Published in final edited form as:
Res Microbiol. 2008 ; 159(7-8): 562–568. doi:10.1016/j.resmic.2008.07.003.

Catabolite repression control of flagellum production by *Serratia marcescens*

Nicholas A. Stella[a], **Eric J. Kalivoda**[a], **Dawn M. O'Dee**[b], **Gerard J. Nau**[b], and **Robert M. Q. Shanks**[a,b,*]

a*Charles T. Campbell Laboratory of Ophthalmic Microbiology, Department of Ophthalmology, University of Pittsburgh Eye Center, EEI 1020, 203 Lothrop St., Pittsburgh, PA 15213, USA*

b*Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA 15261 USA*

Abstract

Serratia marcescens is an emerging opportunistic pathogen with a remarkably broad host range. The cAMP-regulated catabolite repression system of *S. marcescens* has recently been identified and demonstrated to regulate biofilm formation through the production of surface adhesions. Here we report that mutations in components of the catabolite repression system (*cyaA* and *crp*) eliminate flagellum production and swimming motility. Exogenous cAMP was able to restore flagellum production to adenylate cyclase mutants, as determined by transmission electron microscopy and PAGE analysis. A transposon-generated suppressor mutation of the *crp* motility defect mapped to upstream of the *flhDC* operon. This suppressor mutation resulted in an upregulation of *flhD* expression and flagellum production, indicating that *flhDC* expression is sufficient to restore flagellum production to *crp* mutants. Lastly, and contrary to a previous report, we found that *flhD* expression is controlled by the catabolite repression system using quantitative RT-PCR. Together, these data indicate that flagellum production is regulated by the cAMP-dependent catabolite repression system. Given the role of flagella in bacterial pathogenicity, the regulatory pathway described here may assist us in better understanding the putative role of motility in dissemination and virulence of this opportunistic pathogen.

Keywords

Flagella; Catabolite repression; cAMP; Biofilm; *Serratia*

1. Introduction

Serratia marcescens is a Gram-negative bacterium that has a significant health and economic impact as an agent of hospital infections and as a plant pathogen [12,25]. *S. marcescens* infects a broad range of hosts from insects to coral [8,26]. Bacterial motility and surface behaviors such as biofilm formation and swarming may play a role in *S. marcescens* pathogenesis and dissemination [13,37].

*correspondence and reprints.

Swimming motility in bacteria is facilitated by formation of an intricate surface organelle called the flagellum, reviewed by Chilcott and Hughes [6]. Flagella-based motility is thought to contribute to the pathogenic capacity of several bacterial species and is often cited as a virulence factor [29]. The regulation of flagellum production is well characterized in *Escherichia coli* and *Salmonella typhimurium* [6]. In these organisms there is a flagellum production regulatory hierarchy, with the master regulator operon being *flhDC* [6]. *S. marcescens* was shown to have a homologous *flhDC* operon that codes for transcriptional activators that control swimming, swarming and exoenzyme production [11,22,36].

In a previous study, we identified and mutated components of the cAMP-dependent catabolite repression system (CRS) in *S. marcescens* [16]. Catabolite repression systems inhibit the production of proteins involved in the use of less efficiently metabolized carbon sources when more favorable carbon sources are available [4]. cAMP-dependent CRS uses cyclic nucleotide cAMP as a signaling molecule. Adenylate cyclase (*cyaA*) generates cAMP in response to environmental carbon sources, reviewed by Botsford and Harman [4]. The cAMP-receptor protein CRP is a transcription factor, coded by the *crp* gene, which binds to cAMP and can either activate or inhibit expression of multiple genes [4]. In *E. coli* and *S. typhimurium*, the reach of the CRS goes well beyond regulation of carbon sources to include virulence, motility, and cell division [4]. In *S. marcescens*, the CRS functions to control fimbriae production, such that mutations in adenylate cyclase (*cyaA*) or cAMP-receptor protein (CRP) lead to a dramatic increase in biofilm formation [16]. This trend differs from what is seen in *E. coli* and *Pseudomonas aeruginosa* where CRS systems positively regulate biofilm formation [14,23]. While working with mutants of the *S. marcescens* CRS, we noticed that swimming motility was defective. In this study, we provide evidence that the CRS is a positive regulator of flagellum production and that *flhDC* is sufficient to restore swimming motility to CRS mutants. Furthermore, *flhDC* transcription was significantly reduced in *cyaA* and *crp* mutants. A model for the differential regulation of flagellum-based motility versus biofilm formation is presented.

2. Material and methods

2.1 Bacterial strains and growth conditions

All bacteria used in this study are derived from a *S. marcescens* strain from Presque Isle cultures (Presque Isle, PA), strain number 3611. Construction and analysis of the *cyaA-2* and *crp-1* mutation have been described previously [16]. The *scrp31* mutation was derived from a mariner transposon mutation made with the pBT20 delivery vector [18] using previously reported methods [31]. Bacteria were grown in LB broth in all cases. Swimming medium consisted of LB with a 0.3% (w/v) agar concentration. Swarming medium was identical but with an agar concentration of 0.5–1.2% (w/v), and swarming experiments were performed as previously reported [31]. Kanamycin was supplemented at 100 μg/ml. 3’-5’ cyclic AMP (cAMP, Sigma-Aldrich, St. Louis, MO) was added to LB directly and then filter-sterilized at concentrations up to 10 mM. All experiments were performed at 30°C at least two times with multiple independent biological replicates.

2.2. Surface fractions and PAGE analysis

Surface fractions were performed as Labbate and colleagues [19] with the following exceptions. Bacteria, three independent cultures per strain, were grown in culture for 16 h, washed in PBS and adjusted to five A_{600} units in 1 ml of PBS. Aliquots were vortexed for two min on a Turbomix attachment on a Genie vortex unit (Scientific Industries, Bohemia, NY), and bacteria were pelleted with a microcentrifuge. The supernatant was filtered with a PVDF 0.22 micron filter (Millipore item number SLGV033RS, Cork Ireland), then proteins were precipitated with TCA (Sigma-Aldrich, St. Louis, MO). One half of the sample was loaded onto an 8–16% polyacrylamide gel (Precise Protein Gel, Pierce, Rockford IL), using a Minigel

format (Mini Protean 3, Biorad, CA), and stained with Coomassie brilliant blue. Image J software (NIH) was used to quantify protein levels of scanned gels, using at least three independent samples. Mass spectroscopy and peptide identification were performed by the University of Pittsburgh Genomics and Proteomics core facility.

