An sRNA controls a bacterium's social life

May 20 2010

For the first time, biologists have directly shown how spontaneous mutation of a small RNA (sRNA) regulatory molecule can provide an evolutionary advantage. Reporting in this week's *Science*, Indiana University Bloomington scientists also identify the sRNA as a key regulator of social behavior in *Myxococcus xanthus*, a soil bacterium widely studied for its ability to cooperatively construct fruiting bodies that house stress-resistant spores when food runs out.

"We'd been asking how one of our experimental lineages had re-evolved the ability to make fruiting bodies and ended up discovering a completely new aspect of *Myxococcus* biology," said IU Bloomington evolutionary biologist Gregory Velicer.

A genetic change in the sRNA of interest, 'Pxr', had been previously found to give an evolved mutant of M. xanthus a competitive edge over both the mutant's immediate parent, a social "cheater" that does not make fruiting bodies, and that cheater's own ancestor, a cooperative wild-type strain that does construct fruiting bodies. IU Bloomington molecular biologist Yuen-Tsu Nicco Yu and Velicer had been investigating how the mutation converted the socially inept parental cheater into a new strain with a restored capacity to make fruiting bodies.

The scientists learned that the mutation of interest lay within the gene for an sRNA, a class of genetic elements in bacteria new enough to science that their molecular and evolutionary roles are only beginning to be understood.

"It's been known for some time that sRNAs are important regulators of gene expression in other species of bacteria," Velicer said. "However, we did not know that an sRNA plays a central role in controlling the main thing that makes *Myxococcus* interesting -- its incredibly sophisticated multicellular behavior."

Small RNA molecules are transcripts of an organism's genome, similar to the messenger RNAs (mRNAs) that encode proteins. Unlike mRNAs, however, the main function of sRNAs is to regulate the expression of other genes, which they accomplish by binding to target mRNAs or by interacting with proteins.

Scientists have known for decades that sRNAs regulate biological processes inside of cells, but speculation about how sRNAs evolve to increase evolutionary fitness has been based on inferences from comparing sequence differences that originated in the distant past, not on direct observation.

Unlike cells of its troglodytic bacterial cousins that merely cluster in biofilms, M. xanthus cells interact more intricately. Under favorable conditions, wild-type M. xanthus cells swarm in coordinated social groups, moving about and dividing to take advantage of available food, which is often other microbes that M. xanthus kills and consumes as prey. When stressed by lack of food, M. xanthus cells aggregate together and exchange chemical signals to form fruiting bodies, nub-shaped structures that may contain the progeny of several different lineages. Inside the nub, some cells are directed to form spores. The hardy spores have a drastically reduced metabolism and resist various stresses such as starvation, dehydration and temperatures that would kill growing cells.

Because some M. xanthus cells are directed to support the structure of the fruiting body while other, arguably more fortunate cells are directed to form surviving spores, many scientists believe M. xanthus represents a

good model for studying the origins of cell differentiation within microbial social groups.

In previous research, Velicer had found that some M. xanthus strains behave as social cheaters that do not form fruiting bodies on their own but can instead exploit the benefits of fruiting bodies built by other cells. One such cheater named OC, or "obligate cheater", had spontaneously evolved in a previous experiment. OC cells produce as many as 100 times more spores than wild-type cells within fruiting bodies composed of both cell types, even though OC makes far fewer spores than wild-type cells in separate pure groups. But OC's cheating ways have a downside. As OC cells outcompete wild-type cells in mixed groups, subsequent generations have fewer wild-type cells. As the wild-type cooperators diminish in number, so do OC's opportunities for making spores. Eventually, populations in which OC and wild type are allowed to compete with one another over several cycles of development and growth crash to low numbers when the cheater cells become common.

During one such competition, a spontaneously mutated descendent of OC emerged in the population that not only outcompeted both OC and wild-type cells, but also demonstrated renewed capacity for making fruiting bodies and spores in pure culture. Velicer named the mutant PX (Phoenix) and used recently developed genome sequencing technology to identify a single point mutation (cytosine to adenine) that distinguishes PX from OC. Yu then performed experiments showing that this one mutation was indeed the direct cause of PX's emergence and success. However, the mechanism by which this mutation restored fruiting body development in PX remained a mystery.

In their current study, Yu and Velicer show that the single base mutation in PX restores fruiting body development by turning off a negative regulatory function of the sRNA Pxr. They found that in the wild-type strain, Pxr prevents fruiting bodies from forming when food remains

abundant. However, when food runs low, wild-type cells open the gate for fruiting body formation to proceed by removing the blockage of development imposed by Pxr during growth. In contrast, in pure cultures of the cheater OC, Pxr prevents cells from ever triggering development because OC is defective at responding to a signal that would normally relax Pxr's blockage of development upon starvation.

Yu and Velicer further show that the single mutation in strain PX destroys the negative regulatory function of Pxr that OC is unable to remove upon starvation. This mutation in PX gives it a large advantage over both OC and wild-type in mixed cultures and even causes PX to make about eight times more spores than wild-type in pure cultures. Deletion of the entire gene encoding Pxr was found to have a similar effect.

Yu and Velicer, along with graduate student Xi Yuan, also learned that Pxr has two forms, long and short, possibly because of an RNA nuclease that chews away the end of the long form. The scientists report preliminary evidence that the short version of Pxr may be the active form. If these hypotheses are confirmed, it would appear to be the first instance of a bacterial sRNA that must be processed to perform its function. In eukaryotes, processing of non-coding regulatory RNAs into active forms is common.

"Evolutionary studies like this one can provide powerful insights into the fundamental mechanisms by which model organisms work," Velicer said. "Now that genome sequencing is fast and cheap, we are going to see more reports showing exactly what mutations underlie evolutionary changes in fitness, behavior and metabolic abilities. We expect that many similar evolutionary studies in the future will discover important aspects of how organisms work that had not been revealed by traditional molecular genetic approaches."

Provided by Indiana University