SYSTEMS BIOLOGY
FELLOWSHIP PROGRAM

Investigation of *T. thermophilus* Type III CRISPR-Cas Mediated Bacterial Defense Mechanisms

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Fellowship 2019

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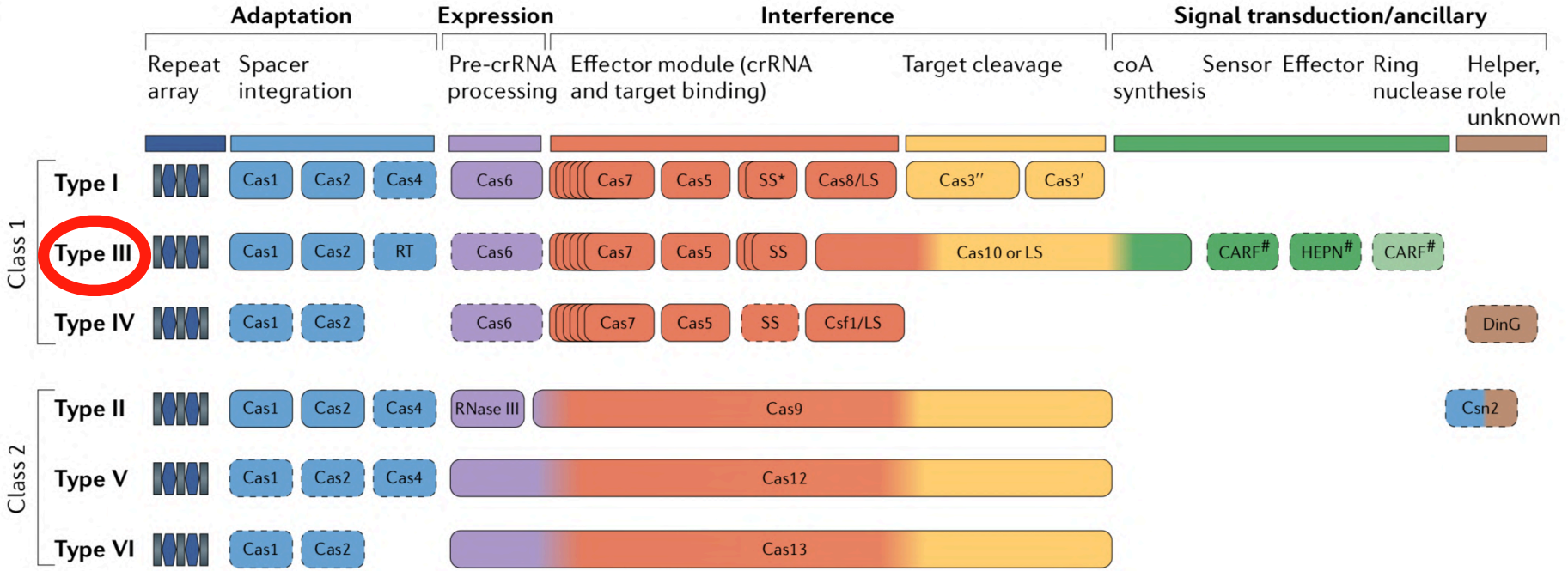
Diversity of CRISPR-Cas systems



adapted from Makarova *et al.*, *Nat. Rev. Microbiol.*, 2020

Investigation of *T. thermophilus* Type III CRISPR-Cas Mediated Bacterial Defense Mechanisms