2.3 Transmission electron microscopy (TEM)

These experiments were performed as previously described [31]. Briefly, bacteria were taken from liquid cultures rotated at high speed on a tissue culture roller (TC-7, New Brunswick Scientific, NJ) for 14–16 h, washed once with PBS, applied to formvar coated copper grids and stained with uranyl acetate (2%). Images were obtained using a JEOL-1011 microscope at the University of Pittsburgh Center for Biological Imaging.

2.4. Genetic manipulations, quantitative-RT-PCR and statistical analysis

Transposon mutagenesis, quantitative RT-PCR (Q-PCR) and arbitrary PCR were performed as previously described [31]. Statistical analysis was performed using Student's T-tests with Excel software. Plasmids were generated and described previously, and had a medium copy number, pBBR1-based, replicon [16,30]. The *crp-1* mutation was generated by integration of pMQ118 into the *crp* gene as directed by an internal fragment of *crp* that disrupts the *crp* open reading frame [16,30]. All experiments with the *crp-1* mutation were performed with kanamycin to ensure a homogenous *crp-1* culture. To restore the wild-type *crp* gene function in the *crp-1 scrp31* double mutant background, we grew the strain in culture to saturation three times in LB medium without antibiotic selection, which maintains pMQ118 integration. Aliquots were then plated on minimal medium with 0.3% glycerol as a sole carbon source to select for bacteria that had experienced a recombination event restoring the *crp* open reading frame (PCR verified), as *crp* mutants cannot grow with glycerol as a sole carbon source [16, 30].

3. Results

3.1 Mutation of catabolite repression system genes inhibits swimming motility and flagellum production

Mutation of the adenylate cyclase gene, *cyaA*, prevents swimming motility through a semi-solid agar matrix (Fig. 1A). The addition of wild-type *cyaA* on a plasmid was able to restore swimming to the *cyaA-2* mutant (Fig. 1A). In a similar experiment where swim zones were measured at 7.5 h, the *cyaA* mutant exhibited no detectable zone of swimming compared to the wild type, which exhibited a swim zone of 7.7 ± 1.5 mm. A *cyaA-2* + p*cyaA* had a 6.05 ± 0.8 mm swim zone, with the wild type + p*cyaA* producing a swim zone of 8.6 ± 1.4 mm swim zone. Mutation of the *crp* gene which codes for a cAMP-binding transcription factor led to a similar lack of swimming motility (described below).

TEM was used to determine if there was an obvious alteration in surface morphology of the *cyaA-2* mutant strain compared to the wild type. We observed that wild-type cells commonly had one or more flagella whereas the *cyaA-2* and *crp* mutants had no flagella (n>400 per strain), yet were covered with fimbriae (Fig. 1B–C, Fig.2A).

To provide further evidence that CyaA is a positive regulator of flagella biosynthesis, a biochemical approach was taken. Sheared surface protein fractions were separated on a polyacrylamide gel. A protein was observed to be absent from *cyaA-2* mutant fractions that migrated at approximately 40 kD (Fig. 1D). This band was excised and determined by mass spectroscopy to be the flagellin subunit. When the amount of flagellin was determined using Image J software, the wild-type strain with the vector alone had a relative flagellin level of 25851 ± 26, the wild-type with p*cyaA* had levels of 19593 ± 3861, the *cyaA-2* mutant with the

vector had only 1177 ± 358, and the *cyaA-2* mutant with wild-type *cya* in trans (p*cyaA*) exhibited flagellin levels of 16098 ± 3232 (n=3 independent cultures per strain). These data show that flagellin levels were significantly reduced in the *cyaA-2* mutant relative to the wild type ($p<0.01$), and could be complemented by the wild-type *cyaA* gene added *in trans*.

3.2. Flagellum production can be restored to the *cyaA* mutant with exogenous cAMP

The enzymatic product of the CyaA protein of *E. coli* was demonstrated to be cAMP [4]. To determine whether the lack of flagella was dependent upon cAMP, the effect of exogenous cAMP on flagellum production was assessed. Flagellum production was restored to the *cyaA* mutant by exogenous cAMP, as determined by TEM analysis (Fig. 2A). WT cells were generally associated with a single flagellum (23% had ≥2 flagella/cell, n=341), and the addition of 10 mM cAMP to the growth medium led to an increased number of flagella relative to the wild type, with 40% of cells associated with 2 or more flagella (n=359). The addition of 2 mM cAMP to the growth medium of *cyaA* mutants led to only 0.7% of cells exhibiting 2 or more flagella (n=576). Rescue of flagellin production by the *cyaA* mutant was also observed on polyacrylamide gels (Fig. 2B). Consistent with cAMP stimulating flagellin production, the addition of 10 mM cAMP to wild-type cells led to a significant ($p<0.01$) 3.3-fold increase in flagellin production compared to the wild-type levels without the addition of 10 mM cAMP.

3.3. High-level expression of *flhDC* suppressed motility defects of a *crp* mutant

Like *cyaA* mutants, disruption of the *crp* gene leads to a defect in swimming motility and flagellum production (Fig. 3A, Fig.4A–B). We identified a transposon-induced mutation that suppressed the swimming deficiency of a *crp* mutant. This mutation, called *scrp31* (for suppressor of *crp*), restored swimming motility to the *crp-1* mutant strain (Fig. 3A). The swimming motility of the wild type was similar to that of the double mutant, suggesting that there are not multiple factors preventing swimming motility in a CRS-deficient strain (Fig. 3A).

The chromosomal locus of the transposon in *scrp31* was mapped using arbitrary PCR to an intergenic region 291 base pairs upstream of the predicted *flhD* start codon (Fig. 3B). The transposon used in this study can upregulate genes near its insertion site via a *Ptac* promoter at one end of the transposon; in the case of *scrp31* the *Ptac* promoter is directed toward the *flhDC* operon (Fig. 3B). Q-RT-PCR was used to determine that *flhD* expression was significantly upregulated 10.96 ± 1.90-fold in the *crp-1 scrp31* mutant compared to the wild type ($p<0.01$). An alternative mechanism for the increase in *flhD* expression is that a negative regulatory region is disrupted by the transposon mutation, as has been recently reported in *Proteus mirabilis* [7].

