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Research Highlights

A novel strategy to protect prokaryotic cells from virus infection

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ABSTRACT

The recent discovery of the CRISPR-Cas-mediated acquired immunity system highlights the fact that our knowledge of phage/virus defense mechanisms encoded in bacterial and archaeal genomes is far from complete. Indeed, new prokaryotic immune systems are now continually being discovered. A recent report described a novel glycosylase that recognizes α-glycosyl-hydroxymethyl cytosin (α-Glu-hmC), a modified base observed in the T4 phage genome, where it produces an abasic site, thereby inhibiting the phage propagation.

Prokaryotic immune systems protect bacteria/archaea from phage/virus infection [1]. Restriction-modification (R-M) [2] and abortive infection (Abi) [3] systems have been studied for many years. The CRISPR-Cas (Clustered Regularly Interspaced Palindromic Repeats/CRISPR-associated) system was discovered 16 years ago [4], representing the only known prokaryotic adaptive immunity. Studies on CRISPR-Cas systems have not only facilitated the development of versatile CRISPR-based genome-editing technologies [5,6], but also reignited the research into other prokaryotic immune systems. As a result, new information has recently been rapidly obtained [7,8]. It is now recognized that prokaryotic immunity is far more complex than previously thought. A broad category of immune systems is shown in Fig. 1, some with known functions while others with as yet unknown functions, and there may be additional ones remain to be discovered, and these discoveries have made this topic one of the hottest research areas in recent times.

In this context, a recent paper published in *Nature*, in which L. Marraffini group in Rockefeller University and their collaborators describes a new glycosylase that cleaves the glycosidic linkage connecting a modified base in bacteriophage DNA to its backbone sugar [9]. This enzyme was discovered by screening *Escherichia coli* for mechanisms of defense against T4 phage infection. The novel glycosylase was identified by introducing an eDNA library of microbial DNA obtained from dry soil in Arizona, USA, and cloned into a cosmid vector, into T4 phage-susceptible *E. coli* cells, and selecting clones showing resistance to infection. The success of this method completely depends on the expression of the genes encoded in the DNA fragments in the cosmid vector in the transformed *E. coli* cells, but the eDNAs are derived from a variety of bacterial species and many genes on the inserted DNA may not be expressed in the heterologous host cells. This is a point of concern of this method. Nevertheless, this strategy has already been successfully used to screen metagenomic DNA [10,11]. Furthermore, before the metagenomics era, a similar strategy was developed for screening DNA transaction enzymes encoded in *Pyrococcus furiosus*. The genomic DNA fragments of the hyperthermophilic archaeon were inserted into a cosmid vector, yielding a cosmid library, and *E. coli* transformants were then obtained. The cell extracts from each transformant cell were heat-treated to inactivate *E. coli* proteins. Thermostable proteins were selectively separated by centrifugation, allowing screening for activities of interest. Using this procedure, genes encoding archaea-specific enzymes such as DNA polymerase D [12,13] and endonuclease MS [14,15] have been identified.

In this study, a more efficient method of selection was utilized, in which clones showing resistance to T4 phage infection were selected among transformants with a cosmid-based eDNA library rather than screened for via enzyme activity in eDNA library cell extracts. Ten million transformants were generated using the *E. coli* EC100 strain as the host. Transformants were infected with T4 phage, and 12 clones with a resistant phenotype were observed. They were not resistant to λ phage, indicating specificity for a particular infecting phage. The DNA fragments in the cosmids recovered from the T4-resistant clones appeared to originate in the *Actinobacteria* phylum. Further analysis showed that one gene (gene c) was involved in protection against T4 phage infection. Based on the encoded amino acid sequence, the gene product was predicted to belong to the uracil DNA glycosylase superfamily [16]. Furthermore, sequences similar to Thoeris ThsA [17] and Wadjet [18] are encoded near this gene, indicating that this gene is involved in the bacterial defense island [7,19,20]. The gene was named *brig1* (bacteriophage replication inhibition DNA glycosylase 1) because expression of this gene significantly reduced T4 DNA in infected *E. coli* cells.

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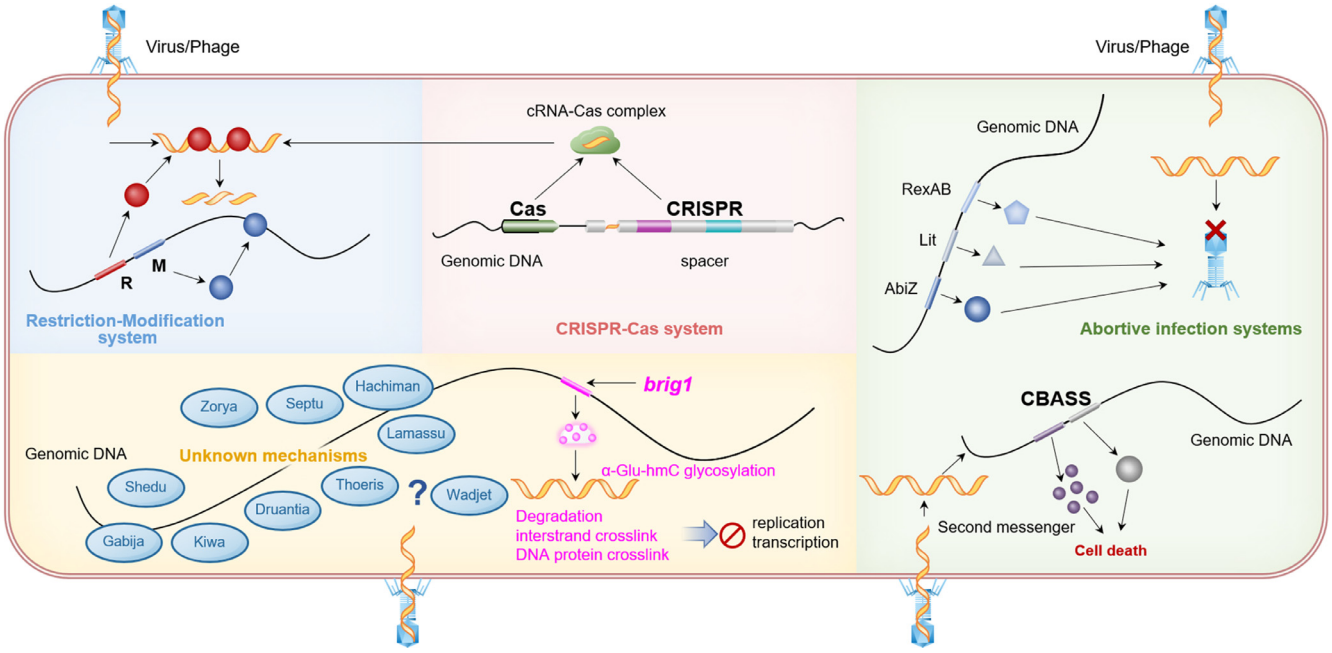

