Constraints on horizontal gene acquisition in bacteria: genetic analysis of a novel restriction-modification system.

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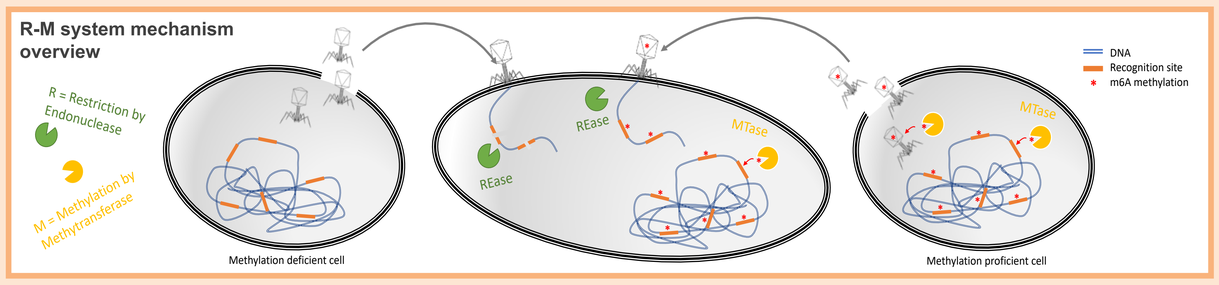
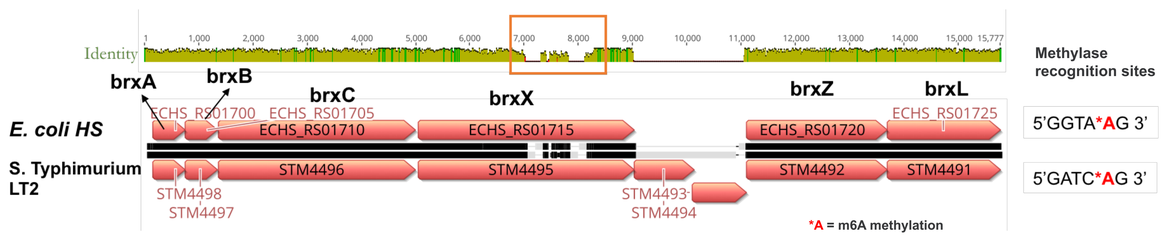
Bacterial lineages respond to changes in their physical and biotic environment by acquisition of niche-adaptive functions via **Horizontal Gene Transfer** (HGT). They are specified in lineage-variable segments called genome islands. For about half of these islands, the RecA-independent mechanism of assembly and dissemination is obscure, while site-specific recombinases and transposases play a role in circulation of others. We study an **island region bearing highly variable restriction-modification (RM) systems**. The island (dubbed the Immigration Control Region, ICR) varies within and between enteric species to protect against exogenous DNA entrance. In addition, unidentified site-specific HGT mechanisms may act here.

To initiate study of the mechanism of intergeneric transfer between *E. coli* and *Salmonella enterica* sv Typhimurium LT2, we chose a **restriction-disabled derivative (LB5000)** often used for molecular genetic constructions. Sequencing of this strain allowed identification of the mutations that potentially result in restriction-deficiency in three RM systems: two well-studied (SenLT2I (LT, StyLT in the early literature) and SenLT2II (SB, StySB)) and one poorly characterized, SenLT2III (SA, StySA). Surprisingly, in the genetic region expected for the StySA system, multiple mutations were found in domains of two separate genes. These identified homologs of a **BREX-like architecture for the StySA** system. Mutational states of the 8 gene cluster were tested for site-specific **methylation level** (PacBio RSII) **and bacteriophage restriction**. We compared wild type alleles with engineered deletions of individual genes and with the multiply mutated conserved domains of two genes. In addition, we performed **transcriptomic** analysis (Cappable-Seq and RACE) in order to unravel the operon structure of this gene cluster. This work should contribute to understanding the role played by this recently-discovered but widespread family of prokaryotic genome defense activities.

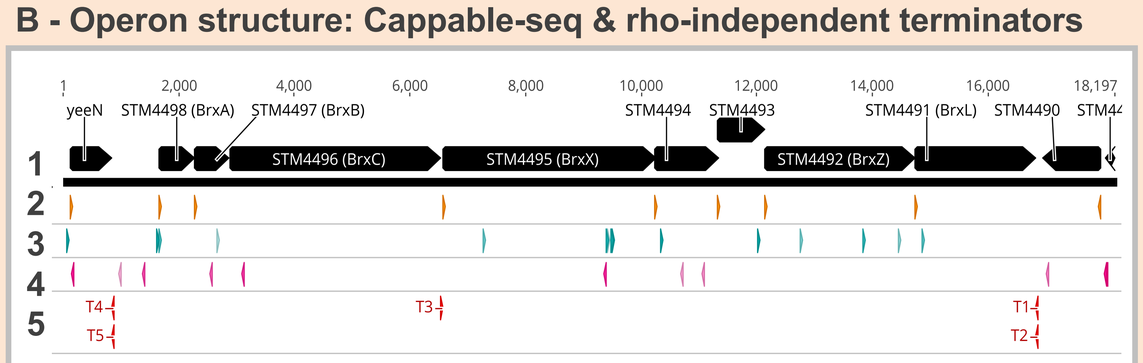


A - StySA: a new BREX system?