We next sought to characterize the effect of the *scrp31* mutation on flagellum production. TEM analysis revealed a hyperflagella phenotype in both the *crp-1 scrp31* (Fig. 4A) and *scrp31* mutant (not shown), with >99% of the cells exhibiting flagella (n>100 cells per strain) compared to ~60% for the wild type (Fig. 2A) and <0.1% for the *crp-1* mutant (n>400). The increase in flagellum production in *crp-1 scrp31* and *scrp31* mutants was clearly observed in separated surface protein fractions (Fig. 4B). Quantification of this difference using Image J software to analyze PAGE flagellin levels from four independent experiments indicated a significant increase in flagellin production conferred by the *scrp31* mutation compared to the wild type (Fig. 4C). There was no statistical difference in flagellin production between the *crp-1 scrp31* double mutant and the *scrp31* mutant ($p=0.499$). Nor did addition of cAMP (10 mM) alter flagellin production by either the *scrp31* or *crp-1 scrp31* double mutant ($p= 0.31$ and 0.40 respectively, data not shown). These data suggest that the *scrp31* mutation renders flagellum production insensitive to catabolite repression.

The multiple flagella associated with the *scrp31* mutation were suggestive of the hyperflagellated bacteria induced by growth on surfaces associated with swarming conditions [2]. We tested whether the *scrp31* mutation altered swarming by *S. marcescens*. The wild-type strain swarmed on the surface of LB plates with 0.5–0.8% agar, the *crp* mutant did not swarm under any condition, and the *scrp31* and the *crp scrp31* double mutants were able to swarm on plates with 0.5–1.2% agar, suggesting that the *scrp31* mutation confers a hyperswarming phenotype (Fig. 4D–E, and data not shown).

3.4. The flagella regulator *flhD* is controlled by the CRS

Sequence analysis revealed a predicted CRP binding site (tTGTGActatgTCACAt) 316 base pairs upstream of the *flhDC* operon in the sequenced Db11 strain (Sanger Center) (Fig. 3B). This suggests that flagella biosynthesis may be regulated by cAMP through transcription of the flagella master regulator operon. The *flhDC* operon is transcriptionally regulated in a positive manner by CRP in *Escherichia coli* [34]. The motility defect of *S. marcescens cyaA* mutants was hypothesized to be a result of a loss of positive regulation of *flhDC* expression by the CRS. This prediction was tested using Q-RT-PCR. Transcript of the *flhD* gene was down >10-fold in the *cyaA-2* strain compared to the wild type ($p<0.01$)(Fig. 5A). A consistent prediction would be that exogenous cAMP should restore *flhD* expression to a *cyaA* mutant. We found a dose-responsive increase in *flhD* levels with the addition of exogenous cAMP, which partially complemented the *cyaA* mutant defect at 10 mM cAMP (Figure 5). Mutation of the cAMP-binding transcription factor *crp* also led to a significant decrease in *flhD* transcript levels compared to the wild type ($p<0.01$). The *scrp31* mutation led to elevated *flhD* levels and was sufficient to restore *flhD* levels in a *crp* mutant background.

4. Discussion

The results of this study support a model whereby the cAMP-dependent catabolite repression system of *S. marcescens* is required for flagella-based motility and flagellum production through control of the master regulator *flhDC*. The expression of *flhDC* conferred by the *scrp31* mutation was able to restore swimming and swarming motility to the non-motile *crp* mutant. This suggests that a lack of *flhDC* expression is the reason for the loss of flagellum production and flagella-based motility conferred by mutations in the CRS. Consistently, flagellin production and *flhDC* expression defects of the *cyaA* mutant can be rescued by exogenous cAMP.

The connection between carbon and swimming motility has been established in other Enterobacteria. It was observed that high levels of glucose inhibit swimming motility by *E. coli* [1]. Later, the catabolite repression machinery was found to be necessary for swimming in both *E. coli* and *S. typhimurium* [17,32]. It was also demonstrated that *flhDC* is regulated by CRP in *E. coli* [3,34]. This has led to the model in which increased cAMP levels caused by the presence of less efficiently metabolized carbon sources leads to an increase in flagellum production, whereas conditions that decrease cAMP levels, namely high glucose, inhibit flagellum production in order to keep the bacterium in this favorable environment. Therefore, the bacteria will become less motile in environments with high levels of glucose and become more motile in environments with less efficient carbon sources. Evidence in support of this model was elegantly shown in a recent report using *E. coli* as a model organism [40].

We have previously reported that cAMP production inhibits *S. marcescens* biofilm formation through negative regulation of surface adhesin production [16]. In that report, it was shown that *S. marcescens* with mutations in genes whose products positively regulate cAMP production (*cyaA* and *crr*) and *crp*, whose gene product responds to cAMP levels, exhibited a major increase in biofilm formation and that this effect was mediated through type 1 fimbriae [16] (Fig. 6). Together, these studies suggest a model by which environments with unfavorable

carbon sources lead to a coordinated decrease in attachment factor production and an increase in motility function, and that these changes are mediated by intracellular cAMP levels (Fig. 6). In the opposite setting of environments with favorable carbon sources, biofilm formation is promoted through deregulation of adhesin production and a reduction in positive regulation of motility (Fig. 6). This system of attachment and motility should serve to help bacteria achieve suitable niches to localize and proliferate.

Whereas the flagellum appears to be a major initiator of biofilm formation for *Pseudomonas* species [24], *V. cholerae* [38] and other bacteria [5,27], we did not find a role for flagella in *S. marcescens* biofilm formation in a genetic screen for biofilm determinants [31]. Given the large target size of the flagellum biosynthetic machinery, it is likely that we screened many motility-defective mutants that were proficient in biofilm formation [31]. Consistently, mutants in the CRS system are unable to swim and form hyperbiofilms [16]. Together, these data suggest but do not prove that flagella are not a major biofilm determinant in *S. marcescens*.

