Regulation of gene expression during swarmer cell differentiation in *Proteus mirabilis*

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Abstract

The gram-negative bacterium *Proteus mirabilis* can exist in either of two cell types, a vegetative cell characterized as a short rod and a highly elongated and hyperflagellated swarmer cell. This differentiation is triggered by growth on solid surfaces and multiple inputs are sensed by the cell to initiate the differentiation process. These include the inhibition of flagellar rotation, the accumulation of extracellular putrescine and O-antigen interactions with a surface. A key event in the differentiation process is the upregulation of FlhD2C2, which activates the flagellar regulon and additional genes required for differentiation. There are a number of genes that influence FlhD2C2 expression and the function of these genes, if known, will be discussed in this review. Additional genes that have been shown to regulate gene expression during swarming will also be reviewed. Although *P. mirabilis* represents an excellent system to study microbial differentiation, it is largely understudied relative to other systems. Therefore, this review will also discuss some of the unanswered questions that are central to understanding this process in *P. mirabilis*.

Introduction

When cultured on agar plates, *Proteus mirabilis* exhibits a striking form of motility, termed swarming, that results in the formation of motility waves forming distinct terraces on agar plates (Fig. 1a; Mobley & Belas, 1995; Rauprich et al., 1996; Rather, 2005). This distinctive swarming behavior allows for the rapid identification of *P. mirabilis* in clinical microbiology labs by the characteristic bulls-eye pattern of agar-grown colonies. Swarming in *P. mirabilis* was originally described in 1885 by Gustav Hauser, who gave this organism the name *Proteus* based on its ability to change shape. It is now known that this shape change is the result of a complex differentiation process that converts vegetative cells, with a morphology typical of gram-negative members of the *Enterobacteriaceae*, to highly elongated swarmer cells (Fig. 1b) (Rather, 2005). Swarmer cells express levels of flagellin, encoded by the *flaA* locus, that are 10-fold higher than vegetative cells (Belas, 1994). The process of swarming requires that swarmer cells align together to form multi-cellular rafts that translocate across solid surfaces (Jones et al., 2004). The migration of swarmer cells is a transient process and after a period of migration, typically 1–2 h at 37 °C, cells dedifferentiate back to the vegetative form and movement ceases in a process termed consolidation. After a period of growth in the consolidated state, the vegetative cells differentiate back to swarmer cells and a new round of migration initiates. This cycle can repeat multiple times, resulting in the formation of distinct terraces that represent a period of swarming and consolidation and appear as a bulls-eye colony phenotype on agar plates (Fig. 1a). A number of theories have been put forward to explain the cyclic nature of swarming and the onset of consolidation, including changes in gene expression and mathematical models involving changes in population density or water activity at the periphery of the expanding cells (Rauprich et al., 1996; Esipov & Shapiro, 1998; Matsuyama et al., 2000; Medvedev et al., 2000; Arouh, 2001; Lahaye et al., 2007). The nature of consolidation is without question a fascinating area of study, but is outside the scope of this review, which will focus on the regulation of gene expression during swarming.

As a human pathogen, *P. mirabilis* is well known for its ability to cause urinary-tract infections (Mobley & Belas, 1995; Rozalski et al., 1997). These infections primarily occur...
in patients undergoing extended periods of catheterization, such as the elderly or patients with spinal cord injuries. Some aspects of virulence are associated with the swarmer cell state. For example, swarmer cells express virulence factors such as urease, IgA protease, and hemolysin at higher levels than vegetative cells (Allison et al., 1992; Walker et al., 1999; Fraser et al., 2002). Studies have demonstrated that swarmer cells are more invasive of uroepithelial cells than vegetative cells (Allison et al., 1992). In addition, in mouse models of virulence, intravenously injected motile, but nonswarming mutants of \textit{P. mirabilis} were impaired in killing (Allison et al., 1994). In a separate study, a non-motile \textit{flaD} mutant exhibited a 100-fold decrease in colonization in a mouse model of urinary tract infection (Mobley et al., 1996). However, in the above study, a \textit{flaD} mutant would also be unable to swim. Therefore, it is difficult to separate the roles of swimming and swarming in virulence. Taken together, these results suggested that swarmer cells were more virulent than vegetative cells. However, subsequent studies have indicated that the relationship between swarmer cells and virulence is less clear. For example, strains lacking flagella are capable of causing human infections (Zunino et al., 1994; Legnani-Fajardo et al., 1996). In addition, using \textit{P. mirabilis} cells tagged with green fluorescent protein, it was shown that swarmer cells are rarely observed in the urinary tract during infection (Jansen et al., 2003). One possibility is that the role of swarmer cells in virulence and colonization is tissue specific. Further studies will be required to clarify the role of swarmer cells in the pathogenicity of \textit{P. mirabilis}.

