Effects of the Global Regulator CsrA on the BarA/UvrY Two-Component Signaling System

Martha I. Camacho, Adrian F. Alvarez, Ricardo Gonzalez Chavez, Tony Romeo, Enrique Merino, Dimitris Georgellis

The hybrid sensor kinase BarA and its cognate response regulator UvrY, members of the two-component signal transduction family, activate transcription of CsrB and CsrC noncoding RNAs. These two small RNAs act by sequestering the RNA binding protein CsrA, which posttranscriptionally regulates translation and/or stability of its target mRNAs. Here, we provide evidence that CsrA positively affects, although indirectly, uvrY expression, at both the transcriptional and translational levels. We also demonstrate that CsrA is required for properly switching BarA from its phosphatase to its kinase activity. Thus, the existence of a feedback loop mechanism that involves the Csr and BarA/UvrY global regulatory systems is exposed.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The strains and plasmids used in this work are listed in Table 1. Strains IFC5010 (csrA::Kan’ csrB-lacZ) and IFC5016 (uvrY::Cam’ csrA::Kan’ csrB-lacZ) were constructed by P1vir transduction of the ackA::tetR::pta allele from strain ECL5336 (27) into strain IFC5010. For strain IFC5017 (barA::Cam’csrA::Kan’ csrB-lacZ) construction, the barA gene was deleted by homologous recombination using the lambda Red recombinase system (28). Briefly, a fragment amplified by PCR, using primers barA-Adel-Fw (5’-ATTTAACGTGTGAACCTACTTGCCATACACGAA CGTCCAAGGCTGCTTC-3’) and barA-Adel-Rv (5’-CATAA ACACAGGCACTTTGTCAACATCTGAAACCCAGCTATGATATC CTCTCTACTCC-3’) and plasmid pKD3 (28) as the template, was used to replace the barA allele with a chloramphenicol cassette in strain IFC5010.

To construct Amp’-linked lacZ operon fusions, plasmid pAH125-blalacZ was first generated by replacing the kanamycin resistance cassette of pAH125 (29) with an ampicillin resistance cassette. To this end, a bla PCR product was generated, using primers Amp-Prom-Fw (5’-GGCGGCC CTCCCAATATGTATCCGGTTGATG-3’) and Amp-Rv (5’-GGC GGGCC CGTGTCGACGTATTACCAAAGTG-3’) and plasmid pUC18 as the template, and cloned into the NarI-NotI sites of pAH125. Helper plasmid pINT-cat was constructed by replacing the ampicillin resistance cassette of plasmid pINT-ts (29) with the chloramphenicol resistance cassette. A 1.1-kb DNA fragment containing the cat gene, obtained from plasmid pKD3 (28) by HindIII digestion, was blunt ended and cloned into blunt-ended Smal-NotI sites of pINT-ts. Plasmid pAH-uvrY, containing an uvrY-lacZ operon fusion, was constructed by cloning a PCR-amplified fragment containing the upstream noncoding region through the first 4 codons (nucleotides [nt] −409 to +12 relative to the start of translation) (using primers uvrYP-fw-Pst [5’-AACCTGAGGGCCGGAGATATC CATAAG-3’] and uvrYP-Rv-BamHI [5’-GGGATCCGAGAAGTTGAT CAAAGGAATATG-3’]) into the PstI-BamHI sites of pAH125-blalacZ. Plasmid pUV-uvrY22, containing a translational uvrY-lacZ fusion under the control of the lacUV5 promoter, was constructed by cloning the region

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TABLE 1 E. coli strains and plasmids

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from nt −47 to +66 relative to the start of uvrY translation, amplified by PCR using primers uvrY-lacZ-Fw (5’-GCCGCCATCGTACCACGAGTAG GATCAGCAGC-3’) and UvrY-Rv-HindIII (5’-CCCAGCTCCGTAACCCGACGATC-3’), into the EcoRI-BamHI sites of plasmid pUV5 (30). All fusions were integrated into the CF7789 and TR1-SCF7789 chromosomes, as previously described (29), to generate strains IFC5011 to IFC5014.