Given the previously described role of the catabolite repression system in regulating motility in other Gram-negative bacteria, it may not be surprising that *S. marcescens* uses a similar mechanism [21,32,39]; however, this report may be controversial regarding the role of catabolite repression and flagella regulation by *S. marcescens*. Two previous reports suggest that swimming motility [20] and *flhDC* expression [22] were not controlled by “glucose catabolite repression”. One reason for the discrepancy between our data and that of Liu et al. [22], could be attributed to strain differences, which are common among *Serratia* strains. Other differences between the Liu study [22] and this report are that in the former study, a luciferase-reporter system was used to measure *flhDC* transcription, and glucose-rich (2%) medium was used to elicit the catabolite repression response. In our strain, we have found that, whereas 2% glucose is sufficient to elicit a catabolite repression response, this effect is not as robust as mutation of *cyaA* or *crp* [16].

The *flhDC* master regulator of flagella has also been demonstrated to regulate production of two extracellular enzymes, phospholipase A and nuclease [9,10,22]. A role for phospholipases in pathogenesis had been demonstrated in a range of bacteria [28,33,35]. Similarly, flagella are a virulence factor for many Gram-negative and -positive bacterial species [15,38]. The CRS is likely to have an important role in bacterial pathogenesis in response to local carbon sources. Future experiments will be directed to determining the roles of the CRS and *flhDC* in bacterial virulence.

Acknowledgements

The authors thank the Campbell and Kinchington lab and Stu Powers for help and discussion, Daniel Kadouri and George O’Toole for critical reading of the manuscript, and James Fender for photography. This work was done with support from an NIH core grant for vision research, EY08098, the Eye and Ear Institute of Pittsburgh, Research to Prevent Blindness, and the Charles T. Campbell Laboratory of Ocular Microbiology.

References

1. Adler J, Templeton B. The effect of environmental conditions on the motility of *Escherichia coli*. J Gen Microbiol 1967;46:175–184. [PubMed: 4961758]
2. Alberti L, Harshey RM. Differentiation of *Serratia marcescens* 274 into swimmer and swarmer cells. J Bacteriol 1990;172:4322–4328. [PubMed: 2198253]
3. Bartlett DH, Frantz BB, Matsumura P. Flagellar transcriptional activators FlbB and FlaI: gene sequences and 5′ consensus sequences of operons under FlbB and FlaI control. J Bacteriol 1988;170:1575–1581. [PubMed: 2832369]
4. Botsford JL, Harman JG. Cyclic AMP in prokaryotes. Microbiol Rev 1992;56:100–122. [PubMed: 1315922]

5. Chagneau C, Saier MH Jr. Biofilm-defective mutants of *Bacillus subtilis*. J Mol Microbiol Biotechnol 2004;8:177–188. [PubMed: 16088219]
6. Chilcott GS, Hughes KT. Coupling of flagellar gene expression to flagellar assembly in *Salmonella enterica* serovar *typhimurium* and *Escherichia coli*. Microb Mol Biol Rev 2000;64:694–708.
7. Clemmer KM, Rather PN. Regulation of *flhDC* expression in *Proteus mirabilis*. Res Microbiol 2007;158:295–302. [PubMed: 17320355]
8. Flyg C, Kenne K, Boman HG. Insect pathogenic properties of *Serratia marcescens*: phage-resistant mutants with a decreased resistance to Cecropia immunity and a decreased virulence to Drosophila. J Gen Microbiol 1980;120:173–181. [PubMed: 7012273]
9. Givskov M, Eberl L, Christiansen G, Benedik MJ, Molin S. Induction of phospholipase- and flagellar synthesis in *Serratia liquefaciens* is controlled by expression of the flagellar master operon *flhD*. Mol Microbiol 1995;15:445–454. [PubMed: 7783615]
10. Givskov M, Molin S. Secretion of *Serratia liquefaciens* phospholipase from *Escherichia coli*. Mol Microbiol 1993;8:229–242. [PubMed: 8316077]
11. Givskov M, Ostling J, Eberl L, Lindum PW, Christensen AB, Christiansen G, Molin S, Kjelleberg S. Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1. J Bacteriol 1998;180:742–745. [PubMed: 9457883]
12. Hejazi A, Falkiner FR. Serratia marcescens. J Med Microbiol 1997;46:903–912. [PubMed: 9368530]
13. Hume EB, Willcox MD. Emergence of *Serratia marcescens* as an ocular surface pathogen. Arch Soc Esp Oftalmol 2004;79:475–477. [PubMed: 15523567]
14. Jackson DW, Simecka JW, Romeo T. Catabolite repression of *Escherichia coli* biofilm formation. J Bacteriol 2002;184:3406–3410. [PubMed: 12029060]
15. Josenhans C, Suerbaum S. The role of motility as a virulence factor in bacteria. Int J Med Microbiol 2002;291:605–614. [PubMed: 12008914]
16. Kalivoda EJ, Stella NA, O'Dee DM, Nau GJ, Shanks RMQ. The cAMP-dependent catabolite repression system of *Serratia marcescens* mediates biofilm formation through regulation of type 1 fimbriae. Appl Environ Microbiol 2008;74:3461–3470. [PubMed: 18424546]
17. Komeda Y, Suzuki H, Ishidsu I, Iino T. The role of cAMP in flagellation of *Salmonella typhimurium*. Mol Gen Genet 1975;142:289–298. [PubMed: 178991]
18. Kulasekara HD, Ventre I, Kulasekara BR, Lazdunski A, Filloux A, Lory S. A novel two-component system controls the expression of *Pseudomonas aeruginosa* fimbrial *cup* genes. Mol Microbiol 2005;55:368–380. [PubMed: 15659157]
19. Labbate M, Zhu H, Thung L, Bandara R, Larsen MR, Willcox MDP, Givskov M, Rice SA, Kjelleberg S. Quorum-sensing regulation of adhesion in *Serratia marcescens* MG1 is surface dependent. J Bacteriol 2007;189:2702–2711. [PubMed: 17237163]
20. Lai HC, Shu JC, Ang S, Lai MJ, Fruta B, Lin S, Lu KT, Ho SW. Effect of glucose concentration on swimming motility in enterobacteria. Biochem Biophys Res Commun 1997;231:692–695. [PubMed: 9070873]
21. Liang W, Pascual-Montano A, Silva AJ, Benitez JA. The cyclic AMP receptor protein modulates quorum sensing, motility and multiple genes that affect intestinal colonization in *Vibrio cholerae*. Microbiology 2007;153:2964–2975. [PubMed: 17768239]
22. Liu JH, Lai MJ, Ang S, Shu JC, Soo PC, Horng YT, Yi WC, Lai HC, Luh KT, Ho SW, Swift S. Role of *flhDC* in the expression of the nuclease gene *nucA*, cell division and flagellar synthesis in *Serratia marcescens*. J Biomed Sci 2000;7:475–483. [PubMed: 11060496]
23. O'Toole GA, Gibbs KA, Hagar PW, Phibbs PV, Kolter R. The global carbon metabolism regulator Crc is a component of a signal transduction pathway required for biofilm development by *Pseudomonas aeruginosa*. J Bactiol 2000;182:425–431.
24. O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol Microbiol 1998;30:295–304. [PubMed: 9791175]
25. Pair SD, Bruton BD, Mitchell F, Fletcher J, Wayadande A, Melcher U. Overwintering squash bugs harbor and transmit the causal agent of cucurbit yellow vine disease. J Econ Entomol 2004;97:74–78. [PubMed: 14998129]