**Requirements for the initiation of swarming**

**Surface sensing**

\textit{Proteus mirabilis} only forms swarmer cells when grown on a solid surface; therefore, the ability to sense surfaces is the first requirement for swarmer cell differentiation. Because \textit{P. mirabilis} is a gram-negative, peritrichously flagellated bacteria that expresses flagella in undifferentiated vegetative cells, Belas and colleagues proposed that inhibition of flagella rotation was a physical signal for swarmer cell differentiation in a manner possibly similar to that described for \textit{Vibrio parahaemolyticus} (McCarter & Silverman, 1990; Alavi & Belas, 2001). In liquid media, flagella are able to freely rotate. However, on a solid surface, it is proposed that the rotation of flagella is inhibited leading to activation of swarmer cell differentiation by an unknown mechanism. Several lines of data support the role of flagellar inhibition in swarmer cell differentiation. First, the addition of a thickening agent (polyvinylpyrrolidone) to liquid media resulted in
the formation of swarmer cells (Belas & Suvanasuthi, 2005). The addition of antiflagellar antibody was also able to cause differentiation in liquid media, presumably by interfering with flagellar rotation (Belas & Suvanasuthi, 2005). Mutations in genes involved in construction of the flagella (secretion), or in the expressed copy of flaA, encoding flagellin, result in the inability to differentiate (Belas, 1994; Belas & Suvanasuthi, 2005). Interestingly, some mutations in the flagellar complex lead to differentiation in liquid, a noninducing condition. For example, mutations in FljG (motor-switch complex) and Flil (unknown) lead to elongation in liquid, while being defective in swarming (Belas & Suvanasuthi, 2005). The flil mutation results in nonflagellated cells that constitutively express genes normally upregulated only during swarming. It is not known how Flil works or how it affects swarming, however it is proposed that Flil helps to stabilize the motor complex and the absence of Flil causes stimulation similar to flagella inhibition (Belas & Suvanasuthi, 2005).

Another cellular component that is exposed to a surface is the outer membrane. Previous studies have implicated lipopolysaccharides (LPS) in P. mirabilis swarming (Armitage et al., 1979; Armitage, 1982; Belas et al., 1995; Gue et al., 2001), but the exact role for LPS in swarming is unclear. Upon contact with a solid surface, the LPS undergoes biochemical changes where swarmer cells have a higher proportion of lipid bilayer in the outer membrane than swimmer cells, along with more long chain O-antigen units (Armitage et al., 1979; Armitage, 1982). More recent techniques have been used to show that there are changes in the LPS during differentiation (Gue et al., 2001). Genetic approaches have also succeeded in showing the importance of LPS. Mutants have been found in cld (wzz) (O-antigen chain length determinant), along with genes required for inner core synthesis (waaD, waaC) that are impaired in swarming motility (Belas et al., 1995). Recent studies suggest a more direct role for O-antigen in transmitting surface contact to changes in transcription (Morgenstein et al., 2010). In addition to the outer membrane, an acidic polysaccharide designated Cmf is required for efficient swarming, but not for swarmer cell differentiation (Gygi et al., 1995).

