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Synopsis

CODING AND NON-CODING FUNCTIONS OF THE GENOME

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EPIGENETICS AND CANCER

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DNA AND EPIGENETICS

A GENOME TO CONTROL THEM ALL

Genetic information is stored in DNA, like an instruction manual. But it isn't so easy to read. In general, the same letters appear in books that are as different as a skin cell, liver cell or heart cell. What sets the stories they tell apart then? Epigenetics, the intimate relationship and regulation of the letters and their readers.

Epigenetics is defined as any inheritable biological variation that arises independently from the DNA sequence. Broadly speaking, it encompasses three main blocks: modification of histones (the proteins that coil the genome more tightly or loosely); DNA methylation (a small mark made on one of its letters that conditions how it is read); and non-coding ribonucleic acids (any RNA that isn't involved in protein synthesis and belongs to what is called the dark genome or junk DNA, which makes up 98% of the genetic sequence and until recently was disregarded with mistaken anticipation.

All of these mechanisms allow a single cell to turn into neurons, bones and lymphocytes. And their importance means that errors in the machinery can lead to tumors or even autism. As they are inheritable, if changes occur in the sex cells, some of them could be passed on to an individual's descendants.

To discuss part of the new advances, some of the top international experts in the field met for a B·Debate session, an initiative of Biocat and the "la Caixa" Foundation to promote scientific debate.

CONCLUSIONS:

- Genes only make up 2% of the genome. The other 98% is what has been called *dark* or *junk* DNA. Not recognizing the importance of this 98% was a mistake, as most of it works to regulate how the other 2% behaves.
- *Dark* DNA is one of the keys of epigenetics, the inheritable changes don't depend on the DNA sequence, and they regulate gene activity.
- Epigenetics allows different cells and organs to be created from the same DNA. Epigenetic alterations have been associated with a wide range of illnesses, including cancer and autism, and research is being done to find therapies to undo them.
- Epigenetics can be modified by the environment, and it seems that some of these changes can be passed down to future generations.

EPIGENETICS: THE DARK GENOME AND BEYOND

“Until recently, we had a dogmatic view that established a very simple relationship: one gene equals one protein,” explained Luciano Di Croce, ICREA professor, group leader at the Center for Genomic Regulation in Barcelona and scientific leader of this B·Debate. “That made research very easy, because we could focus on the 2% of the genome that is made up of genes and discard the other 98%. But we were wrong,” he said.

Although scientists had suspected this was a mistake for some time, a large international consortium turned this dogma on its head in 2012. The [Encode project](#) (Encyclopedia of DNA Elements) established that [at least 80% of DNA has some biological function](#). Although it wasn't clear what was meant by function, one thing was clear: the vast majority of the genome is active.

The majority of that activity has to do with DNA transcription. In general, the genome's instructions are related to protein synthesis: the letters of DNA, when transcribed, act as a mold for RNA, which in turn acts as a mold (like Russian nesting dolls) for proteins, the building blocks of cells. This part, however, only makes up 2% of the book: genes. Nearly all the other DNA is transcribed into RNA, although it doesn't synthesize proteins. This is what is known as non-coding RNA. And we now know that the majority of them allow for regular expression (transcription) of genes.

This is one of the pillars of epigenetics, defined as the sum of the inheritable changes that don't depend directly on the DNA sequence, and which act basically by controlling how genes work, switching them on or off. But there are at least two other pillars, and these have to do with histones, the proteins that the genome wraps around, and DNA methylation, small marks on some of the letters.

The genome shouldn't be considered a linear book; it is a 3-D pop-up book. To read its parts (or to encode them, which is sometimes as important as decoding them), the cell machinery has to be able to get to them, and that depends on how exposed it is. By modifying histones (adding or removing chemical marks), it becomes looser or tighter. Methylation of some of the C letters (cytosine) generally tightens the genome making it harder to read.

This is what allows a neuron to be a neuron and a hepatocyte, a hepatocyte, even though their genes are exactly the same.

HISTONES, MORE THAN JUST A PEARL NECKLACE

It may seem simple, but there is a whole orchestra that has to be coordinated so our two arms will be in the right spot, one on either side of the body, or for us to have two symmetrical legs. And for all of this to come about from a single cell after the joining of an egg and a sperm.

Part of this orchestra is the histones, a group of proteins that DNA wraps around in clumps, making it look like a pearl necklace. Depending on how they move through the DNA and how tightly it is wrapped, histones allow some genes to be expressed and others, not. This is done differently for each cell and depending on their point in the lifecycle: regulation in time and space.

One of the most well known roles is in regulating *Hox* genes, located on the edge of the genome, which establish distribution of organs and extremities. (Alterations to the *Hox* genes can cause flies to be born with [legs instead of antennae](#)). To regulate them, there are two main protein groups, Polycomb and Trithorax. Both add marks (methyl groups) to amino acids in the H3 histone. Polycomb to amino acid 27 and Trithorax to amino acid 4. The result? The first represses and the second activates. During the concert, organs and extremities develop according to plan in the pre-established location.

When something is so important to growth, it's logical that something bad would happen if it stopped working. That's what Ali Shilatifard, professor of biochemistry and molecular genetics at Northwestern University in Chicago, explained. Translocations (rearrangement of chromosomes) affecting MLL genes (homolog of the Trithorax group) cause leukemia with a very bad prognosis in children. And alterations in the Polycomb group seem to be [behind some gliomas](#), particularly aggressive brain tumors. This is why Shilatifard believes that Polycomb is a clear "candidate to treat these tumors."

