The term “acetate switch” refers to the transition from acetate production (dissimilation) to acetate utilization (assimilation). Best studied with the enteric bacterium *Escherichia coli*, the acetate switch takes place as cells deplete their environment of acetate-producing (acetogenic) carbon sources, such as D-glucose or L-serine, and begin to scavenge for the previously produced and excreted acetate (reviewed in reference 15).

The term “quorum sensing” refers to the process by which bacteria monitor population density. This ability to “count” ensures that individual cells do not display behaviors that are advantageous only to groups of cells. These group behaviors include antibiotic production, biofilm development, synthesis of certain virulence factors, and bioluminescence (reviewed in reference 14).

In this issue of the *Journal of Bacteriology*, Studer et al. (10) connect these two distinct phenomena. Specifically, they demonstrate that the luminescent marine bacterium *Vibrio fischeri* uses quorum sensing to control its acetate switch. This finding raises the distinct possibility that acetate assimilation is a group behavior that contributes to the symbiosis of *V. fischeri* with its eukaryotic host, the Hawaiian squid *Euprymna scolopes*.

**Acetate switch.** In *E. coli*, acetate dissimilation requires the Pta-AckA pathway (Fig. 1). This is a high-throughput, energy-producing pathway (reviewed in reference 15) that can impact global signaling through its high-energy intermediate acetyl phosphate (acetyl-P) (3). Acetate assimilation requires AMP-forming acetyl coenzyme A (acetyl-CoA) synthetase (Acs). Because it possesses a high affinity for acetate ($K_m$, 200 μM), Acs can scavenge effectively for small amounts of acetate in the environment (reviewed in reference 15).

**Flipping the switch.** In *E. coli*, the physiological switch from acetate dissimilation to acetate assimilation requires the sequential “flipping” of two molecular switches: the induction of *acs* transcription and the activation of Acs.

*acs* transcription initiates only when the small molecule and second messenger cyclic AMP (cAMP) becomes available, which happens as acetogenic carbon sources become depleted. This CAMP-dependent initiation of transcription involves dynamic interactions between at least two dimers of the CAMP receptor protein (the transcription factor CRP, also known as CAP), at least three copies each of two histone-like nucleoid proteins (Fis and IHF) (reviewed in reference 15), and quite possibly the oxygen-sensitive two-component response regulator ArcA (1).

Acs activity also depends on the availability of small molecules. With acetyl-CoA as its acetyl donor, protein acetyltransferase (Pat) acylates Acs, inhibiting the first, rate-limiting step (9). Using NAD$^+$ as its substrate, the sirtuin CobB deacylates Acs, reactivating it (reviewed in reference 8).

Because glycolysis depends on NAD$^+$ and generates acetyl-CoA, exposure to large amounts of an acetogenic carbon source inhibits both *acs* transcription and Acs activity. As this carbon source becomes depleted, both cAMP and NAD$^+$ accumulate, *acs* transcription initiates, and CobB reactivates Acs. The resultant acetyl-CoA replenishes the NAD$^+$-dependent tricarboxylic acid (TCA) cycle, which delivers electrons to the electron transport chain (ETC) in the form of NADH (reviewed in reference 15).

**Quorum sensing.** *V. fischeri* style. *V. fischeri* possesses three distinct quorum-sensing systems. Two of them, AinS-AinR and LuxI-LuxR, work in sequence to detect and respond to three distinct *V. fischeri* population densities: low, medium, and high. Each pathway uses a signal synthase to produce a distinct acyl-homoserine lactone (HSL), a small molecule that diffuses freely into the environment. As the population increases, each HSL accumulates, diffuses back into the cell, and binds to its receptor, initiating signaling (reviewed in reference 11).