To construct plasmids pMX539 and pMX541, the uvrY open reading frame and its promoter region were PCR amplified using primers uvrY-lead-Fw (5’-CCCAAGCTTCCGTACCACCAGCATCG-3’) and UvrY-lead22-Rv (5’-CGGGATCCTCTTCCAGAATGAGTAGC-3’), into the EcoRI-BamHI sites of plasmid pUV5 (30). All fusions were integrated into the CF7789 and TR1-SCF7789 chromosomes, as previously described (29), to generate strains IFC5011 to IFC5014.

RNA extraction and Northern blotting. Total RNA was purified from samples taken at the indicated times by the hot-phenol extraction method, as described previously (33). Northern blot analysis was performed by fractionation of the purified RNA samples (5 μg) on 1.2% agarose-formaldehyde gels and transfer onto nitrocellulose membranes.
(Amersham XL) by capillary transfer by using 20X SSC (1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Membranes were cross-linked using a cross-linking device (Stratalinker; Stratagene) and prehybridized for 3 h at 42°C in a buffer containing 5X Denhardt solution (34), 5X SSC, 0.2% SDS, 5% formamide, and 250 mg of sheared salmon sperm DNA per ml. Subsequently, a radiolabeled csrB-specific DNA probe, denatured at 90°C for 5 min, was added to the prehybridization buffer, and the membranes were incubated at 42°C overnight. The csrB-specific probe was obtained by digestion of plasmid pMX543 with EcoRI, separation of the fragments on agarose gels, and purification of the csrB-specific band by using the Qiagen agarose purification kit. Probe labeling was performed by using [α-32P]dCTP and the Radiprime kit (Invitrogen), according to the manufacturer’s instructions. Membranes were washed twice with 50 ml of 2X SSC and 0.1% SDS at 37°C and twice with 0.2X SSC and 0.1% SDS at 42°C. Images were obtained using phosphorimager screens and analyzed using the Typhoon image scanner (Amersham Biosciences).

β-Galactosidase activity. β-Galactosidase activity was assayed and expressed in Miller units as described previously (32). Cells were grown in LB broth, or LB with the pH adjusted and buffered to pH 5.0 with 0.1 M homopiperazine-N,N’-bis(2-ethanesulfonic acid) (HOMOPIPES). When indicated, acetate or formate was used at a concentration of 7 mM.

Immunoblotting and generation of polyclonal anti-UvrY and anti-BarA. Cultures for Western blot analyses were grown aerobically at 37°C and harvested by centrifugation during mid-exponential growth. The cell pellet was resuspended in 100 μl lysis buffer (50 mM Tris-HCl, 4% SDS, pH 6.8) and boiled for 5 min. Aliquots of 10 μl were separated by SDS-PAGE (15% polyacrylamide gels for UvrY and 8% polyacrylamide gels for BarA), and the proteins were transferred to a Hybond-ECL filter (Amersham Biosciences). The filter was equilibrated in TTBS buffer (25 mM Tris, 150 mM NaCl, and 0.05% Tween 20) for 10 min and incubated in blocking buffer (1% milk in TTBS) for 1 h at room temperature. Polyclonal antibodies against UvrY and BarA, raised by subcutaneous immunization of rabbits with His6-UvrY and His6-BarA, were added at dilutions of 1:2,000 and 1:10,000, respectively, to the filter and incubated for 1 h at room temperature. The bound antibody was detected by using anti-rabbit IgG antibody conjugated to horseradish peroxidase and the ECL detection system (Amersham Biosciences).