- StySA genes are homologous to BREX system of E. coli HS
- STM4493/94 are a toxin/antitoxin pair integrated after divergence of the two species

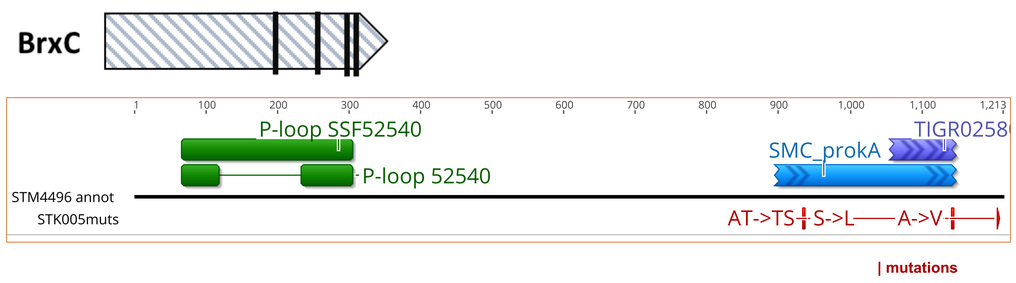
B - Operon structure: Cappable-seq & rho-independent terminators

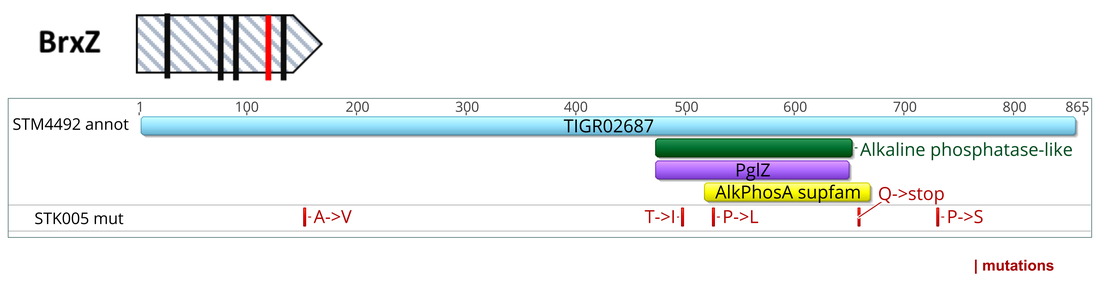


1. Locus organization (CDS)
2. Annotated translation regulatory sequences (LT2 genome)
3. Transcription Start Sites (TSS) reverse (Experimentally determined by Cappable-seq)
4. TSS forward (Experimental determined by Cappable-seq)
5. Predicted rho-independent transcription terminators (*in sillico*)

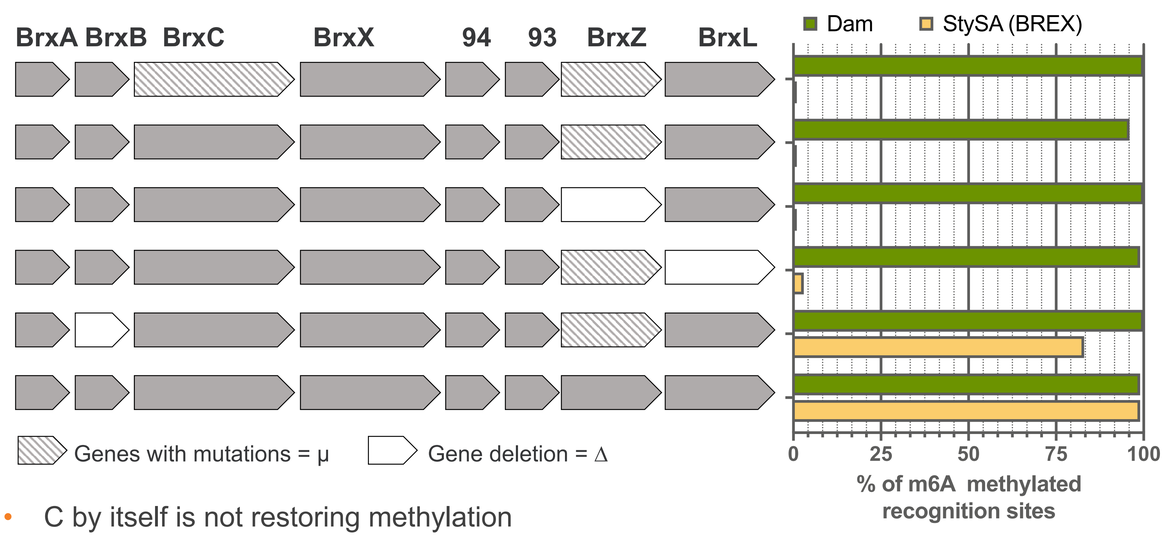
- StySA is insulated from the surrounding genes and vis versa
- Several strong TTS and regulatory sequences can be found along StySA
- A strong transcription terminator has been predicted after BrxC