Fig. 1. Summary of our current knowledge about prokaryotic immune systems. Restriction modification systems, CRISPR-Cas systems, and abortive infection systems are well-known antiviral defense systems in prokaryotes. Their molecular mechanisms are simplified and schematically depicted. Recent research has discovered many more defense systems, but their mechanisms remain unknown. This highlight focuses on the novel glycosylase Brig 1 (shown in magenta), which attacks specifically modified bases with the phage genomes and stops their growth.

The function of the *brig1* gene product was investigated in detail. Genetic and biochemical analyses concluded that the Brig1 protein is a DNA glycosylase that cleaves α-glycosyl-hydroxymethyl cytosine (α-Glu-hmC), a modified base present in the T4 phage genome [21], from its backbone sugar, yielding an abasic site. The authors concluded that Brig1 is a DNA glycosylase that stereo-specifically removes α-Glu-hmC, but not β-Glu-hmC, from the DNA strand. No lyase activity was detected for Brig1, indicating that it is a monofunctional DNA glycosylase.

Brig1 was experimentally proven to encode an immune mechanism that confers resistance against T2 and T6 phages with α-Glu-hmC in their genomes. In the T6 phage, most α-Glu-hmC bases are further converted to gentiobiosyl-hmC, in which glucose is β-linked to α-Glu-hmC, and it is in contrast to the T2 genome that is largely α-Glu-hmC modified. Since *E. coli* is highly susceptible both to T6 and to T2 phages, it is very likely that Brig1 works at the intermediate α-Glu-hmC stage during the T6 genome maturation [21,22].

To investigate *brig1* gene conservation, they searched for homologs in prokaryotic genomes using PSI-BLAST and observed 42 non-redundant homologs. Most of these appeared to be in *Actinobacteria,* and they were located in a region presumed to be an anti-phage defense island. Among them, homologs in the genus *Nocardioides* were observed to confer resistance to T4 and T6 phages upon *E. coli* cells.

Brig1 probably evolved from a member of the uracil DNA glycosylase superfamily [16,23], as high concentrations of Brig1 exhibit uracil-depleting activity *in vitro*. However, as there is no evidence that α-Glu-hmC is incorporated into the host genome, Brig1 is not involved in base excision repair in *E. coli* cells and appears to be a *bona fide* antiviral effector. The gene encoding the α-glucosyl transferase (α-GT) that binds glucose to hmC is observed in the genome of phages infecting diverse hosts. Brig1 does not cleave β-Glu-hmC or gentiobiosyl-hmC *in vitro*. This specificity may indicate that the T-even phage engages in an arms race with its host cells by diversifying its hmC modification pattern to circumvent the restriction of its infectivity by the DNA glycosylase activity of Brig1. In this arms race, the host cells probably evolved a larger Brig DNA glycosylase family with activity against different hmC nucleobases.

The discovery of a modified base-specific glycosylase that prevents phage infection has led to a new concept of the immune system using the mechanism of base excision repair, which causes damaged DNAs to be abasic forms. Brig1 acts on single-stranded DNA, and therefore, gene products involved in base excision repair (*xthA* [24], *nfo* [25]) and recombination repair (*recBCD, recQ, recJ, recA* [26]) may be implicated in the Brig1-based defense systems. However, the deletion of any of these genes did not affect the plaque-forming capability of the T4 phage in the presence of Brig1, suggesting that these repair-related genes are not required for Brig1-mediated immunity.

The discovery of Brig1 glycosylase is reminiscent of another recently reported example of a link between DNA repair and antiphage defense. As mentioned above, the mismatch-specific endonuclease EndoMS, which was discovered in a screen using a cosmid-based library of the *P. furiosus* genome, is conserved in most archaea and certain bacteria that do not possess the well-known MutS/MutL-mediated mismatch repair pathway [14,15]. The Shedu system, a recently discovered prokaryotic defense system, contains a single gene, *sduA* [7], whose product has a hallmark domain with predicted nuclease activity (DUF4263) [27]. The sequence of the SduA nuclease is homologous to that of EndoMS suggesting a predicted functional mechanism whereby Shedu directly targets invading DNA via the SduA nuclease.

In summary, studies of the prokaryotic immune system will continue to yield exciting discoveries of proteins that act on DNA transactions. These findings may lead to the development of novel genetic engineering technologies.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Given his role as editorial board member, Dr. Yoshizumi Ishino, had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Dr. Qunxin She.

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Yoshizumi Ishino: Writing – review & editing, Writing – original draft.

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