The Bordetella Bacteriophage DGR Employs Similar Mechanisms for Retrotransposition in Heterologous Species

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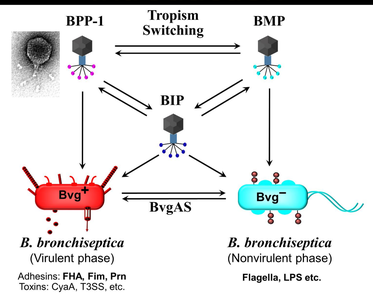
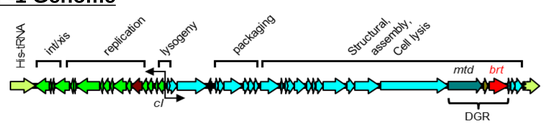
Abstract

Diversity-generating retroelements (DGRs) are a unique group of retroelements found in bacteria, archaea and their viruses. They mediate hyperdiversification of protein-encoding DNA sequences to facilitate the adaptation of their hosts to changing environments. The prototype DGR was discovered in the *Bordetella* bacteriophage BPP-1 and consists of three genes, *mtd* (major tropism determinant), *avd* (accessory variability determinant) and *brt* (*Bordetella* reverse transcriptase), and two imperfect repeats, variable repeat (VR) and template repeat (TR). VR is located at the 3' end of *mtd*, which encodes the phage distal tail fiber protein responsible for receptor recognition. Diversification of *mtd* results from unidirectional transfer of sequence information from TR to VR during which adenine residues in TR are converted into random nucleotides in VR, leading to phage tropic variants that recognize different receptor molecules. Here, we show that the BPP-1 DGR is also functional in heterologous bacterial species - *Escherichia coli* and *Pseudomonas aeruginosa*, and uses a similar mechanism for cDNA synthesis. However, efficiency of DGR mutagenic homing is affected by target sequence orientation in plasmids. Interestingly, overexpression of Avd and bRT has differential effects on DGR homing into targets inserted in different vectors. Surprisingly, homing into plasmid targets in *E. coli* is found to be largely independent of IMH (initiation of mutagenic homing) and the DNA stem-loop, elements important for its homing into native phage targets.

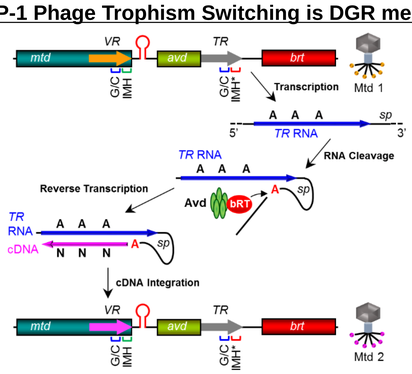
Introduction



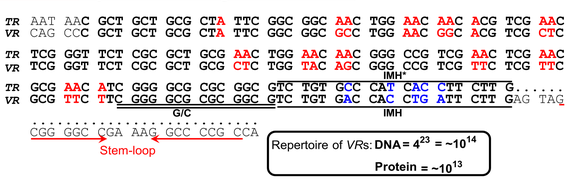
BPP-1 Genome



BPP-1 Phage Trophism Switching is DGR mediated



Adenine-specific mutagenesis in VR



Objectives

- To study BPP-1 DGR cDNA synthesis and mutagenic homing in *E. coli* and other species,
- To determine the mechanism of BPP-1 DGR cDNA integration.

Results

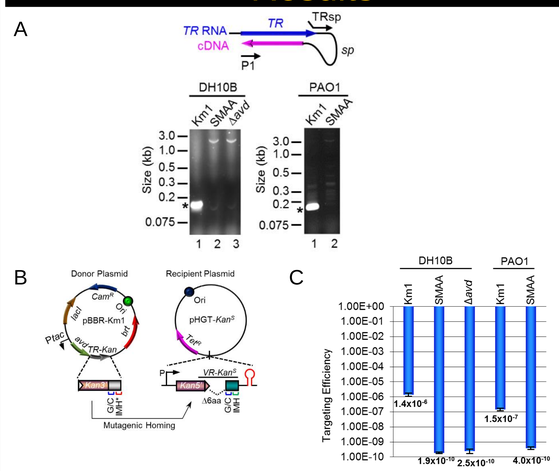


Figure 1. cDNA synthesis and homing of the BPP-1 DGR in heterologous hosts.(A) Top, diagram of RT-PCR assay to detect cDNA synthesis; bottom, cDNA was detected in *Escherichia coli* DH10B and *Pseudomonas aeruginosa* PAO1 but not in RT-deficient (SMAA) and *avd* deletion mutant (Δ*avd).* (B) Diagrams of donor and recipient plasmids of kanamycin reporter assay. (C) Results of kanamycin reporter assay in DH10B and PAO1.