26. Patterson KL, Porter JW, Ritchie KB, Polson SW, Mueller E, Peters EC, Santavy DL, Smith GW. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. Proc Natl Acad Sci USA 2002;99:8725–8730. [PubMed: 12077296]
27. Pratt LA, Kolter R. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol 1998;30:285–293. [PubMed: 9791174]
28. Schmiel DH, Young GM, Miller VL. The *Yersinia enterocolitica* phospholipase gene *yplA* is part of the flagellar regulon. J Bacteriol 2000;182:2314–2320. [PubMed: 10735878]
29. Schmitt CK, Darnell SC, Tesh VL, Stocker BA, O'Brien AD. Mutation of *flgM* attenuates virulence of *Salmonella typhimurium* and mutation of *fliA* represses the attenuated phenotype. J Bacteriol 1994;176:368–377. [PubMed: 8288531]
30. Shanks RM, Caiazza NC, Hinsa SM, Toutain CM, O'Toole GA. *Saccharomyces cerevisiae*-based molecular tool kit for manipulation of genes from Gram-negative bacteria. Appl Environ Microbiol 2006;72:5027–5036. [PubMed: 16820502]
31. Shanks RM, Stella NA, Kalivoda EJ, Doe MR, M ODD, Lathrop KL, Guo FL, Nau GJ. A *Serratia marcescens* OxyR homolog mediates surface attachment and biofilm formation. J Bacteriol 2007;189:7262–7272. [PubMed: 17675374]
32. Silverman M, Simon M. Characterization of *Escherichia coli* flagellar mutants that are insensitive to catabolite repression. J Bacteriol 1974;120:1196–1203. [PubMed: 4373438]
33. Sitkiewicz I, Stockbauer KE, Musser JM. Secreted bacterial phospholipase A2 enzymes: better living through phospholipolysis. Trends Microbiol 2007;15:63–69. [PubMed: 17194592]
34. Soutourina O, Kolb A, Krin E, Laurent-Winter C, Rimsky S, Danchin A, Bertin P. Multiple control of flagellum biosynthesis in *Escherichia coli*: role of H-NS protein and the cyclic AMP-catabolite activator protein complex in transcription of the *flhDC* master operon. J Bacteriol 1999;181:7500–7508. [PubMed: 10601207]
35. Tam C, Lewis SE, Li WY, Lee E, Evans DJ, Fleiszig SM. Mutation of the phospholipase catalytic domain of the *Pseudomonas aeruginosa* cytotoxin ExoU abolishes colonization promoting activity and reduces corneal disease severity. Exp Eye Res 2007;85:799–805. [PubMed: 17905228]
36. Tolker-Nielsen T, Christensen AB, Holmstrom K, Eberl L, Rasmussen TB, Sternberg C, Heydorn A, Molin S, Givskov M. Assessment of *flhDC* mRNA levels in *Serratia liquefaciens* swarm cells. J Bacteriol 2000;182:2680–2686. [PubMed: 10781533]
37. Van Houdt R, Givskov M, Michiels CW. Quorum sensing in *Serratia*. FEMS Microbiol Rev 2007;31:407–424. [PubMed: 17459113]
38. Watnick PI, Lauriano CM, Klose KE, Croal L, Kolter R. The absence of a flagellum leads to altered colony morphology, biofilm development and virulence in *Vibrio cholerae* O139. Mol Microbiol 2001;39:223–235. [PubMed: 11136445]
39. Yokota T, Gots JS. Requirement of adenosine 3′,5′-cyclic phosphate for flagellum formation in *Escherichia coli* and *Salmonella typhimurium*. J Bacteriol 1970;103:513–516. [PubMed: 4317316]
40. Zhao K, Liu M, Burgess RR. Adaptation in bacterial flagellar and motility systems: from regulon members to 'foraging'-like behavior in *E. coli*. Nucleic Acids Research 2007;35:4441–4452. [PubMed: 17576668]

