Revealing the evolutionary origin of genomic imprinting

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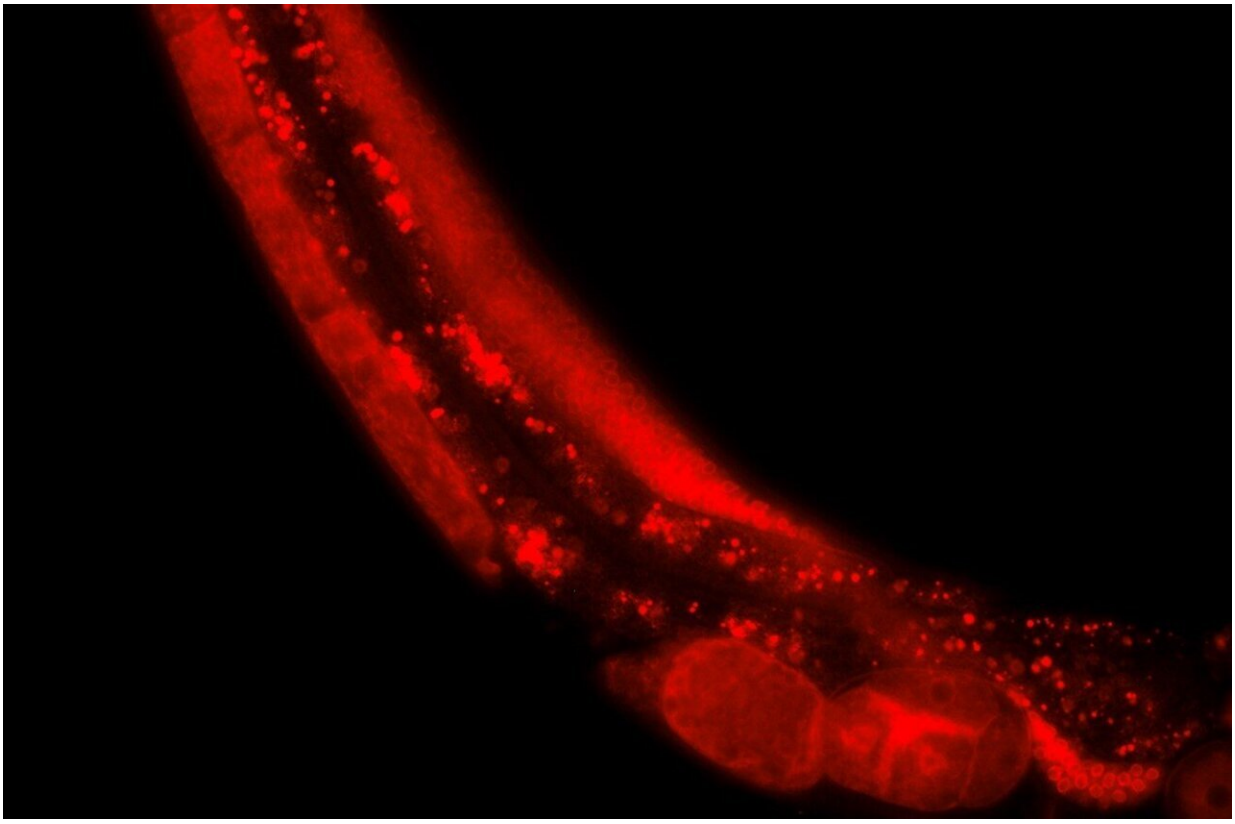

Microscopic fluorescence image of a C.tropicalis mother worm. The worm carries eggs loaded with a toxin (red) which is expressed from a Toxin-Antidote selfish genetic element only when it was maternally inherited. Credit: Pinelopi Pliota/IMBA

Some of our genes can be expressed or silenced depending on whether

we inherited them from our mother or our father. The mechanism behind this phenomenon, known as genomic imprinting, is determined by DNA modifications during egg and sperm production.

The Burga Lab at the Institute of Molecular Biotechnology (IMBA) of the Austrian Academy of Sciences uncovered a novel gene regulation process, associated with the silencing of selfish genes, that could represent the first step in the evolution of imprinting. Their discovery could begin to solve the mystery of how and why imprinting first evolved.

Alejandro Burga and his lab at IMBA, in collaboration with the lab of Eyal Ben-David at the Hebrew University, have reported the discovery of the first parent-of-origin effect in nematodes, in a study published in *Nature* on March 6, 2024.

In diploid organisms, one set of chromosomes is inherited from each parent. However, not all of the genes contained within will be expressed equally; instead, some may be silenced depending on whether they were inherited from the mother or the father. This phenomenon, known as genomic imprinting, depends on DNA methylation, an epigenetic signal that is erased and rewritten in every generation.

