DARK SIDE OF THE GENOME

Juan Pablo Unfried explores the important role of a specific strand of 'junk DNA' in both health and disease

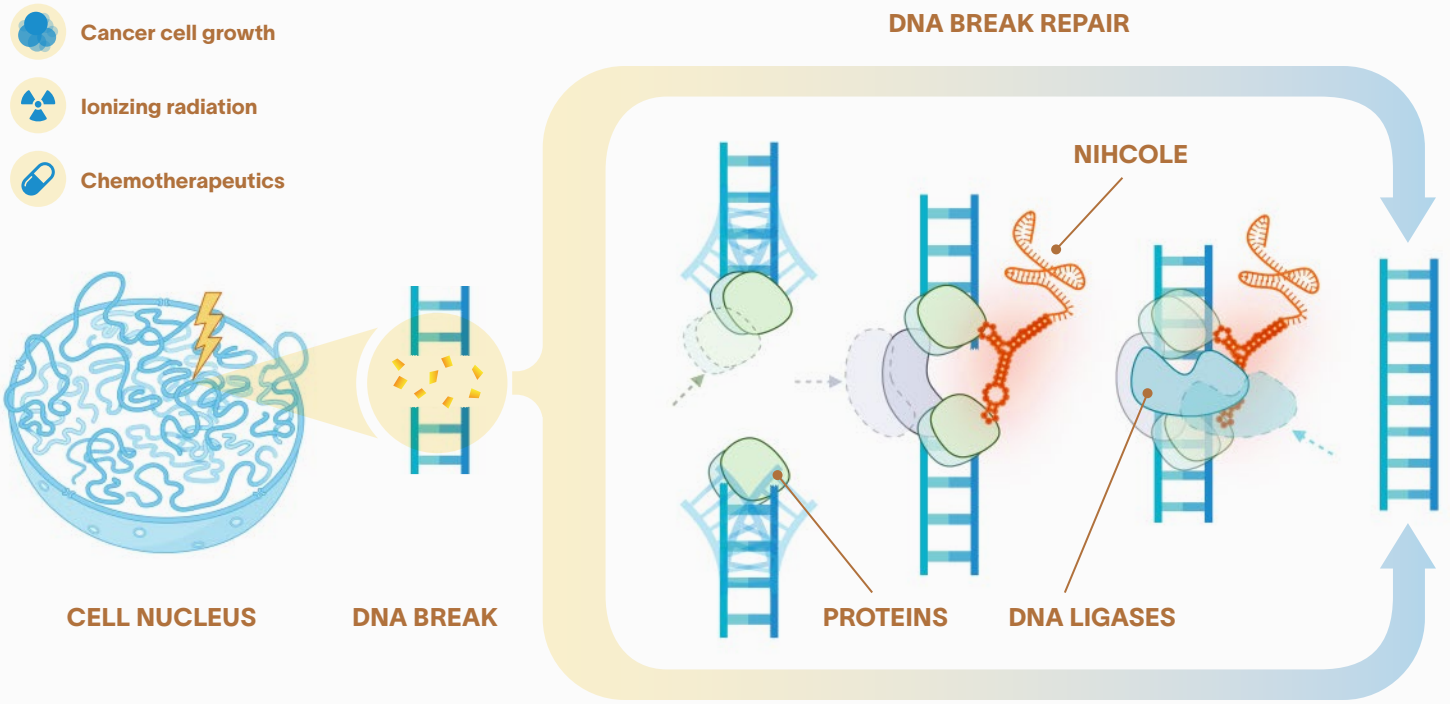
By Diana Kwon

The human genome contains three billion base pairs of DNA, but a mere one per cent of that produces proteins, the building blocks of cells. For a long time, scientists referred to the remaining 99 per cent as "junk DNA," thinking that it consisted simply of meaningless genetic code. In recent years, however, it is becoming increasingly clear that many of these DNA sequences are far from disposable. Instead, they appear to be involved in a multitude of cellular processes. Much about how these sequences work remains unknown, but Juan Pablo Unfried is striving to uncover their secrets by studying this enigmatic side of the genome.

One of the biggest hints that the genetic sequences that do not encode proteins, which are also known as non-coding DNA, could be functional was the discovery around 10 years ago that approximately three-quarters of our DNA is transcribed into RNA. This is a resource-heavy process for the cell. "That's when we started realizing that non-coding RNA isn't junk," says Unfried, a biomedical researcher and Azrieli International Postdoctoral Fellow at the Weizmann Institute of Science. "Otherwise, the cell wouldn't invest so much energy into the production of these RNAs."

Scientists have since learned that non-coding RNA comes in a variety of forms and serves many different biological functions. One example is ribosomal RNA, a key component of the protein-assembling molecular machines known as ribosomes. Unfried is particularly interested in long-noncoding RNAs (lncRNAs). These are at least 200 nucleotides in length and chemically identical to protein-producing messenger RNAs. To date, more than 20,000 lncRNAs have been found within the human genome and more than twice as many are predicted to exist. Although their functions have yet to be fully elucidated, a growing body of evidence suggests that many are important for a wide range of biological processes, such as the regulation of protein production, and are relevant to human diseases like cancer.