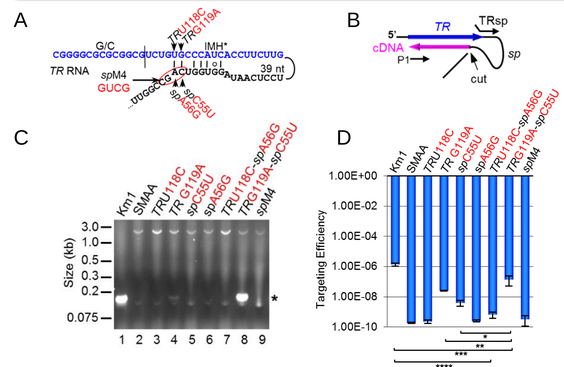


Figure 2. Effects of *TR* RNA mutations on DGR function in *E. coli* DH10B. (A) Diagram to show the positions of single and complementary mutations in *TR* and spacer (*sp*) region. (B) Diagram to show primers used for RT-PCR assay. (C) RT-PCR assay to analyze cDNA synthesis in single and complementary mutants. The expected cDNA products of ~140 bp are indicated by asterisk (*). (*D*) Kanamycin reporter assay of *TR* and *sp* mutants.

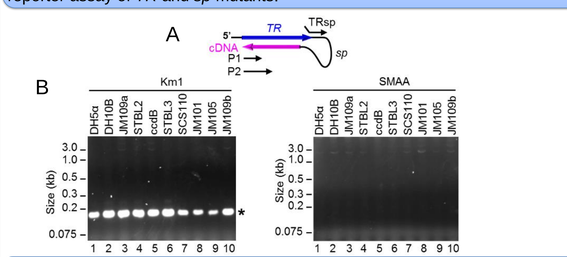


Figure 3. cDNA synthesis in different *E. coli* strains. (A) Diagram to show primers used for RT-PCR assay. (B) RT-PCR assays to analyze cDNA synthesis in 10 different *E.coli* strains. The expected cDNA products of ~140 bp are indicated by asterisk (*). Km1, functional construct with wt RT; SMAA, RT-deficient construct.

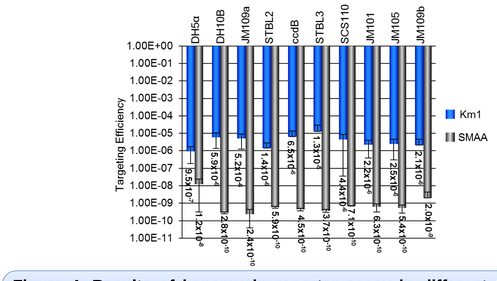


Figure 4. Results of kanamycin reporter assay in different *E. coli* strains. *E.coli* strains tested support DGR homing. Km1 (Blue columns), functional constructs with wt RT and SMAA (grey columns), non-functional RT-deficient constructs.

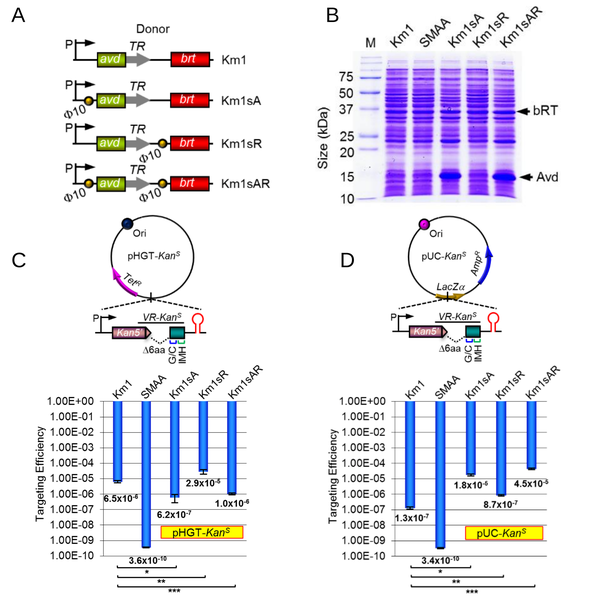


Figure 5. Overproduction of Avd and bRT has differential effects on DGR homing into recipient sequence inserted in different vectors. (A) Diagram to show the insertion mutants having strong Shine-Dalgarno (SD) sequence of gene 10 (Φ10) from phage T7 upstream of *avd* (Km1sA), *brt* (Km1sR) and, both *avd* and *brt* (Km1sAR). Km1; donor plasmid pBBR-Km1 with no SD sequence insertion.(B) SDS-PAGE to show the overexpression of Avd and bRT.(C) Top, diagram of pHGT-*Kan^S* recipient; bottom, effects of Avd and bRT overexpression on DGR homing into recipient plasmids pHGT-*Kan^S* with the RSF1010 origin. *p = 0.0005, **p < 0.05, ***p = 0.0006. (D) Top, diagram of pUC-*Kan^S* recipient; bottom, effects of Avd and bRT overexpression on DGR homing into the recipient plasmid pUC-*Kan^S* with ColE1 origin. *p = 0.0004, **p = 0.0001, and ***p = 0.0001.