RESULTS AND DISCUSSION

CsrA is required for activation of csrB transcription. It has been previously reported that acetate and formate, as well as short-chain fatty acids, act as stimuli for the sensor kinase BarA (4), leading to its autophosphorylation and transphosphorylation of the cognate response regulator UvrY (1). Phosphorylated UvrY (UvrY-P), in turn, activates transcription of the CsrB and CsrC small untranslated RNAs, which act by sequestering the CsrA global regulatory protein and antagonizing its regulatory activity (25, 35). Curiously, UvrY-dependent activation of csrB transcription, which takes place at the transition from exponential to stationary growth phase (25), was found not to occur in a csrA mutant as judged by Northern blotting and by csrB-lacZ (located at attK) reporter expression (Fig. 1A and B), in agreement with a previous report (25). Moreover, it was found that csrB expression in the csrA mutant was fully restored by ectopic expression of CsrA using plasmid pMX544 (Fig. 1A and B). Because CsrA does not affect csrB stability (26) and because csrB transcription is activated directly by UvrY-P, it can be inferred that CsrA affects the activity and/or the expression of the components of the BarA/UvrY signaling system.

Acetate and formate are unable to activate BarA in a csrA mutant. Considering that a role of CsrA, an RNA binding protein, in the control of the BarA/UvrY phosphorelay cascade as not very probable, we hypothesized that CsrA might be required either for the production of the BarA-specific stimulus and thereby activation of the BarA/UvrY signaling cascade or for the expression of the barA and/or uvrY genes. To test the first possibility, we took advantage of the fact that although the BarA/UvrY TCS remains inactive when cells are grown at pH 5.0 (36), addition of acetate or formate to the growth medium results in the immediate activation of BarA/UvrY and thereby activation of csrB transcription (4). Therefore, strains KSB837 (wild type), IFC5010 (the isogenic csrA mutant), and IFC5010 carrying the csrA-expressing plasmid pMX544 were grown in LB medium, and total RNA isolated from samples that were harvested throughout the growth curve (optical density at 600 nm [OD600] of 0.3 to 2.0) was probed for the CsrB transcript. Experiments were repeated three times in their entirety with essentially identical results. (B) Overnight cultures of the wild-type strain (squares) and its isogenic uvrY (circles) and csrA (diamonds) mutant strains and the csrA mutant strain carrying the csrA expressing plasmid pMX544 (closed squares), all carrying the csrB-lacZ transcriptional fusion, were diluted to an OD600 of ~0.05 in LB medium, and the β-galactosidase activity was followed for 300 min. Note that the circles and diamonds extensively overlap. The average from four independent experiments is presented (standard deviations were less than 5% from the mean).

FIG 1 Effects of csrA and uvrY on csrB transcription. (A) Northern blot analysis of CsrB levels in the isogenic strains KSB837 (wild type [wt]), UVKSB837 (uvrY), IFC5010 (csrA), and IFC5010 carrying the csrA-expressing plasmid pMX544 (indicated as pCsrA). Cultures of these strains were grown in LB medium, and total RNA isolated from samples that were harvested throughout the growth curve (optical density at 600 nm [OD600] of 0.3 to 2.0) was probed for the CsrB transcript. Experiments were repeated three times in their entirety with essentially identical results. (B) Overnight cultures of the wild-type strain (squares) and its isogenic uvrY (circles) and csrA (diamonds) mutant strains and the csrA mutant strain carrying the csrA expressing plasmid pMX544 (closed squares), all carrying the csrB-lacZ transcriptional fusion, were diluted to an OD600 of ~0.05 in LB medium, and the β-galactosidase activity was followed for 300 min. Note that the circles and diamonds extensively overlap. The average from four independent experiments is presented (standard deviations were less than 5% from the mean).