HOW NIHCOLE REPAIRS DNA BREAKS



The nucleus of a cell and the DNA packed inside it are incredibly dynamic. When DNA is damaged — which can happen for a range of reasons, such as exposure to ionizing radiation — the cell triggers a step-by-step repair process. First, the broken DNA ends are capped by proteins; second, the DNA ends are brought into proximity and aligned; and third, DNA ligases (a DNA-joining enzyme) perform the re-ligation of the broken ends. Using "molecular forceps" with single molecules of DNA, Juan Pablo Unfried and his collaborators have recently found that the long-noncoding RNA they call NIHCOLE is able to stabilize and increase the duration of DNA-end synapses required for ligation. Moreover, NIHCOLE has been shown to sustain the formation and recruitment of DNA repair factors, favouring faster repair kinetics and allowing unchecked cell growth and cancer progression. This research has implications for the development of chemotherapeutics.

Using a variety of methods, Juan Pablo Unfried hopes to ultimately generate a 'codebook' that will lay out the diverse functions of long-noncoding RNAs and their mechanisms of action.

Unfried grew up in Costa Rica and discovered his passion for research while studying the dengue virus as an undergraduate student in biochemistry. He first encountered lncRNAs as a doctoral student in the laboratory of Puri Fortes, a principal investigator at the University of Navarra in Spain. While probing for lncRNAs involved in cancer, Unfried zeroed in on one particular lncRNA that was associated with worse outcomes in patients with liver cancer. This molecule, which he dubbed NIHCOLE (short for "noncoding RNA induced in hepatocellular carcinoma with an oncogenic role in ligation efficiency"), appeared to only be highly expressed in liver cancer cells. Tissue and disease specificity is a common characteristic of lncRNAs, according to Unfried, making them ideal potential therapeutic targets.

PHOTOGRAPH BY BOAZ PERLSTEIN

Although the functions of long-noncoding RNAs or lncRNAs studied by Juan Pablo Unfried have yet to be fully elucidated, many appear to be important for a wide range of biological processes, such as the regulation of protein production, and are relevant to human diseases like cancer

Further assessments revealed that NIHCOLE was involved in repairing double-stranded breaks in DNA, a common (and the most toxic) form of DNA damage. The rapid proliferation of cancer cells makes them susceptible to this kind of impairment, but by protecting these cells against such harm, NIHCOLE actually enables them to proliferate unchecked, Unfried explains. By helping repair DNA damage, NIHCOLE also enables cancer cells to resist treatments such as radiotherapy, according to Fortes. "Our dream would be to give radiotherapy to patients while we decrease the levels of NIHCOLE, because this is really catastrophic for the cancer cells," she says. "The experiments from Juan Pablo allowed us to propose a new therapy for liver cancer."

The characteristics of NIHCOLE left Unfried wondering why this molecule was selected for during the process of evolution if all it does is help a tumour survive. What Unfried suspects is that lncRNAs like NIHCOLE, which appear to be specific in diseases, may play important roles during early development — particularly in highly proliferative tissue like testis, where sperm is produced, or in energy-guzzling organs such as the brain — then become silenced before reappearing in tumours or other diseases later in life. Supporting this notion, Unfried and his colleagues identified overlaps between the lncRNAs expressed in cancers and those found in healthy tissues in testis and the brain. "We think that these lncRNAs offer a fitness advantage and thus became part of the genome during evolution," he says.

In his current position as a postdoc with Igor Ulitsky in the Department of Biological Regulation at Weizmann, Unfried is focused on identifying what lncRNAs do and how they carry out those functions. In the case of NIHCOLE, he has used atomic force microscopy to find that loops within its structure are key to its ability to bind the DNA repair machinery. Using a variety of methods, Ulitsky and Unfried hope to ultimately generate a kind of "codebook" that will lay out the diverse functions of lncRNAs and their mechanisms of action. Messenger RNAs are relatively simple in that their main function — protein production — is known. The repertoire of lncRNAs, on the other hand, remains largely unknown. "The possibilities are so endless," Unfried says.

Unfried also suspects that lncRNAs may have played a central role in the evolution of higher primates. While many organisms, including some plants, have far more protein-coding genes than we do, the number of non-coding sequences tends to increase with organismal complexity. "The size of the non-coding genome is a better predictor of organismal complexity than protein-coding genes," Unfried says.

Preliminary evidence suggests lncRNAs may be the primary route through which organisms evolve new traits. "It's a bit like the stock market," Unfried explains. "It's a very risky game to play with your building blocks, proteins, because they are essential, but you can mutate a lncRNA many times without consequences." For this reason, some scientists hypothesize that lncRNAs may have been crucial to the evolution of complex organisms like humans. When Unfried eventually sets up his own lab, he hopes that a central focus will be trying to understand how lncRNAs allow the cell to play the evolution game. That, Unfried says, is "the wildest dream of my future." ▲●■