Swarming motility

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Swarming involves differentiation of vegetative cells into hyper-flagellated swarm cells that undergo rapid and coordinated population migration across solid surfaces. Cell density, surface contact, and physiological signals all provide critical stimuli, and close cell alignment and the production of secreted migration factors facilitate mass translocation. Flagella biogenesis is central to swarming, and the *flhDC* flagellar master operon is the focal point of a regulatory network governing differentiation and migration.

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Abbreviations

AHL	*N*-acyl-homoserine lactone
Lrp	leucine-responsive regulatory protein

Introduction

Swarming is a powerful means of rapidly colonising nutrient-rich environments, facilitating colony spread and accelerating biomass production. Several flagellated genera typically swarm, for example, *Proteus*, *Vibrio*, *Bacillus* and *Clostridium*, and a comparable, although usually less vigorous behaviour can be induced in the genera *Serratia*, *Salmonella*, *Escherichia*, *Rhodospirillum*, *Azospirillum*, *Aeromonas*, and *Yersinia* (reviewed in [1–3]). Swarming has been extensively studied in *Proteus mirabilis*, in which it is characterised by differentiation of short motile vegetative cells at the colony margin into elongated polyploid hyper-flagellated swarm cells (Figure 1). These differentiated cells align closely along their long axis, forming rafts that migrate as a population by coordinated flagellar action. Regular cycles of mass migration interspersed by population growth without colony expansion (consolidation) result in characteristic large colonies marked by concentric zones, or terraces [4]. *Vibrio parahaemolyticus* can exhibit similar periodic behaviour (reviewed in [5••]), but other swarming bacteria (e.g. *Serratia*, reviewed in [6••]) do not usually display pronounced phases. While the swarming phenomenon has been reported in the literature for over a century, this review describes recent work that indicates the molecular mechanisms underlying swarming differentiation and migration.

Swarming and virulence

Bacterial swimming motility is influential in many pathogen–host interactions [7], and several pathogens are additionally capable of multicellular swarming migration [1,3,8,9•]. Swarming facilitates ascending colonisation of the urinary tract by *P. mirabilis* [10] and may also be coupled to biofilm formation on catheters [11]. A potential survival disadvantage of swarm cell hyper-flagellation (i.e. recognition by the host immune system) may be overcome by flagellin antigen variation, effected by gene conversion [12•].

In addition to enabling rapid population migration, differentiation into hyper-flagellated swarm cells is coupled to the ability of *P. mirabilis* to enter host cells, and to upregulate virulence proteins, including haemolysin, urease and protease [13,14,15•]. Coordinated expression of flagella and virulence factors is also observed in *Serratia liquefaciens*, in which the *phlAB* phospholipase operon is transcribed from a putative σ28 promoter as part of the flagellar regulon [16]. Flagella biogenesis and virulence may even be coupled at the level of protein export, as it is suggested that the *Yersinia enterocolitica* flagellar export apparatus secretes virulence factors [17]. Although flagella and virulence proteins are maximally expressed in *P. mirabilis* swarm cells, these cells do not express non-agglutinating fimbriae (NAF) or mannose-resistant/*Proteus*-like (MR/P) pili [18]. These adhesins are, however, expressed on non-motile cells during the very late stages of swarm colony development [18], suggesting that they might function to maintain *P. mirabilis* biofilms on host tissues.

Swarming stimuli

Swarming is neither a starvation response nor an obligatory development stage [4]. It is nonetheless a radical and reversible change in behaviour in response to the environment. The social nature of swarming indicates that extracellular and possibly cell–cell signals are central stimuli, as are intracellular physiological parameters and contact with a surface. These signals might be sensed and transmitted by two-component regulatory systems, cytosolic regulators, and even cell-surface flagella.

Extracellular chemical signals

Cell density is critical to swarming as the duration of the lag phase that precedes *P. mirabilis* migration is strongly influenced by inoculum density [4,19••], and in *Salmonella enteritidis* the ability to grow to high densities is correlated to virulence, cell elongation and hyper-flagellation [20]. Indeed, 'quorum sensing' mediated by extracellular *N*-acyl-homoserine lactones (AHLs) promotes swarming migration of *S. liquefaciens* [21•], although AHLs are not required for *Vibrio parahaemolyticus* swarming [22]. In *Proteus*, swarming is stimulated by peptides and amino acids, especially glutamine [1,23]. Because *P. mirabilis* produces at least three broad specificity proteases [15•,24], it is possible that protease could generate an extracellular peptide/amino acid signal, especially as protease activity is maximal in consolidating cells [13,15•]. A possible role for peptide or fatty acid signals is also indicated by the finding

that differentiation and migration are impaired by mutations in a locus [25] that encodes a non-ribosomal peptide/polyketide synthase and a short oligopeptide reminiscent of the *Bacillus subtilis* ComX pheromone [26].

