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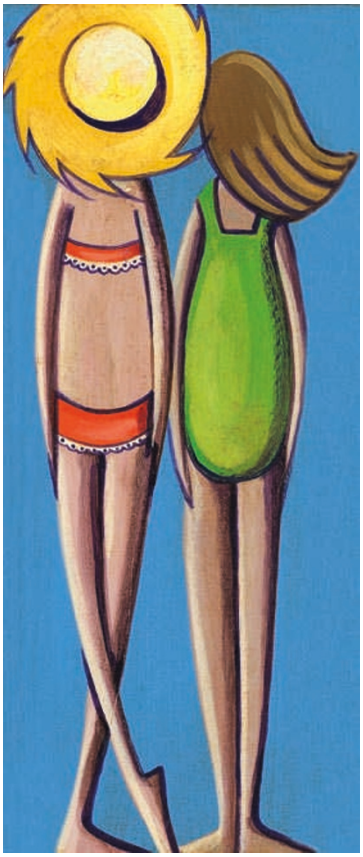
GENE EXPRESSION

UV-induced coupling

> “…DNA damage regulates co-transcriptional AS through inhibition of RNA polymerase II-mediated elongation.”

Alternative splicing (AS) is thought to be a key response to DNA damage because many apoptotic genes are alternatively spliced, but the underlying mechanism is unknown. A new study by Kornblihtt and colleagues reports that ultraviolet (UV) irradiation triggers co-transcriptional AS, through the hyperphosphorylation of the carboxy-terminal domain (CTD) on RNA polymerase II and through the resulting inhibition of transcription elongation.



The authors observed changes in AS patterns in various genes, including the pro-apoptotic *BCLX* gene, in UV-irradiated cells. Similar effects on AS were seen with another DNA-damaging agent, which together suggest that AS is affected by DNA damage. Given that the tumour suppressor p53 is an important regulator of the cellular response to DNA damage, it was somewhat surprising to find UV-induced changes in AS in p53 null cells; the effect is therefore independent of p53.

Splicing can occur both co-transcriptionally and post-transcriptionally, but the effect of UV on AS is strictly co-transcriptional. This led the authors to investigate the effects of UV irradiation on RNA polymerase II-mediated transcription and, specifically, on its CTD, which is hyperphosphorylated in response to UV. Indeed, changes in AS occur after hyperphosphorylation of the CTD, and non-irradiated cells expressing a phosphomimetic CTD mutant duplicate the effect of UV on AS. By contrast, non-phosphorylatable CTD mutants or chemical inhibition of UV-dependent CTD phosphorylation prevent the UV effect on AS. Together, these findings suggest a causal relationship between CTD hyperphosphorylation and a change in AS.

So, how is RNA polymerase II transcription affected by UV irradiation? Using a fluorescence recovery after photobleaching (FRAP) assay to measure RNA polymerase II-mediated elongation *in vivo*, the authors showed that UV-treated cells recovered more slowly compared with untreated cells, suggesting that elongation is inhibited by UV treatment. Consistently, elongation was inhibited in cells that express a phosphomimetic CTD mutant. These findings imply that DNA damage regulates co-transcriptional AS through inhibition of RNA polymerase II-mediated elongation. Furthermore, splicing-sensitive human microarrays showed that the subset of UV-responsive genes that have changed AS overlaps significantly with the subset of genes that have reduced expression, which is consistent with the suggested mechanism.

Finally, the authors analysed whether UV-induced changes in AS of apoptotic genes are physiologically important. The short isoform of BCL-X (BCL-X_S) is known to favour apoptosis over the long isoform, BCL-X_L; indeed, UV-induced apoptosis in cells lacking p53 negatively correlates with the BCL-X_L/BCL-X_S ratio, and overexpressing BCL-X_L blocks the apoptotic response. So, the UV effect on AS of *BCLX* is crucial for the p53-independent apoptotic response to DNA damage.

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ORIGINAL RESEARCH PAPER Muñoz, M. J. *et al.* DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. *Cell* **137**, 708–720 (2009)