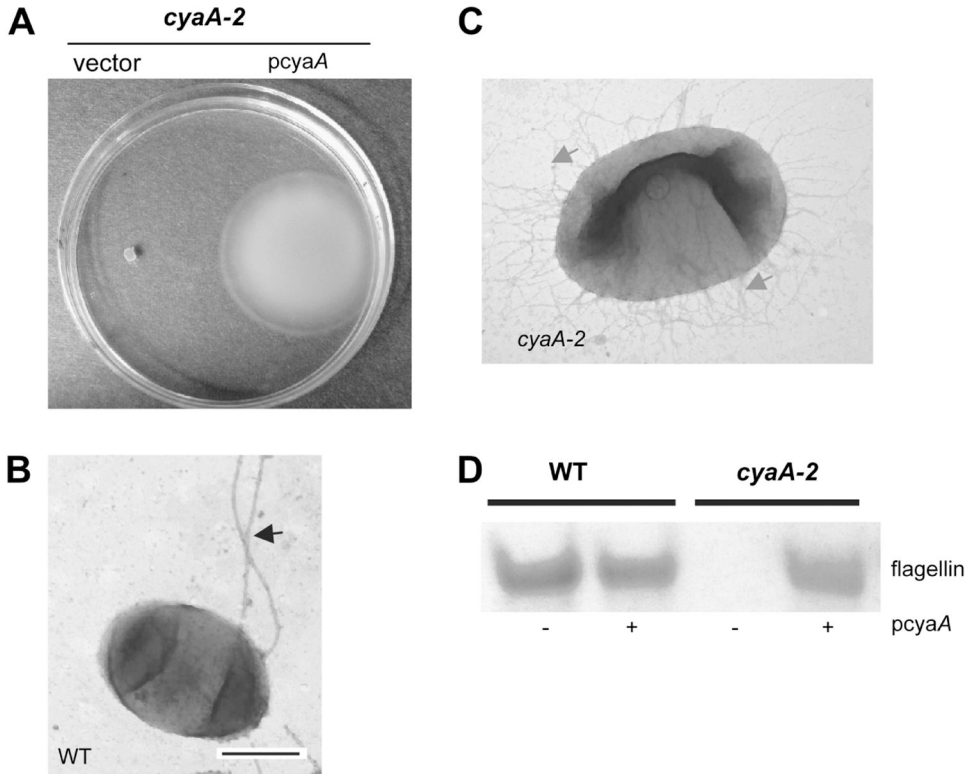

Fig. 1.

Adenylate cyclase is required for swimming motility and flagellum production by *S. marcescens*. A. Photograph of swimming zones through 0.3% agar by the *cyaA* (adenylate cyclase) mutant strain with either an empty vector or the wild-type *cyaA* gene under control of the P_{lac} promoter on a multicopy plasmid (*pcyaA*). B–C. TEM micrographs of wild-type (B) and *cyaA* mutant (C) cells. The black arrow denotes flagella and the gray arrows indicate fimbriae. The bar indicates either 500 nm (B) or 100 nm (C). D. PAGE analysis of surface protein fractions from stationary phase wild-type and *cyaA* cells with vector control or p*cyaA*. The flagellin protein was verified by mass spectroscopy.

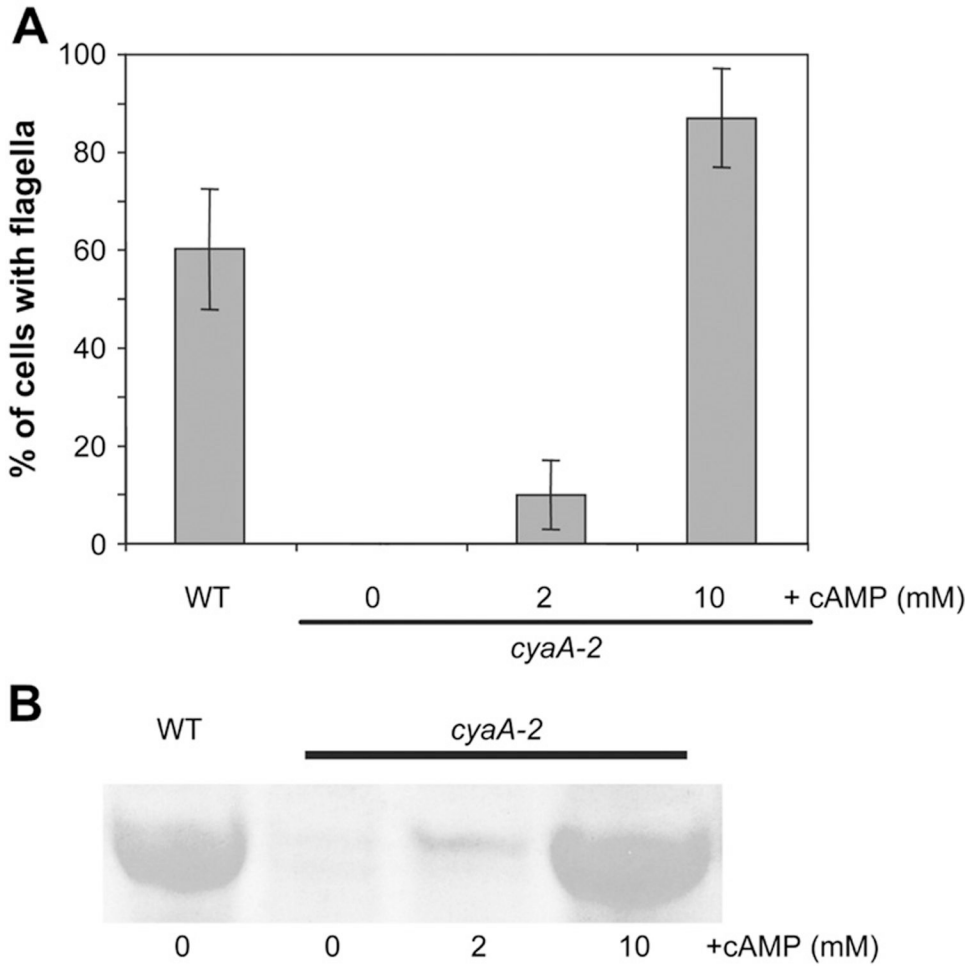

Fig. 2.

Flagellum production can be restored to a *cyaA* mutant by exogenous cAMP. A. The percent of cells with a flagellum observed by TEM microscopy in response to a *cyaA* mutation and increasing amounts of exogenous cAMP (n>200 cells per condition). No flagella were observed among *cyaA* mutant cells without exogenous cAMP (n>400). A statistical difference from the wild type was observed for each condition ($p<0.02$). B. PAGE analysis of surface protein fractions from stationary phase wild-type and *cyaA* cultures grown with increasing concentrations of exogenous cAMP.

