Conditional Essentiality of the csrA Gene in Escherichia coli

Johan Timmermans and Laurence Van Melderen*

Laboratoire de Génétique et de Physiologie Bactérienne, Institut de Biologie et de Médecine Moléculaires, Faculté des Sciences, Université Libre de Bruxelles, 12 rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium.

Received 5 November 2008/Accepted 10 December 2008

CsrA is a global posttranscriptional regulator of numerous physiological processes, such as glycogenesis and glycolysis. Here, we show that the csrA gene of Escherichia coli is essential for growth on LB and on synthetic medium containing glycolytic carbon sources. However, csrA is not necessary for growth on synthetic medium containing pyruvate, showing that the Krebs cycle is functional in the csrA::cat deletion mutant. Deletion of the gIgCIP operon in the csrA::cat mutant restored the ability to grow on LB and on synthetic medium containing glycolytic carbon sources, showing that growth inhibition is due to an excess of glycogen synthesis.

The Escherichia coli csrA (carbon storage regulator A) gene encodes a global regulator controlling a wide variety of biological processes, such as glycogen synthesis, glycolysis, and motility as well as cyclic-di-GMP synthesis and biofilm formation (2, 7, 14, 19, 21, 22, 26). CsrA regulates gene expression both positively and negatively at the posttranscriptional level by modulating translation. It binds as a homodimer to a specific sequence (consensus sequence, ACANGANGA) on both its mRNA targets and its small regulator RNAs (1, 2, 7, 18, 23, 26). Binding of CsrA to the leader sequence of the mRNA target blocks ribosome binding on the Shine-Dalgarno sequence (2, 7, 26) and most likely leads to its rapid degradation (18). As a positive regulator, CsrA enhances translation efficiency by stabilizing its mRNA targets (28).

Homologues of csrA have been identified, and their role in several bacterial species has been studied (for a review, see reference 15). These csrA homologues share regulatory networks with those identified in E. coli, such as motility (17) and biofilm formation (8). These homologues also appear to regulate other processes, such as virulence in Salmonella enterica, Legionella pneumophila, Helicobacter pylori, Proteus mirabilis, and Yersinia pseudotuberculosis (3, 9, 13, 17, 24); quorum sensing in Erwinia carotovora and Vibrio cholerae (5, 16); and oxidative stress resistance in Campylobacter jejuni (8).

In E. coli, the csrA gene was identified by transposon mutagenesis in a screen for mutants with altered glycogen accumulation. The kanamycin transposon insertion site is located at codon 51 in the csrA coding sequence (which encodes a protein of 61 amino acids). In addition to an increased glycogen accumulation, the csrA::kan mutant is affected for motility, biofilm formation, and glycolysis (2, 19, 21, 22, 28). In association with an rpoS defective mutation, the csrA::kan mutation led to an extended lag phase upon growth on acetate (27). Suppressor mutations restoring growth of the double mutant on acetate were mapped in the glg locus (27). This indicates that the growth defect presented by the double csrA::kan rpoS mutant was associated with a high level of glycogen accumulation (27). Acetate sensitivity appears to require both the csrA::kan and the rpoS mutations since single mutants were not sensitive to acetate and did not evolve suppressor mutations (27).

The E. coli csrA gene is essential under certain growth conditions. In an attempt to construct a csrA deletion mutant of E. coli, large series of homologous recombination experiments were carried out with the DJ624 strain (MG1655 lacX74 malP::lacIq) (12) to replace the csrA open reading frame (ORF) with a chloramphenicol resistance cassette (6). Despite many attempts under various conditions, no deletion mutants were obtained, suggesting that csrA is essential for growth. To test this hypothesis, various rescue plasmids were constructed. The csrA gene was cloned in the pBAD33 (11) and pKK223-3 (4) expression vectors. The expression of the rescue copy of csrA was induced in LB medium by the addition of arabinose (0.4%) and isopropyl β-D-1-thiogalactopyranoside (IPTG) (1 mM). These constructs were checked by sequencing, and their ability to complement a csrA::kan mutation was tested. Using these plasmids as a csrA rescue copy, attempts to delete csrA in DJ624 were not successful, indicating that high expression levels of CsrA might be deleterious under these conditions. To bypass this problem, the csrA gene was cloned in the low-copy number pWSK129 vector under the control of the lac promoter (25). DJ624 was transformed by this construct, and csrA expression was induced by the addition of 0.1 mM IPTG in the culture medium. Under these conditions, csrA deletion mutants were obtained on LB plates containing chloramphenicol (20 μg/ml) and kanamycin (50 μg/ml) at 37°C (ΔcsrA::cat). The replacement of the csrA ORF with the chloramphenicol resistance cassette was checked by PCR using primers flanking the csrA ORF. The ΔcsrA::cat mutant strain containing the pWSK- csrA plasmid was able to grow in the presence of 0.1 mM IPTG. No growth was observed on LB plates without IPTG, showing that the csrA gene is essential for growth in LB medium (Fig. 1, upper panels).

The CsrA1-50 peptide is partially active. The kan transposon is inserted at codon 51 in the csrA gene (21). Since this insertion mutant is viable on LB medium, it leaves the possibility that the 50 amino-terminal amino acids might retain some partial activity as proposed by Romeo and coworkers (21). To

* Corresponding author. Mailing address: Laboratoire de Génétique et de Physiologie Bactérienne, Institut de Biologie et de Médecine Moléculaires, Faculté des Sciences, Université Libre de Bruxelles, 12 rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium. Phone: 32 2 650 97 78. Fax: 32 2 650 97 70. E-mail: lvmelder@ulb.ac.be.

