Poster 27

Regulatory elements in the genetic switch region of the genome of the temperate *Streptococcus thermophilus* bacteriophage TP-J34 from a yoghurt starter strain

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Within the lysogeny module of the genome of the temperate *Streptococcus thermophilus* phage TP-J34, all but four adjacent open reading frames (orfs) are transcribed in one direction [1]. They are separated from the potential lytic cycle-promoting genes *cro* and *ant* by a genetic switch region, which contains the divergently oriented promoters P_1 and P_2 and several predicted operator sites. The orfs putatively code for the repressor Crh, a metalloproteinase Orf3, a superinfection exclusion (sie) mediating lipoprotein Ltp [2], and the integrase. Genes *crh*, *orf3* and *ltp* are transcribed as one polycistronic mRNA starting from promoter P_2. RT-PCR and Northern blot experiments suggested own promoters for *ltp* and *int* (confirmed with 5`RACE PCR obtaining the 5`end of transcripts). The repressor gene *crh* (essential for the establishment of lysogenization by suppressing lytic genes) was overproduced by heterologous expression in *E. coli* to perform electrophoretic mobility shift assays. Three operator sites in the intergenic regions between *crh* and *cro* (O_{1A}, O_2, O_3) and one between *cro* and *ant* (O_{1B}), respectively, were confirmed by competition assays with synthetic oligonucleotides. Glutaraldehyde was used as cross-linking reagent for Crh oligomerization (i.e., formation of dimers, tetramers and higher complexes). Knock-out experiments with *orf3* gene revealed a key role in induction of the lytic cycle. Studies on the interaction between Crh and Orf3 indicated that Orf3 prevents binding of the Crh repressor to its operator sites. Cro, the putative repressor of lysogenic genes, only bound to operator O_3 (probably resulting in repression of lysogenic promoter P_2). This would explain its antagonistic role.

[1] Sun, Goehler, Heller, Neve (2006) *Virology* **350**, 146-57.
[2] Ali, Koberg, Heßner, Sun, Rabe, Back, Neve, Heller (2014) *Frontiers in Microbiology* **5** (no. 98), 1-23.