Supporting Information

CsrA activates *flhDC* expression by protecting *flhDC* mRNA from RNase E-mediated cleavage

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Bacterial strains. Strains CF7789 [F⁻ λ⁻ Δ*lacI-lacZ* (MluI)] (Wei *et al.*, 2001), TRCF7789 (CF7789/*csrA::kan*) (Wei *et al.*, 2001), UYCF7789 (CF7789/*uvrY::cam*) (Suzuki *et al.*, 2002), and SK4390 (F⁻ λ⁻ *thyA715 rph-1 rppH::kan*) (Mohanty and Kushner, 2010) have been described. Strains CF7789 and TRCF7789 were transformed with plasmid pFDCZ6 (*flhDC'-'lacZ*), resulting in strains PLB931 and PLB932, respectively. Selection was for resistance to ampicillin (100 μg/ml). CF7789 was used as the parental strain to generate chromosomal insertions of *flhD'-'lacZ* translational fusions carried on plasmids pCSB76 (WT *flhD'-'lacZ*), pCSB77 (ΔBS1 *flhD'-'lacZ*), pCSB78 (ΔBS2 *flhD'-'lacZ*), pCSB79 (ΔBS1 ΔBS2 *flhD'-'lacZ*), and pAY144 (ΔE *flhD'-'lacZ*) according to the CRIM protocol (Haldimann and Wanner, 2001), resulting in strains PLB1258 (WT *flhD'-'lacZ*), PLB1259 (ΔBS1 *flhD'-'lacZ*), PLB1260 (ΔBS2 *flhD'-'lacZ*), PLB1262 (ΔBS1 ΔBS2 *flhD'-'lacZ*), and PLB1759 (ΔE *flhD'-'lacZ*), respectively. Selection was for resistance to ampicillin (100 μg/ml). Isogenic *csrA::kan* strains were generated by $P1_{vir}$ transduction using TRCF7789 (*csrA::kan*) as the donor and PLB1258, PLB1259, PLB1260, PLB1262, and PLB1759 as recipient strains, resulting in strains PLB1263 (WT *flhD'-'lacZ csrA::kan*), PLB1264 (ΔBS1 *flhD'-'lacZ csrA::kan*), PLB1265 (ΔBS2 *flhD'-'lacZ csrA::kan*), PLB1266 (ΔBS1 ΔBS2 *flhD'-'lacZ csrA::kan*), and PLB1760 (ΔE *flhD'-'lacZ*), respectively. Selection was for resistance to kanamycin (50 μg/ml). Isogenic *rppH::kan* strains were generated by $P1_{vir}$ transduction using SK4390 (*rppH::kan*) as the donor and UYCF7789 and CF7789 as the recipient strains, resulting in PLB1601 (*uvrY::cam rppH::kan*) and PLB1762 (*rppH::kan*), respectively. Selection was for resistance to kanamycin (50 μg/ml). The WT *flhD'-'lacZ* translational fusion carried on plasmid pCSB76 was integrated into strains UYCF7789, PLB1601 and PLB1762 according to the CRIM protocol, resulting in strains PLB1768 (*uvrY::cam flhD'-'lacZ*), PLB1773 (*uvrY::cam rppH::kan flhD'-'lacZ*), and PLB1776 (*rppH::kan*

flhD'-'lacZ), respectively. Selection was for resistance to ampicillin (100 μg/ml). The ΔBS1 ΔBS2 *flhD'-'lacZ* translational fusion carried on plasmid pCSB79 was integrated into the same three strains, resulting in strains PLB1770 (*uvrY::cam* ΔBS1 ΔBS2 *flhD'-'lacZ*), PLB1774 (*uvrY::cam rppH::kan* ΔBS1 ΔBS2 *flhD'-'lacZ*), and PLB1777 (*rppH::kan* ΔBS1 ΔBS2 *flhD'-'lacZ*), respectively. Similarly, the ΔE *flhD'-'lacZ* translational fusion carried on plasmid pAY144 was integrated into the same three strains, resulting in strains PLB1771 (*uvrY::cam* ΔE *flhD'-'lacZ*), PLB1775 (*uvrY::cam rppH::kan* ΔE *flhD'-'lacZ*), and PLB1778 (*rppH::kan* ΔE *flhD'-'lacZ*), respectively. Strains PLB931, PLB932, PLB1258-PLB1260, PLB1262-PLB1266, PLB1759, PLB1760, PLB1768, PLB1770, PLB1771, and PLB1773-PLB1778 were used for in vivo expression studies.

