

Sorvall Rt6000d Manual

Julio E. Celis

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Regulation of the DnaA Promoter in Escherichia Coli Victoria Goehner Newman,1999 The timing of initiation of DNA replication from oriC in E coli is regulated by the concentration of the DnaA protein Increases in the intracellular DnaA protein concentration over 1 5 fold normal levels causes asynchronous timing of initiation of DNA replication The regulators of the dnaA operon must keep the DnaA concentration within tight limits yet be able to respond to rapidly changing environmental conditions Here it is shown that Fis and DnaA proteins interact in conjunction with other factors to regulate the expression of dnaA Binding of DnaA to the DnaA box in the dnaA promoter region is destabilized by the binding of Fis to

its binding site in dnaAp2 However DnaA94 containing only the C terminal 94 amino acids of the DnaA protein and retaining specific binding activity to the DnaA box of the dnaA promoter region is not destabilized by Fis binding DNase I and copper phenanthroline Cu op footprinting studies of DnaA and DnaA94 support the existence of a second weak DnaA box which abuts the DNase I and Cu op footprints of Fis in the dnaAp2 promoter We determined that the GC rich region of the dnaAp2 promoter is analogous to the discriminator sequence DS of rRNA promoters in that the GC rich sequence mediates the repression caused by elevated levels of guanosine tetraphosphate ppGpp During starvation conditions the alarmone ppGpp is synthesized Overexpression of ppGpp causes inhibition of dnaAp2 promoter transcription which is alleviated when the GC rich DS is mutated to an AT rich sequence We conclude that this site is responsible for mediating the stringent control of the dnaAp2 promoter Incorrect timing of initiation in the cell cycle results in asynchronous replication Strains with a mutated DS DnaA box or Fis binding site in the dnaA promoter region on the chromosome have increased levels of dnaA gene expression These mutants initiate DNA replication synchronously in minimal M9 medium as shown by flow cytometric assays In rich medium the DS mutant showed very limited asynchrony whereas the other mutants replicated synchronously

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