A methylation-directed, synthetic pap switch based on self-complementary regulatory DNA reconstituted in an all E. coli cell-free expression system

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Pyelonephritis-associated pili (pap) enable migration of the uropathogenic Escherichia coli strain (UPEC) through the urinary tract. UPEC can switch between a stable 'ON phase' where the corresponding pap genes are expressed and a stable 'OFF phase' where their transcription is repressed. Hereditary DNA methylation of either one of two GATC motives within the regulatory region stabilizes the respective phase over many generations. The underlying molecular mechanism is only partly understood. Previous investigations suggest that in vivo phase-variation stability results from cooperative action of the transcriptional regulators Lrp and PapI. Here, we use an E. coli cell-free expression system to study molecular functions of the pap regulatory region based on a specially designed, synthetic construct flanked by two reporter genes encoding fluorescent proteins for simple readout. Based on our observations we suggest that Lrp and the conformation of the self-complementary regulatory DNA play a strong role in the regulation of phase-variation. Our work not only contributes to better understand the phase variation mechanism, but it represents a successful start for mimicking stable, hereditary and strong expression control based on methylation. Since the regulatory DNA cruciform conformation corresponds to a Holliday Junction, gene expression is expected to respond if opposite arms of the junction are separated.