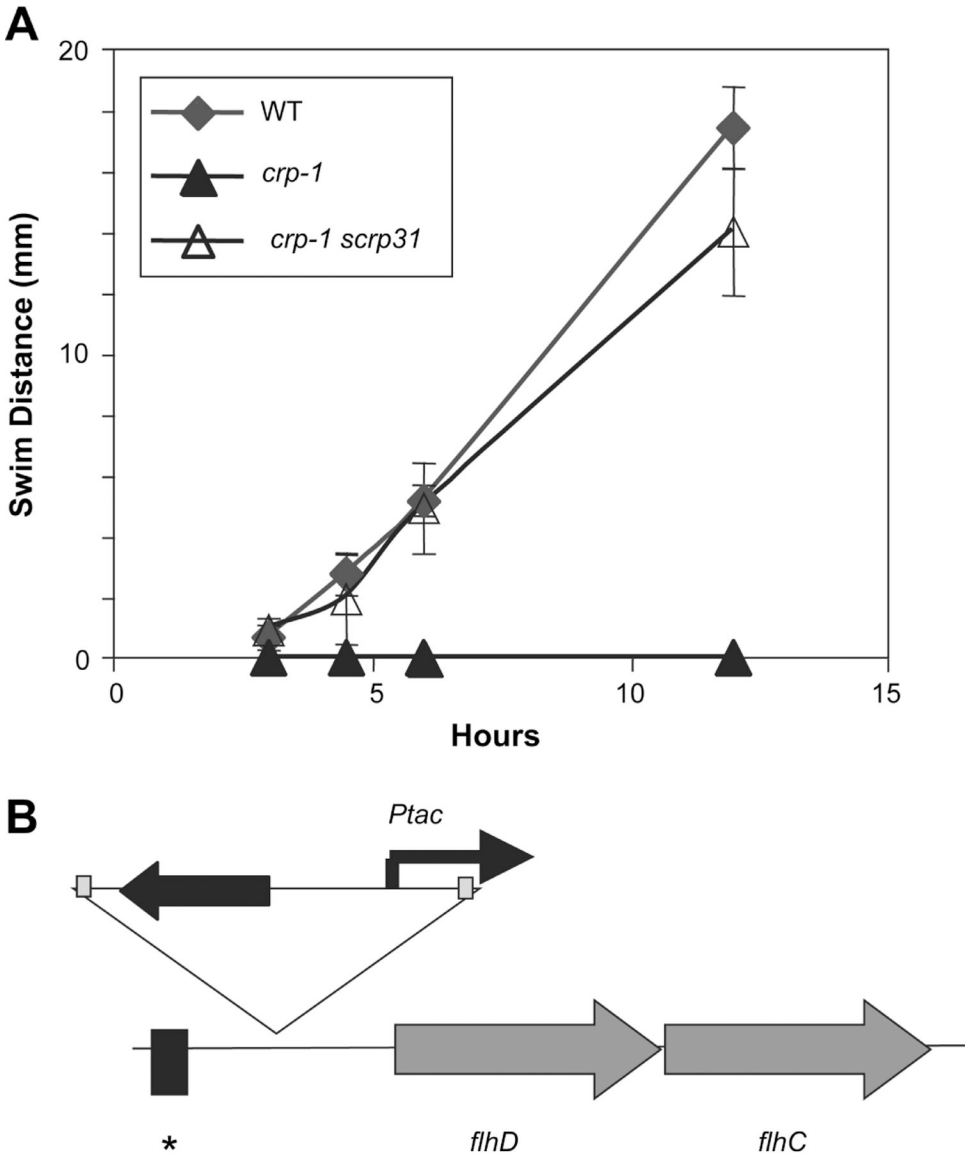

Fig. 3.

The *scrp31* mutation suppresses the swimming and swarming deficiency phenotypes of a *crp* mutant strain. A. Swimming motility charted as a function of time. The *crp scrp31* double mutant is able to swim, whereas the *crp* mutant strain is non-motile. B. Genetic map of the *flhDC* locus with the location of a predicted CRP binding site (black box with asterisk) and the location of a transposon insertion (*scrp31*), not drawn to scale. The transposon bears a P_{tac} promoter, which is directed toward the *flhDC* operon.

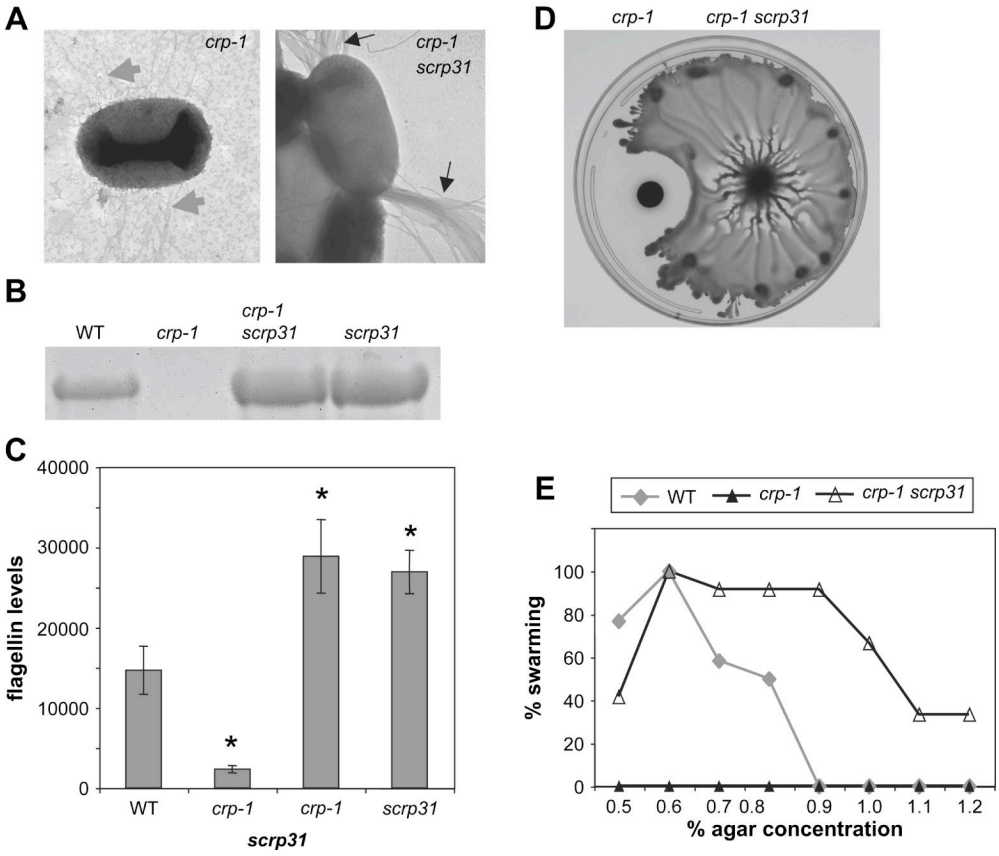

Fig. 4.

