Characterization of bacteriophage T5 pre-early genes: elucidating the minimal set of genes for host takeover.

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Résumé

T5 is a lytic bacteriophage that infects *Escherichia coli*. T5 genome delivery is carried out in two steps:(1) first, only 8% of the whole genome enter the host cell. The corresponding phage pre-early genes are quickly expressed: they inactivate host defense systems, and trigger massive host genome degradation. Then, during the second step, the remaining genome, including mid and late genes, is transferred into the host, thus resuming the phage replication cycle. Because of this unique mechanism of DNA delivery, T5 is an attractive model to study the role of pre-early genes in the host takeover process.
The First Step Transfer (FST) region, injected during the first step, includes sixteen genes. Thus far, only two of them, A1 and A2, are known to be essential for infection. A1 has DNase activity in vitro and in vivo, while A2 seems to act as a transcriptional regulator. However, it is still not clear whether the rest of the FST genes are dispensable and what their function is.

In addition to A1 and A2, genome analysis identified six FST genes, which are conserved in almost all T5-like phages: 02, 05, 07, 10, 13, 14. To assess the role of 02, 05 and 07, we are currently using two strategies: (i) studying the effects of gene deletion in the T5 genome on the infection process and (ii) testing the impact of phage gene overexpression over host fitness.

To accomplish the first aim, we optimized the CRISPR/Cas9 system (2) to select T5 mutants engineered by homologous recombination and we improved the yields up to 1 mutant per 10 lysis plaques. T5 mutants deleted in genes 02 or 05 were obtained, indicating that both genes are not essential for infection under laboratory conditions. We are currently studying their impact on T5 infection kinetics.

In our second parallel approach, we found that overexpression of gene 05, but not genes 02 or 07, inhibits growth of *E. coli*. These results suggest that the putative transmembrane protein 05, while not essential to T5 multiplication, might be toxic to the host during infection. Future work will aim at understanding the function of the minimal set of T5 pre-early genes in the host takeover.

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