Regulation of the flagellar gene cascade in P. mirabilis

After surface contact and growth, one of the hallmark events associated with the initiation of swarmer cell differentiation is the upregulation of flagellin (FlaA) expression. The regulatory proteins that control flagellin expression in P. mirabilis appear to be conserved with those in Escherichia coli and Salmonella typhimurium and the flagellar regulatory pathway in these organisms are composed of Class 1, 2, and 3 genes (Chilcott & Hughes, 2000; Pearson et al., 2008). The primary Class 1 gene, flhDC, encodes the FlhD2C2 complex, a heterotetrameric transcriptional regulator. The FlhD2C2 complex is central to swarmer cell differentiation and is required for the copious amounts of flagellin produced in swarmer cells. FlhD2C2 also likely regulates additional genes required for swarmer cell differentiation and null alleles in flhDC prevent swarmer cell differentiation in P. mirabilis (Claret & Hughes, 2000). The expression of flhDC is regulated by a variety of environmental conditions and regulatory genes and these are discussed in subsequent sections. During the initiation of swarmer cell differentiation, the levels of flhDC expression increase 10-fold and FlhD2C2 activates the promoters for Class 2 genes in the flagellar cascade that encode the flagellar basal body and hook proteins and the sigma factor σ28 (Claret & Hughes, 2000). The expression of σ28 allows RNA polymerase to transcribe the Class 3 genes, which include genes required for flagellar assembly and the flagellin structural gene, designated flaA in P. mirabilis (Belas, 1994).

The regulation of flhDC is central to swarmer cell differentiation. In synchronously differentiating cells on agar plates, the levels of flhDC typically rise 10-fold at a time point approximately 3–4 h after cells have been plated. Then, after 6–7 h of growth, the levels decrease significantly during the process of consolidation. Mutations have been isolated that result in the failure to decrease flhDC expression during consolidation. These mutations are defined by transposon insertions located at −325 or −740 bp upstream of the start site for flhDC transcription (Clemmer & Rather, 2007). There are no obvious ORFs disrupted by these insertions and they do not change the start site of transcription. These insertions appear to be cis-acting mutations that alter the binding of one or more regulatory proteins that serve to downregulate flhDC during consolidation.

Regulation of gene expression during swarming

Two-component systems (TCS)

TCS are one of the most common ways bacteria control gene expression (Stock et al., 2000). The canonical TCS is made up of a sensor kinase, which senses stimuli and a response regulator, a DNA-binding protein that transcriptionally controls gene expression and is activated by the sensor kinase. Proteus mirabilis has 16 predicted TCS (Pearson et al., 2008), yet as discussed below, only two (Rcs and Rpp) have been shown to directly play a role in swarming.

The RcsCDB phosphorelay

The Rcs system is a complex regulatory system consisting of the RcsC sensor kinase, the response regulator RcsB,
RcsD, a protein that serves as an intermediate in the transfer of phosphate to the response regulator RcsB. Studies in *E. coli* and *Salmonella enterica* serovar Typhimurium have revealed that the system is activated upon surface growth and membrane or peptidoglycan stresses (Sledjeski & Gottesman, 1996; Ferrières & Clarke, 2003; Hagiwara et al., 2003; Laubacher & Ades, 2008). An additional protein in the Rcs phosphorelay is RcsF, an outer membrane lipoprotein that influences RcsC phosphorylation, possibly by transmitting stress signals from the outer membrane (Majdalani et al., 2005; Castanie-Cornet et al., 2006).

In *P. mirabilis*, mutations in the Rcs system result in a hyperswarming phenotype. This was first shown with *rcsD* (rsbA) mutants (Belas et al., 1998; Liaw et al., 2001), but has more recently been shown for *rcsB* mutants (Clemmer & Rather, 2008). This hyperswarming phenotype is most likely due to an increased expression of *flhDC*, although direct binding of RcsB to the *flhDC* promoter has not been established in *P. mirabilis*. An interesting phenotype of mutations in the Rcs system is an elongation phenotype in liquid media, a condition normally nonpermissive for elongation. Mutations that cause overexpression of *flhDC* do not result in a similar phenotype, suggesting that the Rcs regulon may include additional genes involved with cellular elongation (Clemmer & Rather, 2008).

