA triggers degradation through a mechanism known as nonsense-mediated mRNA decay (NMD) in WT cells. Therefore, retention of intron 2 in normal cells reduces DVL2 levels, whereas removal of intron 2 in CRC cell precursors, through the epigenetic mechanism elucidated by Yuan et al. (17), increased DVL2 levels, augmenting Wnt signaling and leading to malignant transformation (Figure 1).

Conclusions

Together, the results of this study highlight the importance of the coupling among the deployment of specific histone marks, alternative splicing regulation, and cell fate. Interestingly, another histone methyl transferase, G9a, that is specific for dimethylation of H3K9 has recently been shown to participate in an alternative splicing positive loop that enhances neuron differentiation (18). It is now more evident that when studying the molecular bases of cancer, the view should not be restricted to transcriptional regulation or to epigenetic alterations at the promoter and regulatory regions of genes. Instead, the scope must be widened to fully see how epigenetic changes within gene bodies regulate splicing decisions.

Acknowledgments

(3. AUTHOR: Please verify that all funding sources are properly acknowledged and that grant numbers are provided if appropriate.)

The author is a Senior International Research Scholar of the Howard Hughes Medical Institute and his laboratory is supported by the Agencia Nacional de Promoción Científica y Tecnológica of Argentina, UBA, and the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (CONICET).

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1. Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. *Nat Rev Genet.* 2010;11(5):345-355.
2. Barash Y, et al. Deciphering the splicing code. *Nature.* 2010;465(7294):53-59.
3. Oesterreich FC, Herzel L, Straube K, Hujer K, Howard J, Neugebauer KM. Splicing of Nascent RNA Coincides with Intron Exit from RNA Polymerase II. *Cell.* 2016;165(2):372-381.
4. Tilgner H, et al. Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs. *Genome Res.* 2012;22(9):1616-1625.
5. Tilgner H, et al. Nucleosome positioning as a determinant of exon recognition. *Nat Struct Mol Biol.* 2009;16(9):996-1001.
6. Schwartz S, Meshorer E, Ast G. Chromatin organization marks exon-intron structure. *Nat Struct Mol Biol.* 2009;16(9):990-995.

7. de la Mata M, et al. A slow RNA polymerase II affects alternative splicing in vivo. *Mol Cell.* 2003;12(2):525-532.
8. Dujardin G, et al. How slow RNA polymerase II elongation favors alternative exon skipping. *Mol Cell.* 2014;54(4):683-690.
9. Fong N, et al. Pre-mRNA splicing is facilitated by an optimal RNA polymerase II elongation rate. *Genes Dev.* 2014;28(23):2663-2676.
10. Fuchs G, Hollander D, Voichek Y, Ast G, Oren M, Kornblihtt AR. Cotranscriptional histone H2B monoubiquitylation is tightly coupled with RNA polymerase II elongation rate. *Genome Res.* 2014;24(10):1572-1583.
11. Schor IE, Rascovan N, Pelisch F, Alló M, Kornblihtt AR. Neuronal cell depolarization induces intragenic chromatin modifications affecting NCAM alternative splicing. *Proc Natl Acad Sci U S A.* 2009;106(11):4325-4330.
12. Schor IE, Fiszbein A, Petrillo E, Kornblihtt AR. Intragenic epigenetic changes modulate NCAM alternative splicing in neuronal differentiation. *EMBO J.* 2013;32(16):2264-2274.
13. Sims RJ 3rd, et al. Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. *Mol Cell.* 2007;28(4):665-676.
14. Naftelberg S, Schor IE, Ast G, Kornblihtt AR. Regulation of alternative splicing through coupling with transcription and chromatin structure. *Annu Rev Biochem.* 2015;84:165-198.
15. Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T. Epigenetics in alternative pre-mRNA splicing. *Cell.* 2011;144(1):16-26.
16. Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM, Misteli T. Regulation of alternative splicing by histone modifications. *Science.* 2010;327(5968):996-1000.
17. Yuan H, et al. Histone methyltransferase SETD2 modulates alternative splicing to inhibit intestinal tumorigenesis. *J Clin Invest.* 2017;127(9):XXXX-YYYY.
18. Fiszbein A, et al. Alternative splicing of G9a regulates neuronal differentiation. *Cell Rep.* 2016;14(12):2797-2808.

Ciudad de Buenos Aires, **07 MAY 2018**

VISTO lo dispuesto en el artículo 50° del Estatuto Universitario que instituye el Año Sabático para profesores regulares de la Universidad,

CONSIDERANDO:

Que por Resolución CD N° 1841/16 se solicitó al Consejo Superior se autorice al Dr. Alberto Rodolfo Kornblihtt, Profesor Regular Titular Plenario con dedicación exclusiva del Departamento de Fisiología, Biología Molecular y Celular a hacer uso del Año Sabático,

Que por Resolución CS N° 5968/16 se aprobó dicha solicitud otorgando licencia entre el 1 de enero de 2017 y hasta el 31 de diciembre de 2017,

Que en cumplimiento con el Art. 12° de la Resolución CS N° 4518/93 el Dr. Alberto Rodolfo Kornblihtt presentó su informe de actividades,

Que es necesario cumplir con lo establecido por los Art. 13° y 14° de la citada resolución,

Lo aconsejado por la Comisión de Enseñanza, Programas y Planes de Estudio,

Lo actuado por este cuerpo en la sesión realizada en el día de la fecha,

En uso de las atribuciones que le confiere el art. 113° del Estatuto Universitario,

**EL CONSEJO DIRECTIVO DE LA FACULTAD DE CIENCIAS
EXACTAS Y NATURALES
RESUELVE:**

Artículo 1°: Aprobar el informe correspondiente a las actividades desempeñadas por el Dr. Alberto Rodolfo Kornblihtt durante su Año Sabático.

Artículo 2°: Enviar un ejemplar del informe a la Biblioteca de esta Facultad.

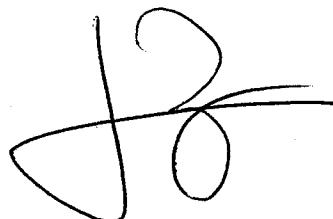
Artículo 3°: Regístrese, notifíquese a quienes corresponda, elévese al Consejo Superior y cumplido, archívese.

RESOLUCIÓN CD N°

0960



Dra. ADALI PECCI
SECRETARIA ACADEMICA ADJUNTA



Dr. LUIS M. BARALDO VICTORICA
VICEDECANO