† Published ahead of print on 19 December 2008.
test this hypothesis, the first 150 5’ nucleotides of the csrA
coding sequence, followed by a stop codon, were cloned in
pKK223-3. The csrA deletion mutant containing the pWSK-
csrA plasmid was transformed with the pKK-csrA1-50 plasmid.
To displace the pWSK-csrA plasmid, we used the incompati-
bility properties of pWSK129 and pMLO59 (both plasmids
have a pSC101 replication origin but different antibiotic resis-
tance genes; pWSK129 confers resistance to kanamycin and
pMLO59 to spectinomycin). The ΔcsrA::cat pWSK-csrA pKK-
csrA1-50 strain was transformed by the pMLO59 vector, and the
resulting transformants were plated on LB plates containing
spectinomycin (100 μg/mL), chloramphenicol (20 μg/mL), and
0.1 mM IPTG to induce the expression of the csrA1-50 polypep-
tide. Transformants were checked for kanamycin sensitivity
(loss of the pWSK-csrA plasmid). The ΔcsrA::cat mutant contain-
ing the pKK-csrA1-50 plasmid was able to form colonies on LB
plates containing 0.1 mM IPTG but not on plates without
IPTG (Fig. 1, lower panels), showing that the CsrA1-50 peptide
is functional. On IPTG plates, the colonies had an abnormal
phenotype (forming heterogeneous and “sticky” colonies). The
molecular mechanisms underlying this phenotype are un-
known. This phenotype does not appear to be dependent on
the CsrA1-50 amount, since it has also been observed at higher
IPTG concentrations (data not shown). As the carboxy-termi-
nal part of CsrA (amino acids 51 to 54) is involved in RNA
binding (20), the truncated form of CsrA might have a reduced
affinity for RNA (another RNA binding site has been mapped
in the amino-terminal region of CsrA). This might provide an
explanation for the phenotype of the csrA::kan mutant.

An excess of glycogen accumulation impairs the growth of
the csrA::cat mutant. CsrA controls the intracellular carbon
flux by positively regulating the glycolysis pathway and nega-
tively regulating the gluconeogenesis pathway and glycogen
synthesis (Fig. 2) (22). In the absence of CsrA, glycogen syn-
thesis is strongly favored. Indeed, the csrA::kan insertion
mutation resulted in 20-fold-higher levels of glycogen (21). The
csrA::cat mutant might thus be unable to direct the carbon flux
to the Krebs cycle, because of a reduced glycolysis and an
enhanced gluconeogenesis. To test this hypothesis, the ΔcsrA::cat
mutant was plated on Ceria synthetic medium (10) containing
amino acids and glycolytic carbon sources. The ΔcsrA::cat mu-
tant was unable to grow on glucose and on fructose-6-phos-
phate as sole carbon sources (Fig. 3B and C). Interestingly, it
was able to grow on pyruvate, which is the carbon source at
the junction between glycolysis and the Krebs cycle (Fig. 3D).
On fructose-6-phosphate plates, very small colonies appeared after
16 h at 37°C (too small to be seen in Fig. 3C). These data
indicate that the early steps of glycolysis are not fully functional
in the ΔcsrA::cat mutant. With glucose and fructose-6-phos-
phate as sole carbon sources, the carbon flux might be essen-
tially directed to glycogen synthesis, thereby impairing glycol-
ysis and the Krebs cycle. We tested whether the deletion of the
glgCAP operon, which is involved in the first two steps of
glycogen synthesis, would restore the ability to grow on glyco-
lytic carbon sources. The glgCAP operon was deleted in the
DJ624 strain by using the method described in reference 6. The
csrA::cat deletion was then transduced by P1 in the ΔglgCAP
strain containing the pWSK-csrA plasmid in the presence of 0.1
mM IPTG. Transductants were obtained and checked by PCR
with primers flanking the csrA ORF. Interestingly, the double
ΔglgCAPΔcsrA::cat pWSK-csrA mutant was able to form colo-
nies on LB plates without IPTG (Fig. 3E) as well as on glucose
and fructose-6-phosphate plates (Fig. 3F and G), unlike the
ΔcsrA::cat pWSK-csrA mutant (Fig. 3A, B, and C). Both strains
were able to grow in the presence of pyruvate as the sole
carbon source (Fig. 3D and H). Our data show that in the
csrA::cat mutant, glycogen synthesis is favored and that glyco-
gen accumulation impairs viability. In the absence of the gly-

FIG. 1. The csrA gene is essential in E. coli. The ΔcsrA::cat mutant
containing either the pWSK-csrA (upper panels) or the pKK-csrA1-50
(lower panels) plasmids were grown on LB plates containing the ap-
propriate antibiotics with 0.1 mM IPTG (left panels) and without
IPTG (right panels).

FIG. 2. CsrA-mediated regulation of the central carbon flux. CsrA
positively regulates glycolysis and negatively regulates gluconeogenesis
and glycogen synthesis. Negative and positive regulations of target
genes are indicated by blocked arrows ( ) and arrows, respectively.
Adapted from reference 22.
Identification of a global repressor gene, csrA, of Erwinia carotovora subsp. carotovora that controls extracellular enzymes, N-(3-oxohexanoyl)-L-homo-

Datsenko, K. A., and B. L. Wanner. 2000. One-step inactivation of chromo-


Romeo, T., M. Gong, M. Y. Liu, and A. M. Brun-Zinkernagel. 1993. Identifi-