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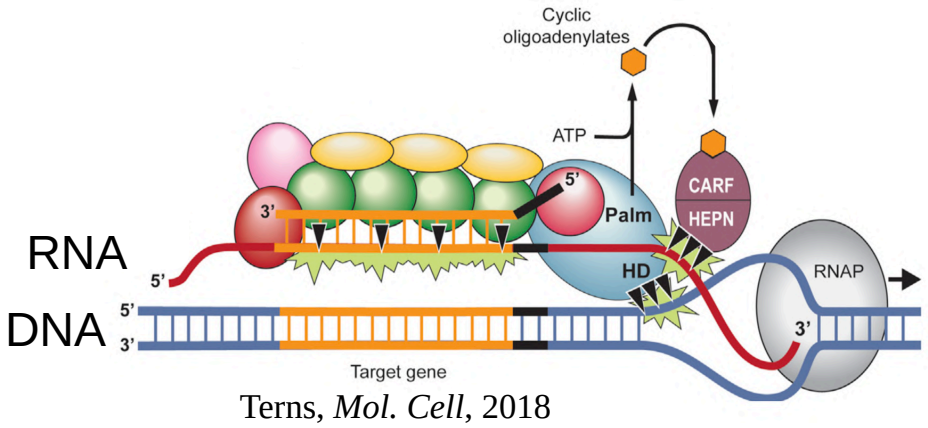
What are the distinctive features of Type III CRISPR-Cas systems?



Terns, *Mol. Cell*, 2018

➤ Transcription-dependent complicated mechanism of interference, both RNA and DNA are targets

➤ adaptation in the presence of unique Cas1-RT

Questions:

1. Can adaptation occur in the presence of regular Cas1?

2. Can escaper viruses appear?

3. DNA damage: A local cleavage or a large scale processive DNA destruction?

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Results

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Plan of the study for the first year:

Investigation of Type III system action in the conditions of infection with siphovirus phiFa

- → **set up the optimal conditions for adaptation** ✓
- → **HTS analysis of acquired spacers** ✓
- → **△*cas1* or/and △*cas2 T. thermopilus* strains creation** ✓
- → **adaptation in △*cas1*/△*cas2* strains** ✓
- → **re-infection of resistant strains** ✓
- → **search for escaper viruses** ✓

✓ completely done

✓ partly done

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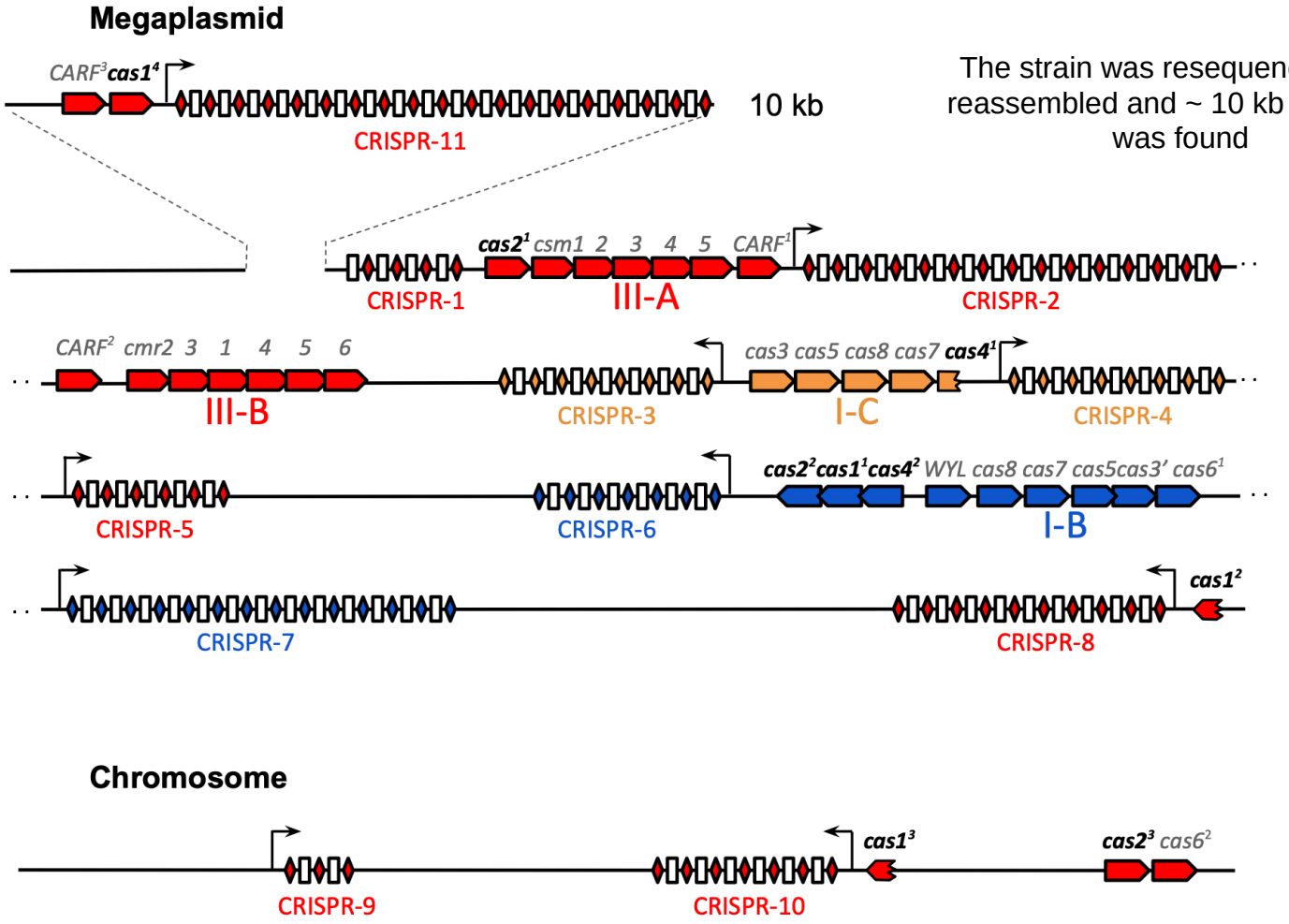
CRISPR-Cas systems of a model organism *Thermus thermopilus* HB27c