CsrA is required for proper uvrY expression. Next, we tested the possibility that CsrA affects the expression of barA and/or...
FIG 2 The synthesis of the BarA stimulus does not appear to be affected in the csrA mutant strain. (A) The isogenic strains KSB837 (wild type), IFC5010 (csrA), and IFC5010 carrying the csrA-expressing plasmid pMX544 (indicated as pCsrA) were grown in LB medium, the pH of which had been adjusted and buffered to 5.0 using 0.1 M homopiperazine-N,N′-bis-2-(ethanesulfonic acid) (HOMOPIPES). At an OD₆₀₀ of 0.2, a sample was withdrawn, 7 mM acetate or formate was added to the medium, and samples were withdrawn every 10 min. Total RNA isolated from these samples was analyzed by Northern blotting using a CsrB-specific probe. The experiment was repeated three times in its entirety with essentially identical results. (B) Cultures of the isogenic strains KSB837 (wild type), IFC5010 (csrA), and IFC5010 harboring plasmid pMX544 (indicated as pCsrA), all carrying the csrB-lacZ transcriptional fusion, were diluted to an OD₆₀₀ of ~0.05 in LB medium at pH 5.0 as described for Fig. 1B alone (squares) or in the presence of acetate (circles) or formate (diamonds), and the β-galactosidase activity was followed. The average from four independent experiments is presented (standard deviations were less than 5% from the mean). (C) Concentration of extracellular acetate. The KSB837 wild-type strain and IFC5010, its isogenic csrA mutant strain, were grown in LB at pH 7.0 or 5.0. Samples were withdrawn either at early exponential growth phase (designated 1) or at late exponential phase (designated 2), and the concentration of acetate was determined with the R-Biopharm acetic acid determination kit (Boehringer Mannheim). The averages from three independent experiments are presented and the standard deviations are indicated.

**FIG 3** CsrA is required for proper uvrY expression. (A) Levels of BarA protein (102.550 Da) in the KSB837 (wild-type) and IFC5010 (csrA) strains as determined by Western blot analyses using BarA polyclonal antibodies. (B) Levels of UvrY protein (23.890 Da) in the KSB837 (wild-type) and IFC5010 (csrA) strains and in IFC5010 complemented with the csrA-expressing plasmid pMX544 (indicated as pCsrA), as determined by Western blot analyses using UvrY polyclonal antibodies. Purified His-tagged Bar’ (81.550 Da), a BarA version lacking the 198 first amino acid residues, and His-tagged UvrY (25.380 Da) (25), and extracts from barA (BAKSB837) and uvrY (UYKSB837) mutant cells were used in the first lanes of each Western blot. The molecular mass differences between purified and wild-type BarA and UvrY proteins are due to the His tag and the absence of the first 198 amino acids in BarA. (C and D) Effects of csrA on uvrY transcription and translation. Wild-type (wt) and csrA mutant cells carrying either a chromosomal PlacUV5-uvrY’-lacZ translational fusion (strains IFC5013 and IFC5014, respectively) (C) or a chromosomal uvrY’-lacZ operon fusion (strains IFC5011 and IFC5012, respectively) (D) were harvested at various times throughout growth and assayed for β-galactosidase activity. The β-galactosidase activity is presented as a function of growth density (OD₆₀₀). The averages from four independent experiments are presented (standard deviations were less than 5% from the mean).

**lacUV5** promoter replaced the native promoter, was monitored in the wild-type and csrA mutant strains. In this case, β-galactosidase activity in the csrA mutant was found to be approximately 50% of that in the wild-type strain (Fig. 3C). As mentioned above, CsrA regulates translation of its target mRNAs by interacting with their 5′ untranslated regions (9–11). However, analyses *in silico* did not reveal any apparent CsrA binding sites in the 5′-UTR of the *uvrY* transcript, which is suggestive of an indirect effect of CsrA on *uvrY* expression. The effect of CsrA on *uvrY* transcription was tested by using a chromosomal *uvrY’*-lacZ translational fusion. It was observed that the β-galactosidase activity in the wild-type strain was almost 2-fold higher than the in the csrA mutant strain (Fig. 3D), suggesting that CsrA indirectly affects the transcription of *uvrY*. It should be noted that the *uvrY* mutation, an insertion of a kanamycin cassette 10 amino acid residues before the stop codon of CsrA (21, 37), decreases but does not entirely eliminate CsrA activity (38). Therefore, the actual effect of CsrA on *uvrY* transcription and translation may be significantly greater than observed. Nonetheless, the above results suggest that CsrA is required for proper *uvrY* expression, affecting both transcription and translation of *uvrY*.

