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Scientists Find RNA Surprises in *Listeria* Bacteria

The bacterium *Listeria monocytogenes* lives happily in soil and in your compost heap, but also in water, processed meats, milk and cheese. When humans eat food contaminated with *Listeria*, they can develop listeriosis, an infection that triggers miscarriage in women and kills people whose immune systems are weak. Scientists would like to understand the molecular mechanisms that transform this bacterium from a harmless soil-dweller to a dangerous human pathogen.

Now, a team at the Pasteur Institute in Paris has taken a major step towards realizing that goal, by mapping the genes that *Listeria* expresses under different environmental conditions. The research is reported in an advance online publication in the journal *Nature* on May 17, 2009.

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— Pascale F. Cossart

As head of the Pasteur Institute's Unit of Bacteria-Cell Interactions, Howard Hughes Medical Institute international research scholar Pascale F. Cossart is proud of what she refers to as the first complete bacterial operon map. Pasteur scientists François Jacob and Jacques Monod first described the concept of the operon in 1960. Both were awarded the Nobel Prize in Physiology or Medicine in 1965 for their seminal work on operons. Operons are functional units of DNA that consist of several adjacent genes controlled by a common promoter—a piece of DNA that determines where and when a gene is active. The genes in operons are transcribed into a single piece of messenger RNA (mRNA).

Since Jacob and Monod first coined the term operon, scientists' understanding of gene regulation has evolved considerably. Researchers now know, for example, that what was once called "junk" RNA because it wasn't translated into protein, can nevertheless fulfill important functions. Cossart's group had previously identified a piece of such non-coding RNA that

regulates *Listeria*'s ability to infect cells, which suggested to them that RNA regulation might be widely exploited by *Listeria* to aid survival. Cossart and her colleagues decided to map *Listeria*'s transcriptional program in a systematic way in order to identify as many of those RNA switches as possible.

The biotechnology company Affymetrix built Cossart customized tiling microarrays—that is, arrays of DNA probes that correspond to overlapping stretches of the *Listeria* genome. Armed with these arrays, a small army of researchers from Cossart's and other labs, led by postdoctoral fellow Alejandro Toledo-Arana, compared bacteria grown in the lab with bacteria extracted from the intestine of *Listeria*-inoculated mice or with bacteria from inoculated samples of human blood. They also compared normal or wild-type bacteria with mutants that had been genetically altered so that they lacked certain known virulence factors.

Their analysis turned up many surprises, one of the biggest of which was how the bacterium's transcriptome shifts between its soil-dwelling and intestinal modes. "When it arrives in the intestine it turns up the activity of many genes and turns down others, so we see a dramatic reshaping of the transcriptional program. Strikingly, a series of non-coding RNAs are expressed more often in the intestine or in the blood," Cossart says. The researchers identified one particular protein, SigB, that controls a series of genes that are needed for *Listeria* to adapt to the human gut, whereas a different protein, PrfA, switches on genes needed for survival and replication in the blood. By comparing mutant and wild-type bacteria, they identified two non-coding RNAs that appear to contribute to the virulence of *L. monocytogenes*.

And there were more surprises to come. The researchers found very long untranslated regions (UTRs) of RNA—that is, part of an RNA that is not translated into protein—that overlapped with several genes on the opposite strand and regulated their expression. This was the case, for example, for three genes that are involved in the manufacture of the *Listeria* flagella, the tiny protrusions that allow it to move and find its way in different environments. A known repressor of flagellum synthesis, MogR, turns out to have one very long UTR that spans all three flagellum genes and acts as an antisense RNA, which can block mRNA from being translated into a protein.

Cossart's team also identified about 40 riboswitches, RNA structures at the front of genes that act as sensors, stopping translation or expression of the RNA when enough of the gene's protein product has been made. Some of these riboswitches controlled expression of the gene downstream of them—as had previously been reported—but also the gene upstream. In other words, a riboswitch can extend its influence in both directions, a finding contrary to what anyone had suspected.

These and other regulatory mechanisms will almost certainly turn up in other microorganisms, Cossart says. She believes her group's paper is likely to be the first of many that will describe, in increasingly minute detail, the complex transcriptional checks and balances that in the case of *Listeria* make it such a

versatile organism.

In the next 10 years, she predicts, the study of bacteria in all their habitats—not just the pathogenic ones—will become a hot topic in research. And the concept of junk in molecular biology will finally be buried, as people realize that when it comes to the genome, nothing is wasted.