The strain was resequenced and reassembled and ~ 10 kb insertion was found

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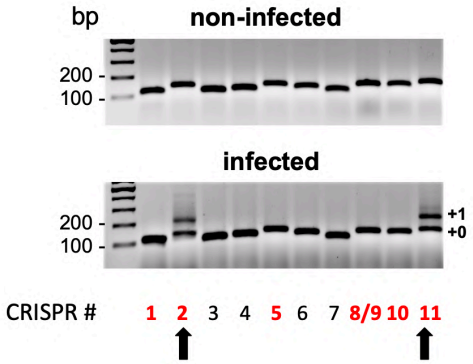
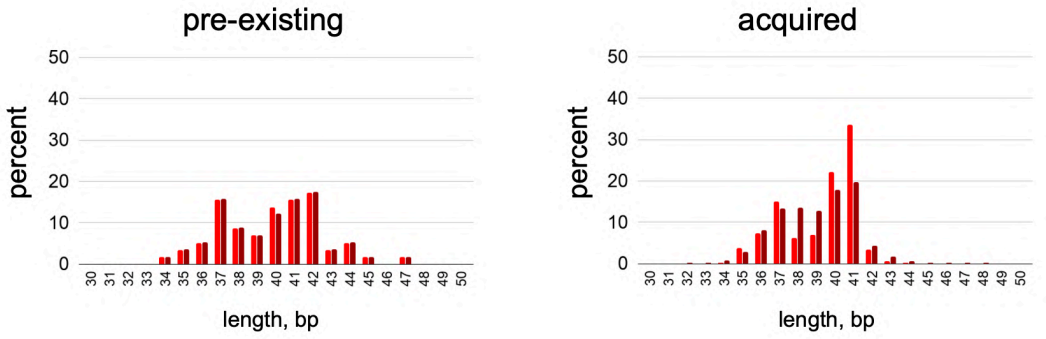
T. thermophilus reverse-transcriptase-free Type III CRISPR-Cas systems are able to incorporate new spacers during infection with siphovirus phiFa

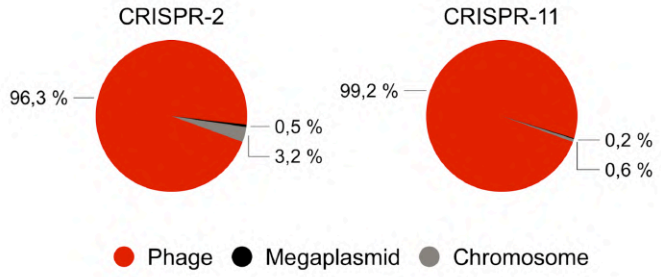
Expansion of some of Type III CRISPR arrays