The *scrp31* mutation restores flagellum production to a *crp* mutant strain. A. TEM micrographs of a *crp-1* mutant with no flagellum, but numerous fimbriae (gray arrows) and *crp scrp31* double mutant cells covered with numerous flagella (black arrows). The size bar represents 500 and 100 nm respectively. B. PAGE analysis of surface protein fractions from wild-type culture, *crp*, *crp scrp31* and *scrp31* cultures showing flagellin production. C. Quantitation of flagellin levels using Image J analysis software. Surface fractions were taken from stationary phase cultures. Asterisks represent a statistically significant difference from the wild-type ($p<0.01$). D. Swarming motility on LB with 0.7% agar at 48 h. The *crp* mutant swarming defect is rescued by the *scrp31* mutation. E. Plotting the percentage of positive swarming motility experiments at 48 h as a function of agar concentration shows that the *scrp31* mutation confers a hyperswarming phenotype ($n\geq12$ plates for WT and *crp-1 scrp31* and $n\geq8$ for the *crp-1* mutant, performed on 4 separate occasions with similar results).

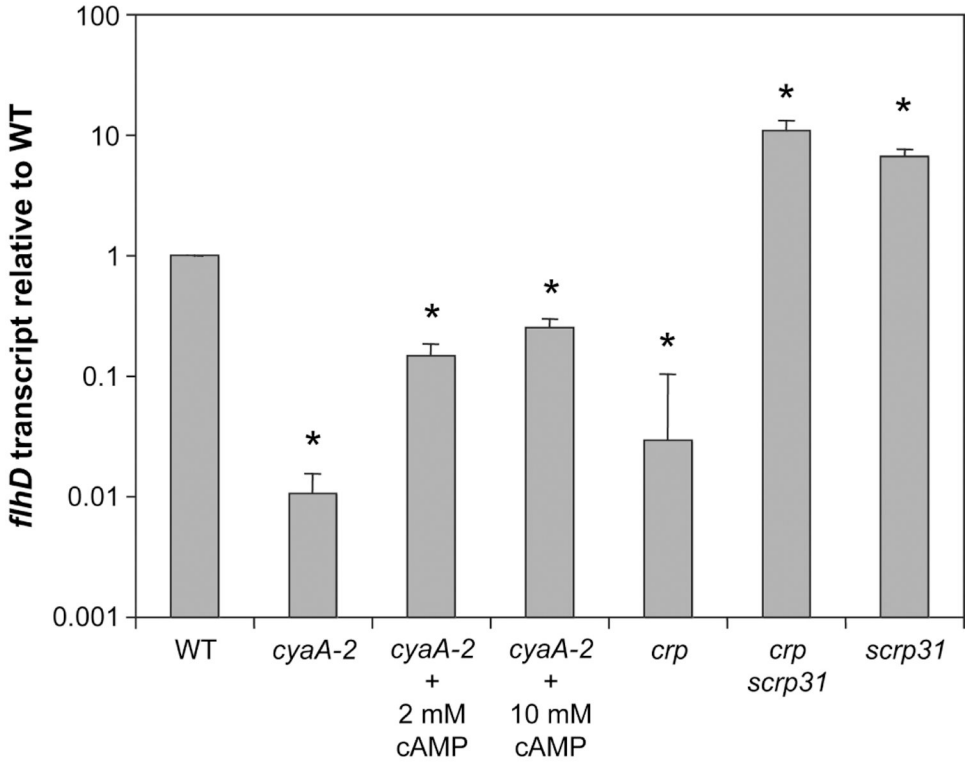

Fig. 5.

Catabolite repression control of *flhD* expression. Quantitative RT-PCR analysis of *flhD* transcript levels relative to wild-type levels shows a significant decrease in transcript produced by the *cyaA* mutant, which is partially restored by the addition of exogenous cAMP to growth medium. The *crp* mutant was similarly reduced in the *flhD* transcript, and was rescued by the *scrp31* mutation which caused a significant increase in *flhD* RNA. RNA was harvested from 3 or more independent cultures at an A_{600} of 1.0. The asterisk indicates a significant difference versus the wild type ($p<0.01$).

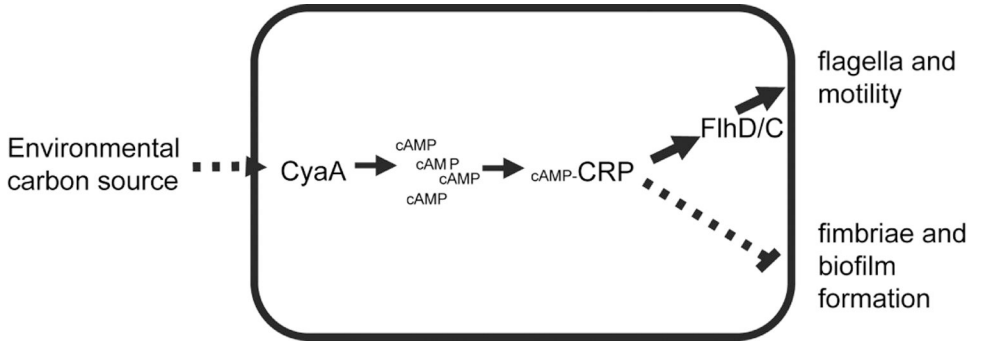

Fig. 6.

Model for coordinated catabolite repression control of attachment and motility processes. The catabolite repression system (CRS) is a signal transduction cascade that responds to environmental carbon. In response to less favorable carbon sources, adenylate cyclase (CyaA) is stimulated to generate cAMP. The activity of the global transcription factor CRP (cAMP receptor protein) is altered through binding with cAMP. The cAMP-CRP complex activates expression of *flhDC*, which in turn activates flagellum synthesis. At the same time, the cAMP-CRP complex directly or indirectly inhibits fimbriae production. Growth conditions with favorable carbon sources led to decreased adenylate cyclase activity and cAMP levels causing a decrease in production of flagella and derepression of attachment factor expression. The result of growth in an environment with a favorable carbon source(s) is decreased production of the flagellum and increased production of fimbriae steering a bacterium toward attachment and biofilm formation. Growth with less favorable carbon source(s) stimulates production of flagella and inhibits production of fimbriae to generate a motile bacterium that is more able to encounter hospitable environments.