**Overexpression of UvrY alone but not concurrently with BarA restores csrB transcription in a csrA mutant.** We then asked whether ectopic expression of *uvrY* and/or *barA* from plasmids...
pMX539 and pBA29 (25) restores csrB expression in the csrA mutant strain. It was found that expression of uvrY but not barA did restore csrB expression (Fig. 4A and B), in agreement with a previous report (25). Notably, the presence of the uvrY-expressing plasmid pMX539 (expressing uvrY and indicated as pUvrY), pMX540 (expressing uvrYD54Q, having Q substituted for the conserved phosphorylatable D, and indicated as pUvrYD54Q), pBA29 (expressing barA and indicated as pBarA), or pMX541 (expressing both uvrY and barA and indicated as pUvrY-BarA), and IFC5015, the isogenic csrA pta ackA triple mutant strain, harboring pMX539 (indicated as pUvrY) were grown in LB medium. Total RNA isolated from samples that were harvested throughout the growth curve (OD600 of 0.3 to 2.0) was probed for the CsrB transcript. (B) Overnight cultures of the csrB-lacZ transcriptional fusion-carrying wild type (panel I, closed squares), the csrA mutant strain (panel I, open squares), and the same mutant strain harboring plasmid pMX539 (expressing uvrY) (panel I to IV, circles), pBA29 (expressing barA) (panel I, diamonds), pMX540 (expressing uvrYD54Q) (panel II, triangles), pMX541 (expressing both uvrY and barA) (panel III, triangles), and the isogenic csrA pta ackA triple mutant strain harboring pMX539 (expressing uvrY) (panel IV, triangles) were diluted to an OD600 of 0.05 in LB medium, and the β-galactosidase activity was followed for 300 min. The average from four independent experiments is presented (standard deviations were less than 5% from the mean). (C) Ectopic expression of uvrY and barA by pMX541 (indicated as pUvrY-BarA) restores csrB expression in uvrY and barA mutant strains. Cultures of the csrB-lacZ transcriptional fusion-carrying wild type (KSB837) and the isogenic uvrY (UYKSB837) and barA (BAKSB837) mutant strains both carrying or not the uvrY- and barA-expressing plasmid pMX541 were grown to an OD600 of ~0.2 in LB medium, and the β-galactosidase activity was assayed. The average from two independent experiments is presented. (D) Levels of UvrY (upper panel) and BarA (lower panel) proteins in KSB837 (wild type) and BarA (lower panel) proteins in KSB837 (wild type), IFC5010 (csrA), and IFC5010 harboring either of the following plasmids: pMX539 (indicated as pUvrY), pMX540 (indicated as pUvrYD54Q), pBA29 (indicated as pBarA), pMX541 (indicated as pUvrY-BarA), and csrA pta ackA (IFC5015) harboring pMX539 (indicated as pUvrY) as determined by Western blot analyses. Purified His−tagged BarA and UvrY proteins and extracts from BarA (BAKSB837) and uvrY (UYKSB837) mutant cells were used in the first lanes of each Western blot.
mutant for csrB expression. It was found that csrB expression was restored in both mutant strains (Fig. 4C), indicating that pMX541 expresses functional BarA and UvrY proteins. Moreover, Western blot analyses, using specific UvrY and BarA polyclonal antibodies, revealed that similar amounts of UvrY protein were expressed by pMX539, pMX540, and pMX541 and that similar amounts of BarA were expressed by pBA29 and pMX541 (Fig. 4D). Thus, expression and functionality of the pMX541 expressed BarA and UvrY does not provide an explanation for the above result. The above results, in combination with the fact that BarA, like other tripartite two-component sensors, has been shown to be capable of having both a kinase and a phosphatase activity on it is cognate regulator (39–41), prompted us to speculate that BarA may remain locked in its phosphatase state and fail to be activated as a kinase in the csrA mutant. In such a scenario, phosphorylation of the pMX539-expressed UvrY in the csrA mutant strain should rely on acetyl-P. Moreover, the vast overexpression of UvrY (Fig. 4D) should overwhelm the phosphatase activity of the chromosomally expressed BarA, permitting the accumulation of significant amounts of UvrY-P and culminating in activation of csrB transcription. On the other hand, when comparable amounts of BarA and UvrY proteins are expressed, i.e., when pMX541 was used, the phosphatase activity of BarA should dephosphorylate the acetyl-P-dependent UvrY-P and thereby cancel its transcriptional regulation.

