

Alberto Kornblihtt: Coupling alternative splicing with transcription

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Kornblihtt studies how gene expression is controlled in mammals and plants.

Alberto Kornblihtt was raised in Buenos Aires, Argentina. His parents both taught (mathematics and geography), and his siblings pursued careers in science and teaching, attesting to the passion for knowledge and education instilled by their parents. Kornblihtt always knew that he would be a scientist, and he settled on biology in high school after learning about DNA, RNA, proteins, and the genetic code from the plant taxonomist Rosa Guaglianone. High school also marked his introduction to Joachim Hämmerring's 1943 experiments, which show that the morphology of the single-cell alga *Acetabularia* is controlled by the nucleus. His interest in the regulation of gene expression and the mechanisms eliciting RNA and protein diversity led him to pursue a PhD at the Campomar Foundation, Argentina, under the supervision of Héctor Torres, a trainee of the Argentine biochemist and Nobel laureate Luis Leloir, who was still at the time an active scientist at the institute. During his postdoc with Francisco "Tito" Baralle at the Sir William Dunn School of Pathology in Oxford, England, Kornblihtt cloned the human fibronectin gene and found that it could generate up to 20 polypeptides through alternative pre-mRNA splicing. We contacted him to learn more about his passion for the mechanisms of RNA splicing, his career, and his research.

What are you currently working on?

Epigenetic changes occurring in internal regions of genes affect alternative splicing (AS) decisions, through both the modulation of RNA polymerase II (RNAPII) elongation rates and the recruitment of splicing factors to the nascent pre-mRNA. For example, we found that changes in nerve cell activity and neuron differentiation that relax or tighten chromatin structure promote changes in transcriptional elongation, which in turn affect AS of genes essential for neuronal function. Furthermore, we studied the enzyme responsible for H3K9 dimethylation in mammalian euchromatin, named G9a (1). We found that G9a is required for differentiation of mouse neuronal cells and that inclusion of one of

its alternative exons promotes G9a nuclear localization and neuron differentiation.

Another aspect of our research relates to chromatin and the nuclear effects of the small RNA machinery. The prevalent view is that, in mammalian cells, argonaute proteins only function in the cytoplasm through a mechanism known as posttranscriptional gene silencing. This view neglects the existence of the nuclear mechanism known as transcriptional gene silencing in these cells. However, we showed that siRNAs play nuclear roles in the control of alternative pre-mRNA splicing (2). siRNAs targeting intronic sequences close to an alternative exon regulate the splicing of that exon. The effect depends on argonaute-1 (AGO1) and is mediated by the "writing" heterochromatin marks (H3K9me2 and H3K27me3) at the target site that create roadblocks for RNAPII elongation. Surprisingly, AGO1 binds to active transcriptional enhancers (2). Currently, we are investigating how AGO1 interacts with transcription factors and how these complexes activate transcription of genes controlled by the targeted enhancers.

Lastly, we study DNA damage and AS in human skin cells. UV irradiation affects cotranscriptional AS through the hyperphosphorylation of the RNA PII carboxy-terminal domain and inhibition of transcriptional elongation (3). We now demonstrate that UV-induced DNA damage is not only necessary but sufficient to trigger the AS response and that photolyase-mediated removal of cyclobutane pyrimidine dimers (CPDs) abrogates the global response to UV. In skin cells, RNAPII is the target, but not a sensor, of the signaling cascade initiated by CPDs, and single-stranded DNA stretches generating during lesion (CPD) repair activate the protein kinase ATR that mediates the UV-induced RNAPII hyperphosphorylation. Our results link DNA damage repair to the control of gene expression both at the transcriptional and RNA splicing levels.



Alberto Kornblihtt.

PHOTO COURTESY OF THE BERLIN FEBS MEETING, 2015.

You also study AS in plants, correct?

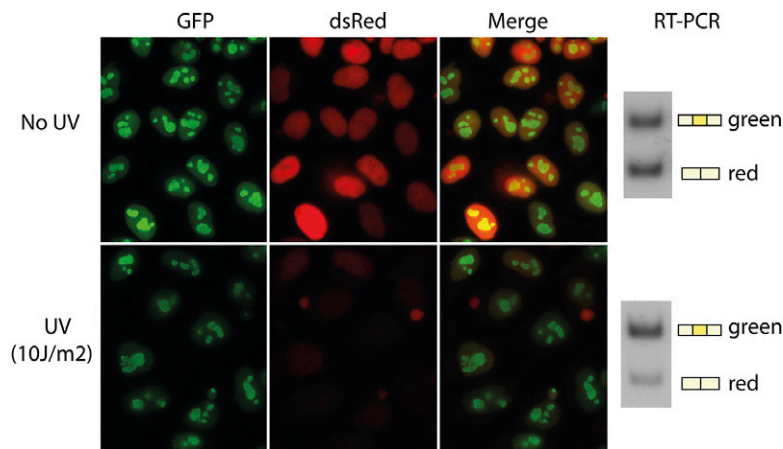
Yes, we showed that light/dark conditions affect AS of a subset of Arabidopsis genes encoding proteins involved in RNA processing (4). The effect requires functional chloroplasts and is observed in roots when the communication with photosynthetic tissues is not interrupted, suggesting a signaling molecule is involved. Using photosynthetic inhibitors with different mechanisms of action, we showed that the reduced pool of plastoquinones initiates chloroplast retrograde signaling that regulates nuclear AS. Surprisingly, we found, but have not yet published, that the effect of light on AS involves changes in transcription elongation. Indeed, in a plant defective in the elongation factor TFIIS, the light/dark effect on AS is completely abolished.