Strains SK5664 (*pyrC::Tn*10) (Arraiano *et al.*, 1988) and SK5665 (*rne-1*) (Babitzke and Kushner, 1991) were described previously. Strain PLB966 (*uvrY::cam csrA::kan*) was generated by $P1_{vir}$ transduction using TRCF7789 (*csrA::kan*) as the donor and UYCF7789 (*uvrY::cam*) as the recipient strain. Selection was for resistance to kanamycin (50 μg/ml). Strains PLB1619 and PLB1620 were generated by transforming strains PLB1601 and UYCF7789 with plasmid pCSB81 (WT *flhDC* operon), respectively. Selection was for resistance to ampicillin (100 μg/ml). Strains PLB1625 and PLB1626 were generated by transforming strains UYCF7789 and PLB1601 with plasmid pCSB83 (ΔBS1 ΔBS2 *flhDC* operon), respectively. Strains PLB1618 and PLB1627 were generated by $P1_{vir}$ transduction using TRCF7789 (*csrA::kan*) as the donor strain and PLB1620 and PLB1625 as the recipient strains, respectively. Strains PLB1618-PLB1620 and PLB1625-PLB1627 were used for mRNA half-life studies.

Strains SK6632 (*pnp-200 rnb-500*) and SK6640 (*pnp-200 rnb-500 rne-1*) containing temperature sensitive alleles of PNPase (*pnp-200*), RNase II (*rnb-500*) and/or RNase E (*rne-1*)

have been described (Yancey and Kushner, 1990). SK6632 and SK6640 were transformed with plasmid pCSB81 (WT *flhDC* operon), resulting in strains PLB1646 and PLB1647, respectively. These strains were used for primer extension studies to identify RNase E cleavage sites in the *flhDC* leader.

Strain UVCF7789 was used as the parental strain to generate a chromosomal insertion of the *flhD'-'lacZ* translational fusion carried on plasmid pYH203 according to the CRIM protocol, resulting in strain PLB1832 (ΔBS1 ΔBS2 ΔE *flhD'-'lacZ*). Selection was for resistance to ampicillin (100 μg/ml). The *pyrC::Tn*10 marker from SK5664 was moved into strains PLB1768, PLB1770, PLB1771, and PLB1832 by $P1_{vir}$ transduction, resulting in strains PLB1812, PLB1813, PLB1814, and PLB1845, respectively. Selection was for resistance to tetracycline (20 μg/ml). The *rne-1* allele from SK5665 was subsequently moved into PLB1812, PLB1813, PLB1814, and PLB1845 by $P1_{vir}$ transduction, resulting in strains PLB1816, PLB1817, PLB1818, and PLB1846, respectively. Selection was for pyrimidine prototrophy. Transductants were screened for the linked temperature sensitive *rne-1* allele by replica plating to 44°C. Strains PLB1768, PLB1770, PLB1771, PLB1816-PLB1818, PLB1832, and PLB1846 were used for quantitative primer extension assays.

The chromosomal *flhDC* leader (-238 to +43 with respect to the start of *flhD* translation) was deleted by targeted substitution via the λ red recombinase system (Datsenko and Wanner, 2000). Plasmids pKD13 and pKD46 were described previously (Datsenko and Wanner, 2000). The kanamycin resistance gene was amplified from pKD13 by PCR, and introduced by electroporation into arabinose-treated MG1655/pKD46, thereby replacing the *flhDC* promoter and leader region with the kanamycin resistance gene, resulting in strain PLB441. Proper gene replacement was confirmed by PCR. The *flhDldr::kan* allele from PLB441 was subsequently

transferred to UYCF7789 by $P1_{vir}$ transduction, resulting in strain PLB442. Strain PLB442 was used as the parental strain to generate chromosomal insertions of *flhDC* promoter and truncated leader regions (–195 to +146 relative to the start of transcription) in which sequences downstream from +146 were replaced by the intrinsic λtR_2 terminator. In this case, plasmids pYH207 (WT *flhDldr*-tR_2), pYH208 (ΔBS1 ΔBS2 *flhDldr*-tR_2), pYH209 (ΔE *flhDldr*-tR_2), and pYH210 (ΔBS1 ΔBS2 ΔE *flhDldr*-tR_2) were integrated via the CRIM protocol, resulting in strains PLB1852, PLB1853, PLB1854, and PLB1855, respectively. Selection was for resistance to ampicillin (100 μg/ml). The *pyrC::Tn*10 marker from SK5664 was moved into PLB1852, PLB1853, PLB1854, and PLB1855 by $P1_{vir}$ transduction, resulting in strains PLB1856, PLB1857, PLB1858, and PLB1859, respectively. Selection was for resistance to tetracycline (20 μg/ml). The *rne-1* allele from SK5665 was subsequently moved into PLB1856, PLB1857, PLB1858, and PLB1859 by $P1_{vir}$ transduction, resulting in strains PLB1860, PLB1861, PLB1862, and PLB1863, respectively. Selection was for pyrimidine prototrophy. Transductants were screened for the linked temperature sensitive *rne-1* allele by replica plating to 44°C. Strains PLB1852-PLB1855 and PLB1860-PLB1863 were used for quantitative primer extension assays.

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