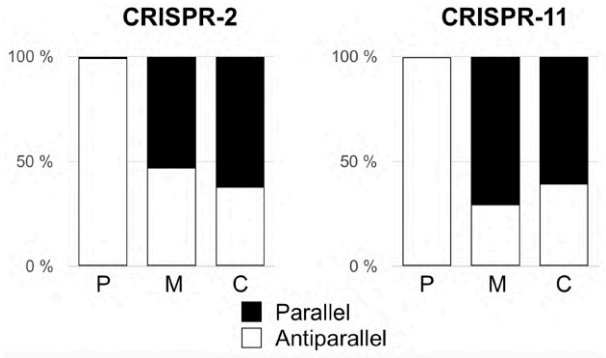
Acquired spacers demonstrate the typical for Type III systems distribution of lengths



The majority of expanded arrays carried phiFa-originated spacers



The direction of acquired spacers is biased



P - phiFa
M - megaplasmid
C - chromosome

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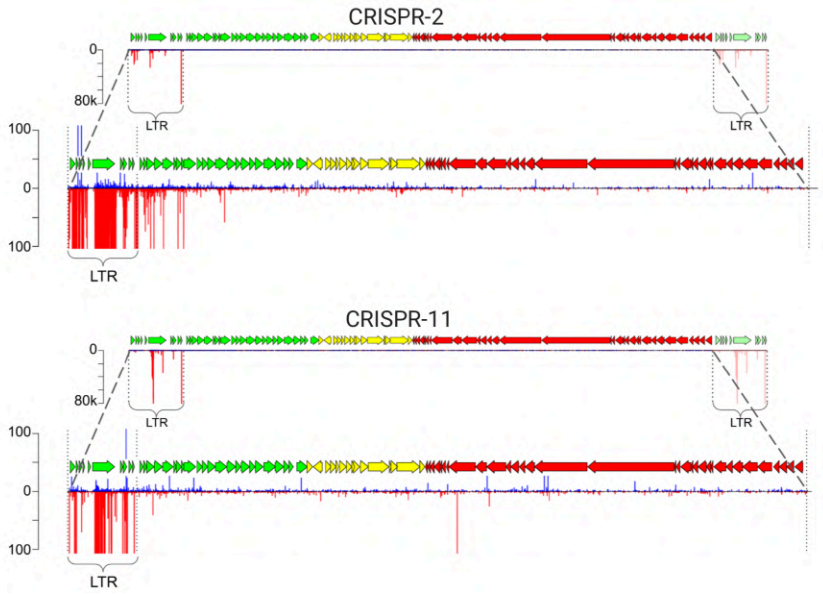
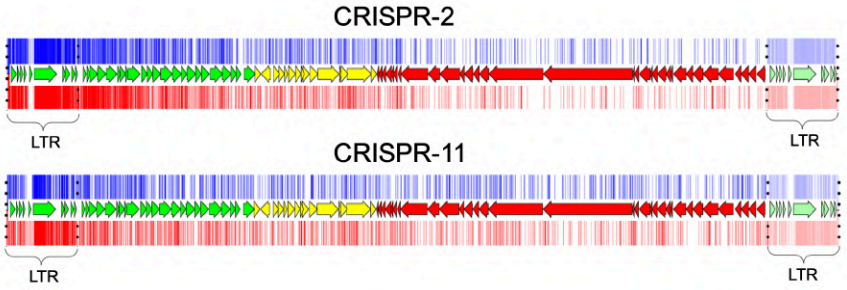
Most newly acquired spacers originate from the early region of phage DNA

Distribution of all spacers on phiFa genome is strongly biased

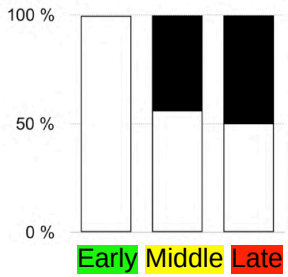


Distribution of unique spacers on phiFa genome is less biased

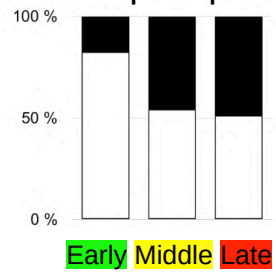


Direction of spacers acquired from phiFa's genes belonging only to early temporal class is biased

All spacers



Unique spacers



parallel to genes
antiparallel to genes

Not real bias but selection of cells acquired protecting spacers?