CsrA is required for activation of the BarA kinase activity. The above hypothesis, that UvrY is autophosphorylated at the expense of acetyl-P and is not transphosphorylated by BarA in the csrA mutant, was then tested. To this end, a p tacackA mutation was inserted into the csrA mutant strain in order to block the synthesis of acetyl-P (42), and the effect of the UvrY-overexpressing plasmid pMX539 on csrB expression was probed. It was found that although similar amounts of UvrY were expressed in the csrA p tacackA triple mutant strain and in the csrA mutant (Fig. 4D), no activation of csrB transcription occurred, as judged by Northern blotting and by the csrB-lacZ reporter fusion (Fig. 4A and B). This suggests that in the csrA mutant strain, UvrY is phosphorylated exclusively at the expense of acetyl-P rather than being transphosphorylated by BarA.

Subsequently, we explored the intriguing possibility that BarA remains inactive as a kinase in the csrA mutant. We argued that if the activity of BarA is not affected by CsrA, then reestablishing the levels of UvrY protein in the csrA mutant should restore csrB transcription. Therefore, we constructed a uvrY gene carrying low-copy-number plasmid (pMX543), where the promoter and 5'-UTR sections of uvrY were replaced with the ones of barA (Fig. 5A), the expression of which was not affected by CsrA (Fig. 3A). This plasmid was transformed into UVKSB837, a uvrY mutant, or IFCS016, a csrA uvrY double mutant strain, and the amount of UvrY was examined by Western blotting (Fig. 5A). It was found that similar amounts of UvrY protein were expressed from the plasmid in both of these strains. Subsequently, we tested whether pMX543 was able to complement csrB expression in these mutant strains. Interestingly, pMX543 restored csrB expression in the uvrY mutant but not in the csrA uvrY mutant (Fig. 5B and C). A possible explanation for this finding is that CsrA is also required for the kinase activity of BarA.

To provide further support to the above conclusions, we argued that ectopic expression of UvrY, but not that of the mutant UvrYD54Q, which is unable to be phosphorylated, in a csrA mutant should restore csrB expression when the cells are grown at pH 5.0 in the presence of acetate, which results in the production of elevated amounts of acetyl-P (43). On the other hand, the addition of formate, which acts exclusively via BarA, should be without effect. Accordingly, overexpression of UvrY in the csrA p tacackA triple mutant, which is not able to convert acetate to acetyl-P, should not restore csrB expression when cells are grown at pH 5.0 in the presence of either acetate or formate. Indeed, csrB transcription in the csrA mutant strain grown at pH 5.0 was restored by the ectopic expression of UvrY in the presence of both acetate and formate, whereas overexpression of UvrYD54Q was without effect (Fig. 6A and B). Also, no activation of csrB transcription was observed in the csrA p tacackA mutant strain transformed with pMX539 when the cells were grown at pH 5.0 in the presence of either acetate or formate (Fig. 6A and B). We therefore concluded that when UvrY is overexpressed in the csrA mutant, acetyl-P-dependent phosphorylation of UvrY is responsible for the activation of csrB transcription.

Finally, we reasoned that if CsrA was required for switching
BarA from its phosphatase to its kinase activity, the simultaneous overexpression of UvrY and BarA in the csrA mutant, using plasmid pMX541, should not restore csrB expression when cells are grown at pH 5.0 in the presence of acetate or formate. The same result should be expected when wild-type levels of UvrY are reestablished in the csrA mutant by plasmid pMX543. This is because BarA should remain locked on as a phosphatase, dephosphorylating the acetyl-P-dependent UvrY-P and thereby cancelling its regulatory effect. In fact, no activation of csrB transcription was detected in the pMX541 or pMX543 carrying csrA mutant strain (Fig. 6A and B). On the other hand, reestablishing the wild-type levels of UvrY by plasmid pMX543 in a csrA barA double mutant, where no UvrY-P-dephosphorylating activity is present, should restore csrB expression in the presence of acetate but not formate. Indeed, csrB transcription in the pMX43-carrying csrA barA double mutant grown at pH 5.0 was restored to wild-type levels in the presence of acetate but not formate (Fig. 6A and B). Taken together, these results indicate that in the csrA mutant, BarA fails to be activated as a kinase but functions as a phosphatase even in the presence of its stimulus. It thus appears that one or more genes, whose expression is regulated by CsrA, may be needed for proper activation of BarA. Thus far, we have been unsuccessful in screening a plasmid-based genomic library for genes that would complement csrB expression in the csrA mutant.