How are extracellular swarm signals sensed? A possible sensory route would be via the chemotaxis system (reviewed in [27]). Chemotaxis components are critical for swarming in *P. mirabilis* and *Serratia marcescens* [28,29]. Although chemotaxis components are not required for differentiation of *V. parahaemolyticus* and *Rhodospirillum centenum*, they are needed for colony migration [30–32]. In *Escherichia coli* and *Salmonella typhimurium*, swarm cell differentiation is abolished in *cheA*, *cheW*, *cheR* and *cheY* mutants [33]; however, non-chemotactic *E. coli* flagellar switch mutants (i.e. that have an intact chemotaxis pathway but cannot change direction of flagellar rotation) can swarm [34••]. These data indicate that although the chemotaxis phosphorelay is essential for *E. coli* differentiation, chemotactic behaviour is not.

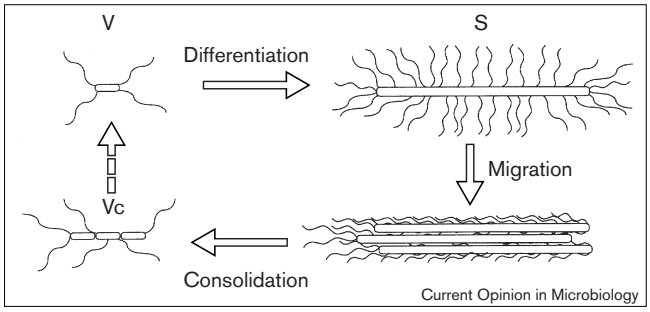
Extracellular signals might also be sensed by two-component regulators, such as RcsC–RcsB in *P. mirabilis* [19••]. Mutations in the *rcsC* sensor kinase gene result in precocious swarming and a constitutive elongated hyper-flagellated phenotype. A similar phenotype is seen when a transposon is inserted in the downstream *rsbA* gene, which encodes a similar membrane sensor kinase. Significantly, *rcsC* and *rsbA* mutants can swarm on minimal media, lacking potential amino acid signals, and *rsbA* mutants initiate migration at a 100-fold lower cell density than wild type. These data indicate that *rcsC* and *rsbA* might encode regulators of swarming behaviour.

Intracellular parameters

Swarming is dependent on the physiological status of cells since high growth rates on nutrient-rich solid media stimulate differentiation and, in some cases, nutrient concentration profoundly influences colony migration [4,35]. Although metabolic cues are important for initiation of *P. mirabilis* differentiation, nutrient (glucose) depletion seems not to be decisive since cells at the centre of a swarm colony are growing exponentially even as the second cycle of differentiation is initiated [4].

Cellular components linking physiological signals to differentiation may include the global transcriptional regulator Lrp (leucine-responsive regulatory protein). A motile but non-swarming *P. mirabilis lrp* mutant does not elongate, hyper-flagellate or hyper-express haemolysin toxin [36]. It is possible that during differentiation Lrp could integrate several signals, particularly as it is known to be responsive to a wide range of metabolites, including amino acids [37]. A role for amino acid concentration is also indicated by the apparent importance of GidA [38], which is encoded by a putative cell division gene that is regulated by ppGpp, the mediator of the stringent response [39]. *P. mirabilis* mutants lacking GidA have a motile but non-swarming phenotype [38]. One further signal may be the level of intracellular cations, as an infrequently consolidating mutant deficient in a P-type ATPase cation transporter is impaired in differentiation and has reduced levels of *lrp* transcript [40].

Figure 1



Stages in the swarming cycle of *Proteus mirabilis*. Vegetative (V) cells at the colony margin differentiate into elongated, hyper-flagellated swarm (S) cells that assemble into multicellular rafts and migrate away from the colony. When migration ceases (consolidation), swarm cells revert to the vegetative (Vc) form.