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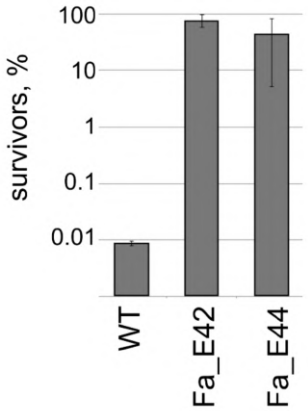
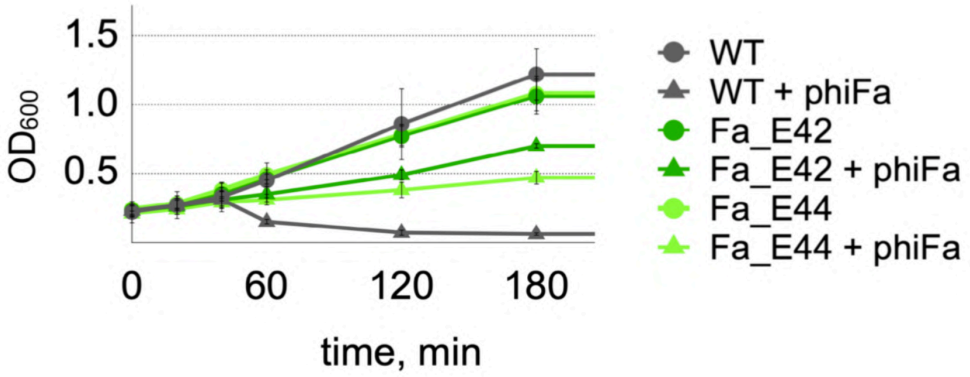
T. thermophilus cells carrying Type III spacers targeting early phiFa genes are resistant to infection

Spacers detected in the resistant *T. thermophilus* HB27c strains recovered after infection originate from early genes located within LTR

T. thermophilus HB27c strains acquired a new spacer and recovered after phage infection are significantly more resistant to re-infection than the wild-type strain





Fa_E42 - strain bearing spacer originated from phiFa's *ORF42* (hypothetical protein)
Fa_E44 - strain bearing spacer originated from phiFa's *ORF44* (RNA polymerase)