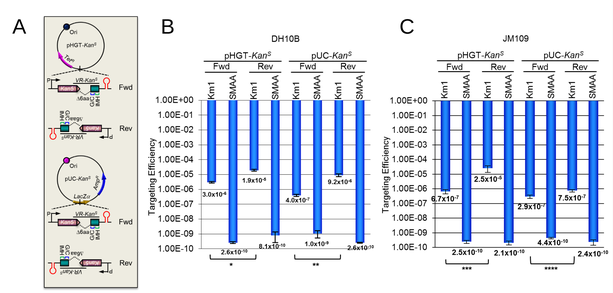


Figure 6. Target DNA replication affects cDNA integration. (A) Diagram of pHGT-Kan^S and pUC-Kan^S recipient plasmids with VR-*Kan^S* cassette in forward (Fwd) and Reverse (Rev) orientation. (B & C) Effect on target sequence orientation on DGR homing with recipient plasmids of pHGT-Kan^S and pUC-Kan^S in DH10B and JM109 respectively.

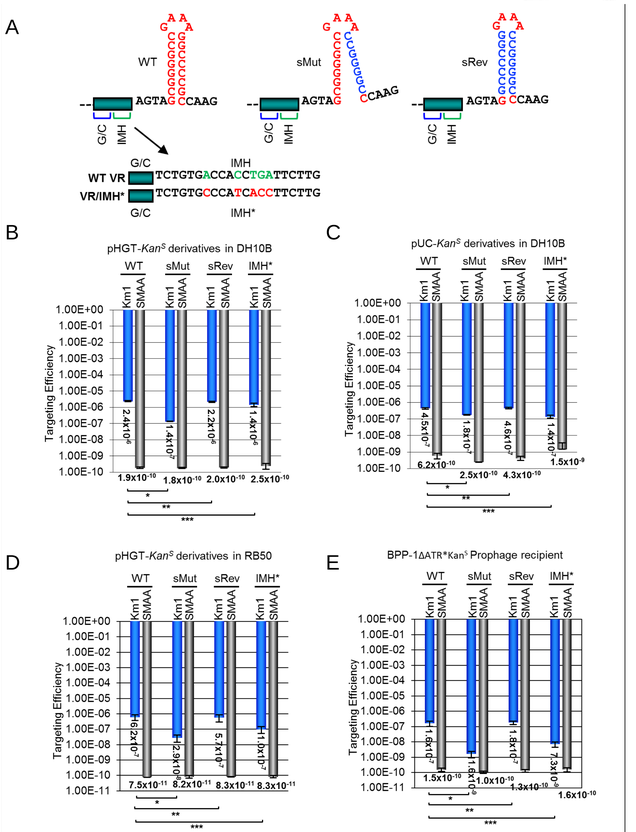


Figure 7. Hairpin/cruciform structure and IMH are not essential for DGR homing into different recipient plasmids and prophage. (A) Top, wild type and mutant hairpin/cruciform structures downstream of $(GC)_{14}$ and IMH of VR. Bottom, nucleotide polymorphism between IMH (Red) and IMH* (Green). VR/IMH* mutant construct has IMH* instead of IMH downstream of $(GC)_{14}$. (B-E) Kanamycin reporter assays with recipient plasmids (pHGT-*Kan^S* and pUC-*Kan^S*) having hairpin/cruciform mutants and VR/IMH* in DH10B; with recipient plasmid pHGT-*Kan^S* and prophage BPP-1ΔATR*Kan^S in RB50 respectively.

Conclusion

1. **BPP-1 DGR can synthesize adenine-mutagenized cDNA and subsequently the cDNA is integrated into target sequence in heterologous bacterial species.**
2. **BPP-1 DGR employs a similar priming mechanism for cDNA synthesis in heterologous species.**
3. **Avd overexpression has differential effects on DGR homing into different recipients suggesting its role in cDNA integration.**
4. **Target sequence orientation in recipient plasmids has an effect on cDNA integration demonstrating that cDNA integration occurs during recipient DNA replication.**
5. **DGR homing into target sequence in different plasmids and prophage does not require hairpin/cruciform structure and IMH*.**

Future Directions

1. **To characterize the amino acid residues that are responsible for adenine-specific mutations during cDNA synthesis in bRT,**
2. **To study the role of Avd in cDNA integration,**
3. **To analyze the function of BPP-1 DGR in higher eukaryotic organisms.**

Acknowledgements

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