SUPPLEMENTARY INFORMATION

Results:

Characterization of the *gcrA* promoter

To study *gcrA* transcriptional regulation, we needed to delineate the region of the *gcrA* promoter that is required for its activity. Because the +1 transcriptional start site was identified previously (Holtzendorff et al. 2004), we assayed transcriptional fusions of different fragments upstream of the +1 site of *gcrA* with a promoterless *lacZ* reporter gene on the low copy plasmid p*LacZ*290 (Fig.3A). The construct containing the -100 to +92 region of the *gcrA* gene showed levels of β-galactosidase activity (3053+/-65 Miller Units) similar to that of the construct containing the -507 to +92 region of the *gcrA* gene (3026+/-74 Miller Units). The construct containing the -78 to +92 region of the *gcrA* gene showed levels of β-galactosidase activity (3308+/-62 Miller Units) slightly higher (~10% higher) than the construct containing the -100 to +92 region of the *gcrA* gene, suggesting that the -100 to -78 region of the *gcrA* gene may contain a weak regulatory sequence. Interestingly, there is an inverted repeated sequence in this region (Fig.3A), which could putatively form a hairpin structure. Nevertheless, since this construct containing the -78 to +92 region of the *gcrA* gene showed levels of β-galactosidase activity quite similar to the other two larger constructs, we concluded that all significant regulatory sequences should be included in the -78 to +1 region of the *gcrA* promoter.

Material and methods:

Site-directed mutagenesis and truncation of the *gcrA*P

Oligos 5`-GGTAAGCTTTCCAGCTCATTACCCGTCTC-3` (gcrA_rev) and 5`-CCGGATCCGGAATCAACATCTTGTTTCT-3` were used to amplify *gcrA*P(-78…+92) and the corresponding PCR product was digested with BamHI and HindIII and cloned into p*LacZ290*, giving rise to p*LacZ290-gcrA*P``(WT). Oligos gcrA_rev and 5`-CCGGATCCTTTTCGCAAGCGCGGGCTTG-3` were used to amplify *gcrA*P(-100…+92) and the corresponding PCR product was cloned into BamHI-HindIII-digested p*LacZ290*, giving rise to p*LacZ290-gcrA*P`(WT).

Site-directed mutations in the CtrA binding site, the two methylation sites and the DnaA box were generated by PCR using the flanking oligos gcrA_rev and 5`-CCGGATCCCTGTTCCAGCGCCGATAG-3` (gcrA_fwd). *gcrA*P(CtrAL) was created

using the mutagenic oligos 5`-CCATTGTGCGTTCCGCCGCATTCTTTACG-3` and 5`-CGTAAAGAATGCGGCGGAACGCACAATGG-3`. *gcrA*P(CtrAM) was created using the mutagenic oligos 5`-TGCGTTTTAACGCAGGCTTTACGATATCTA-3` and 5`-TAGATATCGTAAAGCCTGCGTTAAAACGCA-3`. *gcrA*P(CtrAR) was created using the mutagenic oligos 5`-TTTTAACGCATTCTCTACGATATCTAGTA-3` and 5`-TACTAGATATCGTAGAGAATGCGTTAAAA-3`. *gcrA*P(UM) was created using the mutagenic oligos 5`-CTGTGGACAGAGTTGATGGCGCCCATT-3` , 5`-AATGGGCGCCATCAACTCTGTCCACAG-3`, 5`-GGCTTGCCGGAATTAACATCTTGTTTC-3` and 5`-GAAACAAGATGTTAATTCCGGCAAGCC-3`. *gcrA*P(DnaA) was created using the mutagenic oligos 5`-AACATCTTGTTTCCACGGACAGAGTCGAT-3` and 5`-ATCGACTCTGTCCGTGGAAACAAGATGTT-3`. *gcrA*P(DnaA+CtrAL) was created using the mutagenic oligos used to construct *gcrA*P(DnaA) and *gcrA*P(CtrAL) DNA template. Final mutagenized PCR products were digested by BamHI and HindIII, and cloned into p*LacZ290*, giving rise to p*LacZ290-gcrA*P(CtrAL), p*LacZ290-gcrA*P(CtrAM), p*LacZ290-gcrA*P(CtrAR), p*LacZ290-gcrA*P(UM), p*LacZ290-gcrA*P(DnaA) and p*LacZ290-gcrA*P(DnaA+CtrAL).

Cell cycle transcription and synthesis analysis:

1-ml aliquots of cells grown in M2G were removed and labeled with 10μCi of [S^{35}] methionine (Amersham) for 2 or 5 minutes. Cells were collected by centrifugation and frozen on dry ice. Cell pellets were resuspended in 50 μl of SDS buffer and boiled for 5 minutes to lyse cells. Cell lysates were then mixed with 0.8 ml of RIPA buffer or Wash buffer, and 20 μl of protein A-agarose (Roche) was incubated with each sample for 1 hour to precipitate proteins that bind non-specifically. Equivalent counts of radiolabelled proteins were then used for immunoprecipitation. Anti-GcrA and anti-β galactosidase (Abcam) serums were diluted 1:200 and 1:1000 respectively into samples. Samples were incubated 1 hour before adding 20 μl of protein A-agarose, and incubated for 1 hour again. All incubations were performed at room temperature. Immunoprecipitates were collected by centrifugation, washed 3 times with Wash or RIPA buffer, resuspended in 20 μl of SDS-PAGE sample buffer, and boiled for 5 minutes. Resulting samples were resolved by 8% or 10% SDS-PAGE for β–Galactosidase or 15% SDS-PAGE for GcrA. Gels were dried and exposed against a Phosphor Screen

(Molecular Dynamics). Labelled protein bands were scanned and quantified using a PhosphorImager with ImageQuant software (Molecular Dynamics) and their relative activity was normalized so that the maximum value equals 1.

Buffers composition:

SDS buffer: 10 mM Tris-HCl pH 8, 1% SDS, 1 mM EDTA

RIPA buffer: 50 mM Tris-HCl pH 8, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS

Wash buffer: 50 mM Tris-HCl pH 8, 150 mM NaCl, 0.5% Triton X-100

GcrA half-life determination

Data fitting using time points from three independent experiments was performed with Kaleida Graph 3.5 (Synergy Software), using an exponential decay model. The fraction of residual protein is given by $\exp(-t/\tau)$, where t is the time after the reference time point, t_0, and the sole free parameter, τ, is the characteristic decay time. This expression yields the half-life of the protein, $\tau_{1/2}$, through the relation $\tau_{1/2} = \tau \ln 2$.