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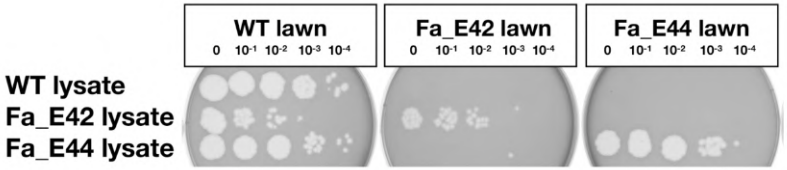
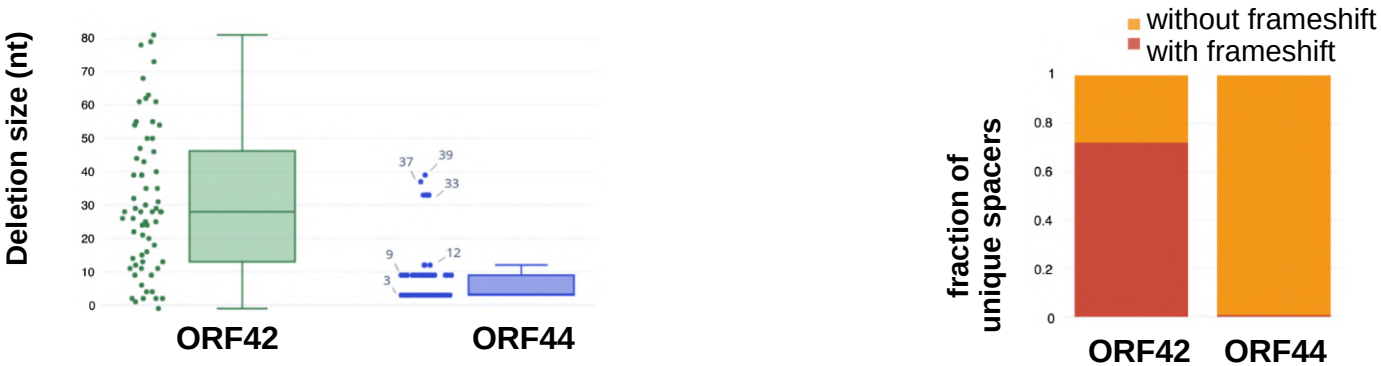
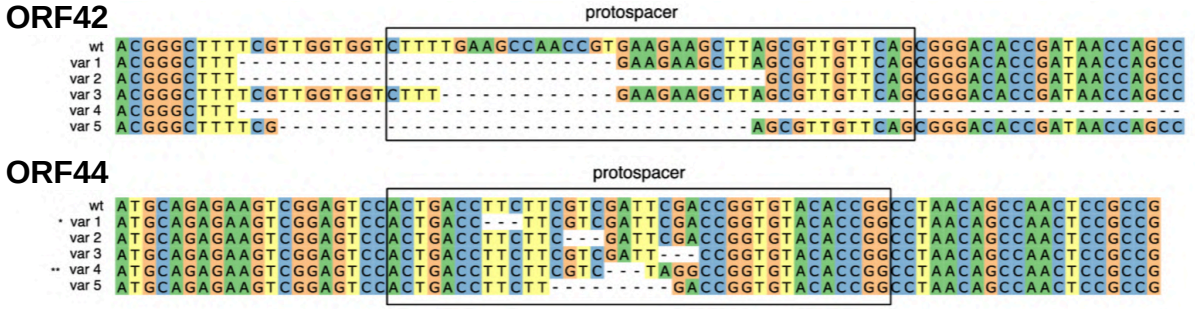
phiFa phage can escape the action of Type III CRISPR-Cas system

phiFa phage escapers accumulate during Type III CRISPR-Cas system action



phiFa escape Type III CRISPR-Cas system by deletions within protospacer region





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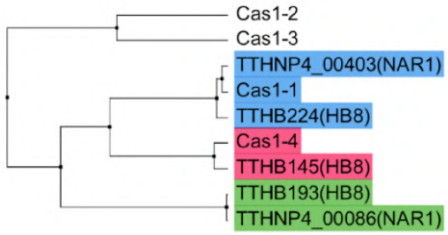
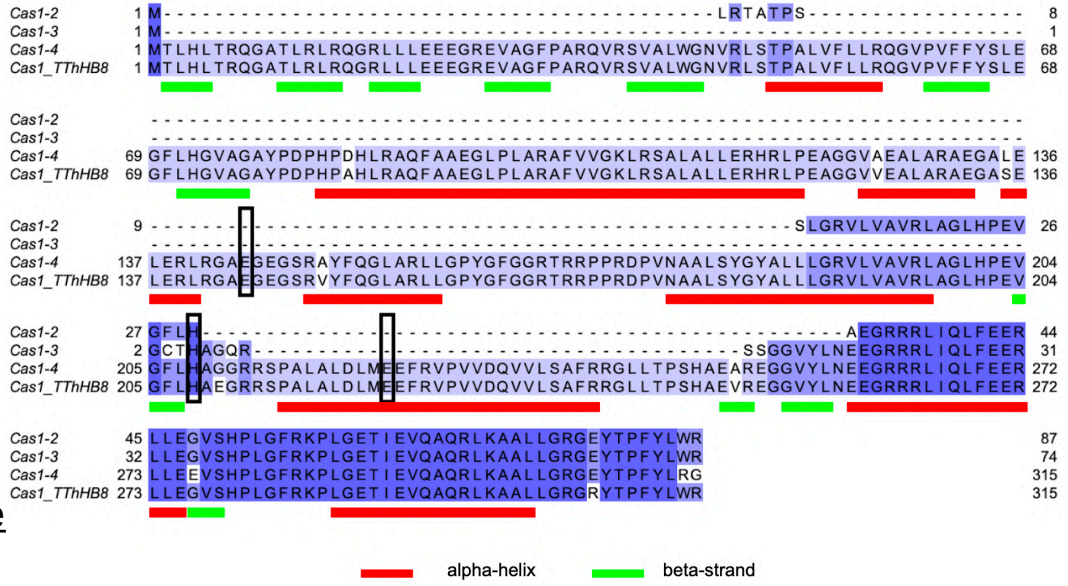
Activity of Cas1 proteins from *T. thermophilus* HB27c strain