**RppAB**

The *rppA* gene encoding a response regulator was found in a screen for transposon insertions that decreased polymyxin B resistance (Wang et al., 2008). Encoded adjacent to *rppA* is a gene designated *rppB*, encoding a gene product similar to members of the histidine sensor kinase family. A null allele in *rppA* exhibited a hyperswarming phenotype and the levels of *flhDC* were elevated approximately twofold at the initiation of swarming (Wang et al., 2008). In addition, the swarmer cells from an *rppA* mutant were longer than wild type, possibly due to the increased amounts of *flhDC*. There was also a concomitant increase in hemolysin, a toxin normally upregulated during swarming. The presence of polymyxin B is able to repress flagellin expression and swarming and this repression was less apparent in the *rppA* mutant. This suggests that polymyxin B may be sensed by the RppB sensor kinase.

**Additional regulators of *flhDC***

**Umo proteins**

The *umo* loci in *P. mirabilis* were identified as genes that when overexpressed could suppress the swarming defect of an *flgN* mutant, defective in a flagellar chaperone (Dufour et al., 1998). Four *umo* genes were identified, *umoA*, *umoB*, *umoC*, and *umoD*, and each was capable of increasing the expression of *flhDC* when overexpressed. The UmoA and UmoC proteins appear to be unique to *P. mirabilis*. However, the UmoD protein is similar to YcfJ of *E. coli* and UmoB is similar to YrF in *E. coli* and IgaA in *S. typhimurium* (Dufour et al., 1998; Cano et al., 2001). In *S. typhimurium*, IgaA has been shown to interact with the Rcs system in a negative manner, possibly by inhibiting the kinase activity of RcsC. In *S. typhimurium*, mutations in *igaA* result in the overexpression of genes in the Rcs regulon and this phenotype is lethal (Cano et al., 2004; Wang et al., 2007). In *P. mirabilis*, a mutation in the *igaA* ortholog, *umoB*, results in a nonswarming phenotype that is likely due to loss of *flhDC* expression (Dufour et al., 1998). The role of the other Umo gene products in swarming is less clear. A mutation in the *umoD* gene results in loss of swarming, but mutations in *umoA* and *umoC* have little effect on swarming (Dufour et al., 1998). It is currently unknown how the Umo proteins upregulate *flhDC* expression.

**Mrp, a family of proteins controlling adherence or motility**

Bacteria can be either motile or sessile, with the genes for one lifestyle expressed when those for the other are repressed. *Proteus mirabilis* makes a variety of fimbriae, with potentially 17 different fimbrial operons representing five different types, present in the genome (Pearson & Mobley, 2008). While flagella mediate motility, fimbriae are required for adhesion. Because adhesion is the opposite phenotype to motility (swimming or swarming) it makes sense that when fimbriae are upregulated, flagella synthesis is downregulated. The MR/P fimbriae in *P. mirabilis* is encoded in a nine-gene operon (Bahrani & Mobley, 1994) with a transcriptional regulator, MrpI, encoded at the end of the operon (Li et al., 2001). MrpI directly binds the *flhDC* promoter region to repress expression (Pearson & Mobley, 2008). This allows the cell to express either adhesion or motility genes, but not both at the same time. The *mrp* gene was also found to have 14 paralogs, 12 of which repressed motility when overexpressed and a subset of these were shown to repress flagellin expression as well (Pearson & Mobley, 2008). Like MrpI, direct binding of the paralog UcaJ to the *flhDC* promoter region has been demonstrated (Pearson & Mobley, 2008).