C - R-M- phenotype of LB5000 is associated with mutated domains in BrxC and BrxZ

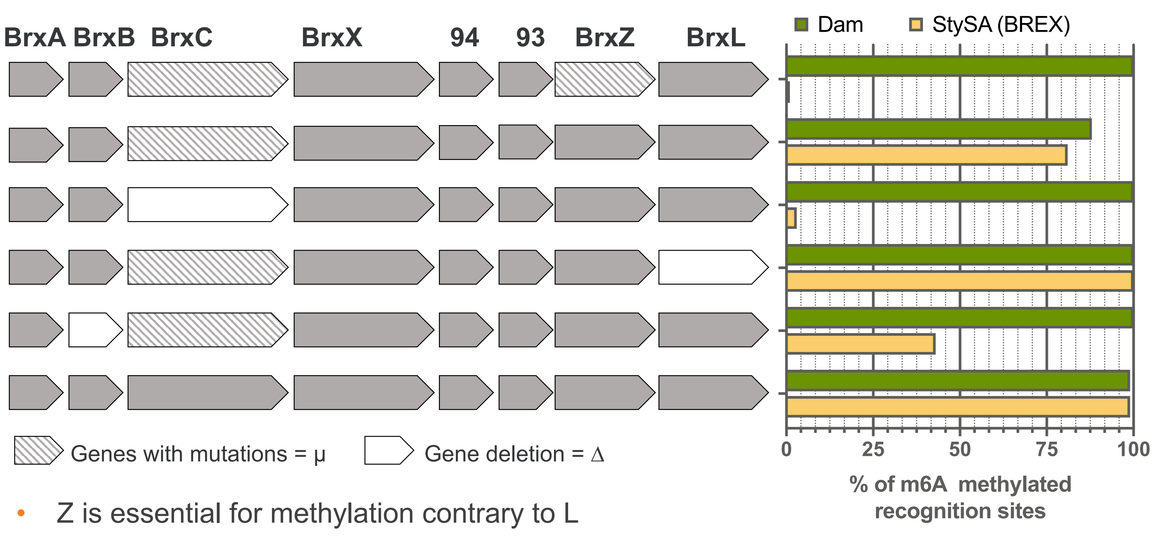




D - Impact of genetic changes on MTase transcription and DNA methylation

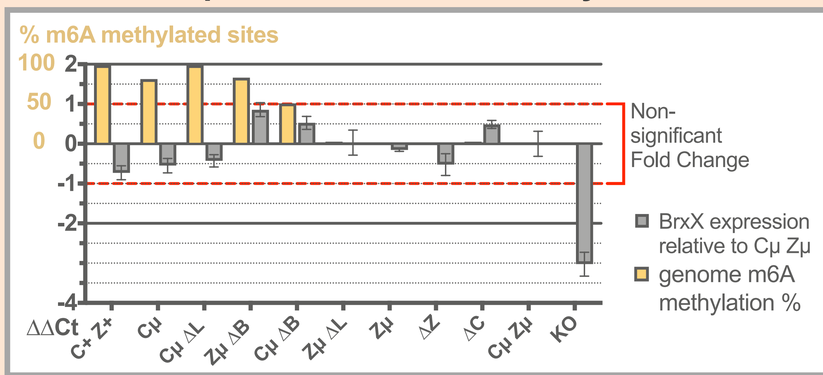


- C by itself is not restoring methylation
- B is potentially regulating the methylation
- Presence or absence of L is not changing methylation



- Z is essential for methylation contrary to L
- B is potentially regulating the methylation
- The P-loop domain of C sounds essential for methylation

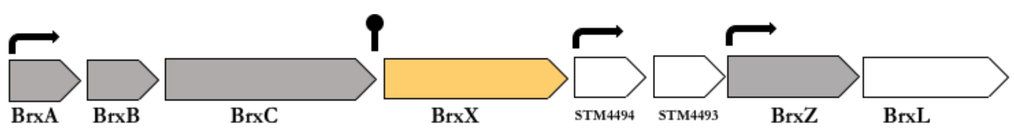
E - BrxX expression and m6A methylation level



- No drastic change in BrxX expression among the strains
- No correlation between methylation level and BrxX transcript level

Conclusions

- The locus may be divided in 2-3 operons
- R^- M^- phenotype may result from mutations in BrxC and BrxZ domains
- BrxB, BrxC, BrxX and BrxZ are necessary for methylation activity
- BrxX activity depends of a post-transcription event



References: Gordeeva et al., 2018 | Goldfarb et al., 2015 | Ettwiller et al. 2016 | Scotto-Lavino et al. 2006