Surface contact

A pivotal stimulus of swarm cell differentiation is surface contact. Differentiation is induced when viscosity of the growth medium is increased, or when flagella are tethered with antibodies [23,41]. In *V. parahaemolyticus*, a polar sheathed flagellum senses viscosity and regulates expression of the structurally distinct lateral flagella that mediate swarming [41,42]. Compromising assembly of the polar flagellum results in constitutive cell elongation and lateral flagella expression (i.e. loss of response to viscosity) [41], as does chemical inhibition of sodium ion flow through the polar flagellum motor [42,43]. In swarming enterobacteria, which produce a single type of flagella, mutations that disrupt flagella assembly prevent differentiation [44–47]. As components of the chemotaxis phosphorelay pathway are closely associated with the rotating flagellum [27], it might be that the response to increased viscosity is transmitted via CheWAY to cytosolic regulators of swarming.

Multicellular migration and consolidation

What are the factors controlling the initiation, velocity and duration of migration? *P. mirabilis* migration requires close cell–cell contact, with cells aligning along their long axis in multicellular rafts [1,3]. This is highlighted by a motile normally differentiating mutant that does not undergo population migration. In this mutant, disruption of the novel *ccmA* gene causes curved morphology of long swarm cells [48•], suggesting that the migration defect results from an inability to align. Two forms of the membrane-associated CcmA protein are hyper-expressed in differentiated cells, and they may function to maintain linearity of highly elongated swarm cells. Intimate

cell–cell contact within swarm rafts might facilitate coordinated flagellar action and the transmission of short-range intercellular signals.

Cell–cell contact is stabilised by the production of exopolymers that encapsulate swarm cell rafts [6••,35,49] and enhance surface fluidity of the growth medium [6••,49]. *P. mirabilis* produces an acidic capsular polysaccharide that is proposed to create a fluid environment by extracting water from the agar medium beneath the colony [49,50•]. This view is supported by observations that compromising polysaccharide biosynthesis or increasing agar concentration, both of which lower the agar/capsular polysaccharide osmotic activity ratio, reduce migration velocity but do not inhibit differentiation [4,49]. Migration is also facilitated by small secreted molecules; for example, *Serratia* and *Bacillus* species produce cyclic lipopeptide biosurfactants that reduce surface tension [6••,35]. In *S. liquefaciens*, synthesis of the cyclic lipodepsipentapeptide serrawettin W2 is regulated in a cell-density-dependent manner by N-butanoylhomoserine lactone (BHL) and N-hexanoylhomoserine lactone (HHL) [51•], and *B. subtilis* surfactin production is similarly regulated by the ComX pheromone [52].

Observations on multicellular swarm cell raft assembly, and the lag time preceding migration [4], indicate that cell density is a major factor influencing migration. A mathematical model of *P. mirabilis* swarm colony development [53••] suggests that swarm cells must reach a minimum age and population density before migration is initiated, and cessation of migration is similarly proposed to be a function of swarm cell age. This model thus predicts that both migration and consolidation are governed by population dynamics rather than by responses to nutrient depletion or accumulation of chemotactic repellents.

Flagella biogenesis is central to swarming differentiation

Hyper-flagellation is the most prominent feature of swarm cells, and differentiation requires efficient flagella assembly [3,5••,6••,44]. The close coupling of flagella biogenesis to other aspects of differentiation is clearly seen in the *P. mirabilis flhA* flagella export mutant and the *flgN* flagellar assembly (chaperone) mutant, neither of which can hyper-flagellate, elongate or upregulate virulence factors [44,45,54•]. Expression of the flagellar regulon is governed by the *flhDC* master operon, which encodes the heterotetrameric FlhD2C2 transcriptional activator [47,55], and this appears to be the principal regulatory fulcrum during swarm cell differentiation.

The *flhDC* flagellar master operon regulates flagella biogenesis and cell division

P. mirabilis swarm cells express 30-fold more *flhDC* mRNA than vegetative cells, and artificial overexpression of *flhDC* promotes differentiation in *S. liquefaciens* and *P. mirabilis* without the normal requirement for surface contact [46,47]. Relatively small changes in *flhDC* expression can have extensive effects on swarm cell development. In the *flhA* mutant, the block in flagella assembly results in a negative feedback that reduces the peak level of *flhDC* mRNA by 7–12 fold [47]. It is this feedback that causes the elongation defect of the *flhA* mutant, as artificial overexpression of FlhDC restores elongation, even in the absence of hyper-flagellation and swarming [47].