All of these marks are different for each cell type. This is why it is so important to understand how they work. For example, it's not possible to generate pluripotent cells (which can turn into many other types of cells) from mature, stable cells, like those in the skin. These are called iPS cells, the great hope for regenerative medicine which Japanese scientist Yamanaka won the Nobel Prize for in 2012. But certain epigenetic barriers, in part due to histones, stop them from being totipotent, or completely flexible in becoming any sort of tissue, as happens with the first cell, the zygote.

This is the topic of study of Maria Elena Torres-Padilla, group leader at the Institut de Génétique et de Biologie Moléculaire et Cellulaire in Strasbourg. Her definition of totipotency is "A cell's ability to create a complete organism all on its own." This ability is lost almost immediately, when the zygote has divided just a couple of times, but her group [has obtained](#) very similar cells by just modifying a gene in later embryo cells. They still don't know if they are truly totipotent, even though they seem to be. But Torres-Padilla wondered if they could be created in the laboratory and "The answer is yes," she said.

It's not all about histone marks, the histones themselves are also important. There are more than thirty different types, with different properties. For example, one called macroH2A. As explained by Marcus Buschbeck, group leader at the Institute for Leukemia Research, they only make up 1% or 2% of all histones, but they stabilize the whole epigenome, as when they are missing, it becomes totally unorganized. Or H2AZ, studied by Sandra B. Hake at the University of Munich, which participates in both disparate and related process like gene regulation, DNA repair, ageing and some tumors.

[Epigenetics is related to genetics, but also to epigenetics itself.](#) Danny Reinberg, of the Howard Hughes Medical Institute in New York and one of the top experts in his field worldwide, showed how some non-coding RNA [activates gene expression](#) by inhibiting the Polycomb group. And how genetic mutations in proteins that go towards the methylated areas of DNA (epigenetics) cause serious neurological disorders and even autism in young girls, as seen in Rett syndrome.

DARK GENOME AND METHYLATION, OTHER TWO PILLARS OF EPIGENETICS

“More than 80% of DNA is transcribed to non-coding RNA,” explained Tony Kouzarides, professor of cancer biology at the University of Cambridge. “And nearly half of that DNA is retrovirus inserted into the genome at some point of evolution,” added Simon J. Elsässer of Karolinska Institutet in Stockholm.

Part of that RNA (called micro RNA) can join with others that do code for proteins. In doing so, the union is destroyed, meaning it serves to regulate how much of each protein there is in a cell. But this is also complicated. Other, longer RNA can work differently. Ramin Shiekhattar, director of the Epigenetics Program at the University of Miami, explained that they can join to the DNA itself, for example in areas that work as switches for expression. One example: there is a type of RNA that can switch on the *Snai1* gene, which is [key](#) to the development of several types of tumors. *Snai1* can't be eliminated completely, though, because healthy cells also need a bit of it. That's why “This RNA makes it possible to control *Snai1* just in tumor cells, because it's almost exclusively found in there,” said Shiekhattar.

The other part of epigenetics is DNA methylation, the union of a chemical group to certain cytosines—one of the four letters—in the ADN. In general, this union involves a closed genome: it impedes its transcription and alterations in these marks can lead to diseases like cancer, schizophrenia or even Alzheimer. Unlike genetics, epigenetics is more flexible, less rigid. Methylation can be reversible, coming and going. These movements are the focus of study for François Fuks, director of the Laboratory of Cancer Epigenetics at the University of Brussels. The TET proteins are very important here, key in removing methylation, and alteration of these proteins seems to be behind some tumors like in some cases of leukemia and melanomas.

To impede transcription, methylation *uses* proteins that join to it and block anything else from entering. Cristina Cardoso, professor of cell biology and epigenetics at the University of Darmstadt, Germany, calls them “the guardians of the epigenome”. They are so important that a simple mutation in one of their genes, for the MeCP2 protein, causes a devastating disease in young girls called Rett syndrome, which leads to respiratory,

neurological (with convulsions and altered movement) and behavioral problems, including traits typically seen in autism.

Epigenetics: Lamarckian inheritance?

According to Darwin's theories, evolution takes place as a result of mutations to DNA that are passed down to future generations. These mutations are a result of random chance and the environment has little or no impact. His contemporary, maligned scientist Lamarck [had a different idea](#): if a giraffe stretched its neck to reach the leaves at the top of a tree, that stretching could be passed down to its offspring. Epigenetics opens the door to Lamarck being somewhat right about this.

Agouti mice normally have yellow fur. But if their diet is rich in methyl groups, their DNA methylation changes and their fur turns brown. That alone can cause their offspring to be born brown. Part of the Netherlands experienced widespread famine during World War II. As a result, it seems, the children of those Dutch mothers were shorter than usual, as were their grandchildren. Some environmental factor, not strictly genetic, seems to have been passed down. Similarly, certain diets seem to make an individual's grandchildren [more prone to diabetes](#).

These mechanisms are possible, although we don't yet know how or to what extent. A majority of epigenetic marks are lost when the zygote is formed: there is something like a reset, known as the Weismann barrier. But some can remain. "Certain methylation marks aren't lost, some histones we thought were substituted in the sperm seem to be preserved, and non-coding RNA found in the egg can be passed on and function in the first moments of life," explained Di Croce. "We don't know to what extent, but yes, epigenetic inheritance may exist."