Genomic imprinting arose independently in mammals and plants over 100 million years ago. However, how this mechanism evolved has, so far, remained largely a mystery. Key to solving this enigma is understanding how parent-of-origin effects, the substrate for the evolution of imprinting, evolved in the first place.

Thirty years ago, Denise Barlow, a pioneer in the study of imprinting working at the IMP, also located at the Vienna BioCenter, hypothesized that imprinting could be evolutionarily related to genome defense mechanisms that silence parasitic DNA elements called selfish genetic

elements.

Selfish elements and the defense mechanisms against them participate in an arms race: each evolves further to outcompete the other. Although much has been discovered about selfish element silencing in the 30 years since Denise Barlow postulated her theory, a direct connection between germline defense mechanisms and the origin of parent-of-origin effects was missing.

The findings by the Burga lab provide the first clear example of how parent-of-origin effects can originate from the host small RNA genome defense pathway. Their findings point to the potential evolutionary origin of imprinting.

Curiosity paves the way for a new discovery

Sometimes in science, curiosity and attention to surprising details can lead to unexpected paths and new discoveries. This was the case when first author Pinelopi Pliota was studying selfish genetic elements in a new nematode model organism called C. tropicalis, a close cousin of the more widely studied C. elegans.

Pliota was investigating toxin-antidote elements (TAs), a type of selfish element that has evolved a fascinating mechanism to ensure its own inheritance. "When a mother carries the TA, it will 'poison' its eggs with a toxin that can only be countered by an antidote which is also present in the TA," she explains, "this way, all descendants that don't inherit the TA will either die or be developmentally delayed."

"To generate the mothers they were studying, the group always crossed a mother C. tropicalis carrying the TA with a father not carrying it. Pinelopi asked me if we had ever done these crossings the other way around," explains Alejandro Burga, corresponding author of the

publication.

Her curiosity led to an interesting discovery. "To our surprise, this reciprocal crossing produced mothers incapable of poisoning their eggs. All of a sudden, there was no effect at all," explains Pliota. Fascinated by this unexpected result, the team decided to study how inheriting the TA from the mother or the father could lead to different effects. "We wanted to understand how this happens, what the molecular basis of this parent-of-origin effect is," says Burga.

Inhibiting the inhibitor: Maternal mRNA licenses toxin expression

To figure out the mechanism of the observed parent-of-origin effect, the Burga group decided to study the main germline defense mechanism against selfish genetic elements, known as the piRNA pathway. In the piRNA pathway, a coordinated effort of different small RNA molecules and proteins silences the expression of selfish elements during germline development to ensure genome stability in reproduction.

The group, collaborating with the lab of Julius Brennecke, also at IMBA, were able to identify the piRNA molecules and proteins involved in silencing the toxin-antidote element. However, all these factors alone didn't explain the parent-of-origin-specific results they were observing. The researchers were missing a piece in this puzzle.

Fortunately, the Burga group had one last trick up their sleeve. "We knew from previous research that worms have evolved various ingenious ways to discriminate their own genes from foreign elements like a virus or a selfish element," Burga says. "We realized that, in this case, the key missing element was maternal RNA which is loaded into eggs."

They proved that, in maternal inheritance, the TA is accompanied by the toxin mRNA, which is expressed in the germline of the mother and loaded into the egg. The Burga group showed that this mRNA marks the TA as "own," avoiding its silencing by the piRNA pathway. This process is called epigenetic licensing, and its balance with the piRNA pathway determines whether a gene is expressed or not.

On the other hand, when the TA is inherited paternally, the lack of maternal mRNA means there is no licensing, leading to a strong repression of the toxin gene and very low levels of toxin being expressed. By default, the piRNA pathway will silence the toxin gene explains Burga. Unless there's maternal mRNA that licenses it by repressing the piRNA pathway. This inhibition of the inhibitor is what causes the toxin gene to be active, and the eggs to be poisoned.

Interestingly, this silencing pattern was observed to last for several generations, meaning that lack of licensing in one generation can even affect their great-grand-daughters. This is not the case in genomic imprinting, which gets reset in each generation.

Explaining the evolution of imprinting

The results from the Burga group cement the evolutionary link between parent-specific gene expression and host defense mechanisms, tracing the origins back to organisms that lack DNA methylation and canonical imprinting.

Despite the differences between these processes in worms and mammals, the Burga group believes that the mechanism they described could represent an evolutionary first step for more advanced forms of inherited silencing. These more advanced forms of silencing ended up regulating the expression of the cell's endogenous genes, leading to the evolution of genomic imprinting.

More information: Alejandro Burga, Selfish conflict underlies RNA-mediated parent-of-origin effects, *Nature* (2024). DOI: 10.1038/s41586-024-07155-z. www.nature.com/articles/s41586-024-07155-z

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