According to the current model (Fig. 2) (reviewed in references 11 and 13), AinS functions as the signal synthase, producing octanoyl-L-HSL (C8-HSL). At low cell density, the C8-HSL receptor AinR (a two-component sensor kinase/response regulator hybrid) remains unbound and, thus, autophosphorylates. The phosphoryl group then transfers in sequence to the histidine phosphotransferase LuxU and the two-component response regulator LuxO. With help from phospho-LuxO, RNA polymerase then initiates transcription of one or more small RNA (sRNA) genes that work in concert with the protein Hfq to destabilize the *luxR* transcript. At medium cell density, C8-HSL accumulates, binds AinR, and converts it to a phosphatase that drains phosphoryl groups via LuxU from LuxO. In the absence of phospho-LuxO, sRNA synthesis does not occur, the *luxR* transcript remains stable, LitR becomes translated, and LuxI-dependent transcription ensues. One result is the transcription of *luxR*. When bound by C8-HSL, LuxR weakly activates transcription of the luminescence (*lux*) genes, including the one that encodes the signal synthase LuxI. This permits low-level production of 3-oxohexanoyl-L-HSL (3O6-HSL). At high cell density, 3O6-HSL accumulates and binds LuxR, which then activates *lux* transcription more

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ishes the NAD$_{\text{A}}$ODPr). The reactivated ACS produces acetyl-CoA, which replen-}

deacetylate ACS, with by-product 2O$^–$/H$^+$-acetyl-ADP-ribose (2O$^–$/H$^+$-acetylase (PTA) converts acetyl-CoA and inorganic phosphate (P$^\text{i}$) requires the Pta-AckA pathway (blue). The enzyme phosphotrans-

disruption of

became excessively acidified. Both behaviors were (i) reversed by the exogenous addition of C8-HSL, (ii) suppressed by the

mutant yielded no detectable CFU, apparently because they

explanation for these observations is that pH homeostasis de-

The AinS-AinR pathway flips the _V. fischeri_ acetate switch.

The AinS-AinR pathway also controls several LuxR-independent

strongly. The result is increased luminescence and other LuxR-

dependent behaviors.

The AinS-AinR pathway flips the _V. fischeri_ acetate switch. The AinS-AinR pathway also controls _acs_ transcription and, hence, the acetate switch (10). This discovery arose from their attempt to understand why the _ainS_ mutant exhibits a growth-yield defect: during growth in unbuffered amino acid-rich, glycerol-supplemented medium, the mutant attains a lower maximum culture density than its wild-type parent (5). In contrast to cultures of the wild-type parent, those of the _ainS_ mutant yielded no detectable CFU, apparently because they became excessively acidified. Both behaviors were (i) reversed by the exogenous addition of C8-HSL, (ii) suppressed by the disruption of _luxO_, and (iii) phenocopied by the disruption of _litR_. They did not, however, depend on LuxR. The simplest explanation for these observations is that pH homeostasis de-

FIG. 1. Acetate switch. Glycolysis (green) metabolizes glucose to acetyl-CoA in an NAD$^+$-dependent manner. Acetate dissimilation requires the Pta-AckA pathway (blue). The enzyme phosphotrans-

zyme ACS. In a two-step process that involves an enzyme-bound in-

intermediate (acetyl-AMP), ACS converts acetate, ATP, and CoA into

Acetate assimilation (red) requires the high-affinity en-

dependent behaviors. The AinS-AinR pathway also controls _luxR_ transcription. _LuxR_ possesses a low affinity for C8-HSL; thus, the _luxR_ transcript. Because _LitR_ is absent, _acs_ transcription remains low; thus, acetate (ace; red diamond) produced by glycolysis and the Pta-AckA pathway (not shown) begins to accumu-

resultant complex can activate _luxR_ transcription. _LuxR_ possesses a low affinity for C8-HSL; thus, the _lux_ operon becomes weakly activated. The result is low-level luminescence (small yellow star) and low-level expression of _luxO_, which encodes the signal synthase LuxI (I). LuxI produces 3OC6-HSL (C6; yellow circle) which, like C8-HSL, freely diffuses in both directions across the cyto-

plasmic membrane (CM) and the outer membrane (OM). At low cell density (LO), the concentration of C8-HSL remains too low to effi-

ciently bind to its receptor AinR (A), which functions as a kinase to

autophosphorylate a conserved histidine residue (H). The phosphoryl
group is passed sequentially from this histidine to a conserved aspartyl

residue (D) within the C-terminal domain of AinR and then to a

conserved histidine (H) in LuxU (U) and finally to a conserved aspartyl

residue in the response regulator LuxO (O). Phospho-LuxO then ac-

tivates transcription of at least one sRNA. In complex with Hfq, the

sRNA(s) destabilizes the _litR_ transcript. Because _LitR_ is absent, _acs_

transcription remains low; thus, acetate (ace; red diamond) produced by glycolysis and the Pta-AckA pathway (not shown) begins to accumu-