While the primary function of the master operon is the control of flagella biogenesis, it is evident that FlhDC also represses cell division [46,47,56]. In *E. coli*, FlhD controls cell division via the CadA lysine decarboxylase, which is a component of the acid response pathway [57], and although the mechanism is unclear, it does not appear to involve modulation of the expression of essential cell division genes, such as *ftsQAZ* [57]. The view that FlhDC is a 'global regulator' controlling flagella biogenesis, cell septation and possibly expression of virulence factors during swarming is supported by the finding that in *S. liquefaciens* the master operon controls the synthesis of at least 62 proteins [21•].

While there is striking transcriptional upregulation and shutdown of *flhDC* during the *P. mirabilis* swarm cycle, it is also clear that following differentiation rapid decay of the FlhD and FlhC proteins is required. Indeed, these proteins do have a very short half-life, especially post-differentiation, and it seems that this lability is the result of proteolytic breakdown (L Claret, C Hughes, unpublished data). Proteolysis is a common mechanism of post-translational control in developmental processes [58], and during *V. parahaemolyticus* swarming, the LonS protease, which is functionally homologous to *E. coli* Lon, regulates cell elongation and lateral flagella expression [59]. Mutants lacking LonS have a constitutive elongated swarmer phenotype [59], and it is possible that homologues of FlhDC are among the targets for proteolysis.

Integration of signals

It seems likely that *flhDC* is a primary site for the integration of signals inducing swarm cell differentiation, and components have been identified that upregulate the flagellar master operon in *P. mirabilis* swarm cells. The effect of the swarming-essential Lrp on elongation and hyper-flagellation is mediated through *flhDC* since transcription of the master operon is strongly reduced in the motile *lrp* mutant [36]. Swarming can be restored by artificial expression of *flhDC*, indicating that the *lrp* mutant is physiologically capable of supporting swarming but fails to upregulate *flhDC*. In contrast, the regulation of haemolysin expression by Lrp is independent of FlhDC [36].

Four novel *P. mirabilis* proteins (UmoA, UmoB, UmoC and UmoD) upregulate the master operon during differentiation [60••]. Each *umo* gene, *in trans* and multicopy, upregulates expression of *flhDC*, while chromosomal

mutations in the *umo* genes reduce swarming and the amount of *flhDC* transcript. Predicted locations for the Umo proteins in the cell envelope are possibly indicative of a role in sensing environmental signals. Although the *umo* genes do not appear to act in sequence within a pathway to upregulate *flhDC*, both *umoA* and *umoD* are themselves reciprocally regulated by FlhDC and are subject to feedback from the *flhA* block in flagella assembly.

FlhDC, Lrp and the Umo proteins probably function as part of a broader regulatory network that might also include RcsBC, RsbA, and the chemotaxis phosphorelay. In *S. typhi*, the RcsB–RcsC proteins modulate capsule synthesis, flagella biogenesis and expression of invasion proteins in response to osmolarity, and *E. coli* RcsB regulates expression of the cell division protein FtsZ [61]. Although the signals detected by RcsB–RcsC and RsbA in *P. mirabilis* are not yet defined [19••], these regulators might similarly control expression of capsular polysaccharide, cell division, virulence and flagellar genes. Surface contact might be detected by the chemotaxis phosphorelay sensing impedance of flagellar motor function or changes in local proton gradients. The CheWAY proteins could additionally respond to metabolic signals detected by the Tsr and Aer membrane kinases that sense the proton motive force and redox changes in the electron transport chain, respectively [62].

Conclusions

Swarming is a tractable model of bacterial differentiation and multicellularity within a growing colony. Differentiation to swarm cells is based on widely conserved pathways governing flagella biogenesis, motility and septation, rather than the evolution of a distinct developmental programme. Nevertheless, these pathways are subject to modulation during swarming, by altered sensitivity to physiological and environmental signals through known and novel regulators (e.g. RcsBC and RsbA, and the Lrp and UmoA–D proteins) that form an extensive network and act primarily through the *flhDC* master operon. Swarming cells also require extracellular components (e.g. polysaccharide and surfactants) that allow mass migration of differentiated cells over difficult terrain. The hyper-expression of the flagellar gene hierarchy in *Proteus* has highlighted induction and negative regulation barely evident in undifferentiated enterobacterial cells, and the coupling of swarming to virulence, whether through an intrinsic role in colonisation or coregulation of motility and virulence genes, adds an additional level of significance. While several signals are believed to induce differentiation (e.g. surface contact, cell density and amino acids) the pathways of signal integration are still poorly understood, in particular the apparent surface contact-sensing by flagellar filaments and the basis of the cell–cell communication assumed to underlie coordinated migration.

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