What is up next for you?

Spinal muscular atrophy is a severe genetic disorder of motor neurons that is caused by loss-of-function mutations in the survival motor neuron 1 (SMN1) gene. Humans have a paralog of SMN1, named SMN2. Because of a variation in a single nucleotide, SMN2 exon 7 (E7) is poorly included in the mature mRNA, so that ~10–20% of SMN2 transcripts encode the fully functional protein, while the remaining 80–90% encode a truncated, nonfunctional protein. Therefore, in the absence of SMN1, SMN2 cannot compensate for SMN protein deficiency. We

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Colorful alternative splicing. HeLa cells were stably transfected with a reporter minigene expressing two alternative splicing mRNA variants differing by the inclusion of a cassette exon. The inclusion and exclusion variants were engineered to specifically express in the nucleus the dsRed and GFP proteins, respectively. In normal conditions (no UV), both mRNA isoforms are produced at equal levels (top RT-PCR gel), giving rise to intense green and red fluorescence (top panels). Upon DNA damage triggered by UV irradiation, inclusion of the alternative exon is up-regulated (bottom RT-PCR gel), which is also indicated by a reduction of red fluorescence compared with green fluorescence (bottom panels).

RESULTS WERE OBTAINED BY GRADUATE STUDENT NICOLÁS NIETO MORENO.

wish to identify drugs that, by affecting RNA PII elongation or chromatin structure, may be used in combination with allele-specific oligonucleotide-driven therapies to improve the inclusion levels of SMN2 E7 in the mature mRNA.

What did you learn that helped prepare you for being a group leader? What were you unprepared for?

During my PhD, I learned a lot about laboratory managing from Héctor Torres, such as how to import reagents and equipment (something critical in Argentina). During my postdoc, I learnt how to persuade instead of giving orders. Tito Baralle taught me that when a disciple comes with questions about how to proceed with a series of experiments, the best form of advice is: “If I were you, I would do such and such...” With both my past mentors, I learned to enjoy experiments and to perform the right controls.

“Learn that it’s your work that is being criticized, not you, your capacities, or your integrity.”

What is the best advice you have been given?

Do not elude responsibilities. Like in Saint-Exupéry’s *The Little Prince*: *You are responsible for your rose...*

What has been the biggest challenge in your career so far?

Overcoming the terrible anguish that invades you when a paper or application is rejected.

Learn that it’s your work that is being criticized, not you, your capacities, or your integrity.

What has been the biggest accomplishment in your career so far?

In 1995, I suggested to a new graduate student in the laboratory to explore if changing the RNAPII promoter of a gene with the RNAPII promoter of another gene would affect the patterns of AS of the produced transcript. She showed that changing promoters caused a 10-fold increase in the inclusion levels of an alternative cassette into the mature mRNA (5). This was the first evidence that transcription and splicing are not independent events, but that the outcome of splicing depends on the parameters of transcription. This coupling involves two non-exclusive working models: AS is affected by the recruitment of splicing factors to the transcription apparatus (recruitment coupling) or by the speed of RNAPII elongation (kinetic coupling). We showed how changes in RNAPII elongation promote inclusion (6) or skipping (7) of alternative exons.

What has been your biggest accomplishment outside of the laboratory?

Since 1985, I have taught an introductory course on molecular and cell biology at the University of Buenos Aires to ~400 undergraduate students every year. I feel proud that many generations of now successful scientists, here and abroad, were inspired by this course. Further outside of the laboratory, I feel proud of my two sons—the younger one is a photographer and the older

one is a historian—and of the always renewed relationship with my wife, who is a psychoanalyst.

What are your hobbies?

I am a cinephile. I keep a record of all the movies I’ve seen in my life (~2,000 so far). I also love cooking, creating conceptual art, etymology, playing Scrabble, and listening to classical music. I love reading movie scripts, texts in Latin, poetry, and novels, if possible in their original languages.

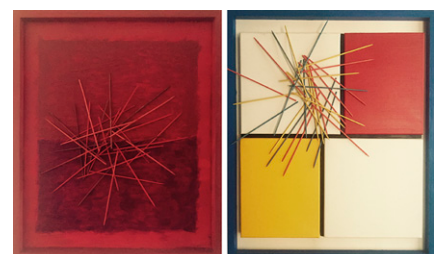
What would you be if you were not a scientist?

Within science, I would have loved to be a geologist or to study linguistics and the origin of languages. Outside of science, I love architecture.

Any tips for a successful research career?

Hard work. Honesty. Respect for others. Do not read too much scientific literature; it limits your creativity. Explore your own ideas and only then, before doing the experiments, check if someone else has already published something similar. Team work. Do not act as the “boss” of your graduate students and postdocs. They are not working for you, they are working for themselves. They are not your employees. You are their advisor, their mentor. Be consistent along time with the subject of your research; do not switch from subject to subject. Publish few but sound papers in which your leadership and contribution are clear.

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Conceptual paintings. Mikado on Rothko (left) and on Mondrian (right).