ulate. At medium cell density (MED), the acetate has accumulated to a high concentration. However, C8-HSL has also accumulated to a concentration large enough to efficiently bind to AinR, switching it from a kinase to a phosphatase. The _AinR_ phosphatase activity drains phosphoryl groups from LuxO. In the absence of LuxO and hence the inhibitory sRNA(s), _LitR_ can become translated and, via a currently

unknown mechanism, can activate _acs_ transcription. The resultant _Acs_
can begin to assimilate the acetate, converting it to acetyl-CoA. Simulta-

neously, _LitR_ can directly activate _luxR_ transcription. _LuxR_ possesses a low affinity for C8-HSL; thus, the _lux_ operon becomes weakly activated. The result is low-level luminescence (small yellow star) and low-level expression of _luxO_, which encodes the signal synthase LuxI (I). LuxI produces 3OC6-HSL (C6; yellow circle) which, like C8-HSL, freely diffuses in both directions across the CM and OM. At high cell density (HI), 3OC6-

HSL has attained a large enough concentration to efficiently bind to

LuxR. The resultant complex can activate _lux_ transcription efficiently; thus, stronger luminescence ensues (large yellow star). Because _Acs_ has been expressed since the cells were at medium density, the acetate concen-

tration has dramatically diminished.

To identify the AinS-dependent pH effector, the authors analyzed the conditioned media and found large amounts of acetate associated with growth of the \textit{V. fischeri} population. The authors then used a time course experimental design that showed that the wild-type \textit{V. fischeri} experiences the physiological acetate switch. In contrast, the \textit{ainS} mutant does not: it excretes acetate into the environment but does not remove it. Since this behavior resembled that of the \textit{E. coli} \textit{acs} mutant, the authors asked if a \textit{V. fischeri} \textit{acs} mutant behaved like the \textit{ainS} and \textit{litR} mutants. It did. Furthermore, \textit{acs} transcription required \textit{AinS} and \textit{LitR} but not \textit{LuxR}. Moreover, C8-HSL, but not 3OC6-HSL, rescued \textit{acs} transcription in the \textit{ainS} mutant but not in the \textit{litR} mutant. These observations are fully consistent with a model in which the AinS-AinR-LitR pathway controls \textit{acs} transcription and, hence, the acetate switch.

**Role for the acetate switch in symbiosis?** The fact that quorum sensing controls \textit{acs} suggests that the acetate switch of \textit{V. fischeri} is a group behavior and led Studer et al. to test the ability of the \textit{acs} mutant to establish a symbiotic relationship (10). When inoculated alone, the mutant did not exhibit a defect. When placed in competition with its wild-type parent, however, the \textit{acs} mutant lost. From these observations, one can conclude that Acs-dependent acetate assimilation by \textit{acs} (i) exerts little impact at low density and (ii) is not required for establishment of the symbiosis but (iii) plays a key role in the symbiont’s adaptation to its host environment.

**Significance of this work.** The impact of this discovery is manifold. It (i) explains why the \textit{ainS} mutant exhibits a growth-yield defect; (ii) lends support to the hypotheses that LitR functions as a global regulator and that some LitR-dependent regulation is independent of LuxR; (iii) demonstrates that an environmental signal, which is not a nutrient source, can “flip” the acetate switch; and (iv) permits speculation that the acetate switch plays a key role in the establishment of symbiosis. Of course, many questions remain unanswered. For example, does LitR control \textit{acs} transcription directly or indirectly? How does \textit{V. fischeri} integrate LitR-dependent information concerning population density with information concerning nutritional status? At what stage of symbiosis does the acetate switch exert its impact? Does the acetate produced by the bacterial symbiont influence the physiology of its eukaryotic host? Does the host contribute to the acetate switch?

The potential impact of these studies could be quite extensive. The \textit{V. fischeri-E. scolopes} association is monospecific, and the symbiont is genetically facile (6, 7, 12). As such, it represents a stellar laboratory model for learning how considerably more complex symbiotic relationships—such as the mammalian colon with its hundreds of resident bacterial species—generate, exchange, and consume organic acids.

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**REFERENCES**


\textit{The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.}