**WosA**

The *wosA* gene was identified by the hyperswarming phenotype conferred by overexpression (Hatt & Rather, 2008). Interestingly, in *wosA* overexpressing strains, the initiation of swarming was similar to wild type, which resulted in the designation *wos* (wild type onset with superswarming). The WosA protein is 321 amino acids and predicted to have a cytosolic C-terminus and a transmembrane domain near its
Repressor of secondary metabolites (RsmA)

RsmA is a homolog of the E. coli CsrA protein, a member of a critical global regulatory system that controls the expression of a variety of stationary-phase genes, such as glycogen biosynthesis, catabolism, and biofilm formation (Romeo et al., 1993) by affecting the stability of mRNA (Liu et al., 1995, 1998). In several Enterobacteriaceae, a counterpart of RsmA, RsmB, has been identified. RsmB is an untranslated regulatory RNA that binds and neutralizes RsmA (Liu et al., 1998). CsrA and RsmA are found in many gram-negative bacteria, including Erwinia carotovora ssp. carotovora, Serratia marcescens, and P. mirabilis, and have been linked to swarming regulation and virulence factor expression in these organisms (Cui et al., 1995; Mukherjee et al., 1996; Liu et al., 1998; Wei et al., 2001; Liaw et al., 2003).

In P. mirabilis, RsmA is a 62-amino acid, 6.8-kDa protein with 96% and 94% identity to the E. coli CsrA and E. carotovora ssp. carotovora RsmA, respectively (Liaw, 2003). Proteus mirabilis RsmA also contains the KH motif characteristic of proteins associated with RNA (Siomi et al., 1994) and conserved within other homologs. It has been shown that P. mirabilis rsmA is able to reduce the over-production of glycogen in an E. coli csrA strain to near wild-type levels, supporting the suggestion that Proteus' RsmA is a functional homolog of CsrA. However, CsrA and RsmA do not have the same regulatory affects in all strains and this may be due to differences in the swarming process in various bacteria. In E. coli, CsrA positively regulates swarming and flhDC expression (Wei et al., 2001), while in the Erwinia species, RsmA represses swarming and virulence factor expression (Cui et al., 1995; Mukherjee et al., 1996; Liu et al., 1998). Increasing the expression of RsmA in P. mirabilis inhibits swarming, differentiation of swarmer cells, and the expression of virulence factors, including hemolysin, protease, urease, and flagellin (Liaw, 2003). Hemolysin mRNA was shown to be fully degraded at 8-min post-rifampicin treatment in P. mirabilis overexpressing rsmA, suggesting that RsmA functions by affecting mRNA stability in P. mirabilis, as it does in other gram-negative bacteria. Highly overexpressing rsmA in Proteus leads to complete growth inhibition. Furthermore, overexpressing rsmA from Erwinia or Serratia in a P. mirabilis strain lacking rsmA induces the same inhibition of swarming, differentiation, and virulence factor expression as overexpressing the native P. mirabilis rsmA (Liaw et al., 2003). Finally, overexpressing rsmA in a strain mutant for rsbA (rcsD), a protein required for RcsB phosphorylation and subsequent repression of flhDC (Liaw et al., 2001), suppresses the hyper-swarming phenotype of the rsbA mutant (Liaw et al., 2003).
This observation suggests that the rsmA/B system may interact with the Rcs regulatory pathway.

**Regulation of FlhD2C2 activity**

**DisA, a decarboxylase inhibitor of swarming**

The disA gene was identified by transposon insertion that suppressed the swarming defect in a strain deficient in the production of putrescine (Stevenson & Rather, 2006). However, this suppression is independent of putrescine because both overexpression and mutation of disA has no effect on putrescine levels. In addition, insertions in disA have been isolated in wild-type cells based on the resulting hyperswarming phenotype. Mutation of disA induces cells to undergo early swarming initiation, increases the distance migrated each cycle by 30–35%, and causes cells to leave the consolidation phase at least one and half hours before wild type (Stevenson & Rather, 2006). However, swarmer cell morphology is unchanged in disA mutants and expression of Class 1 genes, specifically flhDC, is largely unaffected (1.4–1.5 fold). Conversely, a significant increase in Class 2 and 3 genes is seen in a disA mutant, with a 16–32-fold increase in flaA (Class 3) mRNA. Overexpression of disA in high or medium copy number leads to total inhibition of swarming and differentiation, and completely blocks mRNA synthesis of Class 2 and 3 genes without significantly reducing flhDC mRNA levels. However, disA overexpression in a strain also overexpressing flhDC still results in complete swarming inhibition.

