The Puzzle of Prophage λ Stability

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After infection of the host, bacteriophage λ makes a decision between two of its life styles: lysis of the host with production of progeny phage or integration of its DNA as a prophage into the host chromosome with its lytic genes being repressed by the λ cI protein made in the prophage. The prophage state of λ can be induced to a lytic state by DNA damaging agents. However, quantitative analyses revealed that the prophage state of λ is extremely stable in the absence of inducing agents; spontaneous phage production occurs at a rate of 10^{-7} per lysogen, which is less than the mutational rate and cannot be explained by stochastic fluctuations in gene regulation. It has been shown recently that cI protein bound to right (O_R) operators and left (O_L) operators interact to form a DNA loop in the prophage. The loop formation is mediated by octamerization of cI bound to O_{R1}-O_{R2} and O_{L1}-O_{L2} operators at right and left, respectively by cooperative interactions. Mathematical modeling suggested that the co-operative binding-mediated DNA looping makes lysogen extremely stabile. We investigated the contribution of DNA looping in the prophage stability by *in vitro* experiments: First, *in vitro* transcription assays of the phage promoters under various conditions showed that DNA looping results in only a small enhancement of repression of the lytic promoters, P_R and P_L, and auto-activation of the cI maintenance promoter, P_{RM}. Second, transcription assays showed that DNA looping is essential for auto-repression of P_{RM}, which contributes to the stability of the prophage as well as narrows the cI concentration-range in a lysogen so as to fix the "set-point" for prophage induction when needed. Third, quantitative analysis of λ looping dynamics by tethered particle motion experiments (TPM) using single molecules showed that the O_{R1}-O_{R2}/O_{L1}-O_{L2} looping frequency is very low; an additionalO_{R3}/O_{L3} interaction stabilizes the loop. We conclude that cI binding to O_{R1}, O_{R2}, O_{L1} and O_{L2}, and multi-point contacts at O_{R1}-O_{R2}/O_{L1}-O_{L2} and O_{R3}-O_{L3} via cI make the prophage highly stable, whose spontaneous de-repression requiring multiple steps.