New clues on the regulation of the CRISPR-Cas immune system

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Research into the CRISPR-Cas immune system of prokaryotes is progressing at a tremendous pace given both its important biological function and its role as a source of new genetic tools. However, a few areas of the field have remained largely unaddressed. A recent report provides information on one such overlooked area: how the cell regulates the CRISPR-Cas immune system. The processes, despite their importance, have remained illusive. In *Pectobacterium atrosepticum* regulation is, perhaps surprisingly, based on metabolic factors responding to glucose levels in the cell. Regulators include both activators and repressors of *cas* gene expression. It remains an open question why and how this regulatory system have evolved, and if it is a typical example of how CRISPR-as systems are regulated or not.

The CRISPR-Cas system is an adaptive and heritable immune system found in bacteria and archaea. The system comes in different flavors with 5 major types identified so far.¹ The CRISPR-Cas systems are based on collecting genetic information from *e.g.* viruses and plasmids and then storing that information as so-called spacers in the CRISPR locus. The spacer sequences stored in the cell's genome are transcribed and processed into CRISPR RNA (crRNA), these RNA mugshots are then used by CRISPR-associated (Cas) proteins to locate and destroy the matching target.^{2,3}

The discovery of the CRISPR-Cas system adds to our understanding of the complexity of virus-host interaction, one of the key factors in evolution and ecology of life. There is a large interest in the system, both because of its important biological function and its use as a source of

tools for genome editing and control of gene expression in eukaryotes.^{2,4}

The majority of the research in the field has been directed toward understanding the mechanisms of the systems in terms of adaptation, crRNA processing and target interference, for which there is now a considerable amount of detailed knowledge. However, for understanding how CRISPR-Cas systems are actually used, a key factor is the characterization of their regulation. This has been a surprising blind spot in the field with only a few studies published.

The CRISPR-Cas system is in itself a mobile genetic element with frequent horizontal transfers, though the mechanism of transfer is unknown. This adds complexity to the question of regulation, as the system cannot co-evolve with a specific host. Is there perhaps some feature of CRISPR-Cas regulation that allows the system to function independently of cellular settings, or does the system need to pick up a regulatory system in every host it encounters?

The first characterization of CRISPR-Cas regulatory pathway was done on the Type I-E system in *E. coli*, in part to figure out why the system had little natural activity. It was demonstrated to be the result of silencing by H-NS repressor⁵ although the activity of the system can be increased by membrane stress⁶ or increased expression of the LeuO regulator.⁷ The CRP transcription factor is suggested to act as an anti-activator by blocking access of LeuO to its binding site.⁸

In the study by Patterson *et al.* they go for investigating the regulation of a naturally active Type I-F CRISPR-Cas system in *Pectobacterium atrosepticum*, a potato-destroying bacterium.⁹ It might seem like an odd model system, but the group of Peter Fineran has, basically single-handed,

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made it into an important model system for understanding fundamental questions in CRISPR-Cas biology.

The initial screen for regulatory factors were performed by a classical transposon mutagenesis in a strain where *lacZ* is co-transcribed with *cas1*, allowing for an easy blue-white screen for mutants with altered *cas1* expression. After inspecting no less than 32,000 colonies, two *bona fide cas* regulators were suggested. The first was *crp* where transposon insertion decreased *cas1-lacZ* expression, indicating that CRP activated *cas* transcription. The second was *galM*, where transposon insertion increased *cas1-lacZ* expression, making GalM a *cas* repressor (Fig. 1).

CRP is a transcription factor that is activated by cyclic AMP (cAMP). Indeed, when deleting *cyaA*, the gene that code for the protein that synthesizes cAMP, a reduction in *cas1* expression was observed.

Mutation of a predicted CRP binding site in the *cas* promoter also reduced *cas1* expression.

GalM is not a transcription factor, suggesting that its effect on *cas* expression is indirect. In other organisms, GalM is involved in galactose metabolism so the authors speculated that if GalM increased glucose levels then CyaA activity, and *cas* expression, should be reduced. The authors demonstrate this idea by creating a strain with mutations in *galM*, *cyaA* and *crp*, and the increase in *cas1* expression observed in the *galM*-only mutant was lost, indicating that the *galM* effect is indeed mediated through CyaA and CRP.

Another test of involvement of glucose in regulation of *cas* expression was done by growing the strains in either glucose or glycerol as carbon source, where glycerol should not affect genes involved in glucose metabolism. As expected, *cas* expression

was reduced in cultures grown in glucose compared to glycerol.

Of key importance, the observed changes in *cas* expression affected the function of the CRISPR-Cas system. Both adaptation, where new spacers are acquired, and interference, where existing spacers are used to degrade target DNA, was affected. No acquisition of spacers from a target plasmid was observed in *crp* or *cyaA* mutants, while *galM* mutants had an increased rate of acquisition compared to the wildtype strain. Using a conjugating plasmid as target for the CRISPR-Cas system, *crp* or *cyaA* mutants were not very good at destroying the incoming plasmid compared to the wildtype strain, while *galM* mutants were much better at it.

The authors conclude that they have unraveled a regulatory system for the CRISPR-Cas system in *P. atrosepticum*, but they point out that additional regulators are not ruled out, that were not affected by transposon insertion during the assay and thus missed.

The outstanding question is why an immune system is regulated by metabolic factors. The authors have no clear answer but discuss it extensively and list several testable reasons. Infection may disrupt the normal metabolic state of the cell, directly resulting in a drop in glucose levels, which would activate the CRISPR-Cas system. A drop in glucose level could also be a cellular response to various types of stress to the cell. Personally, I would add the incidental reason to the list, where CRP regulation just happened and was sufficient for activity, an example of evolution by tinkering.¹⁰ The fact that CRP is a regulator of CRISPR-Cas also in *E. coli*, although there it is a repressor, is intriguing and suggests that CRP may have a central role.

In conclusion, the thorough work by Patterson *et al.* is a welcome addition to the understanding of CRISPR-Cas regulation. Hopefully, more researchers will draw inspiration from this work and elucidate how the system is regulated in their own favorite organism. Eventually, we will come to some general understanding of how this important system is used by cells in their ongoing battle with virus predators.

Figure 1. Model of CRISPR-Cas regulation in the potato-infecting bacterium *P. atrosepticum*. Lines with arrow indicate production, activation or induction of transcription. Lines with bar indicate inactivation, reduction or interference.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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