DisA is homologous to amino acid decarboxylases and most closely resembles those involved in phenylalanine and tyrosine decarboxylation. Because of the similarity of DisA to amino acid decarboxylases, the effects of different decarboxylated amino acids on swarming were tested. The decarboxylated product of phenylalanine, phenethylamine, was able to inhibit swarming by 50% when present at a concentration of 1 mM and completely abolished swarming at 4 mM (Stevenson & Rather, 2006). Additionally, phenethylamine also inhibited the expression of Class 2 and 3 genes while having little affect on Class 1 expression, a phenotype consistent with overexpression of disA. It is proposed that DisA is a phenylalanine decarboxylase and that phenethylamine inhibits swarming by affecting the expression of Class 2 and 3 genes. The fact that flhDC mRNA levels are not altered by the overexpression or absence of disA when Class 2 and 3 genes are affected suggests that inhibition mediated by DisA must occur downstream of flhDC transcription. Based on this data, it has been proposed that DisA targets FlhD and/or FlhC, either by preventing the assembly of the heterotetramer or by inhibiting binding of the heterotetramer to DNA. However, these hypotheses remain to be proven. These models, in conjunction with the fact that DisA expression and swarmer cell development are concomitant, suggest that DisA acts to decrease the expression of genes involved in swarming preparation for the next cycle of dedifferentiation (Stevenson & Rather, 2006).

**Lon protease**

Lon is an ATP-dependent protease that is highly conserved in bacteria (Tsilibaris et al., 2006). In P. mirabilis, a role for the Lon protease in the regulation of gene expression during swarming was revealed by the hyperswarming phenotype of a mini-Tn5 transposon insertion in the lon gene (Clemmer & Rather, 2008). In addition, the lon mutation resulted in the formation of swarmer cells in liquid, which is normally nonpermissive for differentiation. At least one target of the Lon protease appears to be the FlhD protein. The half-life of this protein increased from 8 min in wild-type cells to 32 min in the lon mutant and the increased levels of FlhD likely account for the increased flagellin expression in the lon mutant. However, the ability to differentiate in liquid is probably due to the accumulation of another protein, because mutants that overexpress FlhDC to levels that are higher than the lon mutant do not differentiate in liquid (Clemmer & Rather, 2008). The lon mutation also increased the expression of the virulence genes zapA and hmpBA encoding an IgA protease and hemolysin, respectively. These genes are under FlhDC control (Allison et al., 1992; Walker et al., 1999; Fraser et al., 2002).

**Cell–cell signaling and the regulation of swarming**

**Role of AHL signals and Al-2**

The regulation of gene expression by the secretion of small chemical signals is a process termed quorum sensing (Waters & Bassler, 2005). In several types of bacteria, quorum sensing is required for swarming motility (Lindum et al., 1998; Daniels et al., 2006). The roles of cell–cell signaling in the swarming process of P. mirabilis are just beginning to be addressed. Studies using a luxS mutant of P. mirabilis revealed that the extracellular signal Al-2 did not have a role in the process of swarming (Schneider et al., 2002). In addition, the recently completed sequence of the P. mirabilis genome revealed that the canonical LuxI or LuxM proteins that produce N-acyl homoserine lactone signals are not encoded in the P. mirabilis genome (Pearson et al., 2008). Based on this information, it is unlikely that N-acyl homoserine lactone signaling molecules are produced by P. mirabilis.