Average distance phylogenetic tree of Cas1 proteins from *T. thermophilus* HB27c, HB8 and NAR1 strains

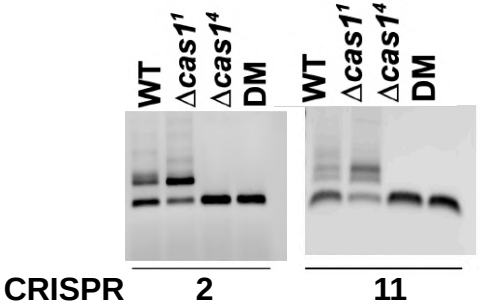


blue - I-B CRISPR-Cas system
red - III CRISPR-Cas system
green - I-E CRISPR-Cas system

Amino acids alignment of potential Type III Cas1 proteins from *T. thermophilus* HB27c and a homologue from HB8



Ability of different *T. thermophilus* HB27c mutants to integrate new spacers into Type III CRISPR arrays



Only one of four *T. thermophilus* HB27c *cas1* genes - *cas1⁴* - is essential for Type III adaptation

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Results

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Summary of results:

- robust adaptation by the Type III CRISPR-Cas systems of *T. thermophilus* HB27c that lacks either RT-Cas1 fusions or standalone RT genes was demonstrated during infection with siphovirus phiFa
- HTS analysis of acquired spacers revealed a very strong strand and coordinate bias of spacer distribution which seems to be a consequence of phage-resistant cells selection rather than a special mechanism of spacer acquisition
- strains acquired spacers targeting transcripts of a subset of phiFa's early genes were isolated during infection; their ability to survive the infection indicates that Type III immunity does not operate through altruistic suicide
- phiFa phages that escaped Type III interference accumulated deletions of integral number of codons in an essential gene and much longer deletions in a non-essential gene were isolated
- potential activity of two Cas1 proteins encoded in *T. thermophilus* HB27c genome was predicted and strains with deletions of the corresponding genes were constructed
- c*as1* gene responsible for Type III adaptation was established

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Future plans

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Plan for the second year:

I. To establish the essential for Type III CRISPR-Cas adaptation *cas2* gene(s)

II. To study features of adaptation during infection of *T. thermophilus* HB27c with a different bacteriophage - tectivirus phiKo

→ HTS analysis of acquired spacers
→ isolation and re-infection of strains with new spacers
→ search for escaper viruses

III. To investigate adaptation in conditions of *T. thermophilus* HB27c transformation with a plasmid

Achievements

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Papers:

Spacer Acquisition by Type III CRISPR-Cas System During Bacteriophage Infection of *Thermus thermophilus*.

Daria Artamonova, Karyna Karneyeva, Sofia Medvedeva, Evgeny Klimuk, Matvey Kolesnik, Anna Yasinskaya, and Konstantin Severinov. ***Nucleic Acids Res*, in review**.

Conferences:

Adaptation By *T. thermophilus* Type III CRISPR-Cas System in the Presence of Different Phages and Appearance of Viral Escapers.

Daria Artamonova, Karyna Karneyeva, Anna Yasinskaya, Alexander Milenkin, Konstantin Severinov. **CRISPR Technologies Conference**. Wurzburg, Germany, Sept 16-18, 2019.