

P042 Dissecting the DNA-binding and regulatory properties of ParB homologue KorB of IncP1 broad host range plasmid RK2

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IncP1 (incompatibility group P) plasmids are low copy number multi resistant plasmids. They are responsible for spread and maintenance of important properties (antibiotic resistance and degradation function) among bacteria. Gene regulation in IncP1 plasmid is controlled by cooperative interaction of four repressors i.e. KorA, KorB, KorC and TrbA; two of them act as global regulators i.e. KorA and KorB and work together at five promoters which results in the more stringent control of genes involved in replication, stable inheritance and conjugative transfer. KorB (358aa) has dual role as a global regulator and active partitioning protein. The aim of this work is to dissect the regions of KorB involved in DNA binding, repression and cooperativity with KorA and TrbA. Negatively charged KorB (-21) binds DNA through a more positively charged surface including a Helix-turn-Helix motif (171-192 aa) but previous studies indicated a critical role for R240 in operator recognition. Mutational studies reported here show that the region 255-285aa can be deleted with little phenotypic consequence while the region from 225-255aa modulates DNA binding, spreading on DNA and activity as a repressor. KorB mutants that are defective in spreading can still repress at a distance so long as a partner protein (KorA or TrbA) binds proximal to the promoter with WT KorB repressors. KorA and TrbA can also promote silencing at a distance irrespective of the positioning of the KorA/TrbA binding site relative to the target for silencing. The results suggest a model in which KorB organises DNA loosely over a long region through a loose wrapping in a way that can accommodate other regulatory proteins. This nucleoprotein complex may also be critical for plasmid partitioning.