**Glutamine**

When grown on minimal media, P. mirabilis is unable to differentiate into swarmer cells. However, the presence of
glutamine allows swarming to initiate on minimal media (Allison et al., 1993). Interestingly, the swarming behavior on minimal media with glutamine is very different than on rich media and is characterized by an absence of the concentric rings seen with swarming on rich media. The mechanisms by which glutamine restores swarming on minimal media is unknown. In addition, this effect may be strain specific, as the swarming of PM7002 is not rescued by glutamine on minimal media (P. Rather, unpublished data).

**Fatty acids**

Liaw et al. (2004) have shown that swarming behavior can be modified by external fatty acids, such as oleic acid, which stimulated swarming and lauric acid and myristic acid, which inhibited swarming. Interestingly, some of these signals were dependent on a functional rsbA (yojN, rcsD) gene for the inhibitory effect, suggesting that these fatty acids may be sensed by the RcsCDB phosphorelay. However, the role of fatty acids as signals for cell–cell communication has not been established in *P. mirabilis* and the physiological role of fatty acids in swarming remains to be determined.

**Putrescine**

A role for putrescine in the regulation of swarmer cell differentiation was revealed by the isolation of mutations in the speA and speB genes that act in a pathway to produce putrescine (Sturgill & Rather, 2004). The SpeA protein is arginine decarboxylase and SpeB is agmatine ureohydrolase that function together to convert arginine to putrescine. Mutations in either speA or speB resulted in a 2–3 h delay in differentiation to swarmer cells. This swarming delay was rescued by adding putrescine to the media or by extracellular complementation via adjacent cells (Sturgill & Rather, 2004). The residual swarming that was present in the speA or speB mutant was likely due to low-level production of putrescine by the SpeC-dependent pathway. Consistent with this, a speA/speC double mutant is unable to swarm (unpublished data).

The mechanism by which putrescine regulates swarming is currently unknown. However, the requirement for putrescine appears to be after activation of the flagellar cascade, as flagellin expression is activated in a normal manner in a speA/speC double mutant during swarmer cell differentiation. Therefore, the putrescine-regulated genes may have a direct role in the cell elongation process.

**Conclusions**

Over the past 10 years, our understanding of the control of gene expression during swarming in *P. mirabilis* has grown significantly. A summary of the genes identified to date that either directly regulate or influence gene expression during swarming are shown in Fig. 2. A central regulatory event during swarmer cell differentiation is the activation of *flhDC* expression during growth on surfaces. As discussed in this review, a variety of gene products have been identified that influence *flhDC* expression, both positively (Lrp, Umo, WosA) and negatively (RcsCDB, RppAB, MrpJ, RsmA, Lon, DisA). Mutations that uncouple swarmer cell differentiation from growth on solid surfaces have been identified and these

![Fig. 2. Key regulators of gene expression during swarming. A summary of the genes that are known to regulate gene expression during swarming in *Proteus mirabilis* is shown. The predicted location of each gene product in the outer membrane (OM), inner cytoplasmic membrane (IM), or cytoplasm is shown.](https://academic.oup.com/femsre/article-abstract/34/5/753/797539)
mutants differentiate to swarm cells in liquid, a normally nonpermissive condition. These mutations include rscC, rcsD, rcsB, lon, and fliL, although in the case of fliL, the differentiated cells do not swarm due to nonfunctional flagella. This suggests that the Rs pathway functions to repress flhDC and additional genes for differentiation and this repression is relieved during growth on surfaces. Incorrect swarm cell differentiation in liquid media is also triggered by overexpression of the WosA protein (Hatt & Rather, 2008). A common feature of both wosA overexpression and mutations in rcs or lon is that they increase flhDC expression 5–20-fold during swarming. However, this alone cannot account for the differentiation in liquid, as other mutants that overexpress flhDC do not have this phenotype, including those that overexpress flhDC to far greater levels than the rcs or lon mutations (Clemmer & Rather, 2007, 2008). Therefore, additional targets of RcsB and Lon likely include those directly involved with cell elongation and/or inhibition of cell division.