Conclusions. In this study, we investigated the effect of the CsrA global regulator on the expression and activity of the components of the BarA/UvrY signaling system. This was motivated by the earlier observation that csrB expression, which relies on the BarA-to-UvrY phosphorelay, did not take place in a csrA mutant (25). Our results demonstrate that the CsrA protein is required for the proper expression of the UvrY response regulator and also for the adequate switch from the phosphatase to the kinase activity of the BarA sensor kinase (Fig. 7), providing an explanation for the above observation.

We provided evidence that CsrA positively affects uvrY expression at both the transcriptional and posttranscriptional levels. The effect of CsrA, an RNA binding protein, on uvrY transcription plausibly may be mediated via the regulation of expression of a transcriptional factor. Previously, SdiA and Crp have been reported to activate, respectively, uvrY transcription in E. coli (46) and csrB transcription in Yersinia pseudotuberculosis (44, 45). However, CsrA modestly represses sdiA translation in E. coli (46), and Crp does not activate csrB expression (A. Pannuri and T. Romeo, unpublished data). Thus, these two regulators cannot account for the positive effects of CsrA on uvrY transcription. Another candidate is LexA, which coordinates the SOS response (47, 48), because a LexA binding site is predicted to be located between nt −120 and −139 upstream of the uvrY start site (49). CsrA-dependent modulation of uvrY translation might also be indirect, because no apparent CsrA binding sequences are present in the 5′-UTR of the uvrY transcript. In fact, the RNA DEAD box helicase DeaD was recently shown to be required for uvrY translation (50), although no link between CsrA and deaD expression is known at this time. Therefore, it is of great importance to clarify how these
proteins are integrated into the Csr/UvrY circuitry, which allows for a global response through the CsrA protein.

Finally, we provide evidence that CsrA plays a significant role in the mechanism that enables BarA to switch from its phosphatase activity to its kinase activity. It is therefore tempting to speculate that, in addition to the BarA stimulus, a protein whose expression is regulated by CsrA is needed for proper activation of BarA. In this respect, it is relevant to mention that proper regulation of the kinase activity of GacS, the BarA homolog in Pseudomonas aeruginosa, requires the presence of the hybrid sensor kinases RstS and LadS (51–53). However, no homologs of these proteins exist in E. coli. Hence, identification of the protein(s) or other factors involved in the regulation of BarA signaling would greatly enhance our understanding of the Csr/BarA-UvrY regulatory network.

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FIG 7 Model for the regulatory circuitry of the BarA/UvrY TCS and Car system. Under stimulatory conditions, acetate and formate act as physiological signals that activate BarA (1), leading to its autophosphorylation at the expense of ATP (2) and transphosphorylation of UvrY (3). UvrY can also autophosphorylate at the expense of acetyl-P (5), which is produced from acetate (4). Phosphorylated UvrY (UvrY-P) activates expression of the noncoding CsrB and CsrC RNAs (6), which bind and sequester the CsrA protein (7) and thereby prevent its regulatory interaction with the mRNA targets. On the other hand, free CsrA positively regulates UvrY-P expression (8) (11), which is required for properly switching BarA from its phosphatase to its kinase activity (9). At the same time, CsrA positively regulates uvrY expression (11) by controlling the expression of the regulator(s) (X) (10). Finally, under nonstimulatory growth conditions or in a csrA mutant strain, BarA acts as a UvrY-P phosphatase (12), enabling the silencing of the system. Reactions under stimulatory and nonstimulatory conditions are indicated with solid and dotted lines, respectively. Double lines indicate effects on gene expression.

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