Surface sensing

An additional important question that remains unanswered is how growth on a solid surface triggers flhDC activation and additional genes required for cell elongation/inhibition of cell division. The actual sensing of surfaces likely involves multiple mechanisms. Clearly, one aspect of surface sensing involves inhibition of flagellar rotation, however, the regulatory target(s) that are triggered by this inhibition are unknown. Moreover, recent data indicates that a motA mutant, which is unable to rotate its flagella, is still able to activate the flhDC operon during swarming. This suggests that at least one additional pathway exists for surface sensing (R.M. Morgenstein & P. Rather, unpublished data).

A second mechanism for surface sensing may involve O-antigen contact with surfaces. A mutation in O-antigen ligase (waaL) or the O-antigen chain length determinant (wzz) results in the inability to upregulate flhDC on solid surfaces and a failure to swarm (Morgenstein et al., 2010). Interestingly, waaL mutants are able to swim normally, suggesting that the requirement for WaaL is specific to solid surfaces. We propose that O-antigen is acting separately from flagella inhibition as a surface sensor to control flhDC expression and flagella inhibition acts on a different part of the differentiation pathway. How O-antigen can control flhDC expression is not known, but it may work through the Rcs phosphorelay.

How and why do cells consolidate during swarming?

An additional aspect of swarming that is largely unexplored is how and why cells decide to stop swarming and undergo dedifferentiation back to vegetative cells during the process of consolidation. One class of regulatory mutants that control flhDC expression has provided some information regarding this issue. Transposon insertions upstream of the flhDC promoter at positions −325 or −740 result in a novel swarming phenotype, where the mutants fail to consolidate and swarm as a rapidly spreading thin film that does not contain the characteristic concentric rings (Clemmer & Rather, 2008). Interestingly, in these transposon insertions, the expression of flhDC fails to shut down after 6–7 h of growth on surfaces like wild-type cells. From this information, it can be inferred that a critical step in the consolidation process is the decreased expression of flhDC.

A second mechanism contributing to consolidation may be the expression of DisA, a putative phenylalanine decarboxylase. DisA is activated during swarming and the DisA catalyzed production of phenylethylamine acts as an intracellular inhibitory signal that decreases FlhDC activity by an undefined mechanism.

Additional mechanisms are likely to control the timing of consolidation, including changes in cell density (Rauprich et al., 1996; Esipov & Shapiro, 1998; Matsuyama et al., 2000; Medvedev et al., 2000; Arouh, 2001). An appealing mechanism proposed by Harshey and colleagues is that swelling on solid surfaces is controlled, in part, by the accumulation of extracellular signals that trigger differentiation (Toguchi et al., 2000; Harshey, 2003). This model invokes a buildup of extracellular carbohydrates and other components (slime) during growth, which then act as a differentiation signal. As cells move out in swarming rafts, extracellular slime is depleted during the movement and eventually cells are unable to maintain the differentiated state and dedifferentiate back to vegetative cells. Upon regrowth, slime builds up again and differentiation/swarming proceeds for the second cycle. This model remains to be verified in "P. mirabilis." However, extracellular carbohydrates, such as colony-migration factor (Gygi et al., 1995), accumulate during swarming and may have a role in modulating the swarming cycle. Although putrescine could also be considered a candidate extracellular signal for mediating the cycle of differentiation and consolidation, the addition of putrescine to agar plates had little effect on the timing or extent of the differentiation and consolidation cycles (Sturgill & Rather, 2004).

In summary, "P. mirabilis" is an attractive model system to study microbial differentiation and the regulatory mechanisms that are involved, because unlike other bacteria, "P. mirabilis" swarming is highly coordinated with easily visible periods of swarming and consolidation. The recent availability of genome sequences for "P. mirabilis," along with the use of technologies for global analysis of gene expression should facilitate these studies. As a better understanding of gene expression during swarm cell differentiation is compiled, there will be likely novel aspects of gene regulation.
that are revealed. This information may serve as a framework for other systems that involve complex differentiations.

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**Authors’ contribution**

R.M.M. and B.S. contributed equally to this work.

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